Elucidating the hidden infection dynamics between hairworms and their aquatic and terrestrial hosts

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To the many thousands of parasitic and free-living invertebrates that were sacrificed in the quest of knowledge.

Front page picture: two mature *Gordius* sp. hairworms coiled inside a cave wētā (*Pleioplectron simplex*).

Abstract

Parasitism, the act of acquiring nutrients at the expense of a host organism, has arguably become the most prevalent mode of life on this planet. Despite the remarkable diversity of parasite species, their life strategies can be grouped mainly into only six general categories. This is reflected by a convergent evolution in life cycle attributes and how parasites successfully transition from one development stage to the next. In parallel to the evolution of these general life strategies, many parasite lineages have been selected to adaptively increase the odds of successfully completing their life cycle through phenotypic changes in their hosts, a phenomenon known as host manipulation.

Perhaps one of the best-known examples of parasites capable of host manipulation are freshwater hairworms (phylum Nematomorpha), which somehow cause their terrestrial host to enter water, for the parasite to exit and reproduce. Despite their notoriety, there is still much left to discover about this group of highly specialised organisms. In fact, I show that much of what is known about the host manipulation of hairworms has been largely misrepresented in both the popular media and the scientific literature. Therefore, due to the cryptic nature of parasite life cycles in general, understanding host-parasite interactions, including host manipulation, requires an in-depth investigation.

This thesis aims to elucidate some of the hidden interactions between hairworms and their aquatic and terrestrial hosts in New Zealand. In the core chapters, I take the reader through the complex life cycle of hairworms to answer some of the broad research questions on the ecology and host-parasite interactions of this group. First, by observing naturally infected aquatic hosts, I show that the internal defence reactions of these hosts toward hairworm larvae and cysts are more complex than previously thought, casting doubt on the true lethality of aquatic host immunity towards hairworms.

Then, I quantify the losses of hairworms in dead-end hosts from two communities of aquatic macroinvertebrates, showing that certain aquatic species can represent important population sinks for hairworms. Here, I reveal that, depending on the species, hairworms follow distinct host transmission routes to reach land, depending on where and when they are consumed by the aquatic hosts in the streams. In light of these challenges that

hairworms face in the water, I present some evidence, from a controlled observational study, that they may in turn accelerate their transition to land by decreasing the development time of aquatic hosts, thus increasing the odds of successfully completing their life cycle.

Finally, I show that different species of hairworm are capable of infecting a diverse range of terrestrial hosts through a survey of two communities of terrestrial arthropods in subalpine habitats, which suggests that hairworms may be less host-specific and more widespread than what is currently known in New Zealand. This thesis uncovers some of the complex and hidden interactions between hairworms and their aquatic and terrestrial hosts. Still, there remains much to explore of the ecology and infection dynamics of this fascinating, yet poorly understood group of parasites.

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Chapter 1

General introduction

Mating knot of *Gordius* sp. hairworms in the laboratory. Mated female (large pale worm), producing eggs (white mass), is entangled with several males. The bilobed posterior end (right) of a male is visible.

1.1 Opening statement

If one were to select, at random, a group of 100 individual organisms from Earth, the odds are that the majority of these would fit into the broad definition of a parasite (Windsor, 1998). This would include any organism adapted to acquire energy from living in or on a host organism, exerting a negative fitness effect on the latter. From global warming (Fox et al., 2018) to pandemics (Piret and Boivin, 2021), parasites have often been cast as the enemy. However, their fundamental roles in ecosystem functioning, including host population dynamics and energy flow within trophic networks, have largely been understated (Hudson et al., 2006). In metazoans alone, parasites likely account for a large proportion of all species (Dobson et al., 2008), although only a small number of these have actually been described (Costello, 2016). If there exists at least one host-specific parasite per metazoan species, then this pervasive mode of life may very well represent the dominant form on this planet (Poulin, 2014). Over 200 distinct lineages of animals have evolved parasitic lifestyles (Weinstein and Kuris, 2016). However, the vast majority of these have converged into one of only six general parasitic strategies, which are reflected by a remarkable convergence in life cycles and transmission modes, among other traits (Poulin, 2011).

Because of the cryptic nature of many parasitic lifestyles, most challenges that parasites face go unseen. Therefore, in order to elucidate the hidden interactions between a parasite and its host, an in-depth investigation is necessary. This introductory chapter serves as an exposition of the relevant scientific background that will then be expanded upon in subsequent chapters. First, host manipulation is presented as an adaptive solution that is closely connected to the evolution of different parasite life cycles. Then, the phylum Nematomorpha is brought forward as an infamous example of host manipulation, which is often cited, yet still poorly understood. This is followed by an examination of their general life cycle, which highlights important traits typically observed across members of this phylum, along with some current gaps in the knowledge. Afterwards, an overview of what is known of hairworms in New Zealand, the study system of the current thesis, is brought to light. Finally, the development and structure of the thesis are presented, ending with the main objectives and an outline of the core data chapters.

