# Direct and indirect effects of trematode exposure on the amphipod *Paracalliope fluviatilis* in terms of parasite acquisition, survival and behaviour

Olivia Grace McPherson

A thesis submitted for the degree of
Master of Science (Ecology)
University of Otago
Dunedin, New Zealand

December, 2017

**Abstract**

Parasites are a debilitating threat to animal populations as they often increase host mortality. Since defences against parasites are costly, selection should favour individuals that only exhibit defences during times of high risk. As a danger must be interpreted before it can be counteracted, the specific cues used by hosts for parasite detection must first be determined. Knowing the importance of various cues would allow us to better understand the variability of host susceptibility to parasites. This is imperative as parasites affect not only their hosts but also the wider community and ecosystems. Reductions of host density by parasites can have wide repercussions, including indirect effects on interspecific interactions and ecosystem function.

The present research investigates the effect of priming by chemical cues from the trematode *Coitocaecum parvum* on the ability of the freshwater amphipod *Paracalliope fluviatilis* to subsequently defend itself against C*. parvum* infective stages, i.e. cercariae, in laboratory tests. *Paracalliope fluviatilis* are the most abundant endemic amphipod in New Zealand waters and are a key species of prey and an intermediate host for many parasites. In an experimental infection, amphipods were first exposed to the odour of a source of cercariae (infected snail first intermediate hosts) or to control water (no odour), and then to actual cercariae. Subsequently, *Coitocaecum parvum* abundance (numbers of 'new' *C. parvum* parasites per amphipod) and survival were compared between primed and unprimed amphipods to assess the effects this type of chemical priming had on amphipod defence success against trematodes. A further study using EthoVision XT assessed aspects of *P. fluviatilis* behaviour (distance moved, and time spent in motion) in response to priming and the presence or absence of a live *C. parvum* cercaria.

Overall, exposure to the odour of *C. parvum*-infected snails had no effect on the average parasite acquisition of primed amphipods compared to unprimed amphipods. However, amphipods already harbouring parasites from earlier natural infections were 20% more susceptible to acquiring further parasites than uninfected amphipods. Survival was unaffected by exposure to the chemical cue. Swimming behaviour of amphipods was also not affected by either priming or the presence or absence of a cercaria.

These results suggest that other cues may be more significant than the chemical factors tested here for amphipod recognition and avoidance of trematode parasites. Further research will be necessary to determine which cues are the most important for parasite detection in amphipods to better understand the complexity of such host-parasite interactions.

**Acknowledgments**

Firstly, I would like to extend a big thank you to my supervisors Robert Poulin and Christian Selbach for their advice and support throughout the year. Thank you to Robert for sharing your knowledge about parasites and about designing studies, your prompt and critical feedback, and for your uncanny ability to find a relevant paper. Thank you, Christian, for your encouragement when experiments seemed to fall apart, for teaching me laboratory skills such as how to identify cercariae of different parasite species, and for giving me feedback every step of my journey.

I would also like to thank the members of the Evolutionary and Ecological Parasitology Research Group for the comradeship and fun times; Thank you to Olwyn Friesen for your advice regarding amphipod handling, dissections, and guidance regarding EthoVision XT. Thank you to Clément Lagrue for showing me how to care for amphipods and snails in the laboratory. Thank you to Bronwen Presswell for your company in the department, for answering all my questions, and for providing me with stunning photos of parasites.

Thank you to my family and friends for supporting me this year; Thank you to my grandmother Anna Marsich for inspiration and for editing all my first drafts. Thank you to my mother Syneve Marsich for being my first teacher. Thank you to my siblings Kotahi and Daisy for your encouragement. Thank you to my partner Lisandru Grigorut for keeping me afloat during the rough times

Table of contents

List of figures

**List of tables**

# Chapter 1: General Introduction

**Introduction**

There is a substantial body of literature that demonstrates how parasites play key roles in virtually all ecosystems (Combes 1996, Poulin et al. 1998, Thomas et al. 1998, Mouritsen and Poulin 2002, Hechinger and Lafferty 2005, Mouritsen and Poulin 2005, Thompson et al. 2005), with most animal species being host to one of more parasite species (Petney and Andrews 1998, Cox 2001, Kuris et al. 2008, Rohr et al. 2010, Telfer et al. 2010, Balmer and Tanner 2011). In some ecosystems, the biomass of parasites rivals that of predators (Kuris et al. 2008, Lafferty and Kuris 2009). Parasitism is also increasingly recognised as playing a key role in structuring animal communities (Dobson & Hudson, 1986; Minchella & Scott, 1991; Mouritsen & Poulin, 2002; Marcogliese, 2004). By modifying host physiology, behaviour and survival, parasites can alter the ecological function of their host species (Holt & Lawton, 1994; Hudson & Greenman, 1998; Combes, 2001; Hudson et al., 2002; Wood et al., 2007). At the individual level, the fitness costs associated with contracting a parasite can be comparable to those of predator encounters (Rohr et al. 2009), as parasites impose negative effects on their hosts such as reduced growth or reproductive success, behavioural changes, and sometimes even death (Baudoin 1975, Libersat et al. 2009, Poulin 2010). To mitigate these negative effects many animals have a range of defences against parasites which can include pre-contact avoidance measures (behavioural defences such as mate choice, grooming, and selective foraging)(Hamilton and Zuk 1982, Ezenwa 2004, Beltran-Bech and Richard 2014), and post-contact defensive measures such as immune reactions leading to encapsulation and melanisation of the parasites (Combes 2001, Bryan-Walker et al. 2007, Thieltges and Poulin 2008). As animals are likely to encounter parasites multiple times during their life time (Cox 2001, Kuris et al. 2008, Telfer et al. 2008), a major area of interest within the field of parasitology is assessing whether prior exposure to a certain parasite species influences an animal's defence response and their likelihood of avoiding infection, i.e. their future susceptibility to parasites (Bull et al. 1998, Poulin et al. 1999, James et al. 2008, Leung et al. 2010, Rohr et al. 2010).

There is evidence that indirect exposure to pathogens can influence an animal's susceptibility to parasites as animals utilize social information, including chemical signals (Poulin et al. 1999, James et al. 2008), to recognize and use defences to avoid parasites or conspecifics infected with parasites (Kavaliers et al. 2005). Defences against parasites are costly and animals will only initiate defences when necessary, i.e. when they perceive an imminent risk of infection (Gross 1993, Lochmiller and Deerenberg 2000, Rigby and Jokela 2000, Rigby et al. 2002). Several studies in humans have found that the mere visual perception of disease in other people (e.g. photos of skin discolouration, sneezing and vomit) promotes

immune defence responses through increased levels of proinflammatory white blood cells and changes in the oral immune response to expel and defend against any ingested pathogens (Schaller et al. 2010, Stevenson et al. 2011). Another study of vertebrate hosts focused on the selective grazing response of red-necked wallabies (*Macropus rufogriseus*) when given the choice between unsullied maize or maize contaminated with faeces from either predators (which contain odour signatures), intraspecific faeces (which contain compatible endoparasites) or faeces from other sympatric species of wallabies (which contained a less similar gastrointestinal parasite fauna) (Sharp et al. 2015). Wallabies were found to have aversion to predator and parasite olfactory cues separately and the presence of both had an additive effect (Sharp et al. 2015). *Bufo americanus* tadpoles have shown similar avoidance activities in response to parasites, i.e. by avoiding snails shedding trematode cercariae of *Echinostoma trivolvis* (Rohr et al. 2009). These studies found host aversion in vertebrates to visual and odour cues that indicate the presence of parasites. There is evidence that some invertebrates also respond to these cues (Peng et al. 1987, Kurtz and Franz 2003), however, it is unknown how common this ability is within various sub-groups of invertebrates such as arthropods.

Direct exposure to parasites, i.e. past infections, also has been found to play a role in host susceptibility to further parasite infections (Cohen et al. 1961, Bull et al. 1998, James et al. 2008, Leung et al. 2010). This is a critical issue to investigate as animals are often exposed to parasites multiple times during their life time (Petney and Andrews 1998, Cox 2001, Telfer et al. 2010). On the one hand, past infections by a particular parasite species can produce acquired immunity, and reduce the host's future susceptibility to infection by that parasite (James et al. 2008), or diseases, such as how humans are known to acquire immunity to malaria following an initial infection (Cohen et al. 1961, Bull et al. 1998). On the other hand, past infections can increase susceptibility to future infections if there is no acquired mechanism of immunity (Leung et al. 2010, Telfer et al. 2010). Other factors can also limit a host's ability to defend itself, for example, several studies have been conducted to examine how genetic variation and ploidy impact the immune responses of the snail *Potamopyrgus antipodarum*, an intermediate host for the trematode *Microphallus* sp. (Osnas and Lively 2005, Osnas and Lively 2006). Successful infection of snails was greater in sympatric host-parasite combinations as defence was less effective against the locally adapted sympatric parasites (Osnas and Lively 2005) and triploid snails (which have an extra set of chromosomes in their cells) were shown to be more resistant to allopatric parasites than diploids, due to intrinsic genetic properties rather than to greater allocation to defence cells (Osnas and Lively 2006). Rainbow trout (*Oncorhynchus mykiss*) have been shown to avoid infective stages of the trematode *Diplostomum spathaceum* in laboratory conditions (Karvonen et al. 2004). Field studies where fish were

caged in natural conditions showed that physiological defences alone are insufficient to avoid parasite establishment in this species; physiological and behavioural defences are probably used in combination to defend against parasites (Karvonen et al. 2004).

Practically all studies that have investigated the distribution of parasites within wildlife host populations typically find aggregated distribution of parasites, with many hosts free from parasites and some individuals hosting many parasite individuals (Fisher 1941, Crofton 1971, Pennycuick 1971, Poulin 1993, McCallum and Dobson 1995, Shaw et al. 1998). Fisher (1941) was the first to show that parasite distributions were overdispersed (i.e. the variance was greater than the mean) and that the negative binomial distribution fully described the frequency distribution of ticks on sheep (Fisher 1941). This method of quantifying parasite distribution in hosts was popularised 30 years later (Crofton 1971).

One of the factors that may generate this sort of aggregated distribution of parasites among hosts might be a positive effect of current infection on future infection success (Poulin and FitzGerald 1989b, Poulin et al. 1991b). This may be occurring in juvenile threespined sticklebacks (*Gasterosteus aculeatus*) and their associated ectoparasite *Argulus canadensis* (Poulin and FitzGerald 1989b). In experimental situations, the dispersal of this parasite showed a negative binomial distribution. More importantly, in controlled experiments fish that had acquired one parasite incurred a significantly greater risk of acquiring further parasites than uninfected fish (Poulin and FitzGerald 1989b). This study lay out two possible reasons to account for the aggregation of parasites on few hosts, with parasites either taking a passive or active role. The first explanation is that fish with parasites were more susceptible to further parasites because of the influence of the original parasite infection (such as behavioural alteration of the host); the secondary parasite was just more likely to passively encounter that fish than an uninfected fish. This has been demonstrated in a different fish-ectoparasite model system involving brook trout (*Salvelinus fontinalis*) and the copepod parasite (*Salmincola edwardsii*) (Poulin et al. 1991a). An alternative explanation is that parasites actively sought out infected fish through chemoreception of conspecific cues for breeding purposes (Poulin and FitzGerald 1989b). Such a situation has been shown to explain why ticks aggregate on a few individual hosts (Norval et al. 1989). Whatever the explanation, it is clear that infected hosts can sometimes be more susceptible to parasites although more work needs to be done to elucidate the exact reason why these hosts are preferentially infected by parasites.

There is evidence that susceptibility of hosts to parasite infection may be influenced by other parasite species (Rodriguez et al. 1999, Thiemann and Wassersug 2000, Thomas et al. 2002, Telfer et al. 2010).

One study found that the presence of *Taenia crassiceps* cysticerci in mice modified the immune response and increased the host's susceptibility to *Trypanosoma cruzi*, however, these modifications depended on how advanced the initial infection was when the host was exposed to *Tr. cruzi* (Rodriguez et al. 1999). Studies conducted in wild populations of rabbits, mice and human studies show that there are positive and negative associations occurring between different parasite species (Lello et al. 2004, Booth 2006, Behnke 2008). For instance, voles acquire microparasites (such as cowpox and *Anaplasma phagocytophilum*) from ticks which act as vectors for these diseases (Telfer et al. 2010). A long-term study recaptured voles every month for three different years to examine their infection status over time to determine whether susceptibility to infection by a microparasite was influenced by other microparasite species (Telfer et al. 2010). The study found positive and negative associations between different parasite species such as an individual with a new *Babesia microti* infection was 5 times as likely to become infected with *Anaplasma phagocytophilum* compared to uninfected voles (Telfer et al. 2010). On the other hand, voles infected with *Bartonella* spp. were found to be 25% less likely to become subsequently infected by *B. microti* (Telfer et al. 2010). A study of a host-macroparasite-microparasite system examined the mechanism of ticks feeding on mice and found that tick saliva reduces the efficiency of the host's protective immune system against microparasite infections (Ferreira and Silva 1999). There are also studies which examine the effects of priming of fish and cockles (as secondary intermediate hosts of larval trematodes) on susceptibility. Genotype specific host defence in three-spined stickleback (*Gasterosteus aculeatus*) occured in response to encountering larval *Diplostomum pseudospathaceum* trematodes (Rauch et al. 2006). Migration success of *Diplostomum* spp. to Arctic charr (*Salvelinus alpinus*) decreased success of establishment was found in previously parasitised fish (Voutilainen et al. 2010). Within cockles (*Austrovenus stutchburyi*) it has been shown that previous exposure to *Curtuteria australis* benefits a second parasite *Acanthoparyphium* sp. as *C. austalis* encrusts preferentially in the cockle foot tip making it more available to infection by *Acanthoparyphium* sp. (Leung & Poulin 2011). These complex and numerous effects found across different study systems show the importance of examining multiple parasite species in tandem and the importance of examining parasite infection of hosts over time.

This thesis explores the roles of pre-existing infections and the perceived risk of parasite infection on the susceptibility of hosts to parasites, using a trematode-amphipod model system consisting of the amphipod *Paracalliope fluviatilis* and the trematode *Coitocaecum parvum*.

***Paracalliope fluviatilis***

*Paracalliope fluviatilis* is the most abundant and widespread freshwater amphipod endemic to New Zealand, with densities in Lake Waihola, Otago, fluctuating from 315 to 1130 individuals per $m^2$ depending on the time of year (Chapman et al. 1976). They can grow up to 6mm in length (Daniels et al. 2013). Amphipods are benthic and found in slow-flowing reaches in lakes and streams (Sutherland et al. 2007). *Paracalliope fluviatilis* are positively thigmotactic and thus are typically found clinging to macrophytes such as *Myriophyllum triphyllum* midstream, small rocks or on the shells of the mud snail *P. antipodarum (MacFarlane 1939)*. They are herbivores that graze on epiphytic algae and diatoms among the aquatic plants (Sutherland et al. 2007). Their movement is typically epigean, they crawl around and cannot swim against the current (Fenwick 2001). They prefer darker patches of the aquatic environment as they are negatively phototropic (Fenwick 2001). Overall, these behaviours are anti-predatory strategies which help them to avoid their natural predators which include mysids, fish and waterfowl (MacFarlane 1939).

As *P. fluviatilis* are short-lived (<1 year) (Poulin 2001b), successful breeding is especially critical. The breeding season begins in late winter as the water temperature starts to rise (Lefebvre et al. 2005). There is only a window of several hours where females have the opportunity to reproduce, as they can only breed when they have freshly moulted their hard exoskeleton, before their new hard shell forms, whereas males have no temporal restrictions dictating their ability to copulate (Lefebvre et al. 2005). Consequently, amphipods display common reproductive strategies such as precopula pair-forming (Hartnoll and Smith 1980, Lefebvre et al. 2005). Males will either guard or attach themselves to females until they moult. Large females are more fecund and produce more eggs per brood. Size-assortative patterns have been found in *P. fluviatilis* probably due to the ability of larger males to outcompete smaller males for larger, more fecund females (Sutherland et al. 2007). When a female has had her eggs fertilised they will relocate to her brood pouch (Wade et al. 2004). Once they have developed to the juvenile stage they are released as the female moults and the brood no longer need any parental care (Wade et al. 2004).

Freshwater amphipods can function as keystone species (Savage 1996). For instance, densities of the amphipod *Gammarus tigrinus* in an English lake system regulate the abundance of other amphipod, isopod and insect species through competition and predation (Savage 1996). While *P. fluviatilis* are only available as prey for a short period of the year (as they have a lifespan of <1 year) (Poulin 2003), they are an important prey item for fish predators such as juvenile brown trout, shortfin and longfin eels, and

bullies (Jellyman 1989, Hayes and Rutledge 1991, Sagar and Glova 1995). Due to their close association with other species, and their high density, any impact of parasitism on this host species may have broader implications for aquatic communities at a range of trophic levels. Research conducted in New Zealand aquatic systems has also shown that *P. fluviatilis* is useful for environmental monitoring studies due to its sensitivity to toxins (Hickey and Vickers 1994).

*Paracalliope novizealandiae*, a marine amphipod species which is also infected by trematode parasites, are fairly well-studied and are the closest parallels (at least taxonomically speaking) to the current parasite-host system. Studies of this species and its parasites have included *P. novizealandiae* survival in response to infection by the trematode *Maritrema novaezelandensis* (Fredensborg et al. 2004), the interactions of clonal and non-clonal *M. novaezelandensis* within amphipods and the lasting effects of early competition on size (Keeney et al. 2007) and excystment success and egg production in *M. novaezealandensis* (Fredensborg and Poulin 2005). Further studies have examined the seasonal dynamics of *M. novaezelandensis* host species and infection in the Otago Harbour (Studer and Poulin 2012) as well as the degree to which *P. novizealandiae* may use immune defences against *M. novaezelandensis* and how this relates to previous experience with this trematode (Bryan-Walker et al. 2007).

**Chemical reception by crustaceans**

The most ancient form of perception that evolved in aquatic animals is chemoreception – the ability to sense environmental signals in water to inform animals' adaptive responses (Blinova and Cherkashin 2012). Crustaceans also have visual capabilities, however, these have limited usefulness in an aquatic environment as visual cues can be obstructed by factors such as low light levels, sediment resuspension or physical obstacles (Dodson et al. 1994, Kaufmann 1994, Wisenden et al. 1999).

Olfaction and chemoreception in crustaceans differ from each other in many ways, including organization in the periphery, central projections of sensory neurons, and function (Derby 2016). Olfaction is mediated by olfactory sensilla, called aesthetascs, on the lateral flagella of the first antennae and the olfactory nerve centre of the suprapharyngeal ganglion (Blinova and Cherkashin 2012, Derby et al. 2016). The aesthetascs are innervated by olfactory receptor neurons which respond to substances ranging from inorganic to complex organic molecules (Derby et al. 2016). The largest concentrations of olfactory receptors in amphipods occur in callynophores, which are patches of sensory bristles, on the first articles of the antennular flagella (Kaufmann 1994). Chemoreception of crustaceans is mediated by

chemosensilla which can be located on the surface of specific appendages such as the mouthparts and second antennae and on parts of the body (Derby 2016). The fast and regular beating of pleopods (swimming legs) creates water currents which are used for respiration and chemoreception (Dahl 1978). These sensilla are bimodal, innervated by both chemoreceptor neurons and mechanoreceptor neurons (Derby 2016). By directly contacting with the chemical environment, the chemoreceptor apparatus supplies information about the constantly changing ecological situation in water habitats and allows crustaceans to mediate different behaviours (Schmidt and Mellon Jr 2010, Derby and Weissburg 2014)

The main chemicals that are interpreted are termed semiochemicals - chemicals which convey a signal from one organism to another and which modify the behaviour of the recipient organism (Mitchell 2012). There are two subtypes; the first are pheromones which are used for intraspecific communication (Karlson and Lüscher 1959). These include alarm pheromones, sex pheromones, pheromones that dictate larval release and many others that influence behaviour or development (Wisenden et al. 1999, Breithaupt and Thiel 2010, Sehr and Gall 2016). A study looking at pheromone use in *P. fluviatilis* in particular found that these amphipods have the ability to respond to pheromones of conspecifics released during moulting to preferentially mate with morphologically similar amphipods (Sutherland et al. 2009). Using pheromones, male *P. fluviatilis* can distinguish females from genetically distinct clades but not more closely related ones (Sutherland et al. 2009).

The second subtype of semiochemicals are allelochemicals - chemicals produced by one species which intentionally or inadvertently affect the behaviour of another species (Brown et al. 1970). These can be subcategorized as either allomones (benefit originator but not the receiver), kairomones (benefit receiver, without benefitting the emitter) or synomones (where both the sender and emitter benefit) (Brown et al. 1970, Nordlund and Lewis 1976). Application of these signals is diverse but within crustaceans can include identifying heterospecifics, prey and food location, and predator avoidance (Williams and Moore 1982, 1985, Kaufmann 1994). The amphipod *P. fluviatilis* has the ability to detect signals from both predatory and nonpredatory fish (Lagrue and Poulin 2007).

Elucidating the origin and composition of semiochemicals emitted and received by crustaceans is an ongoing process as crustacean chemoreceptor proteins have barely been explored at the genetic and molecular levels compared to other taxa commonly used in studies of chemoreception, such as insects and mammals (Derby et al. 2016). The types of molecules used as pheromones by crustaceans are likely to be different from terrestrial pheromones of mammals or insects (Breithaupt and Thiel 2010, Derby et

al. 2016). There is evidence that pheromones in crustaceans might be released in urine as it contains body metabolites that mirror the internal processes involved in sexual maturation, aggression, and illness (Kamio et al. 2002, Breithaupt and Thiel 2010). Allelochemicals present within a crustaceans' environment includes an array of chemicals depending on the animal and plant species present. Known allelochemicals encountered by crustaceans include the mucus of predatory and nonpredatory fish, arthropodins of heterospecific arthropods, and chemical defences of plants in response to grazing (Williams and Moore 1982, 1985, Cronin and Hay 1996). Semiochemicals may play a key role in influencing parasite-host relationships, comparable to their established role in predator-prey relationships. A few studies in crustaceans have considered the susceptibility of hosts to both enemies (predators and parasites) in tandem through exposure to cues from these enemies (Lass and Bittner 2002, Hesse et al. 2012). In one study, *Daphnia galeata* were exposed indirectly to predatory fish through chemicals cues and directly exposed to parasite (*Caullerya mesnili*) infections and it was shown that some responses were directly opposed (e.g. in the presence of predator kairomones *Daphnia* increased fecundity, however parasites suppressed this response) (Lass and Bittner 2002). In a second study, *Daphnia magna* was exposed directly to spores of the yeast parasite *Metschnikowia* sp. and it was found that the parasite suppressed several predator-induced defences (Hesse et al. 2012). Thus far no study has examined whether chemical cues associated with the presence of parasites could affect the subsequent anti-parasite defences of amphipods or their susceptibility to infection.

**Parasites of *Paracalliope fluviatilis***

There are four different endoparasites known to infect *P. fluviatilis* (MacFarlane 1939, Hine 1977, Rauque et al. 2011, Presswell et al. 2014). The first is the acanthocephalan *Acanthocephalus galaxii* which infects *P. fluviatilis* through their accidental ingestion of this parasite's eggs (Hine 1977). Here the parasite successively develops into an acanthella and cystacanth stage, until the host is consumed by a suitable fish host, where the parasite develops into an adult. Two trematode species, *C. parvum* and *Maritrema poulini* both infect the amphipod via penetration of the cuticle as free-swimming cercariae shed from their shared first intermediate host - the snail *P. antipodarum* (MacFarlane 1939, Presswell et al. 2014). The fourth species is an unidentified cyclophyllidean cestode which specific infection mechanism and definitive host are currently unknown (Rauque et al. 2011). Infection with any one, or with a combination of these different parasite species can have a range of effects on the amphipod host (Rauque et al. 2011, Friesen et al. 2017). These parasites have different strategies to complete their life cycle due to their unique adaptations and respective definitive hosts, therefore different parasite species do not influence amphipods in the same way (Rauque et al. 2011, Friesen et al. 2017). Infection with

either *A. galaxii* or *C. parvum* has been shown to increase photophilia of amphipods, which may increase the likelihood of becoming eaten by a viable definitive fish host (Rauque et al. 2011). These parasites can work in collusion with each other or antagonistically (Friesen et al. 2017). For example, *P. fluviatilis* survival is reduced when amphipods are infected with both *C. parvum* and *M. poulini* but the presence of the latter stops the former from affecting amphipod photophilia (Friesen et al. 2017).

This study focuses on the trematode *Coitocaecum parvum* (family Opecoelidae) which infects New Zealand freshwater fish as definitive hosts (figure 1.1). Their main definitive host is the common bully, *Gobiomorphus cotidianus* but other known definitive host species include smelt, *Retropinna retropinna*, and the inanga*, Galaxias maculatus* (MacFarlane 1939, Holton 1984). Within the gut of their definitive host, they will reproduce sexually and will produce eggs (MacFarlane 1939). These are periodically released with fish faeces into the aquatic environment. Snails become infected when they are penetrated by miracidia, the small and ciliated infective stages hatched from the parasite's eggs (Lagrue et al. 2007). Within *P. antipodarum* the parasite migrates to the gonad and castrates the snail (McCarthy et al. 2004, Lagrue and Poulin 2007). The parasite will also alter the shape of shell growth to maximise space for its own expanding tissues (Lagrue and Poulin 2007). Here it develops into sporocysts – a "cloning factory" – over the course of a month (Lagrue and Poulin 2007). Once developed the sporocysts will asexually produce and release free-living cercariae into the water to seek a suitable second intermediate host. Cercariae are released periodically (daily or every few days) until the death of the snail. The amphipod *P. fluviatilis* is one of three known second intermediate hosts of *C. parvum*; the others include the amphipod *Paracorophium excavatum* and the mysid *Tenagomysis chiltoni* (MacFarlane 1939, Luque et al. 2007). The cercariae are adapted to the thigmotropic nature of *P. fluviatilis* as they are adapted for crawling (MacFarlane 1939). *Coitocaecum parvum* cercariae can only survive in the open water for about five hours (Lagrue et al. 2007). Once they find a suitable host, they penetrate through the cuticle and develop within its body cavity (MacFarlane 1939). Within this second intermediate host, the *C. parvum* cercaria develops into a metacercaria. *Coitocaecum parvum* metacercariae have the ability to alter their development in response to chemical cues the amphipod receives from predatory fish, their definitive host (Lagrue and Poulin 2007). If there are cues from a suitable fish host, the metacercaria develops normally, however in the absence of such cues, when there is no perceived chance of reaching their definitive fish host, metacercariae will become progenetic. In this case, the metacercariae will grow larger, prematurely reach sexual maturity in the intermediate host and produce as many eggs as they can (Lagrue and Poulin 2007). Upon the death of their amphipod host, they release their eggs into the environment to begin the life cycle again. Progenetic *C. parvum*

metacercariae can reach a length of 2mm which can be >50% of the length of the amphipod host (Lagrue and Poulin 2007).

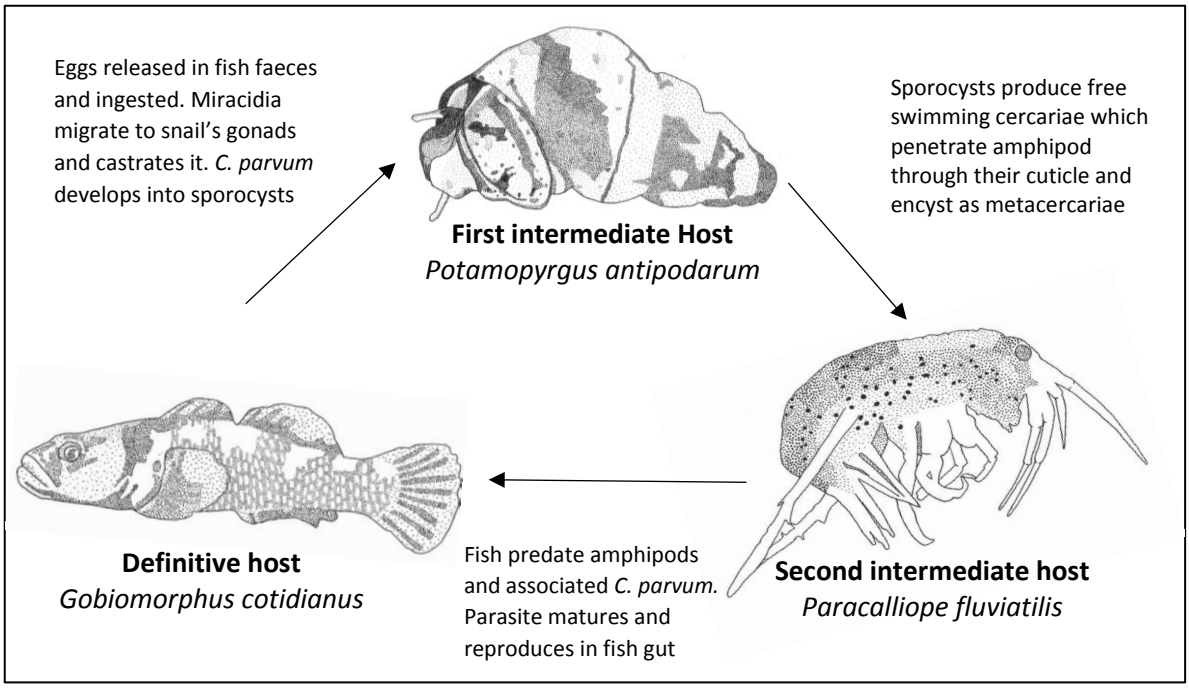


**Figure 1.1 Life cycle diagram of *Coitocaecum parvum***

This study focuses on the stage of the lifecycle where the free-living *C. parvum* cercariae exit the snail host to seek, locate and infect an amphipod host. Amphipods are exposed to parasites directly as cercariae which try to penetrate them, rather than through consumption of parasite eggs (as in their snail host) or through consumption of parasite indirectly through ingestion of prey species (as in the definitive fish host) (MacFarlane 1939). There is no evidence that snails have the ability to distinguish parasite eggs or miracidia in the environment; instead, their adaptive response to a high risk of infection may be to mature rapidly to achieve reproductive success before castration (Lafferty and Kuris 2009). Snails lack adaptive immune systems although some have shown the ability to encapsulate and destroy pathogens with their haemocytes (Horák and Kolářová 2005). *Coitocaecum parvum* seems to have limited direct impact on their definitive hosts as the energy fish save by capturing parasite-debilitated hosts outweighs the cost of infection (Lafferty 1992).

Amphipod hosts have the ability to defend themselves against parasites and suffer from increased mortality if they become infected by trematode cercariae (Friesen et al. 2017). Cercariae are free-living lecithotrophic larvae which typically have an active time of 24 hours or less in which they can penetrate a

host (Anderson and Whitfield 1975, McCarthy 1999, Karvonen et al. 2003); *C. parvum* cercariae are known to stop moving after 5 hours (Lagrue et al. 2017). The presence of trematodes with a free-living cercarial stage is a common feature of most habitats (Derby and Weissburg 2014). Millions of planktonic cercariae enter freshwater habitats on a daily basis (Morley 2012) and possess sophisticated abilities to seek out hosts through visual cues or chemical and tactile cues that indicate host presence (Lewis et al. 1976, Haas 1994, Haas 2003, Chaisson and Hallem 2012). *Coitocaecum parvum* cercariae released from *P. antipodarum* possess a short tail with a sucker to attach to the substrate and spin around to orientate themselves and crawl using their suckers to seek out a suitable host (MacFarlane 1939). As high densities of snails and amphipods are present in macrophyte beds (Hansen and Poulin 2006), this host finding method is appropriate. It has been shown that *C. parvum* abundance within amphipods is not regulated by snail or amphipod densities (Hansen and Poulin 2006), which suggest either limited dispersal of cercariae or local differences of amphipod tolerance or susceptibility to *C. parvum*.

Amphipods have physical, physiological and behavioural defences against parasites (Karvonen et al. 2004, Bryan-Walker et al. 2007). A common behavioural response to the risk of parasites is avoidance of infectious stages (Hart 1994). Grooming is one avoidance behaviour used by marine *Paracalliope* sp. to detach cercariae before they penetrate through their cuticle (Bryan-Walker et al. 2007) and possibly damage cercariae as they are rather fragile (Jewsbury 1985). Another avoidance strategy shown in *Paracalliope* sp. are bursts of movement during incubation with cercariae (Bryan-Walker et al. 2007). This could be adaptive in this case as this could make it harder for the cercariae to find them (Bryan-Walker et al. 2007). Defences also include their hard exoskeleton which functions as a physical barrier against mechanical scratches as well as being a biochemical barrier protecting against microbial invasion (Ashida and Brey 1995, Moret and Moreau 2012). Moulting can also decrease parasite success if an amphipod can shed before a parasite has penetrated through the cuticle (Moret and Moreau 2012). Finally, the crustacean immune system can fight back against parasites that have successfully penetrated through the cuticle through parasite encapsulation and melanisation (Dezfuli et al. 2008).

These defences are expensive, and host's defences may only be used on debilitating parasites. For example, the immune response of the amphipod *Gammarus aequicauda* to the manipulative trematode *Microphallus papillorobustus* has been found to be selectively used based on the associated costs of a parasite (Thomas et al. 2000). This parasite encysts as a metacercaria in either the abdomen of this host or its brain (Thomas et al. 2000). Within the body cavity the parasite has no particular effect, however, if present in the brain, it can strongly alter host behaviour in a way that makes them more vulnerable to

predation by aquatic birds, the parasite's definitive host (Thomas et al. 2000). *Gammarus aequicauda* have been found to preferentially exhibit immune defences against these manipulative parasites with 17% of metacercariae encysted in the brain of hosts being killed by an immune attack leading to encapsulation and melanisation of the parasites, compared to 1% of metacercariae becoming encysted in the body cavity (Thomas et al. 2000). This study found that melanisation can cancel the behavioural alterations induced by the parasite, suggesting that the cause of the manipulation is not the physical presence of metacercariae in the brain (Thomas et al. 2000). Furthermore, it was found that *G. aequicauda* immune system did not attack metacercariae of three other trematode species which all encysted in the amphipod's abdomen, showing that it will only mount an immune response against more costly infection (Thomas et al., 2000).

A further study looked at the amphipod *Gammarus insensibilis* response to four different trematodes (Kostadinova and Mavrodieva 2005). The frequency of melanisation and mean abundance of melanised metacercariae were substantially higher than those observed in the previously mentioned amphipod-trematode system (Thomas et al. 2000). However, the rate of melanisation was lower and showed a significant decrease with amphipod size (Kostadinova and Mavrodieva 2005). Although the four species were differentially melanised, the host response was largely directed against *Microphallus hoffmanni* and *M. subdolum* (Kostadinova and Mavrodieva 2005). It was suggested that the lower melanisation efficiency with age was due to the mode of infection, probably leading to loss of haemolymph and monopolization of the defence resources for wound healing, showing how host response focuses on the most prevalent and abundant species (Kostadinova and Mavrodieva 2005). These studies together show that amphipod hosts' defences are apparently specifically targeted at those metacercariae that debilitate them (Kostadinova and Mavrodieva 2005, Bryan-Walker et al. 2007, Dezfuli et al. 2008).

**The main issue**

As animals are likely to encounter parasites multiple times within their lifetime (Petney and Andrews 1998, Cox 2001, Kuris et al. 2008, Telfer et al. 2010), it is a crucial issue to determine what type of prior experience with a parasite species will affect susceptibility to subsequent parasite infections. There is evidence that both indirect (such as olfactory cues) (Poulin et al. 1999, James et al. 2008) and direct exposure (through parasite infection) (Bull et al. 1998, Leung et al. 2010) to parasites may influence a host's defences when they encounter a parasite again. However, for the majority of host-parasite associations little is known about whether these defences result in an actual decrease in parasite success. Infection by a parasite species has been shown to either increase, decrease or have no influence

on a hosts' susceptibility towards parasites (Coats et al. 2010, Rauque et al. 2011, Friesen et al. 2017). In particular, little is known about how trematode species influence susceptibility of their hosts, both prior to and after initial infection. This study focuses on a common freshwater trematode species, *C. parvum*, and the ability of its second intermediate host, the amphipod *P. fluviatilis*, to defend itself against parasite infection. Amphipods are good models to test for parasite odour recognition and defence behaviours for several reasons. Firstly, amphipods need direct exposure to *C. parvum* to become infected, so it is logical that they would have the time and opportunity to sense the parasite. Secondly, it is known that the amphipod *P. fluviatilis* has a close association with snails from which *C. parvum* cercariae (the stage that infects amphipods) are released; although few snails are infected, cues from the snails may act as cues for infection risk (MacFarlane 1939). Thirdly, *P. fluviatilis* seem to possess a sophisticated olfactory system (Lagrue and Poulin 2007), and it is thus plausible that amphipods are able to discern and avoid snails infected with *C. parvum* based on their "smell", i.e. the chemical cues emitted from an infected snail. Finally, this parasite has negative effects on amphipod fitness, which should have selected for defences against infection. Understanding the influence of both pre-existing infections and perceived infection risk on amphipod susceptibility to further infections is essential to inform a wider understanding of the subtle and diverse effects of parasites on host behaviour and survival.

**Study location**

The current study used one discrete location in Lake Waihola, Otago (46°01'10.2"S 170°05'54.0"E) as a collection site (figure 1.2). Lake Waihola is a freshwater lake with a surface area of 9km$^2$. It is part of a larger wetlands complex (which includes Lake Waipori) with a total area of 2084 ha. Both connected lakes outflow into the Waipori River, then into the Taieri River and into the ocean.


**Figure 1.2 Collection site shown at two scales. A) Lake Waihola and its outflow through the Taieri river into the ocean. B) Details of the exact collection site at Bryan's Bay. The satellite images are taken from Google Maps.**

The Waihola Waipori wetlands are relatively undisturbed and as such represent the best remaining example of a lowland wetland in Otago and one of the largest and most significant remaining areas of wetland in New Zealand (Ausseil et al. 2008). These wetlands are home to a diverse fauna and flora including fifty-five bird species (including the endemic fernbird) and twelve species of native fish (Cromarty and Scott 1995). It is a significant habitat for waterfowl with annual counts for ducks and swans reaching 10,000 and a major breeding and moulting site for waterfowl including Black Swan, Paradise Shelduck, Mallard, Grey Duck, Grey Teal, New Zealand shoveller and New Zealand Scaup (Cromarty and Scott 1995). Several fisheries are maintained in these wetlands such as a whitebait fishery, a commercial eel fishery, and a recreational perch and brown trout fishery (Taylor 2002, Ludgate and Closs 2003, Beentjes 2013).

Lake Waihola is a shallow lake and is therefore strongly influenced by physical forces such as wind-induced sediment resuspension, tidal inputs of water and high inputs of nutrients which shape the water quality and influence community structure of zooplankton and invertebrates, such as amphipods (Schallenberg and Burns 2003). Windy weather has a short-term influence on community structure of small crustaceans as it causes strong waves that toss up sediment, detach animals from the substrate

and reduce light penetration into the water (Schallenberg and Burns 2003). Small crustaceans have a high tolerance for suspended particulate matter (SMP) even in cases when high concentrations of SPM are maintained for several weeks (Schallenberg and Burns 2003). Suspended matter in Lake Waihola contains 30 – 50% organic matter so it is possibly a nutritious food source for some small crustaceans (Schallenberg and Burns 2003). Like many lakes, Lake Waihola receives intermittent tidal inputs of saline water (Moss et al. 1989). Salinity within Lake Waihola is hugely variable as tidal inflows from the Waipori river (of up to 13 ppt) bring high salinity water which can reach 4.6 ppt (Schallenberg and Burns 2003). Freshwater inputs also enter from the Waipori River through regulated discharges associated with the Waipori Hydroelectric Power stations and reverse the salinity gradient (Schallenberg and Burns 2003). The salinity gradients are strongly related to invertebrate community structure, as some small crustaceans, such as cladocerans, can only tolerate a low salinity (Frey 1993, Schallenberg and Burns 2003). Nutrient gradients are also related to community structure but to a lesser degree (Jeppesen et al. 1994). The main riverine source of nutrients to Lake Waihola is from the lower Waipori river as this area has sites of intensive urban and agricultural land use, resulting in the export of large amounts of nutrients, sediment, and other pollutants to the lakes (Schallenberg and Burns 2003).

Lake Waihola has been a sampling location for previous studies on animals of the *C. parvum* study system (Poulin 2003, Lagrue and Poulin 2007, 2008, Friesen et al. 2017). A year-long study investigated the densities of host species across seasons within Lake Waihola, and the prevalence of *C. parvum* within each host species (Lagrue and Poulin 2008). Densities of *P. antipodarum* ranged from 2000-9000 individuals per $m^2$ with peaks in early autumn and early spring. *Paracalliope fluviatilis* fluctuated from 315 to 1130 individuals per $m^2$ in correlation with water temperatures. *Gobiomorphus cotidianus* caught per sampling date ranged from 12 individuals per $m^2$ in winter to 230 in summer with high numbers of juveniles present from January to April (Lagrue and Poulin 2008). In terms of prevalence of *C. parvum*, the snail *P. antipodarum* has natural prevalence of infection of about 1% in the winter months to 5% in summer, but higher infection levels have been occasionally reported in snail populations (see table 1.1). *Paracalliope fluviatilis* have an average *C. parvum* prevalence of 11.2% across all seasons, and in the fish host *G. cotidianus* prevalence of *C. parvum* ranged between 70 to 100% (Lagrue and Poulin 2008). This study found no significant seasonal patterns in *C. parvum* prevalence in any of the host species. Prevalence of *P. antipodarum* in Lake Waihola over different years was averaged at 4.6% (table 1.1).

**Table 1.1 Prevalence data of *Coitocaecum parvum* in *Potamopyrgus antipodarum* from studies in Lake Waihola using data from Lagrue et al. (2007), Lagrue and Poulin (2008)**

| PAPER | YEAR | SNAILS EXAMINED | PREVALENCE |
|---|---|---|---|
| **Current thesis data** | 2017 | 4110 | 8.4% |
| **Christian Selbach (pers. comm.)** | 2017 | 2670 | 0.6% |
| **Lagrue & Poulin** | 2008 | 13797 | 3.4% |
| **Lagrue et al.** | 2007 | 991 | 17.2% |
| | | **Total mean prevalence** | **4.6%** |

**Objectives**

My overall aim was to investigate the effects of pre-exposure to *C. parvum* either directly or indirectly on both behaviour and subsequent acquisition of parasites in the amphipod *P. fluviatilis*. In order to separate out these different types of exposures, and their resulting effects, two objectives were laid out.

Objective one was to investigate whether priming of amphipods through either direct (through pre-existing parasite infections) or indirect exposure to parasites (through exposure to the odour of a source of cercariae) would influence the subsequent likelihood of an amphipod becoming infected by a parasite. Parasite infection levels and survival in amphipods exposed to odour priming were compared to those in control amphipods in standardised exposure trials that accounted for potentially confounding factors, such as amphipod size. My first hypothesis was that there would be a lower prevalence and abundance of parasite infections in amphipod hosts that had been primed to the smell of parasites relative to control amphipods due to higher investment in anti-parasite defences. My second hypothesis was that amphipods with pre-existing parasite infections would accumulate a higher intensity of parasites as infected amphipods, with reduced energy reserves, would be less able to allocate resources towards parasite defence.

Objective two was to investigate the effect of indirect parasite exposure (through exposure to the odour of parasites) on subsequent amphipod swimming behaviour in terms of time spent moving and distance moved. Swimming behaviours of amphipods were assessed through video analysis in EthoVision XT both the presence and absence of parasites. This led to my third hypothesis that amphipods would show marked increase of activity in terms of time spent moving and distance moved as a behavioural response towards parasites in response to the chemical cue compared to control amphipods, and my fourth hypthesis; that no difference in swimming behaviours would be found between amphipods in absence of parasites as natural selection should not favour behavioural changes during times when no cues perceived indicate a threat.

**Overview**

The current study is presented as four chapters consisting of a general introduction (the present chapter), two chapters dedicated to separate laboratory studies and the concluding chapter which presents a general discussion linking the findings of the two experiments together.

The second chapter will focus on whether priming of amphipods through either direct and indirect exposure to parasites influences their subsequent likelihood of becoming infected by a parasite.

The third chapter will focus on whether amphipods can discern the risk of infection through exposure to the odour of a source of cercariae and will assess whether amphipods primed to parasite odour show any difference in swimming behaviour when they are subsequently placed in presence or absence of parasites.

Chapter four integrates the finding of the previous two chapters and provides a general discussion of the findings.

# Chapter 2: Amphipod parasite acquisition and survival in response to direct and indirect exposure to parasites

**Introduction**

It has been established that parasites play key roles in virtually all ecosystems (Combes 1996, Thomas et al. 1998, Mouritsen and Poulin 2002, Hechinger and Lafferty 2005, Mouritsen and Poulin 2005, Thompson et al. 2005), with most animal species becoming infected simultaneously or sequentially by one or more parasite species (Petney and Andrews 1998, Poulin et al. 1998, Cox 2001, Kuris et al. 2008, Telfer et al. 2010, Balmer and Tanner 2011). Trematodes are a ubiquitous class of internal parasite worms which species show a wide range of lifecycles and high diversity (Cribb et al. 2001, Olson et al. 2003). They require a wide range of invertebrate hosts and complete their life cycle within a vertebrate definitive host (Yamaguti 1971, Cribb et al. 2003). Trematodes have been shown in both field and laboratory studies to induce negative effects on their hosts through active manipulation to parasite virulence (Ewald 1995, Koskella and Lively 2007, Lefèvre et al. 2009, Poulin 2010). The various costs involved with trematode infection include impaired development, costly immune responses, and reduced survival (Johnson et al. 1999, Jokela et al. 1999, Sandland and Minchella 2003). These costs increase as the number of parasites present in a given host increases (Crofton 1971, May and Anderson 1978) and have direct effects on host growth, fecundity, behaviour, spatial distribution, and survival, and wider effects on ecological and evolutionary processes (Sousa 1983, Lafferty 1993, Mouritsen and Jensen 1994, Sokolova 1995, Lefèvre et al. 2009).

Trematodes are generally more harmful to their intermediate hosts (Lafferty 1992, Shaw and Dobson 1995). As the definitive host is the site of sexual reproduction for a parasite, natural selection has favoured parasites that limit their pathology to encourage host longevity and allow the parasite to achieve greater fecundity over time (Pennycuick 1971).

On the other hand, larval trematodes typically castrate their gastropod first intermediate host (McClelland and Bourns 1969, Fredensborg et al. 2005, Hechinger and Lafferty 2005), thus greatly reducing the reproductive output of infected individuals (Fredensborg et al. 2005). This can also affect host growth depending on the typical lifespan of the host (Rothschild 1936, Sturrock and Sturrock 1970, Sousa 1983, Mouritsen and Jensen 1994). One common adaptation of female snails to trematode parasites is to mature rapidly (Minchella and Loverde 1981, Jokela and Lively 1995). This enable the host to reproduce prior to infection to ensure at least some degree of reproductive fitness under conditions of intense infection pressure (Sokolova 1995).

Aquatic crustaceans, such as amphipods are common intermediate hosts of trematodes (Thomas et al. 1995a, Bryan-Walker et al. 2007, Luque et al. 2007). Trematodes can influence amphipod behavior, fecundity and survival (Thomas et al. 1995a, Thomas et al. 2002, Lefebvre et al. 2005, Ponton et al. 2005). Since second intermediate hosts are often prey of the parasite's final host, it is a common strategy of trematodes to adaptively facilitate transmission to the definitive host by making the intermediate host more vulnerable to predation by the definitive host (Bethel and Holmes 1977, Lafferty and Morris 1996, Thomas et al. 2002, Ponton et al. 2005, Leung et al. 2010). An example of this is the amphipod *Gammarus insensibilis* which is manipulated by the trematode *Microphallus papillorobustus* found in the brain of the amphipod host (Thomas et al. 2002, Ponton et al. 2005*).* These parasites induce strong behavioural alterations (such as positive phototactism, negative geotactism and an aberrant evasive behaviour) which make the amphipod more vulnerable to predation by aquatic birds, the definitive host of this parasite (Thomas et al. 2002, Ponton et al. 2005, Lefèvre et al. 2009).

To mitigate these negative effects, many amphipod host species have a range of defences against parasites which can include physical, physiological and behavioural defences (Thomas et al. 2000, Bryan-Walker et al. 2007, Thieltges and Poulin 2008). These may include a hard exoskeleton which provides a dual physical and biochemical barrier against parasites, moulting to prevent parasites from penetrating the cuticle, behavioural defences such as selective mate choice, and/or a responsive immune system (Ashida and Brey 1995, Thomas et al. 2000, Moret and Moreau 2012). Since these defences are energetically expensive, they are often only used when the hosts have information that primes them to the high risk of infection, and they are only maintained if they are needed (Thomas et al. 2000, Bryan-Walker et al. 2007).

As animals are likely to encounter parasites multiple times within their life time (Poulin et al. 1998, Cox 2001, Kuris et al. 2008, Telfer et al. 2010), a major area of interest within the field of parasitology is assessing the cues hosts use to detect parasites (Petranka and Fakhoury 1991, Poulin et al. 1999, Kiesecker and Skelly 2000, Van der Wal et al. 2000). Animals can either become aware of a parasite threat through direct contact or indirectly through visual perception of a parasite or through the chemosensory perception of cues released by infected host animals which indicate infection status (Poulin and FitzGerald 1989a, Shaw et al. 1998, Poulin et al. 1999, James et al. 2008).

There is evidence that parasite infection can change the response of a host to future infections by parasites (Bull et al. 1998, James et al. 2008, Leung et al. 2010, Rauque et al. 2011). Direct exposure of the host to parasites may lead to acquired immunity, which develops in response to amassed experience of infection and acts to decrease parasite establishment, survival, reproduction and maturation in future parasite encounters (May and Anderson 1978, Anderson and Crombie 1984, Crombie and Anderson 1985, Dobson et al. 1990, Anderson and May 1991, Wilson et al. 2002).

Increased defences of experienced hosts against future parasite encounters do not have to involve an immune defence response. For example, fathead minnows *Pimephales promelas* have been shown to exhibit no innate ability to recognise or avoid the cercariae of the trematode *Ornithodiplostomum* sp. However, after a single exposure to cercariae experienced *P. promelas* associated parasite risk with the novel chemical and visual cues and reduced their activity, which led to them encountering fewer parasites (James et al. 2008). It remains to be determined whether crustacean hosts can adapt their defences based on experience or whether this experience will result in a reduced success of parasites in the future.

Trematode infections may also increase the vulnerability of hosts to further trematode parasites (Leung et al. 2010, Nissen et al. 2014). It is one of the proposed explanations for the aggregated distribution for patterns of parasites in host populations, where many hosts are parasite-free with a small number of animals hosting a high intensity of parasites (Fisher 1941, Pennycuick 1971, Shaw et al. 1998). Vulnerability to trematode species can vary between taxonomically related host species and may be a determinant of animal community structure, which is why it is imperative to examine the species-specific susceptibility of many different amphipod hosts (Thomas et al. 1995b, Jensen et al. 1998, Friesen et al. 2017). Multiple amphipod-trematode systems have been found to have strongly contrasted patterns of parasite-induced mortality (Thomas et al. 1995b, Jensen et al. 1998, Friesen et al. 2017). For example, in Mediterranean costal lagoons, two French sympatric host species (*Gammarus insensibilis* and *G. aequicauda*), infected by the trematode *Microphallus papillorobustus* show marked differences of decreased survival (Thomas et al. 1995b). Infection by this parasite had no effect on the survival of *Gammarus aequicauda*, but within *G. insensibilis* parasite burden was positively correlated with increased mortality. A similar pattern was found between two New Zealand amphipod species, *Paracalliope fluviatilis* and *Paracorophium excavatum*, infected by two trematodes *Maritrema poulini* and *C. parvum*. It has been found that these trematodes reduced survival of *P. fluviatilis,* but not of *Pr. excavatum* (Friesen et al. 2017). The results of this study suggest that parasite-induced mortality may

influence differences in parasite abundance between amphipod species due to a difference in the virulence of the parasite between hosts as documented in several prior studies both in amphipod hosts and other invertebrates (Park 1948).

There is evidence that host size could play a role in the effect *C. parvum* has on *P. fluviatilis* susceptibility because the probability of *C. parvum* becoming progenetic is related to host size (Daniels et al. 2013). The parasite life history choice within *P. fluviatilis* may influence host susceptibility to further parasite infections, and could potentially have a strong effect on the survival of its host. *Coitocaecum parvum* metacercariae can either grow slowly in anticipation of a transmission event to a definitive host or undergo progenesis, where the immature worms grow rapidly and reach early sexual maturity within this host (Lagrue and Poulin 2007). They will then produce as many eggs as they can, and when their *P.-fluviatilis* host dies they release their eggs into the environment. Normal metacercariae reached an average size of 0.04 mm whereas progenetic metacercariae can reach up to 2 mm depending on the host size (Lagrue and Poulin 2007).