1.2 Parasite life cycles and host manipulation

Parasite life cycles can involve one or multiple host species that serve as sources of energy to further development or as transmission pathways to another host or environment (Poulin, 2007). In turn, the necessity for a parasite to complete its life cycle by successfully transmitting to another host or to an environment suitable for reproduction is ultimately governed by what the host does. Therefore, any change in host phenotype that increases parasite transmission can have fitness benefits for the parasite. In fact, multiple lineages across most major parasitic groups are capable of inducing phenotypic changes in their host (Moore, 2002; Poulin, 2010). This phenomenon, commonly known as host manipulation, usually involves conspicuous changes in host behaviour or appearance (Lefèvre et al., 2009a; Cézilly et al., 2013). Although behavioural change has been the focus of most studies on host manipulation (Poulin, 2010), any aspect of host phenotype may be altered by the parasite, either directly or indirectly (Bhattarai et al., 2021). For instance, intracellular parasites can alter host immune responses to favour their transmission (Christiaansen et al., 2015). Other parasites can induce changes in host reproduction and growth, castrating the host or inducing gigantism (Ebert et al., 2004; Lafferty and Kuris, 2009). All of these changes ultimately facilitate life cycle completion of the parasite and, therefore, confer fitness benefits. Whether host manipulation is adaptive or not has been debated for decades (Poulin, 1995a; Cézilly and Perrot-Minnot, 2005; Bhattarai et al., 2021). Nonetheless, if a change in host phenotype increases the transmission of a parasite, and this change originates either directly or indirectly from the parasite genotype, then this is a case of adaptive host manipulation (Poulin, 2010). Evidently, these phenotypic changes go hand in hand with the type of life cycle of the parasite.

Because of the general life history strategies that have evolved in parasites, it is possible to distinguish between at least four categories of parasite transmission for which changes in host phenotype are beneficial (Figure 1.1). An additional two categories have been suggested, namely diseases transmitted either sexually within a species (Adamo, 2014) or contagiously (by direct contact or close proximity) within or between species (Barton et al., 2020), but there is currently a lack of convincing empirical evidence to support these. Firstly, if an intermediate host needs to be predated upon by the definitive host for

the parasite to complete its life cycle, selection may favour parasites capable of increasing conspicuousness or neutralising anti-predator strategies of intermediate hosts, either through visual cues or behavioural changes in the latter (Figure 1.1A). This type of host manipulation closely follows the evolution of trophic transmission in parasites with complex life cycles (Lafferty, 1999). For instance, trematodes of the genus *Leucochloridium* infect snails and develop into sporocysts with broodsacs (structures filled with the dormant stage of the parasite) that invade the tentacles of the host. These modified tentacles resemble pulsating caterpillars and, paired with behavioural changes observed in infected snails (Wesołowska and Wesołowski, 2014), are more likely to be eaten by insectivorous birds, which happen to be the definitive hosts of the trematode.