A large proportion of research into parasite-host interactions has examined the host seeking ability (Haas 1994, Haas and Haberl 1997, Martin et al. 2010) without consideration for whether hosts also have the ability to sense and avoid parasites. The common method for assessing host response to parasite encounters involves the experimentally infecting hosts (Thiemann and Wassersug 2000, James et al. 2008). However, in the wild a host will not only encounter parasites through infection; there are hosts which avoid areas with parasites (Petranka and Fakhoury 1991, Cooper et al. 2000, Van der Wal et al. 2000, Karvonen et al. 2004) or infected conspecifics innately (Kavaliers et al. 2005). Several studies have examined the ability of fish to avoid trematode cercariae through the perception of chemical cues (Poulin et al. 1999, James et al. 2008). An example of this is the second intermediate host rainbow trout (*Oncorhynchus mykiss),* which cannot detect the odour of *Diplostomum* sp. trematode cercariae directly but can react to alarm cues released by infected conspecifics (Poulin et al. 1999). *Oncorhynchus mykiss* respond to these cues by changing their activity - performing more random darts and increasing the time they spend stationary (Poulin et al. 1999). A second example of indirect response to trematodes was shown in the tadpoles of *Bufo americanus* exposed to a snail releasing *Echinostoma trivolvis* trematode larvae (Rohr et al. 2009). *Bufo americanus* were indifferent to the presence of an uninfected snail, but exhibited avoidance and elevated activity in response to the presence of a snail shedding cercariae, the presence of only cercariae and the alarm cues of conspecifics (Rohr et al. 2009). However, neither of these studies considered whether these changes in behaviour led to a decreased rate of parasite

infection success. Thus far, the ability of amphipods to perceive trematodes indirectly has not been examined.

Laboratory experiments involving experimental infections of hosts by parasites are valuable tools for investigating parasite lifecycles and pathogenesis, and to test treatments for diseases (Holton 1983, Paull and Johnson 2011, Sauerwein et al. 2011). This chapter uses experimental infections to explore the roles of pre-existing infections as well as the perceived risk of parasite infection, through the perception of chemical cues, on the susceptibility of hosts to subsequent parasite infection. This was conducted using the amphipod *P. fluviatilis* and the trematode parasite *C. parvum*. Findings from these experiments can also illuminate our understanding of the role parasites play in shaping ecosystems or the ability of hosts to defend against parasites (Thomas et al. 1999, Poulin 1998).

**Objectives**

There were two main objectives of this chapter. The first objective was to investigate whether direct or indirect exposure to parasites influenced subsequent infection success of amphipods. The second was to assess whether exposure to a perceived risk of infection (odour treatment) had a negative effect on amphipod survival.

To address the first objective, a factorial experimental design was used in which I tested the effects of previous direct exposure of amphipods to parasites (they either had or did not have prior infections obtained in the field) and indirect exposure to perceived infection risk (amphipods were either exposed or not to chemical cues from snail) on their subsequent defense success against parasites. I hypothesised that (A) amphipods with direct exposure to parasites (pre-infections) would accumulate a higher intensity of parasites due to their increased susceptibility and (B) amphipods that had been pre-exposed indirectly to parasite cues would acquire fewer parasite infections as the perception of imminent risk may boost defense efforts.

The second objective was to assess whether perceived infection risk, and the associated stress and increased defensive effort, had a negative impact on survival of amphipods. As reduced survival has already been shown in *P. fluviatilis* in response to *C. parvum* (Friesen et al. 2017), I hypothesised that *P. fluviatilis* exposed to the odour of a source of parasites (through infected snail water) would have lower survival due to stress associated with parasite cues (Friesen et al. 2017).

## Methods

### Animal Collection and maintenance

Snails (*Potamopyrgus antipodarum*) were collected in May 2017, among macrophytes from Lake Waihola, Otago, New Zealand (46◦01'14.1" S, 170◦05.8" E) using a dip net. Snails were divided into separate tanks depending on whether they were infected with the trematode parasite *Coitocaecum parvum* or uninfected by any parasite. Many trematode parasites, such as *C. parvum*, are known to exploit snails they infect by castrating them and altering their shell shape to increase the volume available for parasite growth (McCarthy et al. 2004). It is possible to distinguish (with 90-95% accuracy) between snails infected with *C. parvum* and snails free of this trematode species due to alterations of typical length and width of the shell whorls (figure 2.1) (Lagrue et al. 2007). Shell shape was therefore used as a preliminary sorting method for infection status. Afterwards, snails suspected of being infected were separated into individual wells of tissue culture plates with filtered lake water so that their infection status could be verified using a brief exposure to higher temperature, which is known to induce cercarial release (Fredensborg et al. 2005, Hay et al. 2005). The plates were incubated at 20°C for three to four hours and then each well was examined under a microscope to check whether cercariae had been released. Snails with *C. parvum* infections and uninfected snails were maintained separately in aerated 10L stock tanks filled with aged lake water and macrophytes (*Chara corallina*) until the end of the experiments (200 infected snails, 200 uninfected snails). Snails which harboured other parasite infections were euthanised.

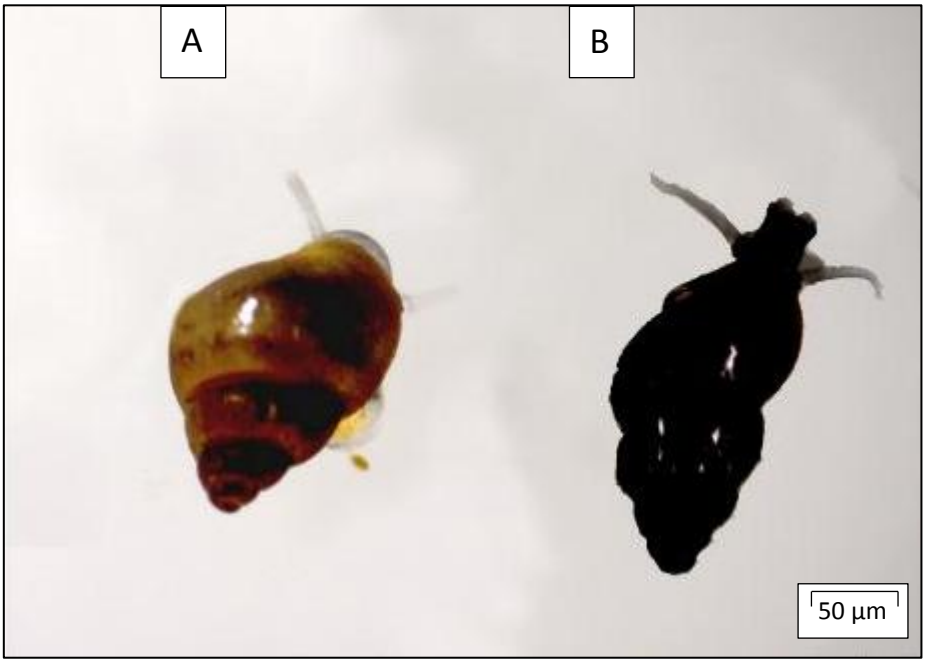


**Figure 2.1 *Potamopyrgus antipodarum* shell morphology difference induced by the trematode *Coitocaecum parvum*. A) snail free of *C. parvum*, and B) snail infected with *C. parvum*. Snails collected from Lake Waihola, Otago.**

Amphipods (*Paracalliope fluviatilis)* were collected as needed throughout May-June 2017. After a 24 hour acclimation period, they were used immediately in the experiment as they cannot easily be maintained for more than two weeks without significant reductions in survival (Friesen et al. 2017). Amphipods were collected concurrently with snails as amphipods are both found in copious quantities clinging to aquatic plants. This population of *P. fluviatilis* has relatively low natural levels of *C. parvum* infection (typically about 11%) (Lagrue and Poulin 2008). Only male amphipods (visually distinguishable from females by typically longer and thinner bodies) were kept for use to eliminate any variation due to sex. Amphipods were kept in the laboratory for six days. They were first maintained in an aerated 2L stock container with aged lake water and macrophytes and left for at least 24 hours to acclimatise. In preparation for odour exposure, amphipods were separated into batches of 20-30 into three 1L containers (one for each treatment group – control, exposed to uninfected snail water, and exposed to infected snail water) (figure 2.2). These containers had similar conditions to the stock container (500ml filtered lake water, aerated and containing macrophytes). Amphipods were acclimatised to these new containers for a further 24 hours prior to the odour exposure.

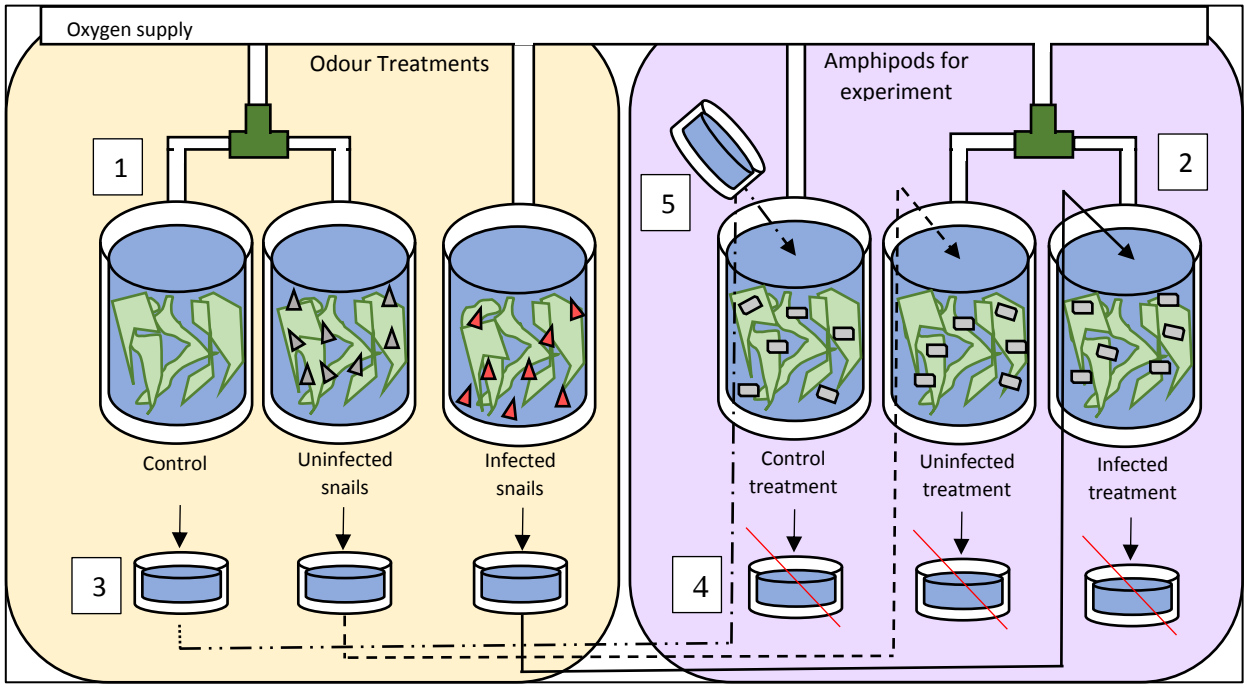


**Figure 2.2 Schematic showing how amphipods (*Paracalliope fluviatilis*) were primed with odour treatments in preparation for experimental infection experiment: 1) Three 1L containers were set up for odour treatments containing 200ml of filtered lake water, macrophytes and a steady oxygen supply. For the control odour treatment, no snails were added, while 15 parasite-free snails (grey triangles) and 15 snails infected with *Coitocaecum parvum* (red triangles) were added to the second and third containers, respectively. Odour treatment containers were left for 48 hours so that water would be conditioned by the snail cues. 2) Three 1L containers were set up for amphipod treatments containing filtered lake water, macrophytes and a steady oxygen supply. Amphipods (rectangles) were added to these containers two days after odour treatments were set up. 3) 200ml of water was removed from each odour treatment container and filtered through a coffee filter to remove any parasites and replaced with 200ml of filtered lake water. The water that was removed was kept aside. 4) Immediately following this, 200ml of water was removed and discarded from the amphipod containers. 5) The water from step 3 was added to the corresponding amphipod containers. 24 hours later steps 3 - 5 were repeated.**

### Odour Treatments

Odour treatments included aged lake water (control), water conditioned with the smell of uninfected snails and water conditioned with the smell of snails infected with *C. parvum*. These treatments will be referred to as control, uninfected snail and infected snail treatment henceforth. The uninfected snail treatment was included so that any specific response of amphipods to indirect exposure to parasites could be separated from any general response they may have to the odour of snails.

Snail density varies throughout Lake Waihola, with average densities of snails found in patches of macrophytes reaching up to 12,477 snails per $m^2$ (Lagrue and Poulin 2015a). The mean infection prevalence of *C. parvum* in the *P. antipodarum* population of Lake Waihola is 4.6% (table 1.1). Snails and

amphipods use macrophyte patches as a food base (Hansen and Poulin 2006). As snails are not very mobile it is assumed that most successful trematode transmissions between hosts would occur in these dense macrophyte rich areas (Hansen and Poulin 2006). A high density of infected snails was used for the odour treatments so that any response of amphipods to trematode infection risk would be clear. The two treatments which used snail conditioned water were prepared by moving 15 infected and 15 uninfected snails respectively from their stock tanks into separate 1L containers filled with 500ml of filtered lake water. The third set of containers were left with only filtered lake water (control) to standardize the treatment. Snails were left in these containers alongside the control container for two days to allow chemical cues to concentrate in the water. Over the next two days, 200ml of water from each snail container and the lake water control was removed and replaced with filtered lake water. Once daily for two days, 200 mL of water was removed from each amphipod container and replaced with the odour treated water from either the control container or from containers conditioned by infected or uninfected snails. The same treatment was applied to a given container for the whole experiment. Odour treatments for the experiment in Chapter 3 were prepared using the same methodology for collection, laboratory maintenance, and odour treatments.

**Obtaining Parasites**

Free-swimming *C. parvum* cercariae were obtained from the stock of infected snails (figure 2.3). One hundred snails were randomly selected from the infected snails' tank and placed in a Petri-dish with 10ml of filtered lake water. To induce cercarial shedding snails were incubated at 20◦C for 30 minutes under constant light (Fredensborg et al. 2005). This usually yielded >100 cercariae per incubation. Cercariae were located using a microscope and transferred using a 20 µL micropipette. Two cercariae were transferred into each 0.6ml Eppendorf tube along with 2.5 µL of lake water. After transport, all cercariae were visually checked and damaged or inactive cercariae were discarded and replaced.


**Figure 2.3 *Coitocaecum parvum* cercaria shed from the snail *Potamopyrgus antipodarum*.**

**Experimental infection**

Amphipods from each treatment group were added to the Eppendorf tubes containing the cercariae immediately after the cercariae were transferred (figure 2.4). The small amount of water used was enough to sustain the amphipods, however, was not enough to allow them to swim freely in the tube. This enabled the parasites to encounter amphipods and limited host defences against parasites to grooming and beating of gills to avoid infection. To ensure no extra water was added to each tube amphipods were first transferred to an individual tissue plate well using a pipette. Any associated water with this transfer was removed and the amphipods were then individually placed into the Eppendorf tubes with an extremely fine paint brush. Amphipods were left in the tube along with the two cercariae for 5 hours at 18◦C, by which time unsuccessful cercariae die (Lagrue et al. 2007). Amphipods which survived the infections were transferred back to their treatment containers and were maintained for a further two days before dissection, to give the successful cercariae time to burrow through the host cuticle and encyst within the body cavity. After this time amphipods were killed in 70% ethanol, rinsed with distilled water and dissected immediately. All parasites found within amphipods were recorded and measured, and the total length of each amphipod was recorded (from rostrum to telson) to the nearest millimeter.


**Figure 2.4 *Paracalliope fluviatilis* maintained in an Eppendorf tube with 2.5 µL for five hours during experimental infection with two *Coitocaecum parvum* cercariae (one is visible and indicated with an arrow).**

Parasites recovered during dissection were catagorised as either new infections (infections of *C. parvum* which occurred during the experimental infection) or pre-existing infections (infections acquired in the field) (Figure 2.5 and 2.6). Infections with other parasite species, i.e. the trematode *Maritrema poulini* or the acanthocephalan *Acanthocephalus galaxii*, were classified as pre-existing infections. *Coitocaecum parvum* parasites were classified as pre-existing if they were larger than 0.33mm as it is unlikely *C. parvum* metacercariae could reach this size a mere two days after infection. *C. parvum* metacercariae were classified as newly acquired if they were 0.1-0.23mm in size. Amphipods containing *C. parvum* in the middle size range 0.24-0.32mm were excluded from analysis as it was unclear whether these parasites were acquired in the field or during experimental infection. This resulted in the exclusion of 10 amphipods in total. The single amphipod found with an *A. galaxii* infection was also excluded from further analysis. The prevalence (percentage of hosts infected) and the abundance (number of metacercariae per host) of parasites per amphipod host were recorded. Amphipods that had pre-existing parasites were classified as having had previous direct exposure to parasites, whereas amphipods that were exposed to the infected snail treatment experienced a perceived risk of infection, i.e. they had indirect exposure to parasites prior to the experimental infections.

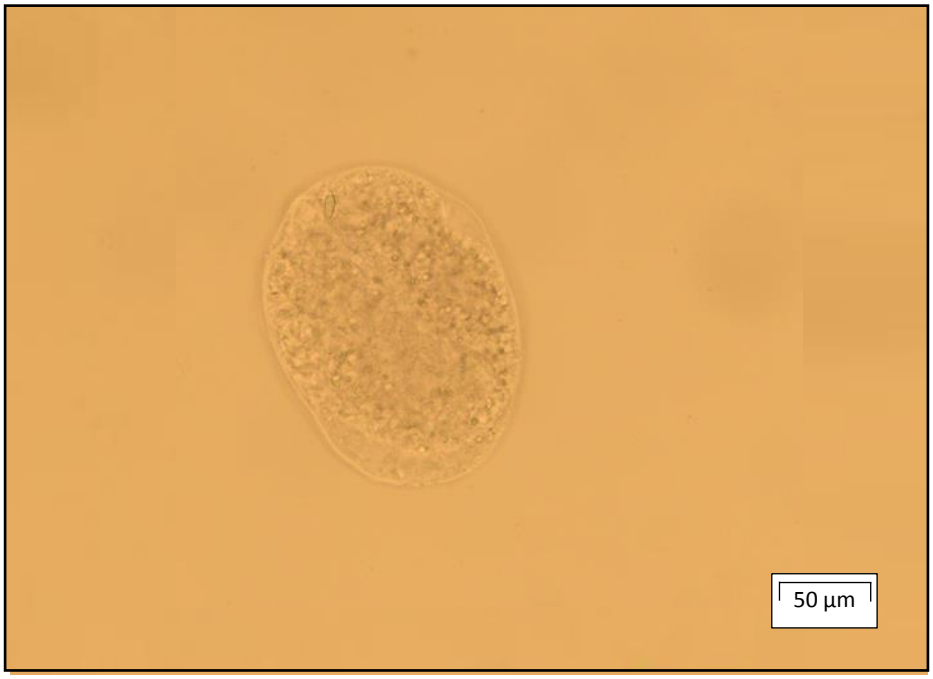


**Figure 2.5 *Coitocaecum parvum* metacercaria classified as a "new infection"**

**Amphipod survival**

As amphipods were exposed to odour treatments in distinct batches, I tested whether any of the specific odour treatments influenced amphipod survival. The fixed effects (see Statistical analysis below) used were treatment and density of amphipods per container (this was originally 20 but was increased to 25, then to 30 so that larger numbers of amphipods could be infected during a single run of the experiment). Several potential confounding batch effects were accounted for by including (as random factors) order of batch for infection (1st, 2nd or 3rd) as *C. parvum* shed from snails at about the same time may have lower success with age and collection date to account for any potential seasonal variance throughout the experiment. Four batches of amphipods were excluded from analysis because fewer than 50% survived. Extremely low survival in these batches may have been due to a problem with the individual container (such as a fault with aeration) rather than an effect of treatment or density.

**Statistical analysis of experimental infections**

A majority of the statistical analyses were carried out using the software package R 3.4.1 (R Core Team 2017). The abundance of new *C. parvum* infections per host was analyzed using a cumulative link mixed model (clmm2) using the R package *ordinal* (Christensen 2015). The main goal was to test the effect of both indirect and direct earlier exposure to parasites on subsequently acquired parasite abundance per host and to test whether these effects varied with respect to the size of the host. Amphipods found to

have pre-existing infections were classified as having had direct exposure to parasites, amphipods that were exposed to the treatment odour (infected snail water) were classified as having had indirect exposure to parasites. To assess whether any effect from the infected snail water was due to the presence of parasites rather than a general response to snails, uninfected snail water was also included as a separate treatment. Treatment, number of pre-existing infections and size of amphipod were included as fixed factors in the model. The interaction between pre-infections and treatments was included in the original model but was excluded from the final model as it was not significant. Batch, the order of batch for infection and date of collection were included as random factors to account for any variance due to these factors. A one-way analysis of variance (ANOVA) was conducted in Microsoft Excel 2016 to determine whether treatment of amphipod influenced the average size of 'new' *C. parvum* infections.

**Statistical analysis of survival**

Survival of amphipods per batch was analyzed in R 3.4.1 with a generalised linear mixed-effects model (GLMM) using package *lme4* (Bates et al. 2014), using the proportion of survivors within a batch as the response variable. My main goal was to test whether there was a relationship between the treatment applied to a batch (control, uninfected or infected treatment) and the survival of amphipods within a batch. The density of amphipods per batch increased over the course of the experiment and so was accounted for as a fixed factor along with treatment. Order of a batch used for infections and date of collection were included as random factors so that any variation caused by these factors would not confound the estimated effects of the fixed factors.

**Results**

**Experimental infections**

The first set of questions aimed to determine whether indirect or direct exposure to parasites influenced the subsequent acquisition of new parasite infections. One third of amphipods were exposed to parasites indirectly through exposure to water conditioned with snails infected with parasites, while the other two-thirds received one of two levels of control – water conditioned with parasite-free snails, or filtered lake water. The final dataset consisted of 396 amphipods in total from the three treatments: *control (131), uninfected snails (108), and infected snails (157).* The number of individuals differs for each treatment as only amphipods that survived to the end of the experiment were used. Some amphipods used were found to have infections that were acquired from the field prior to the experiment. These amphipods were classified as having had direct exposure to parasites. All amphipods were then exposed

to two *C. parvum* cercariae and the ensuing prevalence and abundance of parasite infections were calculated.

**Parasites acquired in the field**

Of the 396 amphipods used, 38 (9.6%) were found to be hosting parasites acquired in the field prior to the infection experiment. Most amphipods found with pre-infections (28, 7.1%) were infected with a single parasite. Seven amphipods (1.8%) hosted two parasites, two amphipods (0.5%) hosting three parasites and a single amphipod had four parasites (0.25%). Pre-existing infections by three different parasite species were found during dissections including *Coitocaecum parvum* metacercariae (found in both normal and progenetic life stages), *Maritrema poulini* metacercariae (figure 2.7), and a juvenile acanthella stage of *Acanthocephalus galaxii* (figure 2.8). Instances of single infection by non-progenetic *C. parvum* were found in fourteen amphipods (3.5%), single progenetic *C. parvum* were found in five amphipods (1.3%), and a single amphipod was infected with A. *galaxii* (0.3%) which was excluded from subsequent analyses. There were three cases of two *M. poulini* individuals infecting a single amphipod (0.75%), four cases of a progenetic and non-progenetic *C. parvum* co-infecting in the same host (1.0%) and one instance where a single amphipod had two *M. poulini* and two non-progenetic *C. parvum* parasites (0.25%). A summary of size, prevalence and abundance data of these parasites is provided in table 2.1.

**Table 2.1 Prevalence and mean abundance of parasites, and their length, present in *Paracalliope fluviatilis* amphipods which were obtained in Lake Waihola prior to the infection experiment.**

| PARASITE | SIZE (mm) | PREVALENCE (%) | MEAN ABUNDANCE +/- SE |
|---|---|---|---|
| **Progenetic *C. parvum* metacercaria** | 0.731 ± 0.114 | 2.3 | 0.028 ± 0.008 |
| ***C. parvum* metacercaria** | 0.554 ± 0.035 | 4.8 | 0.076 ± 0.016 |
| ***M. poulini* metacercaria** | 0.208 ± 0.021 | 5.1 | 0.028 ± 0.009 |
| ***A. galaxii* Acanthellae** | 1.63 | 0.3 | 0.003 ± 0.003 |

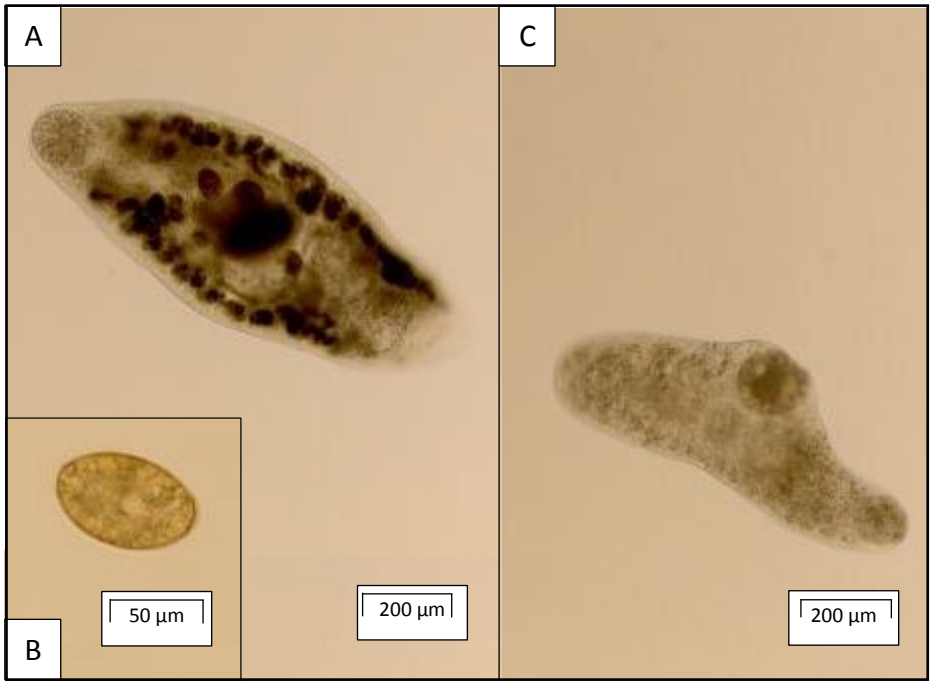


**Figure 2.6 Several life stages of *Coitocaecum parvum* found in *Paracalliope fluviatilis* during dissection: A) progenetic metacercaria; B) egg from a progenetic metacercaria; C) non-progenetic metacercaria acquired in the field.**

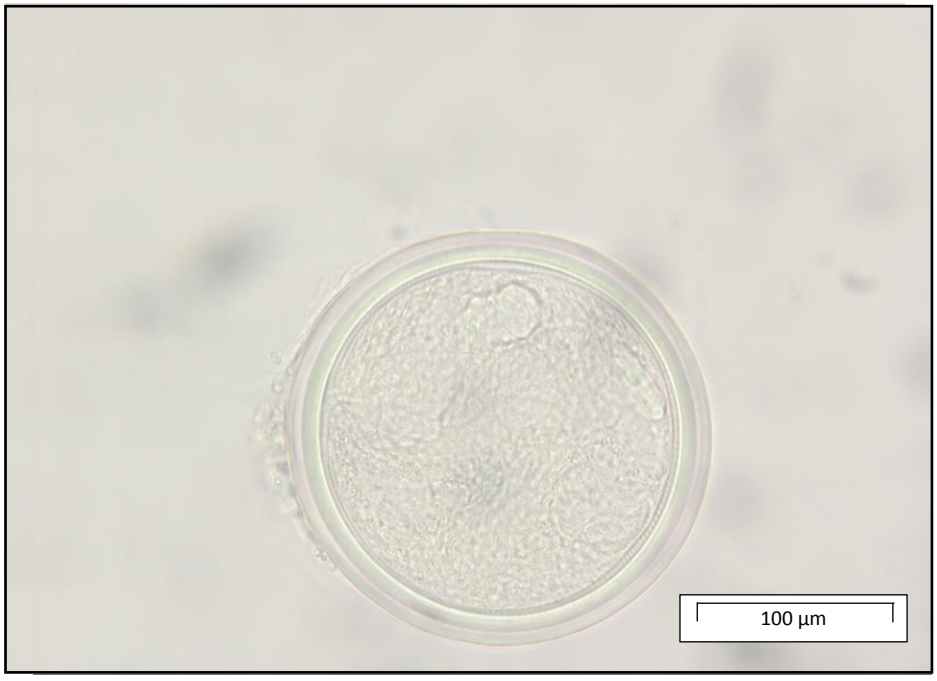


**Figure 2.7 *Maritrema poulini* metacercaria, photo courtesy of Bronwen Presswell.**


**Figure 2.8 *Acanthocephalus galaxii* acanthella found in a *Paracalliope fluviatilis* amphipod during dissection.**

**Infections acquired during the experiment**

Each amphipod was exposed to two *C. parvum* cercariae during the experiment and could, therefore, acquire either no infection, a single infection or a double *C. parvum* infection. The prevelance of new infections for treatments is shown in figure 2.9 and the mean abundance obtained is shown in figure 2.10. The total number of amphipods that acquired new infections during the experimental infection was 207 (52.3%) with 154 (74.4%) of these having one infection and 53 (25.6%) having two infections. The size of newly acquired *C. parvum* cysts ranged from 0.01 – 0.28 mm. The average size of new metacercariae per treatment group was 0.162 mm ± 0.0007 for control, 0.163 mm ± 0.0006 for uninfected snail treatment and 0.163 mm ± 0.0009 for the infected snail treatment.

A one-way analysis of variance (ANOVA) showed that there was no significant difference in the average metacercarial size of new infections between the different treatments ($F_{2,210} = 0.30$, $p=0.738$). Co-infection by two new *C. parvum* did not seem to influence the size of the *C. parvum* metacercariae two days post infection (table 2.2).

**Table 2.2 Results of infection experiment with *Paracalliope fluviatilis* from each treatment acquiring either no *Coitocaecum parvum* metacercaria, a single infection or a double infection; also shown is the average size of *C. parvum* metacercariae. The treatments were: aged lake water (control), uninfected snail water (uninfected) and infected snail water (infected).**

| TREATMENTS | NO INFECTIONS (%) | SINGLE INFECTION (%) | SIZE (mm) MEAN ± SE | DOUBLE INFECTION (%) | SIZE (mm) MEAN ± SE |
|---|---|---|---|---|---|
| **CONTROL** | 48.0 | 37.0 | 0.161 ± 0.0004 | 15.0 | 0.165 ± 0.005 |
| **UNINFECTED** | 45.0 | 42.0 | 0.165 ± 0.0004 | 13.0 | 0.159 ± 0.005 |
| **INFECTED** | 49.0 | 39.0 | 0.167 ± 0.0004 | 12.0 | 0.155 ± 0.003 |

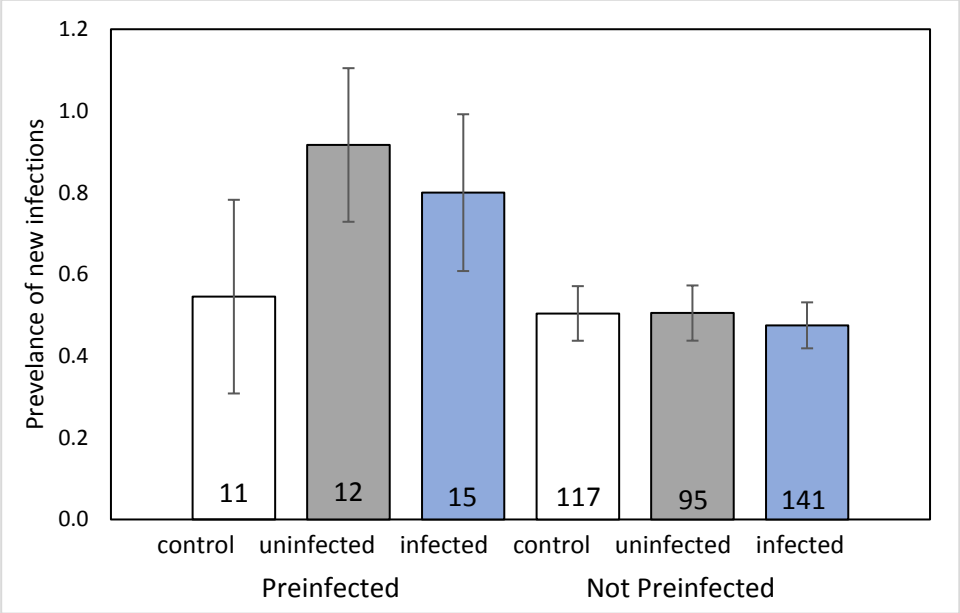


**Figure 2.9 *Coitocaecum parvum* prevalence among treatments creating indirect exposure to parasite cues (control water, uninfected snail water, infected snail water), and between *Paracalliope fluviatilis* which were either or not exposed directly to parasites (through pre-infections) prior to the experimental infections. Numbers at the base of each bar are sample sizes and error bars show standard error.**

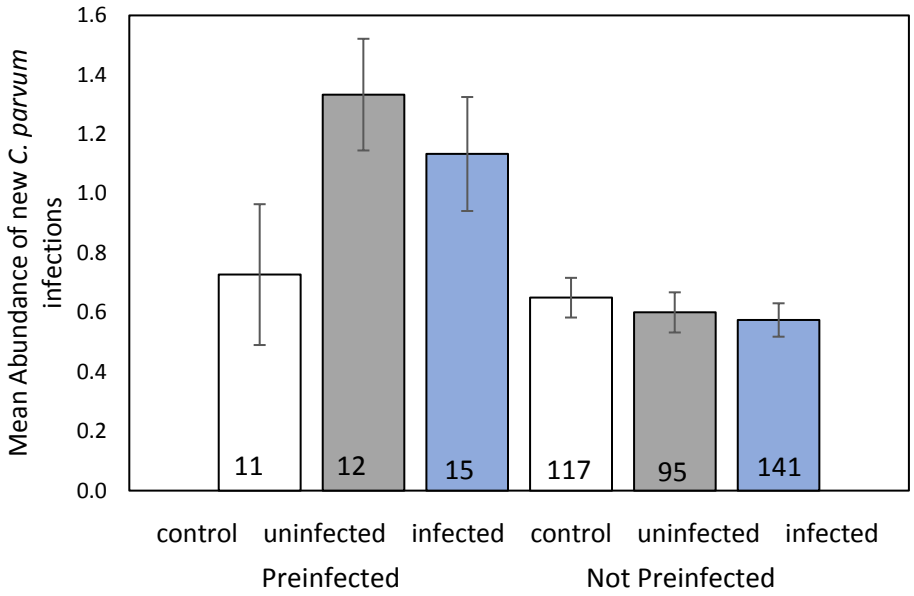


**Figure 2.10 *Coitocaecum parvum* mean abundance among treatments, and between *Paracalliope fluviatilis* which were exposed directly (through pre-infections) and indirectly (through odor treatments) to parasites prior to the experimental infections. Numbers at the base of each bar are sample sizes and error bars show standard error.**

**Cumulative link mixed model**

Treatment and amphipod size had no significant effect on parasite abundance (treatment: $R^2$ =0.00098, p = 0.82, size: $R^2$ = 0.0031, p =0.26). There was a slight trend that amphipods exposed to the infected snail treatment acquired a lower mean abundance of new parasites (0.628 ± 0.5) than either of the control treatments (control: 0.656 ± 0.5, uninfected snail: 0.68 ± 0.5) however as variance was so high it was inconclusive. The number of parasites acquired during the experiment was significantly affected by the presence of pre-infections, however, the effect size was quite small ($R^2$ = 0.04, p =0.000007). On average amphipods with preinfections acquired a mean density of 1.079 ± 0.5 *C. parvum* infections, whereas amphipods free from prior infection acquired a mean density of 0.606 ± 0.5 new infections. Amphipods that had pre-infections and were exposed to either snail treatment (uninfected or infected snail treatments) showed a slight trend toward acquiring more new parasites in terms of prevalence and abundance than amphipods exposed to the control treatment of lake water or amphipods which did not have prior infections (figure 2.9 and 2.10). None of the random factors explained a significant proportion of the variance in parasite density between amphipods. The total variance explained by batch, order and date was less than 1%. A summary of this model is provided in table 2.3.

**Table 2.3 Results of the cumulative mixed-effects model with the number of new *Coitocaecum parvum* infection per *Paracalliope fluviatilis* as the response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors.**

| FIXED FACTORS | ESTIMATE | STD ERROR | Z VALUE | P | RANDOM FACTORS | % VARIANCE |
|---|---|---|---|---|---|---|
| **TREATMENT** | 0.03528 | 0.13155 | 0.27 | 0.7890 | Batch | 0.16 |
| **PREINFECTIONS** | 0.94999 | 0.24047 | 3.95 | 0.000007 | Order | 0.01 |
| **SIZE** | -0.30337 | 0.26695 | -1.14 | 0.2560 | Date | 0.04 |

**Amphipod Survival**

The amphipods used in the experiment were grouped in batches according to the odour treatment they received (control, uninfected snail or infected snail treatment). Throughout the six days they were maintained in the laboratory (one day for acclimation, two days of odour treatments, the day experimental infections occurred and two days following to allow for the parasites to establish) different proportions of amphipods from each batch died (figure 2.11). The survival of amphipods was assessed to establish whether the treatment experienced by amphipods influenced their overall survival. The starting number per container (density) was also related to the number of amphipods that survived two days post-experiment. Of the 24 batches used for the experiment, four were excluded due to >50% of their amphipods dying. Three of these excluded batches were from the uninfected snail treatment and the remaining batch from the control treatment. Survival of these excluded batches was between 0 - 27% survival. The 20 usable batches of amphipods had survival rates of 57 - 97%.

**Linear mixed-effects model**

The model shows that the survival of amphipod batches was not significantly affected by either the odour treatment they were exposed to or the density of amphipods present within an individual container (treatment: $R^2$=0.0483, F=0.432, p=0.656) (figure 2.11, table 2.4). The average survival was 73.5% ± 1.0% for amphipods exposed to the control treatment, 80.3% ± 1.0% for amphipods exposed to the uninfected snail treatment and 76.1% ± 2.4% for amphipods directly exposed to the chemical cues of

parasites, i.e. the infected snail treatment. The random factors of date and order accounted for a very small proportion of variance in the data (<0.001% of variation).

**Table 2.4 Results of the linear mixed-effects model with the percentage of *Paracalliope fluviatilis* surviving (%) as the response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors.**

| FIXED FACTORS | ESTIMATE | STD ERROR | Z VALUE | P | RANDOM FACTORS | % VARIANCE |
|---|---|---|---|---|---|---|
| **TREATMENT** | 0.01423 | 0.03105 | 0.46 | 0.6058 | Date | 0.0001 |
| **DENSITY** | -0.00886 | 0.00636 | -1.39 | 0.1602 | Order | 0.0007 |

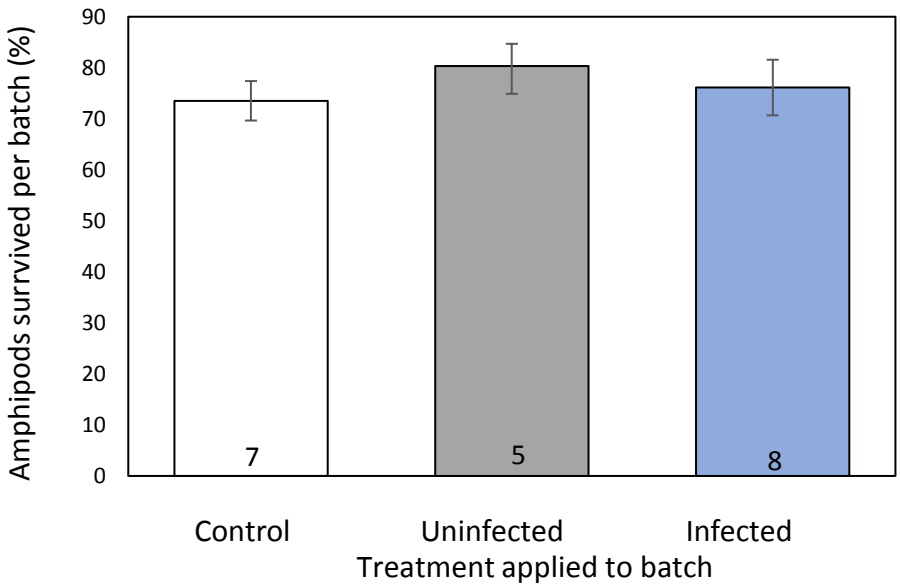


**Figure 2.11 Differences in amphipod survival between batches exposed to the three odor treatments (control, uninfected or infected treatment). Error bar shows standard error and numbers on each bar show number of *Paracalliope fluviatilis* batches per treatment.**

**Discussion**

The overall aim of this experiment was to investigate the effect of previous experience with the trematode *C. parvum* on the subsequent acquisition of parasites in the amphipod *P. fluviatilis*. Amphipods were exposed to trematodes either directly (through pre-existing parasite infections) or indirectly (through exposure to the odour of parasites). It was hypothesised that a lower prevalence and

abundance of new parasite infections would be found in amphipod hosts which had been primed for defence against parasites indirectly (via chemical cues) because their awareness of the imminent risk of infection may trigger anti-parasite defences (Lochmiller and Deerenberg 2000, James et al. 2008). It was also hypothesised that amphipods previously directly exposed to parasites (through pre-existing parasite infections) would accumulate a higher intensity of parasites due to the aggregated distribution of parasites within hosts (Fisher 1941, Pennycuick 1971, Poulin 1993). It was hypothesised that the costs associated with pre-existing parasites (Friesen et al. 2017) would leave amphipods with less available energy to use for subsequent parasite defence. They could, therefore, be more susceptible to further infections (Beldomenico and Begon 2010, Leung et al. 2010). The results of this study did not support the first hypothesis as there was no difference in parasite acquisition between amphipods exposed to the chemical cues of parasites and naïve amphipods. However, the second hypothesis was supported as it was found that amphipods previously infected by parasites acquired more new parasites in terms of prevalence and abundance. Taken together, the results of this experiment suggest that although exposure to indirect parasite cues did not influence the ability of *P. fluviatilis* to evade parasites, the burden of direct exposure to a parasite (through past infection) had a negative effect on the ability of *P.-fluviatilis* to avoid future parasites and could be due to innate reduced resistance.

*Paracalliope fluviatilis* which had direct exposure to *C. parvum* trematodes, through pre-existing parasite infections prior to the experimental infection, tended to accumulate more parasites in terms of both prevalence and abundance than amphipods which did not harbour preinfections. This finding follows a pattern found in the literature regarding the importance of aggregation to the dynamics of animal populations (Skellam 1951, Shaw and Dobson 1995). The distribution of macroparasites among hosts is characteristically aggregated due to an overdispersion of the parasites, where a few hosts harbour a relatively large number of parasites in contrast to the majority of the hosts (Crofton 1971, Pennycuick 1971, May and Anderson 1978, Keymer and Anderson 1979, Anderson and Gordon 1982, Shaw and Dobson 1995, Hutchings et al. 1998, Poulin and Morand 2000).

For example, this pattern of distribution has been observed in the amphipod *Gammarus pulex* infected by trematode metacercariae of *Clinostomum marginatum*, and the acanthocephalan *Polymorphus minutus* (Crofton 1971). Many possible host and parasite factors might be important in influencing patterns of abundance and aggregation including transmission method, parasite type (such as ectoparasite versus endoparasite), parasite taxonomic group, type of life cycle, habitat of hosts, and whether the host migrates (Shaw and Dobson 1995). It has been shown that even small differences in

susceptibility between hosts can rapidly produce non-random, aggregated distributions of parasites (May and Anderson 1978). A potential explanation for the differing susceptibility of animals is that individuals which already pay the cost of infection by a parasite may have less energy to defend themselves against further parasite attacks. This can result in increased rates of infections in hosts which are already debilitated by parasites (Telfer et al. 2010). In other cases, host susceptibility to parasites can decrease over time in systems where acquired immunity against future parasites is important (Crombie and Anderson 1985, Wilson et al. 2002). However, acquired immunity is mostly restricted to vertebrate hosts, which have a complex immune system (Allen and Maizels 2011). Nevertheless, there are examples that show that at least some invertebrates have functional equivalents to the acquired response of vertebrates (Kurtz and Franz 2003, Moret and Siva-Jothy 2003) and both specific and general immunity can also be transferred across generations (Huang and Song 1999, Males 2000, Little et al. 2003). In the current experiment, the immune defence of *P. fluviatilis* was not explored directly. The experimental infection set up in this study allowed for behavioural defences such as grooming as well as potentially seeing some evidence of immune defence response (as amphipods were dissected two days post-infections). This may not have been long enough for the completion of an immune response as other research into *Paracalliope* sp. left them for at least 7 days post-infection before dissection (Bryan-Walker et al. 2007).

In the current study, no significant difference was found between amphipods which were indirectly exposed to the trematode *C. parvum* through chemical cues from snails infected with this parasite and amphipods which had no prior experience with *C. parvum*. This could be because amphipods free from parasite infection were parasite-naïve and did not associate the chemical cues from trematodes with a threat to their health. This is consistent with the results of an earlier study, where fathead minnows *Pimephales promelas* were shown to exhibit no innate ability to recognise or avoid the cercariae of the trematode *Ornithodiplostomum* sp (James et al. 2008). However, after a single exposure to cercariae experienced *P. promelas* associated parasite risk with the novel chemical and visual cues and reduced their activity (James et al. 2008). Although *P. promelas* responded to chemical cues, this was only after the threat of parasites had been experienced directly (James et al. 2008). It is possible that in the present system the impact of the trematode *C. parvum* on the amphipod *P. fluviatilis* host is more severe than the trematode in the minnow host and could limit the ability of the host to avoid or defend against future parasite encounters.

Further experiments should be conducted to examine other indirect ways in which *P. fluviatilis* may become aware of the risk of parasites. One potential way of examining this could be to assess the responses of *P. fluviatilis* to the chemical alarm cues of a conspecific during a parasite encounter such as has been assessed in fish and amphibian hosts (Poulin et al. 1999, Rohr et al. 2009). Even though no difference was observed between the relative success of amphipods to defend against parasites, between individuals exposed to parasite cues and those naïve to parasites, there still may be defence strategies not revealed in the present study, such as behavioural or physiological defences. Constrained within a very small volume of water during experimental exposure to cercariae, the amphipods did not have the ability to behave as they would when encountering parasites in nature. To further investigate this, the next chapter explores whether the behaviour of amphipods changed after exposure to the same indirect chemical cues.

It could be interesting in the future to test the susceptibility of *P. fluviatilis* which have encountered trematodes directly but successfully defended themselves (e.g. through successful grooming or through a well-timed moult) to future parasites (Moret and Moreau 2012). These amphipods would presumably have experienced the risk of parasites without the burden of having acquired an actual parasite. As amphipods encounter and successfully evade parasites in wild populations, exploring the effects of experience with parasites could bring to light some of the more nuanced ways hosts interact with parasites.

The current study found no significant differences in the survival rate of amphipods exposed to water containing indirect chemical cues of snails infected with *C. parvum* compared to amphipods which were exposed to just water, or water conditioned with uninfected snails. The density of amphipods in a container also proved to be non-significant and did not affect amphipod survival. One factor that may have influenced the survival of *P. fluviatilis* was the presence of pre-existing parasite infections as *C. parvum* and *M. poulini* are known to induce higher host mortality in *P. fluviatilis* than mortality rates of parasite-free amphipods (Friesen et al. 2017). The level of natural infection of *P. fluviatilis* hosts could not be assessed until the end of the experiment. It is possible that the subset of amphipods that died included a high proportion of amphipods that hosted pre-existing parasites. The daily survival of *P. fluviatilis* was not examined due to the study design which involved batches of amphipods which meant that individual amphipods could not be assessed as they were not independent. Daily counts would allow more sophisticated survival models to be made, however, this daily disturbance could have impacted the response of remaining amphipods and may have influenced their survival to the end of the

experiment. Friesen (2017) examined daily survival of *P. fluviatilis* and found that *C. parvum* and *M. poulini* reduced survival while *A. galaxii* did not influence amphipod survival. If I were to further assess the effect of exposure to priming cues on *P. fluviatilis* survival in the future, I would plan it such that that Kaplan–Meier survival estimates could be calculated as these generate useful survival curves (Clark et al. 2003, Devevey and Christe 2009, Nissen et al. 2014).

For the current experiment, it was vital that as many *P. fluviatilis* as possible survived until after the experimental infection. As survival of *P. fluviatilis* infected with *C. parvum* has previously been assessed, a future study could assess the effects of stress of indirect parasite cues alone in a similar way to (Friesen et al. 2017) which conducted separate survival tests of *P. fluviatilis* in individual well plates. Assessing the effects of indirect chemical cues would be possible as any mortality associated with the stress of daily disturbance through the removal of dead hosts would not compromise the completion of the study.

The experimental infection component of this study found that pre-infections were more important for subsequent parasite acquisition than the chemical cue examined. Although this was not the main focus of this study it is an essential finding and could be examined in further detail by examining the effects of different parasite species on *P. fluviatilis* susceptibility to further infections. In the current study, a proportion of amphipods were naturally infected prior to the experiment. The parasites which happened to have been acquired prior to the experiment arbitrarily included *C. parvum* and *M. poulini* (and a single *A. galaxii* which was excluded). Since these parasite species have been shown to affect their hosts in different ways and to different degrees (Lefebvre et al. 2005, Rauque et al. 2011, Friesen et al. 2017), and because hosts often acquire parasites of different species naturally (Petney and Andrews 1998, Poulin et al. 1998, Telfer et al. 2010) future laboratory experiments could experimentally infect amphipods with various combinations of parasites and examine their subsequent vulnerability to further parasites. This would allow us to explore the combined effects different parasites have on a host (i.e. antagonistic and cancel out the effects of each other, or co-operative with negative consequences for hosts) (Poulin 2001a).

In conclusion, the main finding of this study was that amphipods with pre-existing infections were more likely to acquire higher numbers of *C. parvum* trematodes. It was also found that *Paracalliope fluviatilis* which had been primed with chemical cues (through exposure to infected snail-conditioned water) did not outperform naïve amphipods in terms of successful parasite defence. As the mechanisms for parasite detection through chemical cues have rarely been studied, the following chapter will build on the

findings of this chapter by examining whether amphipods can respond behaviourally to the presence of this same specific chemical cue.

# Chapter 3: Amphipod behavioural response to an odour of a source of trematode

**Introduction**

Animals constantly accumulate information about their surroundings because this knowledge can inform their strategic decision-making in situations like predator and parasite encounters (Schmidt et al. 2010). Many animals show immune responses to parasites, however, these are costly (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Rolff and Siva-Jothy 2003, Bryan-Walker et al. 2007). In many cases, behavioural reactions to threats are nonspecific and serve to reduce exposure to both parasites and predators as avoiding these threats is less energetically expensive than defending against them directly (Hart 1994, Combes 2001). Avoidance responses can be nonspecific because of the similarities between the ways predators and parasites detect targets. Predators frequently locate their prey by detecting motion or olfactory cues (Lima and Dill 1990, Catania et al. 2008, Hughes et al. 2010). In a similar manner, infective stages of many parasites also detect their host via visual, chemical, and tactile cues which indicate host presence (Lewis et al. 1976, Haas 1994, Haas 2003). A common response by prey or host animals is to lower their activity to help reduce the risk of detection by parasites and predators (Lawrence and Smith 1989, Lima and Dill 1990, Poulin et al. 1991b, Chivers and Smith 1998, Thomas et al. 2000).

Some empirical support exists for the detection and avoidance of less conspicuous larval stages of aquatic parasites (Lowenberger and Rau 1994, Kiesecker and Skelly 2000, Thiemann and Wassersug 2000, Karvonen et al. 2004). Rainbow trout (*Oncorhynchus mykiss*), for example, actively avoid refugia containing cercariae of the trematode *Diplostomum spathaceum* (Karvonen et al. 2004). Larval green frogs (*Rana clamitans*) and wood frogs (*Rana sylvatica*) have been shown to reduce their activity by 25-33% when exposed to cercariae of the trematode *Echinostoma sp*. to avoid infection (Thiemann and Wassersug 2000). Some hosts such as mosquitos (*Aedes aegypti*) and gray tree frogs (*Hyla versicolor*) avoid depositing eggs in water bodies where trematode cercariae or individuals infected with metacercariae are present to save their offspring from infection (Lowenberger and Rau 1994, Kiesecker and Skelly 2000). Extensive studies have been conducted on immune priming in insects investigating different aspects of trans-generational immune priming (where parents invest in their offspring by preparing them for defense against pathogens encountered only by the parent) in bumble bees *Bombus terrestris* (Sadd et al. 2005), beetles such as the mealworm beetle *Tenebrio molitor* (Moret 2006) and the red flour beetle *Tribolium castaneum* (Roth et al. 2009). Immune priming is also shown to show specificity in protection upon secondary pathogen exposure in *B. terrestris* (Sadd et al. 2006). In moths (*Galleria mellonella*) this immune priming is shown to be the result of maternal transfer of bacteria (Freitak et al. 2014).

These studies provide evidence for the existence of general avoidance behaviours of fish and other aquatic animals to infection threats, but too few studies have been completed on too few host/parasite systems to make generalisations, and no study has yet been conducted on the behaviour of amphipods exposed to parasites. Both the degree to which even the most pathogenic aquatic parasites are detectable in the aquatic medium and what cues alert hosts to the risk of parasitism remain to be determined.

In order to avoid a potential parasite, a host must have the ability to detect the parasite directly, or to recognise cues associated with the risk of parasitism (Lima and Dill 1990, Smith 1992, Hart 1994, Kiesecker et al. 1999, Kiesecker and Skelly 2000). In aquatic ecosystems, chemical cues are often an open and accessible source of information (Dodson et al. 1994, Chivers et al. 1996b, Brown and Godin 1997, Kats and Dill 1998, Wisenden 2003, Wisenden and Chivers 2006, Breithaupt and Thiel 2010). For many aquatic prey, anti-predator defences are induced when animals become aware of the threat through exposure to chemical cues from predator kairomones or from substances released by injured conspecifics or other prey species' alarm cues (Blum 1969, Howe and Sheikh 1975, Smith 1992, Chivers et al. 1996b, Hazlett and Schoolmaster 1998, Kats and Dill 1998).

Although a few studies have investigated the avoidance of aquatic hosts in response to parasites, these have not usually considered chemical cues (Karvonen et al. 2004, James et al. 2008). In contrast, the responses of aquatic prey to predator cues have been investigated in many systems (Atema and Stenzler 1977, Hazlett 1997, Barry 2000, Brown 2003, Relyea 2003, Ferrari et al. 2010). More specifically, aquatic crustaceans such as hermit crabs (*Diogenes avarus*) and other crab species (*Petrolisthes elongatus, Halicarcinus innominatus, Notomithrax ursus* and *Cyclograpsus lavauxi*) have been shown to respond to kairomones and alarm cues associated with predation through decreased locomotion and other movements (Hazlett 1994, 1997, Hazlett and McLay 2000). Crayfish (*Orconectes virilis)* switch to a lower posture when detecting predator cues, adopt a 'watchful' pose and cease all movements if detecting alarm cues (Hazlett 1994). Other crustaceans, such as the sympatric crayfish *O. propinquss*, show no response to chemical cues that indicate a considerable risk of predation (Hazlett 1994). It is unclear why some species have evolved high sensitivity to chemical cues and other closely related species have not, although it could be dependent on an animal's active time, as animals which are active during the daytime (such as *O. propinquus*) may rely more on visual cues for information than primarily nocturnal animals (such as *O. virilis*) (Hazlett 1994, Brown 1998). The crab *Heterozius rotundifrons* also does not

respond to chemical cues or visual cues alone but will respond to these in combination with a strong tactile input from a predator (Hazlett 1997). In response, it renders itself hard to swallow by spreading its chelipeds and stopping all movement (Field 1990, Hazlett and McLay 2000). The presence of chemical or visual cues in addition to tactile cues results in this response being held for a significantly longer period (Hazlett and McLay 2000).

Crustaceans, such as amphipods, have sophisticated chemosensory abilities to detect chemical cues and will only exhibit defence responses when the risk of infection is high, as discussed in chapter 1 (Dodson et al. 1994, Kaufmann 1994, Wisenden et al. 1999, Bryan-Walker et al. 2007, Blinova and Cherkashin 2012). For example, individuals of a specific natural population of amphipods *(Paracalliope novizealandiae*), which regularly encounter the debilitating trematode parasite *Maritrema novaezealandensis* have an increased ability to avoid becoming infected and to respond to infection by encapsulating and melanising parasites compared to another parasite naïve population of *P. novizealandiae population* (Bryan-Walker et al. 2007). Most empirical tests regarding the success and range of host avoidance behaviours have examined the avoidance by grazing mammals of faecal patches containing larvae of gastrointestinal nematodes, and the avoidance of parasitoids by insects (Sutherst et al. 1986, Ohsaki and Sato 1994). Much less attention has been devoted to evaluating parasite avoidance behaviours in aquatic systems. Of the studies that have been performed, they have focused on fish and most did not examine chemical cues (Poulin and FitzGerald 1989a, Poulin and FitzGerald 1989c, Poulin et al. 1991b, Barber et al. 2000, Karvonen et al. 2004, James et al. 2008, Wisenden et al. 2009a). For instance, studies on the three-spined stickleback (*Gasterosteus aculeatus*), the blackspotted stickleback (*Gasterosteus wheatlandi*) and brook trout (*Salvelinus fontinalis*) have examined how shoal size increases as a behavioural strategy to reduce the chance of encountering ectoparasites (Crustacea: Branchiura) (Poulin and FitzGerald 1989a, Poulin and FitzGerald 1989c, Poulin et al. 1991b).

Some chemical cues promote avoidance responses innately, while others will only elicit evasive responses when the novel neutral stimulus becomes associated with stimuli correlated with predation risk (Brown 2003). For example, rainbow trout (*O. mykiss*) show an innate avoidance response to the eye fluke *Diplostomum spathaceum* when they are in the direct presence of the cercariae (Karvonen et al. 2004). Another example of an innate response to chemical cues is shown by predator-naïve glowlight tetras (*Hemigrammus erythrozonus*). They significantly increase their antipredator behaviour when exposed to chemical cues of a potential fish predator (convict cichlid*, Cichlasoma nigrofasciatum*) that had been fed conspecific tetras but not swordtails (*Xiphophorus helleri*), (Brown and Godin 1999). On the

other hand, fathead minnows *Pimephales promelas* have a learned response and they can learn to recognise and avoid the cercariae of the trematode *Ornithodiplostomum* sp. once they have encountered them previously (James et al. 2008). Minnows responded to both visual and chemical cues corresponding with the trematode and reduced their activity, which is a useful strategy for evading parasites that detect hosts through movement (Lima and Dill 1990, James et al. 2008). As emphasized earlier, behavioural responses generally occur when the cost of infection is severe (Thomas et al. 2002, Bryan-Walker et al. 2007). This is true in known cases of anti-parasite responses summarised above. *Ornithodiplostomum* sp. encysts in the brains of fathead minnows and induce a significant metabolic cost (Goater et al. 2005). Eye flukes limit the visual capabilities of a host and make them more vulnerable to predation by their definitive hosts (Karvonen et al. 2004).

Varying chemical substances associated with trematode parasites have been used to determine which are important cues for hosts, including live or dead cercariae, water conditioned by either uninfected or infected snails, and exposure to other infected conspecifics (Lowenberger and Rau 1994, Kavaliers et al. 2004, James et al. 2008). Although no previous work has examined the ability of *P. fluviatilis* to sense the presence of parasites, this amphipod can recognise the chemical cues of predatory fish species (Lagrue and Poulin 2007). Other amphipods have been shown to recognise and avoid fish predators when they encounter their associated chemical cues, such as *Gammarus pulex* in response to perch cues (Baldauf et al. 2007).

The amphipod *P. fluviatilis* provides an ideal model since its trematode *C. parvum* can grow up to half the host's body length and reduce its survival (MacFarlane 1939, Lagrue and Poulin 2007, Friesen et al. 2017). Although the chemosensory abilities of crustaceans are sophisticated, no study has examined their ability to sense parasites (Breithaupt and Thiel 2010). A few studies on crustaceans have considered the susceptibility of hosts to both enemies (predators and parasites) in tandem through exposure to cues from these enemies (Lass and Bittner 2002, Hesse et al. 2012). In these studies predator cues are often in the form of chemical cues, however, parasite exposure was tested through infection by parasites rather than to chemical cues (Lass and Bittner 2002, Hesse et al. 2012).There are studies where trematode chemical cues have been shown to elicit behavioural responses from non-crustacean host species (Lowenberger and Rau 1994, Karvonen et al. 2004, James et al. 2008). We may expect animals to show varying responses to parasite cues depending on the extent of the negative impact a given parasite has on its host and depending on the host's previous experience with parasites (Bryan-Walker et al. 2007).

In my study system, it may be adaptive for *P. fluviatilis* to reduce activity to avoid *C. parvum* cercariae. At the same time, as predatory fish such as common bullies (*Gobiomorphus cotidianus*) use nonvisual prey detection methods, such as olfactory, tactile, or lateral-line prey detection, the lack of movement in *P. fluviatilis* may allow them to escape predation as well (Rowe 1999, Rowe et al. 2001). *A*voidance behaviours have been emphasised as an important filter that may limit infection rates (Combes 2001). These behaviours range from simple adjustments of movement and habitat choice to complex avoidance behaviours linked to fine-tuned parasite detection strategies (Hazlett 1994, Mathis 2009, Wisenden et al. 2009a).

The previous chapter explored the roles that pre-existing infections and the perceived risk of new parasite infection (via exposure to chemical cues which indicate the presence of parasites) had on subsequent parasite acquisition in an amphipod intermediate host. It was found that amphipods with pre-existing infections were more likely to acquire higher numbers of *Coitocaecum parvum* trematodes. It was also found that *Paracalliope fluviatilis* which had been primed with chemical cues (through exposure to infected snail-conditioned water) did not outperform naïve amphipods in terms of successful parasite defence. However, the very small volume of water used greatly limited, and probably even eliminated, any use of behavioural defences by the amphipod. As the mechanisms for parasite detection through chemical cues have rarely been studied, this chapter aims to build on the findings of the previous chapter by examining the ability of an amphipod host to sense a trematode parasite indirectly through chemical cues and adjust its behaviour accordingly.

Amphipods will be primed to the presence of parasites using parasite chemical cues and their subsequent swimming behaviour will be measured using EthoVision XT 9.0 (Noldus Information Technology 2015) and compared to the behaviour of parasite-naïve amphipods both in the presence and absence of parasite cercariae. The same trematode-amphipod system as in the previous chapter will be used consisting of the amphipod *P. fluviatilis* and the trematode parasite *C. parvum*.

**Objectives**

The main objective of this chapter was to investigate whether *P. fluviatilis* show sensory recognition of chemical cues which indicate the presence of parasites. I tested this by exposing *P. fluviatilis* to the chemical cues of *C. parvum* (through priming with infected snail-conditioned water) and then measuring their response when they are subsequently exposed to infective stages of *C. parvum*, or in the absence of a parasite. Swimming behaviours measured included time spent moving and distance moved. It was

hypothesised that amphipods primed to the risk of parasites through chemical cues would increase their activity in terms of time spent moving and distance moved compared to naïve amphipods as this is a typical host response to avoid a parasite threat. It was hypothesised that there would be no difference in swimming behaviours between amphipod treatments in the absence of a parasite as only temporary reponses were measured.

**Methods**

All amphipods for this study were collected in one sampling trip in July 2017. Amphipods and snails were collected and maintained using the same methodology as in chapter 2 (figure 2.5). As in the previous chapter, only male amphipods of similar size were used to limit variation in behavioural responses. Amphipods were exposed to odour treatments in a staggered manner over three days (Table 3.1). A total of 30 amphipods were prepared for each odour treatment, for three days (n= 270). All amphipods were only used once and were exposed to either one *C. parvum* cercaria with lake water or lake water alone which created six unique treatment groups (table 3.1). Amphipods which were identified as being injured or otherwise impaired were excluded from the experiment as well as any female amphipods which were selected by accident (identified when under the microscope during later dissections). The treatments were: *control treatment parasite absent* (n=35), *control treatment parasite present* (n=34), *uninfected snail treatment parasite absent* (n=36), *uninfected snail treatment parasite present* (n=36), *infected snail treatment parasite absent* (n=37) and *infected snail treatment parasite present* (n=37). The total number of amphipods used in the final analyses was 215 of the original 270. A summary of the steps of the behavioural assessment is shown in figure 3.1.

**Table 3.1. Test stimuli presented for priming and test trials of *Paracalliope fluviatilis***

| TREATMENT | STIMULUS SET UP | PRIMING STIMULUS | TEST STIMULUS | TREATMENT CODE |
|---|---|---|---|---|
| **WATER CONTROL** | 1L of filtered lake water, topped up to 1L each time priming occurs | 200ml of control water, twice over two days | 5 ml filtered lake water with *C. parvum* cercaria absent or present | C0<br>C1 |
| **UNINFECTED SNAIL TREATMENT** | 1L of filtered lake water, containing 15 parasite-free *P. antipodarum*. Topped up to 1L each time priming occurs | 200ml of uninfected snail-conditioned water, twice over two days | 5 ml filtered lake water with *C. parvum* cercaria absent or present | U0<br>U1 |
| **INFECTED SNAIL TREATMENT** | 1L of filtered lake water, containing 15 *P. antipodarum* infected with *C. parvum*. Topped up to 1L each time priming occurs | 200ml of infected snail-conditioned water, twice over two days | 5 ml filtered lake water with *C. parvum* cercaria absent or present | I0<br>I1 |

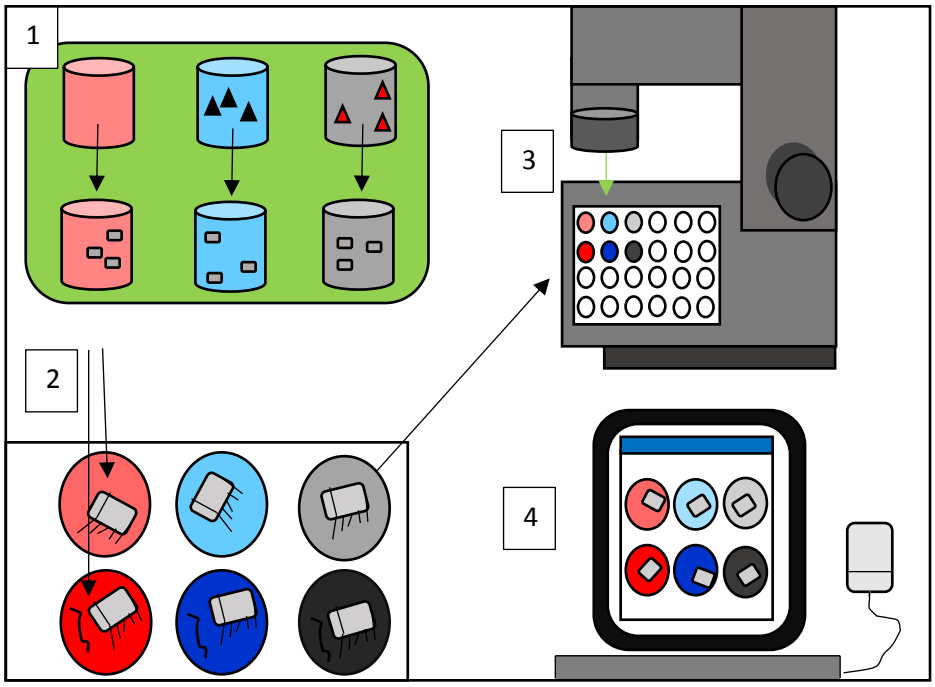


**Figure 3.1 Schematic diagram showing methodology of behavioural assessment: 1) *Paracalliope fluviatilis* were exposed to odour treatments; control, uninfected snails (black triangles) or infected snails (red triangles) using the same methodology as chapter 2 (figure 2.5). The behavioural observations were conducted 24 hours after the last odour treatment. 2) Amphipods from each of the three treatments were transferred to individual wells of a 96 well culture plate containing aged filtered lake water with either no parasite or one *Coitocaecum parvum* cercaria creating six unique treatment groups: control parasite absent (pink), control parasite present (red), uninfected snail parasite absent (light blue), uninfected snail parasite present (dark blue), infected snail parasite absent (grey) and infected snail parasite present (black). Amphipods were left for 30 minutes to acclimatise. 3) Amphipods were then recorded for 5 minutes using a camera mounted on a dissecting microscope and connected to a computer. 4) Video data was analysed in EthoVision XT to quantify the behaviour of amphipods (in terms of distance moved and time spent moving).**

### Behavioural observation

Amphipods from each of the six treatment groups were transferred from odour treatment containers to individual wells of a culture plate using a pipette and left for 30 minutes to acclimatise to their new environments. The rims of the wells were marked using a coding system so that amphipod treatment could be later identified as EthoVision XT flips the video files (figure 3.2). The wells were filled up to the rim with filtered lake water. Half of the wells contained one *C. parvum* cercaria each, and half contained only filtered water. Cercariae were collected through inducing shedding of infected *Potamopyrgus antipodarum* by incubating at 20◦C for 30 minutes under constant light (Fredensborg et al. 2005). Only fresh cercariae were used, and after transfer to the well plates, all cercariae were inspected to ensure they were alive and active. Amphipods were subsequently filmed for 5 minutes with a camera mounted on a dissecting microscope (Olympus SZ61) and recorded using the computer screen recording program

Bandicam. Six amphipods were filmed simultaneously consisting of one amphipod from each treatment group so that any nested trial effects would not confound the results of the experiment. The illumination came from the base light of the microscope and was constant for all trials.

EthoVision XT was subsequently used to assess swimming behaviour of amphipods when alone and when exposed to a parasite (Noldus Information Technology 2015). EthoVision XT is a tool designed to overcome the limitations of manual observations by measuring the behaviour of animals, allowing assessment of movement and behavioural recognition of animals in response to stimulus (Noldus et al. 2001). The program has been utilised for diverse studies such as settlement behaviour of marine larvae, responses of mice and rats to novel objects, and to measure swimming behaviour of fish fry (Spink et al. 2001, Marechal et al. 2004, Delcourt et al. 2006). EthoVision XT has been used to assess amphipod responses to chemical cues such as predator cues in *Gammarus pulex*, and alarm cues of conspecifics (Åbjörnsson et al. 2000, Åbjörnsson et al. 2004, Nilsson and Bengtsson 2004). One particularly relevant study used the program to look at the behaviour of invertebrate communities in response to the direct presence of parasites, including the amphipod *P. fluviatilis* collected from Lake Waihola (Friesen et al. 2017). This study aims to assess the behaviour of amphipods in response to chemical cues from parasites, which is a novel way to assess the reaction of hosts to the presence of parasites.

The specific behaviours measured were general measures of activity including distance moved (tracking movement of the animal's centre point, measured in mm) and mobile duration (more than 20% of the animal has moved within the sample period of 0.05 s), were calculated for each individual over 5 min using EthoVision XT (Noldus Information Technology 2015). EthoVision XT measures the co-ordinates every 0.05s and this data can be used to generate a path illustrating the distance travelled in 5 minutes (figure 3.3). Time spent in different areas can also be visualised in this program by creating a heat map (figure 3.4). Amphipods were then measured and dissected the same day. Any amphipods which had existing parasite infections (acquired in their natural habitat) were excluded from further analysis (n=14). One run of amphipods were also excluded as the video file was corrupted (n=6). In total this resulted in a sample size of 195 individuals.


**Figure 3.2 The six treatment groups of *Paracalliope fluviatilis* during the behavioural assessment. Red dashes indicated control treatment, blue dashes indicated uninfected snail treatment, and black dashes represented the infected snail treatment. One dash was for an amphipod with no parasite and two dashes meant a *Coitocaecum parvum* cercaria was present with the amphipod.**

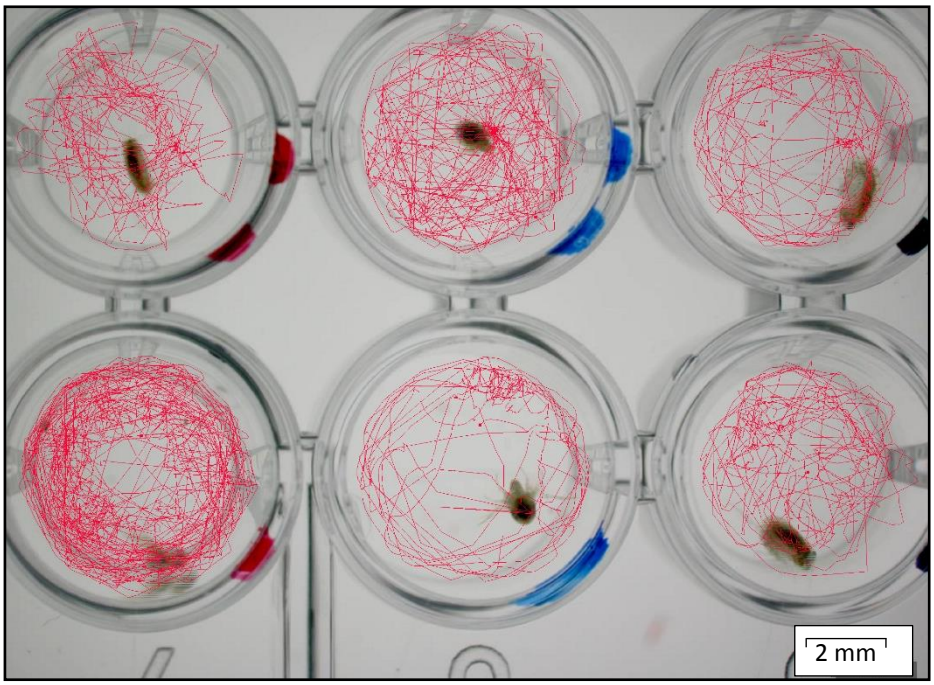


**Figure 3.3 Tracked paths of *Paracalliope fluviatilis* amphipods showing distance moved in five minutes, generated with EthoVision XT.**

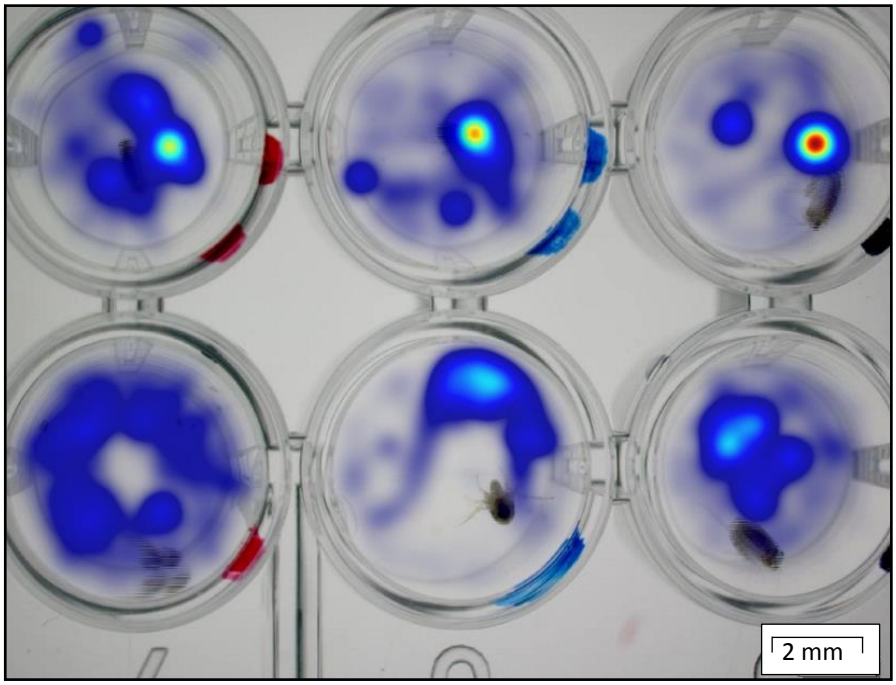


**Figure 3.4 Heat maps showing the area in each well where *Paracalliope fluviatilis* spent the largest proportion of time in five minutes. Blue indicates areas where amphipods spent short amounts of time and red indicates areas where they spent a large proportion of time, generated with Ethovision XT.**

**Statistical analysis**

Swimming behaviours of amphipods were analysed in R 3.4.1 with generalised linear mixed-effects models (GLMM) using package *lme4* (Bates et al. 2014). Total distance moved and mobile duration (which will be referred to as time spent in motion henceforth) were used as response variables, and amphipod size was included as fixed effect. My main goal was to test whether there was a relationship between being primed to the presence of parasites through chemical cues (from water conditioned with *C. parvum* infected snails) and the resulting behaviour of amphipods, therefore treatment (control, uninfected snail, infected snail) and presence/absence of cercariae were included as fixed factors. The planned acclimatisation time for each run of amphipods was 30 minutes but in reality, this varied from 27 – 38 minutes and was therefore accounted for as a random factor along with date collected. Date of use was also included as a random factor as all amphipods were collected on the same day, but the experiment was conducted over three consecutive days and the extra maintenance time for amphipods in the laboratory may cause variation. These random factors were included so that any variation caused by these factors would not confound the estimated effects of the fixed factors.

**Results**

My study aimed to determine whether *P. fluviatilis* showed chemosensory recognition of chemical cues indicating the risk of a parasite by assessing whether they changed their behaviour after being exposed to the chemical cues of parasites (in the form of water conditioned by snails infected with *C. parvum*) or in the presence or absence of a *C. parvum* cercaria. The final dataset consisted of 195 amphipods in total from the six treatments: *control treatment parasite absent* (n=33), *control treatment parasite present* (n=30), *uninfected snail treatment parasite absent* (n=33), *uninfected snail treatment parasite present* (n=31), *infected snail treatment parasite absent* (n=34) and *infected snail treatment parasite present* (n=34). Overall there were no differences in behaviour between amphipods primed with chemical cues and those exposed to a neutral stimulus (filtered lake water or parasite-free snail conditioned water).

**Total distance moved**

There was no relationship between total distance moved and amphipod treatment ($R^2$= -0.2717, p=0.17) (table 3.2). The mean distance moved of all amphipods over the five minute period was 327.0 mm ± 17.2. There was no difference between mean distance moved between amphipods which were primed with parasite cues compared to naïve amphipods (figure 3.5). Furthermore, no difference in mean distance moved was found between amphipods exposed directly to cercariae and those not exposed to cercariae (330.8 mm ± 24.6 compared to 323.4 mm ± 24.1). None of the random factors explained a significant proportion of the variance in distance moved between amphipods. The total variance explained by acclimatisation time and date was less than 1%.

**Table 3.2 Results of the linear mixed-effects model with (a) total distance moved (TDM) and (b) time spent in motion (TSM) of *Paracalliope fluviatilis* as response variables, showing the effects of the main predictors and the percentage of the remaining variance accounted for by the random factors.**

| | FIXED FACTORS | ESTIMATE | STD ERROR | T VALUE | P | RANDOM FACTORS | % VARIANCE |
|---|---|---|---|---|---|---|---|
| **(a)** | **TDM** | | | | | | |
| | Treatment | -27.1730 | 19.7970 | -1.37 | 0.1669 | Acclimatisation | 0.03 |
| | Parasite | 0.04750 | 0.11284 | 0.42 | 0.6670 | Date | 0.08 |
| | Size | 0.07604 | 0.12809 | 0.94 | 0.1707 | | |
| **(b)** | **TSM** | | | | | | |
| | Treatment | -0.14961 | 0.65823 | -0.23 | 0.8176 | Acclimatisation | 0.10 |
| | Parasite | 0.02596 | 1.05930 | 0.02 | 0.9889 | Date | 0.11 |
| | Size | -0.32581 | 1.18577 | -0.28 | 0.7661 | | |

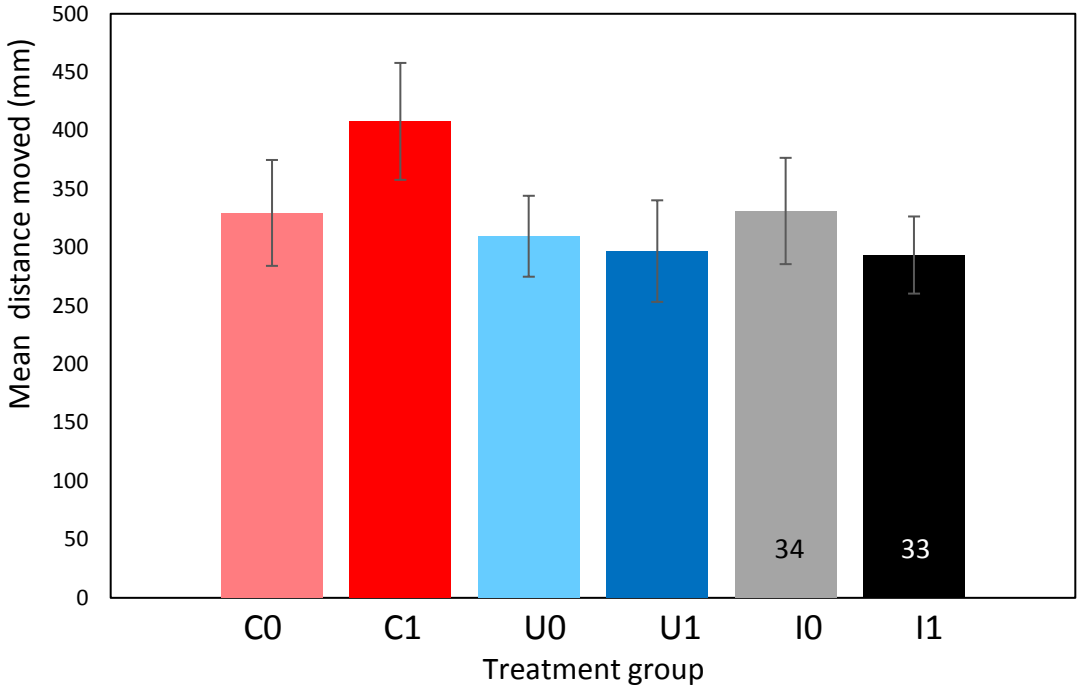


**Figure 3.5 Differences in *Paracalliope fluviatilis* total distance moved in five minutes. Six treatment groups were exposed to the three odour treatments: control, uninfected snail or infected snail treatment (C, U, and I) either in the presence or absence of a *C. parvum* cercaria (0 = no parasite present, 1= parasite present). Error bars show standard error and the number on each bar represents the sample size for each treatment.**

**Time spent moving**

No relationship was found between time spent in motion and amphipod treatment ($R^2$= -0.15, p= 0.82) (table 3.2). The mean time spent in motion of amphipods from all treatments over the five minute period was 10.7 s ± 0.5 (figure 3.6). There was no difference between mean time spent in motion between amphipods which were primed with parasite cues compared to naïve amphipods (10.9 s ± 1.0 vs 10.5 s ± 0.6), or differences between those measured in the presence of a parasite vs the absence of a parasite (10.6 s ± 0.8 vs 10.7 s ± 0.7) (figure 3.6). None of the random factors explained a significant proportion of the variance in time spent in motion between amphipods. The total variance explained by acclimatisation and date was just over 1%.

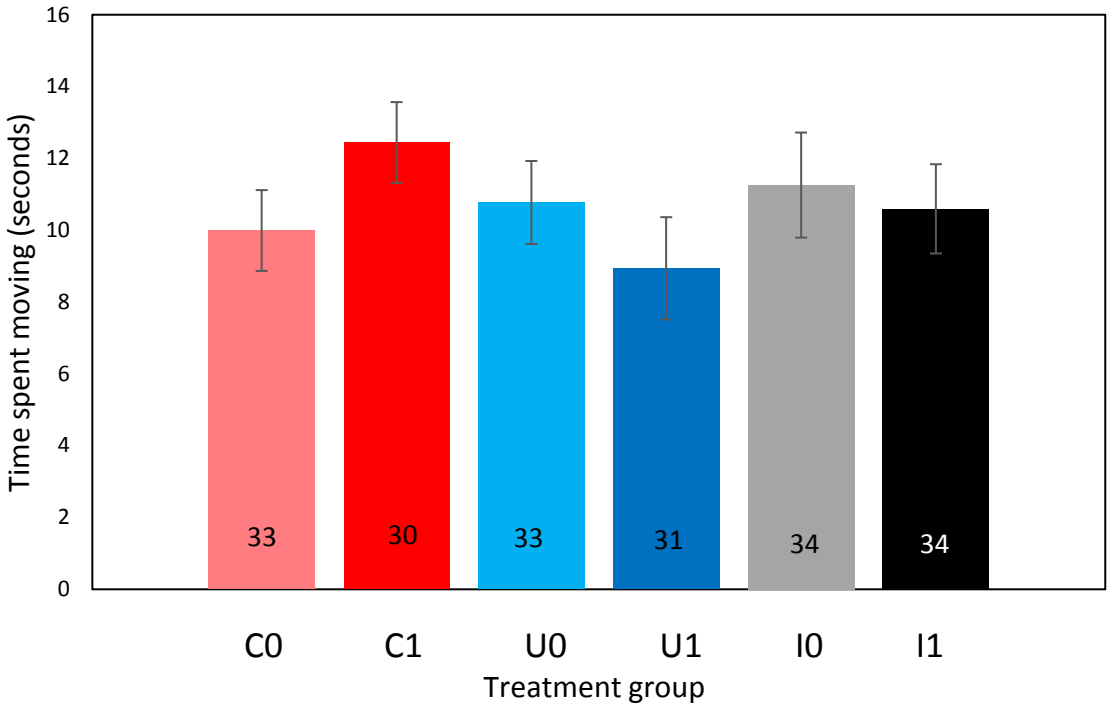


**Figure 3.6 Differences in *Paracalliope fluviatilis* time spent in motion. Six treatment groups were exposed to the three odor treatments: control, uninfected snail or infected snail treatment (C, U, and I) either in the presence or absence of a *C. parvum* cercaria (0 = no parasite present, 1= parasite present). Error bars show standard error and the number on each bar refers to the group size of each treatment.**

**Discussion**

This chapter examines whether *P. fluviatilis* which have been primed to the presence of *C. parvum* cercariae change their swimming behaviour either in the presence or absence of cercariae. It was hypothesised that primed amphipods exposed to a parasite would spend a higher proportion of time moving and would move greater distances compared to parasite naïve amphipods. However, the results of this experiment did not support the original hypothesis as there was no difference in swimming behaviour between treatments. It was also hypothesised that there would be no difference in swimming behaviours between amphipod treatments in the absence of a parasite, because the behaviours measured are temporary. Although amphipods showed no response when there was no direct threat of parasites (i.e. parasite absent treatment), this does not support the original hypothesis because amphipods also showed no response in the presence of *C. parvum*. Taken together these findings suggest that *P. fluviatilis* either cannot sense the chemical cues of snails infected with *C. parvum*, or does not use this cue in isolation to respond to the presence of harmful trematode larvae.

The variables used in this study to evaluate swimming behaviours were distance moved (mm) and time spent moving. These are two general parameters commonly examined to assess the activity of an animal in response to a stimulus such as a predator or a parasite (Marston and Ertle 1973, Persons and Rypstra 2001, Blaser et al. 2010). The current study did not find any change in these variables in amphipods primed to the specific parasite chemical cue used (infected snail conditioned water); in addition, there was no change in behaviour when amphipods were subsequently directly exposed to a cercaria, suggesting that amphipods may not respond to visual cues or contact with parasites. A further study could assess the behavioural responses of *P. fluviatilis* to *C. parvum* in a slightly different way using Ethovision XT by placing a dead cercaria in a known location within a well and seeing if an amphipod avoids crossing that area (figure 3.3) or avoids spending time in that area (figure 3.4). If *P. fluviatilis* did not respond to these types of exposure there are other chemical cues to which they may respond, such as the alarm cues released by conspecifics which have been examined extensively in other studies. Defensive reactions elicited by alarm cues have been documented for several animal groups including terrestrial insects (Huryn and Chivers 1999), crustaceans (Hazlett 1994), arachnids (Machado et al. 2002), gastropods (Stenzler and Atema 1977), echinoderms (Freeman 2005), fish (Pfeiffer 1977, Mathis and Smith 1993, Mathis 2009), amphibians (Chivers et al. 1996a, Kiesecker et al. 1999) and mammals (Kavaliers et al. 2005), although these alarm cues are usually studied in the context of predator threats (Rottman and Snowdown 1972). It is possible that when trematodes such as *C. parvum* bore through the exoskeleton of amphipods they release these injury-induced chemical cues and conspecifics may have the opportunity to exhibit defences or trigger avoidance behaviours, as seen for skin penetrating parasites of juvenile rainbow trout (Poulin et al. 1999).

Several studies have shown that different host species respond to different combinations of parasite cues, predator cues, and alarm cues in various ways. Therefore, in some cases, parasite detection may require a two-component system. A necessary indicator present in the field may not have been included in this experiment such as seasonality, time of day, or multiple chemical cues (Bouwma and Hazlett 2001, Jacobsen and Stabell 2004). Seasonality and associated temperature fluctuations prove important in the case of the marine snail *Tegula funebralis* and its avoidance response to predatory crabs (Jacobsen and Stabell 2004). The predator avoidance strategy of this snail is to climb up walls (Geller 1982). Snails were exposed to a crude extract of conspecifics and water conditioned by predatory and non-predatory crabs (Jacobsen and Stabell 2004). Additional factors other than the chemical cues of predatory crabs proved to be more important than the presence of chemical cues, such as seasonality as *T. funebralis* extract alone induced climbing behaviour in May but not June, and a combination of predatory cues and alarm

cues induced a greater degree of avoidance overall (Jacobsen and Stabell 2004). Seasonality-dependent response to alarm cues has also been shown in other species such as gastropods (Jacobsen and Stabell 1999), cyprinoid fishes (Smith 1977, 1992) and newts (Rohr et al. 2002). In a crayfish (*O. propinquus*) the addition of conspecific alarm cues in combination with tactile and visual cues produced a stronger response in terms of distance retreated (Bouwma and Hazlett 2001). The magnitude and frequency of defence response to combinations of tactile cues and alarm cues was higher but only during the night. This provides evidence that the ecology of prey and threat species may play a role as important as detection of chemical cues.

In Lake Waihola the prevalence of *C. parvum* within the snail *P. antipodarum* ranges from 1% in the winter months to 5% during summer, and densities of *Paracalliope fluviatilis* fluctuate between 315-1130 individuals per $m^2$ in correlation with water temperatures (Lagrue and Poulin 2008). This means that at different times of the year, and at different temperatures, *P. fluviatilis* populations would experience a variable risk of becoming infected by *C. parvum*; therefore these factors could influence the likelihood of *P. fluviatilis* investing in anti-parasite defences. Testing behavioural responses of *P. fluviatilis* at different temperatures or different times of the day may also be important because *C. parvum* cercariae are shed from snails when conditions are warm and bright (MacFarlane 1939). The breeding season for *P. fluviatilis* begins in late winter, at which time it is critical for both sexes to invest in reproduction given their short lifespan and the finite number of clutches that females can produce (Lefebvre et al. 2005). In a further study, *P. fluviatilis* behavioural responses could potentially be re-examined at various times of the year to assess if seasonality and their changing energy investments affect their defences. In a laboratory setup, this could be simulated by different temperatures and light regimes.

It is possible that the chemical cues used were not strong enough to provoke a response from amphipods although the concentration used was higher than that expected in the natural habitat. Although varying concentrations of parasite cues have not been examined previously, concentrations of cues involved with predator encounters (i.e. alarm cues and predator cues) have been examined in a few cases (Hazlett 1997, Bouwma and Hazlett 2001). For example, the defence response of crayfish *Orconectes propinquus* was tested when presented with visual cues of predators in combination with alarm cues at 100, 50, 10, and 1% dilution. There was no difference seen in terms of distance retracted directly related to chemical concentration. However, *O. propinquus* were found to increase their behavioural response to visual cues in combination with higher concentrations of alarm cues (Bouwma

and Hazlett 2001). Another study of a similar design, where conflicting chemicals were provided at increasing concentrations, examined the response of hermit crabs (*Diogenes avarus*) to the smell of degraded snail flesh (which indicates potential empty shells) and predator odour (Hazlett 1997). In this case, the responses to these cues were hierarchical. In the presence of snail flesh odour *D. avarus* would increase locomotion and grasping rate at gastropod shells but if any concentration of predator cue in combination with the snail flesh odour was present, these behaviours were inhibited (Hazlett 1997). Although these studies relate to chemical cues involved with predation events rather than parasite encounters, they are still useful to draw upon as they show how combinations of cues might produce or suppress various behavioural responses.

Priming *P. fluviatilis* to snail conditioned water alone may not be enough to provoke a change in behaviour, as amphipods will not invest in defences against parasites unless the risk of infection is high (Bryan-Walker et al. 2007). As *C. parvum* infected snails typically only shed between 1 - 30 cercariae every couple of days and cercariae are relatively short-lived (MacFarlane 1939), *P. fluviatilis* which live in close proximity may not be constantly at risk of infection. This might explain why amphipods primed with infected snail treatment did not show any response in terms of distance moved or time spent in motion (figure 3.5 & 3.6) As discussed by Bouwma and Hazlett 2001, several cues at once may be necessary for parasite detection. For example, a combination of alarm cues produced from newly infected conspecifics

in combination with parasite chemical cues may be necessary for *P. fluviatilis* to recognize the high risk of infection and cause them to respond defensively, either in a gradual or a hierarchical manner.

The response of *P. fluviatilis* to parasite cues may also be affected by the age and duration of the chemical cues in water. The aging of cues which indicate parasite presence (such as water conditioned with infected snails) has not been examined but it is probable that these could be produced from host animal's metabolites and could, therefore, be of similar composition to chemical signals associated with predation events (kairomones and alarm cues) which could mean parasite cues have similar active times. It has been found that fathead minnows (*Pimephales promelas*) respond to the alarm cues of either conspecifics or heterospecifics (*Gammarus lacustris*) from cues that were newly produced or had an age of three hours, but animals do not respond to cues older than six hours (Wisenden et al. 2009b). A similar pattern was found for all three datasets used in Wisenden's wider examination of cue age (both laboratory and field tests with *P. promelas*, and field tests with northern redbelly dace *Phoxinus eos*) which suggests a common duration of these cues or a common molecular basis of chemical cues

(Wisenden et al. 2009b). It may be possible that animals can detect protein degradation of these cues and use this to assess the risk of a threat (Wisenden et al. 2009b). In the current experiment, *P. fluviatilis* responses were examined 24 hours after amphipods were last exposed to a parasite cue. Perhaps a better time to examine their swimming responses would be at the time of exposure to parasite cues as they might only respond to these in the short term.

Another interesting factor to consider in a further study could be whether behavioural responses to parasite cues were innate or learned. Based on the findings of this study (i.e. no behavioural change observed), *P. Fluviatilis - C. parvum* might not be the best host-parasite system to address this question. In many cases, a single encounter with a novel predator cue is enough for near-permanent recognition of a novel predator (Suboski 1990, Chivers and Smith 1994, Brown 2003). In subsequent encounters with the same predator, indirect indicators of risk such as chemical cues have been shown to be sufficient exposure to elicit an evasive behavioural response. This has been shown in *P. promelas* where one encounter with trematode cercariae is enough to be used for the detection and avoidance of parasitism risks in the future (James et al. 2008). Learned responses to predator cues have been shown in the amphipod *Gammarus minus* using injury-induced chemical cues released by injured conspecifics to alert them to the presence of an actively foraging predator. Amphipods learn to associate the chemical cues of predators such as fathead minnows (*P. promelas*) or other predator kairomones with the risk of predation (Chivers and Smith 1998, Wisenden et al. 1999, Wisenden et al. 2009b, Wisenden et al. 2010). Further investigations into the present study system are necessary to understand the intricate nature of the ways in which recognition of these chemical cues are utilised.

All amphipods used in this experiment were collected from Lake Waihola, a site where the trematode species *C. parvum* and *Maritrema poulini* are common in *Potamopyrgus antipodarum.* The presence of some pre-infections (which were excluded) confirms that these amphipods had encountered parasites in some capacity before the experiment. If a specific parasite cue, or combination of cues is found which does create a defensive response in *P. fluviatilis*, it could be useful to examine whether behavioural responses are innate or learned using laboratory raised amphipods. Additionally, another local amphipod-trematode system could be used in further experiments as a comparison, such as *Paracalliope novizealandiae* (a marine amphipod known to host the *trematode Maritrema novaezealandensis).* This amphipod is abundant in their habitats, easy to handle and can be cultured in the laboratory (King et al. 2006, Bryan-Walker et al. 2007). Comparing the responses of amphipod species within different model systems would, therefore, therefore illuminate how general these responses are.

Another explanation of the lack of behavioural change found in this experiment is that for *P. fluviatilis* chemical cues may not be the most adaptive way of sensing the presence of *C. parvum* cercariae. Snails and *P. fluviatilis* are known to be present in dense congregations in macrophyte beds in Lake Waihola, since both *P. fluviatilis* and snails feed on epiphytic microalgae growing on macrophytes. Therefore amphipods are likely to encounter *C. parvum* cercariae in close quarters (Sutherland et al. 2007, Lagrue and Poulin 2008). Amphipods are known to frequently settle upon *Potamopyrgus* and remain there for several minutes, meaning they are potentially available for parasitization (Macfarlane, 1939).

One possible explaination for why no response was found is that *C. parvum* could be more adapted for finding *P. fluviatilis* than the amphipod is at sensing and avoiding parasite larvae. This trematode cercaria may be adapted to the anti-predatory behaviours of *P. fluviatilis*. The threat-avoiding behaviours of *P. fluviatilis* include negative phototropism and their epigean nature where they will crawl and cling to a substrate such as macrophytes (MacFarlane 1939). The cercaria of *C. parvum* has no eyespots and does not show a definite phototropism. Unlike many trematode cercariae, which have tails allowing them to swim in the water column, *C. parvum* possess a short tail with a sucker to attach to the substrate and make their swinging movement to seek out a suitable host (MacFarlane 1939). They approach amphipods by crawling on the substrate in an adaption to the positively thigmotropic nature of *P. fluviatilis* (Macfarlane, 1939).

In conclusion, the main findings of this experiment were that *P. fluviatilis* did not show behavioural changes in terms of time spent in motion and distance moved when primed with *C. parvum* conditioned snails and when *P. fluviatilis* were exposed to *C. parvum* cercariae directly. This is still an interesting finding as we can conclude that amphipods do not respond to these specific cues in isolation, at the given concentration of parasite cues used. In the next chapter, these results and the results from chapter 2 will be examined together and discussed in a wider ecological context.

# Chapter 4: General Discussion

**Thesis findings**

The objective of this study was to assess the ability of the amphipod *Paracalliope fluviatilis* to recognise and respond to potential chemical cues which indicate the threat of infection by the trematode parasite *Coitocaecum parvum*. This a common freshwater trematode which infects New Zealand freshwater fish as definitive hosts and requires amphipods as a second intermediate host (Lagrue and Poulin 2015b). For most aquatic hosts, including crustaceans, key aspects of their responses to the chemical cues of parasites have not been investigated. These are vital to understand, as hosts are exposed to parasites multiple times over the course of their lifetime, and we might expect natural selection to have favoured adaptations against repeated threats from fitness-reducing parasites. In the case of the trematode *C. parvum* and its relationship with the second intermediate host *P. fluviatilis*, multiple aspects of their interactions have been well explored including *C. parvum* life history, progenesis of *C. parvum* within *P. fluviatilis*, *C. parvum* intra- and interspecific competition within *P. fluviatilis* and the seasonal variation of *C. parvum* infection rates within Lake Waihola (Holton 1984, Lagrue and Poulin 2007, 2008, Daniels et al. 2013). However, despite some studies into the impact of this trematode on assortative pairing, survival and fecundity of amphipods (Lefebvre et al. 2005, Rauque et al. 2011, Friesen et al. 2017), their reaction to cues indicating the presence of parasites remains unknown. In this study, amphipods collected from Lake Waihola were exposed to chemical cues associated with parasites (water conditioned by snails infected with *C. parvum*) and the average number of acquired parasites, and survival of amphipods was compared between primed and un-primed amphipods in an infection experiment (chapter 2). This was to assess whether being primed with chemical cues increased the amphipod's ability to avoid infections in subsequent encounters with the parasite. The second set of experiments (chapter 3) was designed to test whether this priming to parasites had effects on host activity in a behavioural study (distance moved, and time spent in motion).

The infection experiment revealed that priming to the specific chemical cue used did not influence the subsequent acquisition of parasites in the amphipod *P. fluviatilis,* as there was no difference in parasite abundance between amphipods exposed to the chemical cues of parasites and naïve amphipods. This could be because the chemical cue used was not enough on its own to trigger a defence response, as hosts may only learn to avoid parasites after direct exposure proves costly (James et al. 2008). It is also possible that other chemical cues may prove more important, such as alarm cues from conspecifics, which have been examined in the context of parasite infection in fish and amphibian hosts (Poulin et al. 1999, Rohr et al. 2009). An alternative explanation for these results is that *P. fluviatilis* may not have the

necessary energetic requirements to defend against parasites due to trade-offs from investing in feeding activity, predator avoidance or reproduction (Sheldon and Verhulst 1996).

Amphipods which harboured natural infections from the field before this experiment were found to acquire more parasites, in terms of prevalence and mean abundance, over the course of this experiment. This phenomenon may lead to the general pattern found in nature where parasites will typically be aggregated - where most members of a host population are parasite free, and a few animals host a relatively large number of parasites (Crofton 1971, Pennycuick 1971, Shaw and Dobson 1995, Poulin and Morand 2000). It has been suggested that the costs of infection may limit the available energy of a host to combat against future threats (Beldomenico and Begon 2010, Choisy and de Roode 2010). Taken together, the results of this experiment suggest that although exposure to indirect parasite cues (through exposure to water conditioned with snails infected with *C. parvum*) did not influence the ability of *P. fluviatilis* to evade parasites, previous direct exposure to a parasite (through existing infection) had a negative effect on the ability of *P. fluviatilis* to defend itself in future parasite encounters.

To expand upon the investigation of the first experiment, the behavioural study examined whether *P. fluviatilis*, which have been primed to the presence of *C. parvum* cercariae in the same way (exposed to water conditioned by snails infected with *C. parvum*), would change their swimming behaviour either in the presence or absence of an actual *C. parvum* cercaria. It was hypothesised that primed amphipods exposed to a trematode cercaria would reduce their activity in terms of distance moved and time spent in motion to avoid encountering the parasite. However, there was no significant difference in swimming behaviour of amphipods between priming treatments, and no difference in behaviour between amphipods in the presence or absence of a parasite. These findings suggest that *P. fluviatilis* either cannot sense the chemical cue (of snails infected with *C. parvum*), or alternatively does not use this cue in isolation to recognise the presence of harmful trematode larvae. These findings, based on the study of an amphipod-trematode parasite system in the laboratory, could be relevant to other amphipod species as the basis of chemical cues that mediate host awareness may be similar in different systems. This may be indicative of general patterns of amphipod recognition and response to trematode parasites, or a shift from the usual response due to the ecology of both *P. fluviatilis* and *C. parvum.*

**Significance of results**

The results of the current study are important as very few studies have been conducted to examine the specific response of aquatic hosts to chemical cues which indicate trematode presence, and no study has examined an amphipod host specifically (Poulin et al. 1999, James et al. 2008). As animals commonly encounter parasites numerous times within their lifetime, it is a crucial issue to determine which types of cues are present, if any, and how these affect encounter rates, the susceptibility of hosts, and parasite infection success. The amphipod species used, *P. fluviatilis*, is the most abundant and widespread freshwater amphipod endemic to New Zealand (Chapman et al. 1976). This species is, therefore, an important prey item for fish predators, such as juvenile brown trout, shortfin and longfin eels, and bullies (Jellyman 1989, Hayes and Rutledge 1991, Sagar and Glova 1995), and is a common second intermediate host of multiple parasite species other than *C. parvum,* such as *Maritrema poulini*, *Acanthocephalus galaxii* and an unidentified cyclophyllidean cestode (MacFarlane 1939, Hine 1977, Rauque et al. 2011, Presswell et al. 2014). As parasites are known to influence hosts' coexistence with other species in diverse ways, such as through altered levels of competition, predation and herbivory or through increased susceptibility to parasites and disease (Lafferty and Morris 1996, Rodriguez et al. 1999, Beldomenico and Begon 2010, Hatcher et al. 2012), the levels of infection of amphipods in a population may impact the wider aquatic community. The experimental infection component of this study showed that *P. fluviatilis* which were exposed to the chemical cues indicating the risk of trematodes (water conditioned with snails infected with *C. parvum*) did not outperform naïve amphipods in terms of successful parasite defence. It was also found that amphipods with pre-existing infections were more susceptible to further *C. parvum* trematodes. Amphipods are an important food source and host for parasites in this system and therefore these results could prove significant. *Paracalliope fluviatilis* susceptibility to other common parasites such as *M. poulini, A. galaxii,* or the unidentified cyclophyllidean cestode which could cause increases of parasite densities at other trophic levels.

The behavioural component of this study found there was no change in swimming behaviours of amphipods when primed with chemical cues, or when they are directly exposed to *C. parvum*. Studies in the literature have shown that some animals show avoidance to parasites innately either through recognition of their cues (Cooper et al. 2000, Karvonen et al. 2004, Kavaliers et al. 2004) or as a response to alarm cues released by conspecifics during infection. Other animals can learn to avoid parasites through experience. The results of this study suggest that *P. fluviatilis* may lack the ability to sense trematode larvae through the chemical cue used, or alternatively do not respond to this cue with a behavioural defence. This is a significant result as it suggests that, although some animals have the

capability to recognise parasites and respond to them behaviourally prior to infection, this may not be a universal ability. Since no behavioural change was found, it eliminates this chemical cue as the prime signal *P. fluviatilis* use to identify trematode cercariae. Potentially *P. fluviatilis* may not be capable of avoiding infection by trematode cercariae and instead utilise other adaptive defences such as reaching reproductive age earlier.

**Further studies**

The current research sheds new light on the ability of amphipods to respond to chemical cues which indicate the threat of parasites, as well as the effects that exposure to these cues had in terms of survival and behaviour. However, it also identified areas where further research is required.

One of the mechanisms hosts possess to defend themselves against parasites, other than behavioural responses, is an immune response. Amphipods such as *Paracalliope novizealandiae*, have the ability to encapsulate and melanise encysted trematode metacercariae (Bryan-Walker et al. 2007). Although immune responses are expensive, they may be worthwhile when the risk of parasitism is high (Bryan-Walker et al. 2007). In a system like Lake Waihola, *P. fluviatilis* are frequently exposed to *C. parvum* parasites due to the high density of the snail *Potamopyrgus antipodarum* (4.6% see table 1.1) (Lagrue and Poulin 2008). As amphipods and snails share a habitat in macrophyte beds, amphipods would probably encounter cercariae frequently and it is reasonable to assume that amphipods would routinely benefit from immune responses to eliminate parasites (MacFarlane 1939). A further study could investigate the importance of the immune response of *P. fluviatilis* using a similar study design as the experimental infection conducted in the present study with the addition of temporarily anaesthetising *P. fluviatilis* with carbonated water(Kumazawa 2016), or with clove oil (Brown et al. 2003) to knock out behavioural defences (swimming away, grooming). This would allow one to solely examine the impact of immune defences by comparing subsequent cercarial infection success with a control group which is not anaesthetised. Clove oil has been used (Haas 1994) successfully to temporally anaesthetise giant prawn (*Macrobrachium rosenbergii*) with no long term mortality effects on post larval stages, as well as crabs (*Pseudocarcinus gigas*) (Gannon and Gannon 1975, Brown et al. 2003). Carbonated water is also an efficient, gentle narcotic for crustaceans such as Cladocera, Calanoida and Cyclopida (Gannon and Gannon 1975) and cooled carbonated water has been used to temporarily immobilize copepods for infection experiments (Kumazawa 2016). A pilot study would first need to be conducted to assess which substance would be best to use for amphipods, and the appropriate concentration and submergence times.

As *P. fluviatilis* have short lifespans, we may not have seen a defence response because investing energy into breeding may take precedence over defending against trematodes in this host species (Sheldon and Verhulst 1996, Agnew et al. 2000). There seems to be some support for this hypothesis as a recent study found that female *P. fluviatilis* carrying young tend to have higher *Maritrema poulini* and *C. parvum* abundance than those that were not, and yet the number of young carried was not affected by parasite abundance (Friesen et al. 2017). One explanation could be that parasitised females become "handicapped'' with artificially impaired swimming performance, and become therefore easier for males to pair with (Sutherland et al. 2007). Alternatively, this finding could suggest that females may choose to invest more into reproduction if they become infected, a response which has been found in other invertebrate host species (Agnew et al. 2000). An example of this in a crustacean host is shown with female amphipods in an intertidal species (*Corophium volutator*) which respond to trematode infection by reproducing as fast as possible, in contrast to uninfected females which reproduce only once they have reached their maximum body size (McCurdy et al 2001). Any fecundity effects due to seasonality, the age of amphipod and infection status could be examined further in the studied system using laboratory experiments where female *P. fluviatilis* of different ages could be experimentally infected at different times of the year, and their subsequent fecundity and growth could be compared to those of uninfected amphipods.

The specific findings of the experimental infection regarding the increased susceptibility of previously infected *P. fluviatilis* highlight the importance of investigating other host-parasite systems from the same lake (e.g. involving *Maritrema poulini*). There is evidence that *M. poulini* infections are similarly prevalent in *P. fluviatilis* (5.8% in males and 12% in female amphipods) as *C. parvum* infections (11.2% across all seasons) (Lagrue and Poulin 2008b). *Maritrema poulini* is known to increase *P. fluviatilis* mortality and uses different host finding strategies than *C. parvum* to locate hosts and it is possible that *P. fluviatilis* has evolved more obvious defences against this trematode than against *C. parvum* and that these could be examined in further experiments. As there are other potential hosts in the system for these parasites such as *Paracorophium excavatum* and *Austridotea annectens*, which support higher prevalence of *M. poulini* infections (Friesen et al. 2017), it is important to understand the tolerance of these hosts and the larger ecosystem effects different parasite species can have (Friesen 2017). Further research should consider the effect of multiple infections and double infections within *P. fluviatilis* as different parasites have been shown to interact in various ways as has been shown in other systems (antagonistic,

cancelling out the effects of each other, or co-operative with additive negative consequences for hosts) (Poulin et al. 1999, Telfer et al. 2008).

Lastly, to achieve a thorough understanding of a phenomenon such as the cues driving host defence responses, it is often necessary to combine experimental studies in the laboratory with field surveys of wild populations to allow the validation of laboratory results with real-world observations (Shaw and Dobson 1995). There are various limitations to both small-scale laboratory tests (e.g. low genetic variety of assessed species if animals are only collected from one location, difficulty to assess true fitness costs in short term studies) and field studies (e.g. unknown host exposure to parasites, the complexities associated with processing large amounts of data, and patchy parasite densities in the field). These restrictions can be overcome by using laboratory and field studies in combination, such as when attempting to assess fitness consequences (Graham et al. 2011). In this case, future studies could be done to assess how the shared ecology of *C. parvum* and *P. fluviatilis* influences the responses of amphipods. Necessary cues may be discerned if laboratory conditions more closely mirror field conditions by conducting observational measurements such as host fitness and responses in the field (Norris et al. 1994, Stjernman et al. 2008). Studying interactions between the amphipod host and parasite cercariae might help illuminate the range of defences used by *P. fluviatilis*. Further experiments could be conducted in macrophyte beds (either simulated in the laboratory or within isolated sections of naturally occurring macrophyte beds in the field), as large densities of *P. antipodarum* and *P. fluviatilis* congregate in macrophyte beds in Lake Waihola where most natural infection events probably occur. Furthermore, the interaction with the parasite's first intermediate snail hosts could be investigated, since it was noted that amphipods frequently settle on the shells of *P. antipodarum* (MacFarlane 1939). Experiments could determine whether amphipods change this behaviour depending on the snail's parasite infection status. Mechanical cues (such as water movement, or vibrations) are others factor that may be important for trematode detection in this species, especially due to the limited mobility of *C. parvum* cercariae (which move across the substrate using their oral and caudal suckers instead of swimming) (MacFarlane 1939). It has been shown that free-swimming trematode larvae use a combination of visual, tactile and chemical stimuli to detect suitable hosts in their aquatic habitats (e.g. bird schistosome cercariae (Haas 1992, Haas 1994, Haas 2003)). It is, therefore, possible that the host's defence mechanisms rely on a similarly complex combination of cues to detect and avoid potential parasites and pathogens.

**Conclusions**

The experimental and observational studies summarised in the present thesis emphasise the importance of considering the role of different cues on host response and avoidance of trematode parasites. Priming amphipods to the chemical cue examined did not influence the number of parasites acquired or affect the swimming behaviour of *P. fluviatilis.* It was found that amphipods harbouring infections prior to the experiment were 20% more likely to acquire new cercariae compared to parasite-free amphipods during experimental infections. It was also found that survival of amphipods in the laboratory exposed to water conditioned by snails infected with *C. parvum* was comparable to that of naïve amphipods which suggests that exposure to this cue does not stress amphipods enough to impact their short-term survival. Overall, these results suggest that other cues may be more significant than the tested factors for amphipod recognition and avoidance of trematode parasites. It is important to determine which cues play recognition roles to understand the complexity of how hosts encounter and interact with parasites both spatially and temporally. Host-parasite interactions involve many fascinating aspects that must be carefully considered to better understand the complex interactions taking place in ecosystems, many of which we do not yet comprehend.

# References

Agnew, P., J. C. Koella, and Y. Michalakis. 2000. Host life history responses to parasitism. Microbes and Infection **2**:891-896.

Allen, J. E., and R. M. Maizels. 2011. Diversity and dialogue in immunity to helminths. Nature Reviews Immunology **11**:375-388.

Anderson, R., and J. Crombie. 1984. Experimental studies of age-prevalence curves for *Schistosoma mansoni* infections in populations of *Biomphalaria glabrata*. Parasitology **89**:79-106.

Anderson, R., and D. Gordon. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology **85**:373-398.

Anderson, R., and P. Whitfield. 1975. Survival characteristics of the free-living cercarial population of the ectoparasitic digenean *Transversotrema patialensis* (Soparker, 1924). Parasitology **70**:295-310.

Anderson, R. M., and R. M. May. 1991. The spread of HIV-1 in Africa: sexual contact patterns and the predicted demographic impact of AIDS. Nature **352**:581.

Ashida, M., and P. T. Brey. 1995. Role of the integument in insect defense: pro-phenol oxidase cascade in the cuticular matrix. Proceedings of the National Academy of Sciences **92**:10698-10702.

Atema, J., and D. Stenzler. 1977. Alarm substance of the marine mud snail, *Nassarius obsoletus*: biological characterization and possible evolution. Journal of Chemical Ecology **3**:173-187.

Ausseil, A., P. Newsome, and P. Johnson. 2008. Wetland mapping in the Otago Region. Landcare Research Contract Report LC0608/115. Prepared for Otago Regional Council, NZ:355-384

Baldauf, S. A., T. Thünken, J. G. Frommen, T. C. Bakker, O. Heupel, and H. Kullmann. 2007. Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. International journal for parasitology **37**:61-65.

Balmer, O., and M. Tanner. 2011. Prevalence and implications of multiple-strain infections. The Lancet Infectious Diseases **11**:868-878.

Barber, I., D. Hoare, and J. Krause. 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries **10**:131-165.

Barry, M. J. 2000. Effects of endosulfan on Chaoborus-induced life-history shifts and morphological defences in *Daphnia pulex*. Journal of Plankton Research **22**:1705-1718.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. *lme4*: Linear mixed-effects models using Eigen and S4. R package version **1**:1-23.

Baudoin, M. 1975. Host castration as a parasitic strategy. Evolution **29**:335-352.

Beentjes, M. 2013. Monitoring commercial eel fisheries: 2009–10 to 2011–12. New Zealand Fisheries Assessment Report **47**:76.

Behnke, J. 2008. Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions.... or pure randomness? Parasitology **135**:751-766.

Beldomenico, P. M., and M. Begon. 2010. Disease spread, susceptibility and infection intensity: vicious circles? Trends in Ecology & Evolution **25**:21-27.

Beltran-Bech, S., and F.J. Richard. 2014. Impact of infection on mate choice. Animal Behaviour **90**:159-170.

Bethel, W. M., and J. C. Holmes. 1977. Increased vulnerability of amphipods to predation owing to altered behavior induced by larval acanthocephalans. Canadian Journal of Zoology **55**:110-115.

Blaser, R., L. Chadwick, and G. McGinnis. 2010. Behavioral measures of anxiety in zebrafish (*Danio rerio*). Behavioural brain research **208**:56-62.

Blinova, N., and S. Cherkashin. 2012. The olfactory system of crustaceans as a model for ecologo-toxicological studies. Journal of Evolutionary Biochemistry and Physiology **48**:155-165.

Blum, M. S. 1969. Alarm pheromones. Annual review of entomology **14**:57-80.

Booth, M. 2006. The role of residential location in apparent helminth and malaria associations. Trends in parasitology **22**:359-362.

Bouwma, P., and B. A. Hazlett. 2001. Integration of multiple predator cues by the crayfish *Orconectes propinquus*. Animal Behaviour **61**:771-776.

Breithaupt, T., and M. Thiel. 2010. Chemical communication in crustaceans. Springer Science & Business Media. 3-22

Broni, S. 1993. Lakes Waipori and Waihola Wetland - A natural resources inventory. Department of Conservation, editor., Otago Conservancy, Dunedin:6-8

Brown, G. E. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. Fish and Fisheries **4**:227-234.

Brown, G. E., and J.G. J. Godin. 1997. Anti-predator responses to conspecific and heterospecific skin extracts by threespine sticklebacks: alarm pheromones revisited. Behaviour **134**:1123-1134.

Brown, G. E., and J.G. J. Godin. 1999. Who dares, learns: chemical inspection behaviour and acquired predator recognition in a characin fish. Animal Behaviour **57**:475-481.

Brown, J. H., S. McCauley, B. Ross, A. Taylor, and F. Huntingford. 2003. A test of two methods for marking larvae and postlarvae of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture Research **34**:49-54.

Brown, K. 1998. The role of shell strength in selective foraging by crayfish for gastropod prey. Freshwater Biology **40**:255-260.

Brown, W. L., T. Eisner, and R. H. Whittaker. 1970. Allomones and kairomones: transspecific chemical messengers. Bioscience **20**:21-21.

Bryan-Walker, K., T. L. Leung, and R. Poulin. 2007. Local adaptation of immunity against a trematode parasite in marine amphipod populations. Marine Biology **152**:687-695.

Bull, P. C., B. S. Lowe, M. Kortok, C. S. Molyneux, C. I. Newbold, and K. Marsh. 1998. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nature medicine **4**:358-360.

Catania, K. C., J. F. Hare, and K. L. Campbell. 2008. Water shrews detect movement, shape, and smell to find prey underwater. Proceedings of the National Academy of Sciences **105**:571-576.

Chaisson, K. E., and E. A. Hallem. 2012. Chemosensory behaviors of parasites. Trends in parasitology **28**:427-436.

Chapman, M. A., M. H. Lewis, and V. M. Stout. 1976. Introduction to the Freshwater Crustacea of New Zealand. Collins: 171-177

Chivers, D., and R. Smith. 1994. The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. Journal of Fish Biology **44**:273-285.

Chivers, D. P., J. M. Kiesecker, M. T. Anderson, E. L. Wildy, and A. R. Blaustein. 1996a. Avoidance response of a terrestrial salamander (*Ambystoma macrodactylum*) to chemical alarm cues. Journal of Chemical Ecology **22**:1709-1716.

Chivers, D. P., and R. J. F. Smith. 1998. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience **5**:338-352.

Chivers, D. P., B. D. Wisenden, and R. J. F. Smith. 1996b. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. Animal Behaviour **52**:315-320.

Choisy, M., and J. C. de Roode. 2010. Mixed infections and the evolution of virulence: effects of resource competition, parasite plasticity, and impaired host immunity. The American Naturalist **175**:E105-E118.

Christensen, R. H. B. 2015. ordinal: Regression Models for Ordinal Data. R package version 2015.6-28. See http://www.cran.r-project.org/package=ordinal.

Clark, T., M. Bradburn, S. Love, and D. Altman. 2003. Survival analysis part I: basic concepts and first analyses. British Journal of Cancer **89**:232.

Coats, J., R. Poulin, and S. Nakagawa. 2010. The consequences of parasitic infections for host behavioural correlations and repeatability. Behaviour **147**:367-382.

Cohen, S., I. McGregor, and S. Carrington. 1961. Gamma-globulin and acquired immunity to human malaria. Nature **192**:733-737.

Combes, C. 1996. Parasites, biodiversity and ecosystem stability. Biodiversity and Conservation **5**:953-962.

Combes, C. 2001. Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press: 45-50

Cooper, J., I. J. Gordon, and A. W. Pike. 2000. Strategies for the avoidance of faeces by grazing sheep. Applied Animal Behaviour Science **69**:15-33.

Cox, F. 2001. Concomitant infections, parasites and immune responses. Parasitology **122**:S23-S38.

Cribb, T. H., R. A. Bray, D. Littlewood, S. P. Pichelin, and E. A. Herniou. 2001. The digenea. Interrelationships of the Platyhelminthes:168-185.

Cribb, T. H., R. A. Bray, P. D. Olson, D. Timothy, and J. Littlewood. 2003. Life cycle evolution in the Digenea: a new perspective from phylogeny. Advances in Parasitology **54**:197-254.

Crofton, H. 1971. A quantitative approach to parasitism. Parasitology **62**:179-193.

Cromarty , P., and D. A. Scott. 1995. A Directory of Wetlands in New Zealand, New Zealand Department of Conservation, Wellington, New Zealand:348-394

Crombie, J., and R. Anderson. 1985. Population dynamics of *Schistosoma mansoni* in mice repeatedly exposed to infection. Nature **315**:491-493.

Cronin, G., and M. E. Hay. 1996. Induction of seaweed chemical defences by amphipod grazing. Ecology **77**:2287-2301.

Dahl, E. 1978. The amphipod functional model and its bearing upon systematics and phylogeny. Zoologica Scripta **6**:221-228.

Daniels, R. R., S. Beltran, R. Poulin, and C. Lagrue. 2013. Do parasites adopt different strategies in different intermediate hosts? Host size, not host species, influences *Coitocaecum parvum* (Trematoda) life history strategy, size and egg production. Parasitology **140**:275-283.

Derby, C. D., M. T. Kozma, A. Senatore, and M. Schmidt. 2016. Molecular mechanisms of reception and perireception in crustacean chemoreception: a comparative review. Chemical Senses **41**:381-398.

Derby, C. D., and M. J. Weissburg. 2014. The Chemical Senses and Chemosensory Ecology of Crustaceans. Crustacean Nervous Systems and Their Control of Behavior, New York, NY: Springer:263-293.

Devevey, G., and P. Christe. 2009. Flea infestation reduces the life span of the common vole. Parasitology **136**:1351-1355.

Dezfuli, B. S., E. Simoni, L. Duclos, and E. Rossetti. 2008. Crustacean-acanthocephalan interaction and host cell-mediated immunity: parasite encapsulation and melanization. Folia Parasitologica **55**:53.

Dobson, R., P. Waller, and A. Donald. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. International Journal for Parasitology **20**:347-352.

Dodson, S. I., T. A. Crowl, B. L. Peckarsky, L. B. Kats, A. P. Covich, and J. M. Culp. 1994. Non-visual communication in freshwater benthos: an overview. Journal of the North American Benthological Society **13**:268-282.

Ewald, P. W. 1995. The evolution of virulence: a unifying link between parasitology and ecology. The Journal of parasitology **81**:659-669.

Ezenwa, V. O. 2004. Selective defecation and selective foraging: antiparasite behavior in wild ungulates? Ethology **110**:851-862.

Fenwick, G. D. 2001. the freshwater amphipoda (crustacea) of New Zealand: a review. Journal of the Royal Society of New Zealand **31**:341-363.

Ferrari, M. C., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus The present review is one in the special series of reviews on animal–plant interactions. Canadian Journal of Zoology **88**:698-724.

Ferreira, B., and J. Silva. 1999. Successive tick infestations selectively promote a T-helper 2 cytokine profile in mice. Immunology **96**:434.

Field, L. H. 1990. Aberrant defense displays of the big-handed crab, *Heterozius rotundifrons* (Brachyura: Belliidae). New Zealand Journal of Marine and Freshwater Research **24**:211-220.

Fisher, R. A. 1941. The negative binomial distribution. Annals of Human Genetics **11**:182-187.

Fredensborg, B.L. and R. Poulin. 2005. Larval helminths in intermediate hosts: Does competition early in life determine the fitness of adult parasites? International Journal for Parasitology **35**:1061-1070.

Fredensborg, B.L., K.N. Mouritsen, and R. Poulin. 2004. Intensity-dependent mortality of Paracalliope novizealandiae (Amphipoda: Crustacea) infected by trematode: experimental infections and field observations. Journal of Experimental Marine Biology and Ecology **311**:253-265.

Fredensborg, B. L., K. N. Mouritsen, and R. Poulin. 2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. Marine Ecology Progress Series **290**:109-117.

Freeman, A. 2005. Size-dependent trait-mediated indirect interactions among sea urchin herbivores. Behavioral Ecology **17**:182-187.

Freitak, D., H. Schmidtberg, F. Dickel, G. Lochnit, H. Vogel, and A. Vilcinskas. 2014. The maternal transfer of bacteria can mediate trans-generational immune priming in insects. Virulence **5**:547-554.

Frey, D. G. 1993. The penetration of cladocerans into saline waters. Hydrobiologia **267**:233-248.

Friesen, O. C., R. Poulin, and C. Lagrue. 2017. Differential impacts of shared parasites on fitness components among competing hosts. Ecology and Evolution. **7**:4682-4693

Gannon, J. E., and S. A. Gannon. 1975. Observations on the narcotization of crustacean zooplankton. Crustaceana **28**:220-224.

Geller, J. B. 1982. Chemically mediated avoidance response of a gastropod, *Tegula funebralis* (A. Adams), to a predatory crab, *Cancer antennarius* (Stimpson). Journal of Experimental Marine Biology and Ecology **65**:19-27.

Goater, C. P., D. Bray, and D. B. Conn. 2005. Cellular aspects of early development of *Ornithodiplostomum ptychocheilus* metacercariae in the brain of fathead minnows, *Pimephales promelas*. Journal of Parasitology **91**:814-821.

Graham, A. L., D. M. Shuker, L. C. Pollitt, S. K. Auld, A. J. Wilson, and T. J. Little. 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Functional Ecology **25**:5-17.

Gross, P. 1993. Insect behavioral and morphological defences against parasitoids. Annual review of entomology **38**:251-273.

Haas, W. 1992. Physiological analysis of cercarial behavior. Journal of Parasitology:243-255.

Haas, W. 1994. Physiological analyses of host-finding behaviour in trematode cercariae: adaptations for transmission success. Parasitology **109**:S15-S29.

Haas, W. 2003. Parasitic worms: strategies of host finding, recognition and invasion. Zoology **106**:349-364.

Haas, W., and B. Haberl. 1997. Host recognition by trematode miracidia. Advances in trematode biology. CRC Press. Boca Raton:197-227.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science **218**:384-387.

Hansen, E., and R. Poulin. 2006. Spatial covariation between infection levels and intermediate host densities in two trematode species. Journal of Helminthology **80**:255-259.

Hart, B. L. 1994. Behavioural defense against parasites: interaction with parasite invasiveness. Parasitology **109**:S139-S151.

Hartnoll, R., and S. Smith. 1980. An experimental study of sex discrimination and pair formation in *Gammarus duebenii* (Amphipoda). Crustaceana:**253**-264.

Hatcher, M. J., J. T. Dick, and A. M. Dunn. 2012. Diverse effects of parasites in ecosystems: linking interdependent processes. Frontiers in Ecology and the Environment **10**:186-194.

Hay, K., B. L. Fredensborg, and R. Poulin. 2005. Trematode-induced alterations in shell shape of the mud snail *Zeacumantus subcarinatus* (Prosobranchia: Batillariidae). Journal of the Marine Biological Association of the United Kingdom **85**:989-992.

Hayes, J. W., and M. J. Rutledge. 1991. Relationship between turbidity and fish diets in Lakes Waahi and Whangape, New Zealand. New Zealand Journal of Marine and Freshwater Research **25**:297-304.

Hazlett, B. A. 1994. Alarm responses in the crayfish *Orconectes virilis* and *Orconectes propinquus*. Journal of Chemical Ecology **20**:1525-1535.

Hazlett, B. A. 1997. The organisation of behaviour in hermit crabs: responses to variation in stimulus strength. Behaviour **134**:59-70.

Hazlett, B. A., and C. McLay. 2000. Contingencies in the behaviour of the crab *Heterozius rotundifrons*. Animal Behaviour **59**:965-974.

Hazlett, B. A., and D. R. Schoolmaster. 1998. Responses of cambarid crayfish to predator odor. Journal of Chemical Ecology **24**:1757-1770.

Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society of London B: Biological Sciences **272**:1059-1066.

Hesse, O., W. Engelbrecht, C. Laforsch, and J. Wolinska. 2012. Fighting parasites and predators: How to deal with multiple threats? BMC Ecology **12**:12.

Hickey, C., and M. Vickers. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. Archives of Environmental Contamination and Toxicology **26**:292-298.

Hine, P. 1977. *Acanthocephalus galaxii* n. sp. Parasitic in *Galaxias maculatus* (Jenyns, 1842) in the Waimeha Stream, New Zealand. Journal of the Royal Society of New Zealand **7**:51-57.

Holton, A. 1984. A redescription of *Coitocaecum parvum* Crowcroft, 1945 (Digenea: Allocreadiidae) from crustacean and fish hosts in Canterbury. New Zealand Journal of Zoology **11**:1-8.

Holton, A. L. 1983. Life history of *Coitocaecum parvum* Crowcroft, 1944 (Trematoda) from Canterbury.

Horák, P., and L. Kolářová. 2005. Molluscan and vertebrate immune responses to bird schistosomes. Parasite Immunology **27**:247-255.

Howe, N. R., and Y. M. Sheikh. 1975. Anthopleurine: a sea anemone alarm pheromone. Science **189**:386-388.

Huang, C.-C., and Y.-L. Song. 1999. Maternal transmission of immunity to white spot syndrome associated virus (WSSV) in shrimp (*Penaeus monodon*). Developmental & Comparative Immunology **23**:545-552.

Hughes, N. K., C. J. Price, and P. B. Banks. 2010. Predators are attracted to the olfactory signals of prey. PLoS One **5**:e13114.

Huryn, A. D., and D. P. Chivers. 1999. Contrasting behavioral responses by detritivorous and predatory mayflies to chemicals released by injured conspecifics and their predators. Journal of Chemical Ecology **25**:2729-2740.

Hutchings, M. R., I. Kyriazakis, D. Anderson, I. J. Gordon, and R. Coop. 1998. Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. Animal Science **67**:97-106.

Jacobsen, H., and O. Stabell. 1999. Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labelling of predators. Marine Biology **134**:551-557.

Jacobsen, H. P., and O. B. Stabell. 2004. Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. Oikos **104**:43-50.

James, C., K. Noyes, A. Stumbo, B. Wisenden, and C. Goater. 2008. Cost of exposure to trematode cercariae and learned recognition and avoidance of parasitism risk by fathead minnows *Pimephales promelas*. Journal of Fish Biology **73**:2238-2248.

Jellyman, D. J. 1989. Diet of two species of freshwater eel (*Anguilla* spp.) in Lake Pounui, New Zealand. New Zealand Journal of Marine and Freshwater Research **23**:1-10.

Jensen, T., K. T. Jensen, and K. N. Mouritsen. 1998. The influence of the trematode *Microphallus claviformis* on two congeneric intermediate host species (Corophium): infection characteristics and host survival. Journal of Experimental Marine Biology and Ecology **227**:35-48.

Jeppesen, E., M. Søndergaard, E. Kanstrup, B. Petersen, R. Eriksen, M. Hammershøj, E. Mortensen, J. Jensen, and A. Have. 1994. Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? Nutrient Dynamics and Biological Structure in Shallow Freshwater and Brackish Lakes. Springer. **15**-30

Jewsbury, J. 1985. Effects of water velocity on snails and cercariae. Parasitology Today **1**:116-117.

Johnson, P. T., K. B. Lunde, E. G. Ritchie, and A. E. Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science **284**:802-804.

Jokela, J., C. Lively, J. Taskinen, and A. Peters. 1999. Effect of starvation on parasite-induced mortality in a freshwater snail (*Potamopyrgus antipodarum*). Oecologia **119**:320-325.

Jokela, J., and C. M. Lively. 1995. Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution **49**:1268-1271.

Kamio, M., S. Matsunaga, and N. Fusetani. 2002. Copulation pheromone in the crab *Telmessus cheiragonus* (Brachyura: Decapoda). Marine Ecology Progress Series **234**:183-190.

Karlson, P., and M. Lüscher. 1959. 'Pheromones': a new term for a class of biologically active substances. Nature **183**:55-56.

Karvonen, A., S. Paukku, E. Valtonen, and P. Hudson. 2003. Transmission, infectivity and survival of *Diplostomum spathaceum* cercariae. Parasitology **127**:217-224.

Karvonen, A., O. Seppälä, and E. Valtonen. 2004. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology **129**:159-164.

Kats, L. B., and L. M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience **5**:361-394.

Kaufmann, R. S. 1994. Structure and function of chemoreceptors in scavenging lysianassoid amphipods. Journal of Crustacean Biology **14**:54-71.

Kavaliers, M., E. Choleris, A. Ågmo, and D. W. Pfaff. 2004. Olfactory-mediated parasite recognition and avoidance: linking genes to behavior. Hormones and Behavior **46**:272-283.

Kavaliers, M., E. Choleris, and D. W. Pfaff. 2005. Recognition and avoidance of the odors of parasitized conspecifics and predators: differential genomic correlates. Neuroscience & Biobehavioral Reviews **29**:1347-1359.

Keeney, D.B., J.M. Waters, and R. Poulin. 2007. Diversity of trematode genetic clones within amphipods and the timing of same-clone infections. International journal for parasitology **37**:351-357.

Keymer, A. E., and R. Anderson. 1979. The dynamics of infection of *Tribolium confusum* by *Hymenolepis diminuta*: the influence of infective-stage density and spatial distribution. Parasitology **79**:195-207.

Kiesecker, J. M., and D. K. Skelly. 2000. Choice of oviposition site by gray treefrogs: the role of potential parasitic infection. Ecology **81**:2939-2943.

Kiesecker, J. M., D. K. Skelly, K. H. Beard, and E. Preisser. 1999. Behavioral reduction of infection risk. Proceedings of the National Academy of Sciences **96**:9165-9168.

King, C. K., S. A. Gale, R. V. Hyne, J. L. Stauber, S. L. Simpson, and C. W. Hickey. 2006. Sensitivities of Australian and New Zealand amphipods to copper and zinc in waters and metal-spiked sediments. Chemosphere **63**:1466-1476.

Koskella, B., and C. M. Lively. 2007. Advice of the rose: experimental coevolution of a trematode parasite and its snail host. Evolution **61**:152-159.

Kostadinova, A., and R. Mavrodieva. 2005. Microphallids in *Gammarus insensibilis* Stock, 1966 from a Black Sea lagoon: host response to infection. Parasitology **131**:347-354.

Kumazawa, H. 2016. Laboratory propagation of freshwater cyclopoid copepods as an intermediate host for parasitic helminths. Parasitology international **65**:599-602.

Kuris, A. M., R. F. Hechinger, J. C. Shaw, K. L. Whitney, L. Aguirre-Macedo, C. A. Boch, A. P. Dobson, E. J. Dunham, B. L. Fredensborg, and T. C. Huspeni. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature **454**:515.

Kurtz, J., and K. Franz. 2003. Innate defence: evidence for memory in invertebrate immunity. Nature **425**:37-38.

Lafferty, K. D. 1992. Foraging on prey that are modified by parasites. The American Naturalist **140**:854-867.

Lafferty, K. D. 1993. Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail *Cerithidea californica*. Marine Ecology Progress Series:229-237.

Lafferty, K. D., and A. M. Kuris. 2009. Parasitic castration: the evolution and ecology of body snatchers. Trends in parasitology **25**:564-572.

Lafferty, K. D., and A. K. Morris. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology **77**:1390-1397.

Lagrue, C., J. McEwan, R. Poulin, and D. B. Keeney. 2007. Co-occurrences of parasite clones and altered host phenotype in a snail–trematode system. International Journal for Parasitology **37**:1459-1467.

Lagrue, C., and R. Poulin. 2007. Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? Journal of Evolutionary Biology **20**:1189-1195.

Lagrue, C., and R. Poulin. 2008. Lack of seasonal variation in the life-history strategies of the trematode *Coitocaecum parvum:* no apparent environmental effect. Parasitology **135**:1243-1251.

Lagrue, C., and R. Poulin. 2015a. Bottom–up regulation of parasite population densities in freshwater ecosystems. Oikos **124**:1639-1647.

Lagrue, C., and R. Poulin. 2015b. Spatial covariation of local abundance among different parasite species: the effect of shared hosts. Parasitology research **114**:3637-3643.

Lagrue, C., R. Rinnevalli, and R. Poulin. 2017. Smelling the future: subtle life-history adjustments in response to environmental conditions and perceived transmission opportunities in a trematode. Parasitology **144**:464-474.

Lass, S., and K. Bittner. 2002. Facing multiple enemies: parasitised hosts respond to predator kairomones. Oecologia **132**:344-349.

Lawrence, B., and R. Smith. 1989. Behavioral response of solitary fathead minnows, *Pimephales promelas*, to alarm substance. Journal of Chemical Ecology **15**:209-219.

Lefebvre, F., B. Fredensborg, A. Armstrong, E. Hansen, and R. Poulin. 2005. Assortative pairing in the amphipod *Paracalliope fluviatilis*: a role for parasites? Hydrobiologia **545**:65-73.

Lefèvre, T., C. Lebarbenchon, M. Gauthier-Clerc, D. Missé, R. Poulin, and F. Thomas. 2009. The ecological significance of manipulative parasites. Trends in Ecology & Evolution **24**:41-48.

Lello, J., B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson. 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature **428**:840.

Leung, T. L., D. B. Keeney, and R. Poulin. 2010. Genetics, intensity-dependence, and host manipulation in the trematode *Curtuteria australis*: following the strategies of others? Oikos **119**:393-400.

Leung, T. L., & R. Poulin. 2011. Intra-host competition between co-infecting digeneans

within a bivalve second intermediate host: Dominance by priority-effect or taking advantage of others? International journal for parasitology, **41(3)**:449-454.

Lewis, W., R. L. Jones, H. Gross, and D. A. Nordlund. 1976. The role of kairomones and other behavioral chemicals in host finding by parasitic insects. Behavioral Biology **16**:267-289.

Libersat, F., A. Delago, and R. Gal. 2009. Manipulation of host behavior by parasitic insects and insect parasites. Annual review of entomology **54**:189-207.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology **68**:619-640.

Little, T. J., B. O'Connor, N. Colegrave, K. Watt, and A. F. Read. 2003. Maternal transfer of strain-specific immunity in an invertebrate. Current Biology **13**:489-492.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos **88**:87-98.

Lowenberger, C. A., and M. E. Rau. 1994. Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to a larval parasite, *Plagiorchis elegans* (Trematoda: Plagiorchiidae). Environmental Entomology **23**:1269-1276.

Ludgate, B. G., and G. Closs. 2003. Responses of fish communities to sustained removals of perch (*Perca fluviatilis*). Department of Conservation Wellington, New Zealand: 6-32

Luque, J., L. Bannock, C. Lagrue, and R. Poulin. 2007. Larval *Hysterothylacium* sp. (Nematoda, Anisakidae) and trematode metacercariae from the amphipod *Paracorophium excavatum* (Corphiidae) in New Zealand. Acta Parasitologica **52**:146-150.

MacFarlane, W. 1939. Life cycle of *Coitocaecum anaspidis* Hickman, a New Zealand digenetic trematode. Parasitology **31**:172-184.

Machado, G., V. Bonato, and P. S. Oliveira. 2002. Alarm communication: a new function for the scent-gland secretion in harvestmen (Arachnida: Opiliones). Naturwissenschaften **89**:357-360.

Males, W. M. W. 2000. Immune defence in bumble-bee offspring. Vaccine **18**:3091-3096.

Marston, N., and L. R. Ertle. 1973. Host influence on the bionomics of *Trichogramma minutum*. Annals of the Entomological Society of America **66**:1155-1162.

Martin, S. J., J. M. Carruthers, P. H. Williams, and F. P. Drijfhout. 2010. Host specific social parasites (*Psithyrus*) indicate chemical recognition system in bumblebees. Journal of Chemical Ecology **36**:855-863.

Mathis, A. 2009. Alarm responses as a defense: chemical alarm cues in nonostariophysan fishes. Fish defences **2**:323-386.

Mathis, A., and R. J. F. Smith. 1993. Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. Animal Behaviour **46**:645-656.

May, R. M., and R. M. Anderson. 1978. Regulation and stability of host-parasite population interactions: II. Destabilizing processes. The Journal of Animal Ecology:249-267.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology & Evolution **10**:190-194.

McCarthy, A. M. 1999. The influence of temperature on the survival and infectivity of the cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). Parasitology **118**:383-388.

McCarthy, H., S. Fitzpatrick, and S. Irwin. 2004. Parasite alteration of host shape: a quantitative approach to gigantism helps elucidate evolutionary advantages. Parasitology **128**:7-14.

McClelland, G., and T. Bourns. 1969. Effects of *Trichobilharzia ocellata* on growth, reproduction, and survival of *Lymnaea stagnalis*. Experimental Parasitology **24**:137-146.

Minchella, D. J., and P. T. Loverde. 1981. A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. The American Naturalist **118**:876-881.

Mitchell, E. 2012. Management of insect pests with semiochemicals: concepts and practice. Springer Science & Business Media: viii-ix

Moret, Y. 2006. 'Trans-generational immune priming': specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. Proceedings of the Royal Society of London B: Biological Sciences **273**:1399-1405.

Moret, Y., and J. Moreau. 2012. The immune role of the arthropod exoskeleton. Invertebrate Survival Journal **9**:200-206.

Moret, Y., and M. T. Siva-Jothy. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. Proceedings of the Royal Society of London B: Biological Sciences **270**:2475-2480.

Moss, B., I. Booker, H. Balls, and K. Manson. 1989. Phytoplankton distribution in a temperate floodplain lake and river system. Hydrology, nutrient sources and phytoplankton biomass. Journal of Plankton Research **11**:813-838.

Mouritsen, K. N., and K. T. Jensen. 1994. The enigma of gigantism: effect of larval trematodes on growth, fecundity, egestion and locomotion in *Hydrobia ulvae* (Pennant)(Gastropoda: Prosobranchia). Journal of Experimental Marine Biology and Ecology **181**:53-66.

Mouritsen, K. N., and R. Poulin. 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology **124**:101-117.

Mouritsen, K. N., and R. Poulin. 2005. Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. Oikos **108**:344-350.

Nissen, S., L. A. T. Nguyen, S. M. Thamsborg, A. Dalsgaard, and M. V. Johansen. 2014. Reinfection of dogs with fish-borne zoonotic trematodes in northern Vietnam following a single treatment with praziquantel. PLoS Neglected Tropical Diseases **8**:e2625.

Noldus Information Technology. (2015). EthoVision XT 9.0.

Noldus, L. P., A. J. Spink, and R. A. Tegelenbosch. 2001. EthoVision: a versatile video tracking system for automation of behavioral experiments. Behavior Research Methods **33**:398-414.

Nordlund, D. A., and W. Lewis. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. Journal of Chemical Ecology **2**:211-220.

Norris, K., M. Anwar, and A. F. Read. 1994. Reproductive effort influences the prevalence of haematozoan parasites in great tits. Journal of Animal Ecology:601-610.

Norval, R., H. R. Andrew, and C. Yunker. 1989. Pheromone-mediation of host-selection in bont ticks *Amblyomma hebraeum* Koch. Science **243**:364-366.

Ohsaki, N., and Y. Sato. 1994. Food plant choice of pieris butterflies as a trade-off between parasitoid avoidance and quality of plants. Ecology **75**:59-68.

Olson, P., T. Cribb, V. Tkach, R. Bray, and D. Littlewood. 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). International journal for parasitology **33**:733-755.

Osnas, E., and C. Lively. 2006. Host ploidy, parasitism and immune defence in a coevolutionary snail–trematode system. Journal of Evolutionary Biology **19**:42-48.

Osnas, E. E., and C. M. Lively. 2005. Immune response to sympatric and allopatric parasites in a snail-trematode interaction. Frontiers in zoology **2**:8.

Park, T. 1948. Interspecies competition in populations of *Trilobium confusum* Duval and *Trilobium castaneum* Herbst. Ecological Monographs **18**:265-307.

Paull, S. H., and P. T. Johnson. 2011. High temperature enhances host pathology in a snail–trematode system: possible consequences of climate change for the emergence of disease. Freshwater Biology **56**:767-778.

Peng, Y.S. C., Y. Fang, S. Xu, L. Ge, and M. E. Nasr. 1987. Response of foster Asian honeybee (*Apis cerana* Fabr.) colonies to the brood of European honeybee (*Apis mellifera* L.) infested with parasitic mite, *Varroa jacobsoni* Oudemans. Journal of Invertebrate Pathology **49**:259-264.

Pennycuick, L. 1971. Frequency distributions of parasites in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. Parasitology **63**:389-406.

Persons, M. H., and A. L. Rypstra. 2001. Wolf spiders show graded antipredator behavior in the presence of chemical cues from different sized predators. Journal of Chemical Ecology **27**:2493-2504.

Petney, T. N., and R. H. Andrews. 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. International Journal for Parasitology **28**:377-393.

Petranka, J. W., and K. Fakhoury. 1991. Evidence of a chemically-mediated avoidance response of ovipositing insects to blue-gills and green frog tadpoles. Copeia **1991**:234-239.

Pfeiffer, W. 1977. The distribution of fright reaction and alarm substance cells in fishes. Copeia:653-665.

Ponton, F., D. G. Biron, C. Joly, S. Helluy, D. Duneau, and F. Thomas. 2005. Ecology of parasitically modified populations: a case study from a gammarid–trematode system. Marine Ecology Progress Series **299**:205-215.

Poulin, R. 1993. The disparity between observed and uniform distributions: a new look at parasite aggregation. International journal for parasitology **23**:937-944.

Poulin, R. 2001a. Interactions between species and the structure of helminth communities. Parasitology **122**:S3-S11.

Poulin, R. 2001b. Progenesis and reduced virulence as an alternative transmission strategy in a parasitic trematode. Parasitology **123**:623-630.

Poulin, R. 2003. Information about transmission opportunities triggers a life-history switch in a parasite. Evolution **57**:2899-2903.

Poulin, R. 2010. Parasite manipulation of host behavior: an update and frequently asked questions. Advances in the Study of Behavior **41**:151-186.

Poulin, R., M. A. Curtis, and M. E. Rau. 1991a. Size, behaviour, and acquisition of ectoparasitic copepods by brook trout, *Salvelinus fontinalis*. Oikos:169-174.

Poulin, R., and G. FitzGerald. 1989a. Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). Behavioral Ecology and Sociobiology **24**:251-255.

Poulin, R., and G. J. FitzGerald. 1989b. A possible explanation for the aggregated distribution of *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) on juvenile sticklebacks (Gasterosteidae). Journal of Parasitology:58-60.

Poulin, R., and G. J. FitzGerald. 1989c. Risk of parasitism and microhabitat selection in juvenile sticklebacks. Canadian Journal of Zoology **67**:14-18.

Poulin, R., K. Hecker, and F. Thomas. 1998. Hosts manipulated by one parasite incur additional costs from infection by another parasite. Journal of Parasitology:1050-1052.

Poulin, R., D. Marcogliese, and J. McLaughlin. 1999. Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. Journal of Fish Biology **55**:47-53.

Poulin, R., and S. Morand. 2000. Parasite body size and interspecific variation in levels of aggregation among nematodes. Journal of Parasitology **86**:642-647.

Poulin, R., M. E. Rau, and M. A. Curtis. 1991b. Infection of brook trout fry, *Salvelinus fontinalis*, by ectoparasitic copepods: the role of host behaviour and initial parasite load. Animal Behaviour **41**:467-476.

Presswell, B., I. Blasco-Costa, and A. Kostadinova. 2014. Two new species of *Maritrema* Nicoll, 1907 (Digenea: Microphallidae) from New Zealand: morphological and molecular characterisation. Parasitology research **113**:1641-1656.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Australia.

Rauch, G., Kalbe, M., & Reusch, T. B. 2006. One day is enough: rapid and specific host–parasite interactions in a stickleback-trematode system. Biology Letters, **2(3)**:382-384.

Rauque, C., R. Paterson, R. Poulin, and D. Tompkins. 2011. Do different parasite species interact in their effects on host fitness? A case study on parasites of the amphipod *Paracalliope fluviatilis*. Parasitology **138**:1176-1182.

Relyea, R. A. 2003. Predator cues and pesticides: a double dose of danger for amphibians. Ecological Applications **13**:1515-1521.

Rigby, M. C., R. F. Hechinger, and L. Stevens. 2002. Why should parasite resistance be costly? Trends in Parasitology **18**:116-120.

Rigby, M. C., and J. Jokela. 2000. Predator avoidance and immune defence: costs and trade–offs in snails. Proceedings of the Royal Society of London B: Biological Sciences **267**:171-176.

Rodriguez, M., L. I. Terrazas, R. Marquez, and R. Bojalil. 1999. Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection. Parasite Immunology **21**:177-185.

Rohr, J. R., D. M. Madison, and A. M. Sullivan. 2002. The ontogeny of chemically-mediated antipredator behaviours in newts (*Notophthalmus viridescens*): responses to injured and non-injured conspecifics. Behaviour **139**:1043-1060.

Rohr, J. R., T. R. Raffel, and C. A. Hall. 2010. Developmental variation in resistance and tolerance in a multi-host–parasite system. Functional Ecology **24**:1110-1121.

Rohr, J. R., A. Swan, T. R. Raffel, and P. J. Hudson. 2009. Parasites, info-disruption, and the ecology of fear. Oecologia **159**:447-454.

Rolff, J., and M. Siva-Jothy. 2003. Invertebrate ecological immunology. Science **301**:472-475.

Roth O, B.M Sadd, P. Schmid-Hempel, J. Kurtz. 2009. Strain specific priming of resistance in the red flour beetle, *Tribolium castaneum*. Proc Biol Sci **276**:145- 51

Rothschild, M. 1936. Gigantism and Variation in *Peringia ulvæ* Pennant 1777, caused by Infection with Larval Trematodes. Journal of the Marine Biological Association of the United Kingdom **20**:537-546.

Rottman, S. J., and C. T. Snowdown. 1972. Demonstration and analysis of an alarm pheromone in mice. Journal of Comparative and Physiological Psychology **81**:483.

Rowe, D. 1999. Factors influencing the abundance of the common bully, *Gobiomorphus cotidianus* McDowall, in small, North Island, New Zealand, lakes. Fisheries Management and Ecology **6**:377-386.

Rowe, D. K., S. Nichols, and G. R. Kelly. 2001. Depth distribution and abundance of the common bully, *Gobiomorphus cotidianus* (Eleotridae), in three oligotrophic New Zealand lakes, one of which is turbid. Environmental Biology of Fishes **61**:407-418.

Sadd B.M., Y. Kleinlogel, R. Schmid-Hempel, P. Schmid-Hempel. 2005. Trans-generational immune priming in a social insect. Biol Lett **1**:386- 8.

Sadd, B.M. and P. Schmid-Hempel. 2006. Insect immunity shows specificity in protection upon secondary pathogen exposure. Current Biology **16**:1206-1210.

Sagar, P., and G. Glova. 1995. Prey availability and diet of juvenile brown trout (*Salmo trutta*) in relation to riparian willows (*Salix*-spp.) in three New Zealand streams. New Zealand Journal of Marine and Freshwater Research **29**:527-537.

Sandland, G. J., and D. J. Minchella. 2003. Costs of immune defense: an enigma wrapped in an environmental cloak? Trends in Parasitology **19**:571-574.

Sauerwein, R. W., M. Roestenberg, and V. S. Moorthy. 2011. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nature Reviews. Immunology **11**:57.

Savage, A. 1996. Density dependent and density independent relationships during a twenty-seven year study of the population dynamics of the benthic macroinvertebrate community of a chemically unstable lake. Hydrobiologia **335**:115-131.

Schallenberg, M., and C. W. Burns. 2003. A temperate, tidal lake-wetland complex 2. Water quality and implications for zooplankton community structure. New Zealand Journal of Marine and Freshwater Research **37**:429-447.

Schaller, M., G. E. Miller, W. M. Gervais, S. Yager, and E. Chen. 2010. Mere visual perception of other people's disease symptoms facilitates a more aggressive immune response. Psychological Science **21**:649-652.

Schmidt, K. A., S. R. Dall, and J. A. Van Gils. 2010. The ecology of information: an overview on the ecological significance of making informed decisions. Oikos **119**:304-316.

Schmidt, M., and D. Mellon Jr. 2010. Neuronal processing of chemical information in crustaceans. Chemical communication in crustaceans. Springer: 123-147

Sehr, E. K., and B. G. Gall. 2016. Responses of an aquatic isopod and amphipod to chemical alarm cues from damaged conspecifics. Journal of Freshwater Ecology **31**:231-237.

Sharp, J. G., S. Garnick, M. A. Elgar, and G. Coulson. 2015. Parasite and predator risk assessment: nuanced use of olfactory cues. Proceedings of the Royal Society B. **282**: 20151941.

Shaw, D., and A. Dobson. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology **111**:S111-S133.

Shaw, D., B. Grenfell, and A. Dobson. 1998. Patterns of macroparasite aggregation in wildlife host populations. Parasitology **117**:597-610.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution **11**:317-321.

Skellam, J. G. 1951. Random dispersal in theoretical populations. Biometrika **38**:196-218.

Smith, R. J. F. 1977. Chemical communication as adaptation: alarm substance of fish. Chemical signals in vertebrates. Springer: 303-320

Smith, R. J. F. 1992. Alarm signals in fishes. Reviews in Fish Biology and Fisheries **2**:33-63.

Sokolova, I. M. 1995. Influence of trematodes on the demography of *Littorina saxatilis* (Gastropoda: Prosobranchia: Littorinidae) in the White Sea. Diseases of Aquatic Organisms **21**:91-101.

Sousa, W. P. 1983. Host life history and the effect of parasitic castration on growth: a field study of *Cerithidea californica* Haldeman (Gastropoda: Prosobranchia) and its trematode parasites. Journal of Experimental Marine Biology and Ecology **73**:273-296.

Stenzler, D., and J. Atema. 1977. Alarm response of the marine mud snail, *Nassarius obsoletus*: specificity and behavioral priority. Journal of Chemical Ecology **3**:159-171.

Stevenson, R. J., D. Hodgson, M. J. Oaten, J. Barouei, and T. I. Case. 2011. The effect of disgust on oral immune function. Psychophysiology **48**:900-907.

Stjernman, M., L. Råberg, and J.-Å. Nilsson. 2008. Maximum host survival at intermediate parasite infection intensities. PLoS One **3**:e2463.

Studer, A. and R. Poulin. 2012. Seasonal dynamics in an intertidal mudflat: the case of a complex trematode life cycle. Marine Ecology Progress Series **455**:79-93.

Sturrock, B., and R. Sturrock. 1970. Laboratory studies of the host-parasite relationship of *Schistosoma mansoni* and *Biomphalaria glabrata* from St Lucia, West Indies. Annals of Tropical Medicine & Parasitology **64**:357-363.

Suboski, M. D. 1990. Releaser-induced recognition learning. Psychological Review **97**:271.

Sutherland, D. L., I. D. Hogg, and J. R. Waas. 2007. Is size assortative mating in *Paracalliope fluviatilis* (Crustacea: Amphipoda) explained by male–male competition or female choice? Biological Journal of the Linnean Society **92**:173-181.

Sutherland, D. L., I. D. Hogg, and J. R. Waas. 2009. Phylogeography and species discrimination in the *Paracalliope fluviatilis* species complex (Crustacea: Amphipoda): can morphologically similar heterospecifics identify compatible mates? Biological Journal of the Linnean Society **99**:196-205.

Sutherst, R., R. Floyd, A. Bourne, and M. Dallwitz. 1986. Cattle grazing behavior regulates tick populations. Cellular and Molecular Life Sciences **42**:194-196.

Taylor, M. J. 2002. The National Inanga Spawning Database: trends and implications for spawning site management. Department of Conservation: 1-37

Telfer, S., R. Birtles, M. Bennett, X. Lambin, S. Paterson, and M. Begon. 2008. Parasite interactions in natural populations: insights from longitudinal data. Parasitology **135**:767-781.

Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010. Species interactions in a parasite community drive infection risk in a wildlife population. Science **330**:243-246.

Thieltges, D. W., and R. Poulin. 2008. Parasites and Pathogens: Avoidance. eLS: 1-8

Thiemann, G. W., and R. J. Wassersug. 2000. Patterns and consequences of behavioural responses to predators and parasites in *Rana* tadpoles. Biological Journal of the Linnean Society **71**:513-528.

Thomas, F., J. Fauchier, and K. D. Lafferty. 2002. Conflict of interest between a nematode and a trematode in an amphipod host: test of the "sabotage" hypothesis. Behavioral Ecology and Sociobiology **51**:296-301.

Thomas, F., E. Guldner, and F. Renaud. 2000. Differential parasite (Trematoda) encapsulation in *Gammarus aequicauda* (Amphipoda). Journal of Parasitology **86**:650-654.

Thomas, F., R. Poulin, T. de Meeüs, J.F. Guégan, and F. Renaud. 1999. Parasites and ecosystem engineering: what roles could they play? Oikos:167-171.

Thomas, F., F. Renaud, T. De Meeûs, and R. Poulin. 1998. Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? Proceedings of the Royal Society of London B: Biological Sciences **265**:1091-1096.

Thomas, F., F. Renaud, J. Derothe, A. Lambert, T. De Meeüs, and F. Cézilly. 1995a. Assortative pairing in *Gammarus insensibilis* (Amphipoda) infected by a trematode parasite. Oecologia **104**:259-264.

Thomas, F., F. Renaud, F. Rousset, F. Cézilly, and T. De Meeus. 1995b. Differential mortality of two closely related host species induced by one parasite. Proceedings of the Royal Society of London B: Biological Sciences **260**:349-352.

Thompson, R. M., K. N. Mouritsen, and R. Poulin. 2005. Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. Journal of Animal Ecology **74**:77-85.

Van der Wal, R., J. Irvine, A. Stien, N. Shepherd, and S. Albon. 2000. Faecal avoidance and the risk of infection by nematodes in a natural population of reindeer. Oecologia **124**:19-25.

Voutilainen, A., H. Huuskonen, and J.Taskinen. 2010. Penetration and migration success of *Diplostomum* spp. cercariae in Arctic charr. Journal of Parasitology, **96:**232-235.

Wade, S., T. Corbin, and L. McDowell. 2004. Critter catalogue: a guide to the aquatic invertebrates of South Australian inland waters.

Williams, D. D., and K. A. Moore. 1982. The effect of environmental factors on the activity of *Gammarus pseudolimnaeus* (Amphipoda). Hydrobiologia **96**:137-147.

Williams, D. D., and K. A. Moore. 1985. The role of semiochemicals in benthic community relationships of the lotic amphipod *Gammarus pseudolimnaeus*: a laboratory analysis. Oikos:280-286.

Wilson, K., O. Bjørnstad, A. Dobson, S. Merler, G. Poglayen, S. Randolph, A. Read, and A. Skorping. 2002. Heterogeneities in macroparasite infections: patterns and processes. The Ecology of Wildlife Diseases **44**:6-44.

Wisenden, B. D. 2003. Chemically mediated strategies to counter predation. Sensory processing in aquatic environments. Springer:236-251

Wisenden, B. D., C. L. Binstock, K. E. Knoll, A. J. Linke, and B. S. Demuth. 2010. Risk-sensitive information gathering by cyprinids following release of chemical alarm cues. Animal Behaviour **79**:1101-1107.

Wisenden, B. D., and D. P. Chivers. 2006. The role of public chemical information in antipredator behaviour. Communication in fishes. Science Publisher, Enfield:259-278.

Wisenden, B. D., A. Cline, and T. C. Sparkes. 1999. Survival benefit to antipredator behavior in the Amphipod *Gammarus minus* (Crustacea: Amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. Ethology **105**:407-414.

Wisenden, B. D., C. P. Goater, and C. T. James. 2009a. Behavioral defences against parasites and pathogens. Fishes defences (eds Zaccone G., Perrière MA, Kapoor BG):151-168.

Wisenden, B. D., M. L. Rugg, N. L. Korpi, and L. C. Fuselier. 2009b. Lab and field estimates of active time of chemical alarm cues of a cyprinid fish and an amphipod crustacean. Behaviour **146**:1423-1442.
Yamaguti, S. 1971. Synopsis of digenetic trematodes of vertebrates. Vols I and II. Synopsis of digenetic trematodes of vertebrates. Vols I and II.