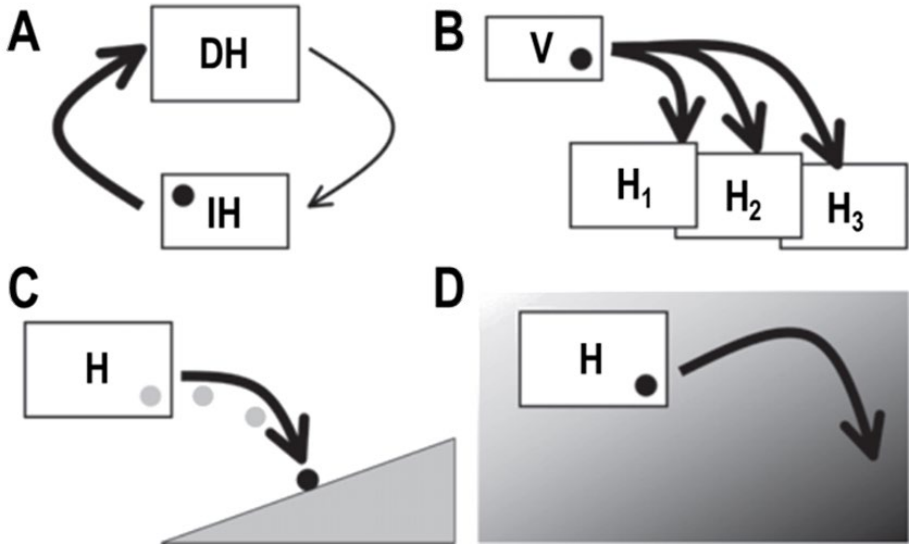


Figure 1.1 The four main manifestations of host manipulation by parasites. Hosts are represented by the boxes and parasites by the grey or black circles. Thick arrows represent the transmission stage of the parasite where host manipulation is observed. (A) Trophically transmitted parasites alter the phenotype of their intermediate host, IH, to increase the odds of transmission to the definitive host, DH, by predation. (B) Vector-borne parasites typically increase the frequency that vectors, V, visit hosts, H_n, resulting in higher transmission rates between hosts. (C) Parasitoid larvae (grey circles), which need to exit their host to pupate on exposed surfaces (black circle), somehow induce "protective" behaviours in the host to reduce the risk of pupae being eaten or attacked by natural enemies. (D) Parasites that need to exit their host in another environment, either to release their propagules or reproduce, somehow cause their host to end up in said environment, which may be unsuitable, if not deadly, for the host. Figure taken from Poulin (2010).

The second category where host manipulation is observed involves parasites that require vector organisms to transmit them between hosts (Figure 1.1B). Here, any change in the frequency at which vectors visit hosts could be beneficial for the parasite. For example, the protist *Leishmania* infects mammals as primary hosts and is transmitted between

mammals via bloodsucking flies. It was shown that parasitised vectors are more persistent when feeding on mammals and are more likely to feed on multiple hosts (Rogers and Bates, 2007), two behaviours that increase the transmission of the protist. Thirdly, insect parasitoids that grow inside their host can induce changes in host behaviour that increase their chances of completing development after they exit their host to pupate on an exposed surface (Figure 1.1C). These involve host behavioural changes that reduce the odds of pupae being attacked or eaten by natural enemies. For instance, when larvae of the braconid wasp *Glyptapanteles* sp. exit their caterpillar host to pupate on a plant, the host remains close to pupae and knocks predators off the plant with vigorous head swings, resulting in a substantial decrease in mortality for the parasitoids (Grosman et al., 2008).

The fourth and final type of parasite transmission in which a change in host phenotype is beneficial, and the focus of this thesis, involves a change in habitat that may be unsuitable, if not deadly, for the host (Figure 1.1D). This environmental change is necessary for the parasite to either exit the host as a free-living organism or to release its propagules. Well-known examples include the parasitic fungus *Ophiocordyceps* that infects ants, causing them to die in areas favourable for the release of fungal spores (Pontoppidan et al., 2009), e.g., hanging from the underside of leaves. This fungus causes the ant to bite down onto the stems of leaves (Mangold et al., 2019), where it dies and the fruiting body of the fungus can grow. In the same vein, mermithid nematodes somehow induce a positive movement towards water in their earwig hosts (Herbison et al., 2019a), which benefits the parasite that needs to exit the host and complete its development in an environment saturated with humidity. This may not be deadly for the host, but the sudden change in their behaviour ultimately benefits the parasite. Another lineage of parasites, which shows signs of convergent evolution with mermithids (Herbison et al., 2019b), requires a drastic environmental change that can be deadly for the host. The remaining sections of this chapter are dedicated to these highly specialised parasites.

1.3 Phylum Nematomorpha

1.3.1 General introduction

The phylum Nematomorpha, commonly known as horsehair worms or hairworms, comprises over 350 described species (Bolek et al., 2015), with an estimated total of approximately 2,000 species globally (Poinar, 2008). To date, all species described are parasitic for part of their life, making this phylum one of the few that are entirely parasitic (Hanelt et al., 2005). The vast majority of species consist of freshwater or gordiid hairworms; only one species of terrestrial hairworm (Anaya et al., 2019; Anaya et al., 2021) and five species (possibly six, see Kakui et al. (2021)) of marine hairworms (Poinar and Brockerhoff, 2001) have been reported. Therefore, much of what is known of the biology of Nematomorpha is based on freshwater species (Schmidt-Rhaesa, 2013), which will be the focus here. Hairworms have complex life cycles, including five recognised life stages and multiple hosts, and a transition from water to land and back (Bolek et al., 2015). The most conspicuous stage in this life cycle is the adult, which are long, slender worms usually measuring between 10 to 30 cm in length and found typically in ponds, streams, or rivers.

Although the full life cycle of hairworms has been resolved empirically within the past two decades (Hanelt and Janovy, 2004a), there are still only a limited number of studies that address the ecology and host-parasite interactions of this highly specialised group (Schmidt-Rhaesa, 2013). Since the start of this project in March 2018, a total of 15 new articles on freshwater hairworms have appeared on Web of Science, from which nine consist of new species descriptions or basic morphology or physiology. In other words, there is still much to discover of this poorly understood group of parasites. The following sections take us through the general life cycle of freshwater hairworms, shedding light on the current gaps of knowledge that need to be addressed, which are formulated as broad research questions in Figure 1.2.

1.3.2 From egg to larva

Free-living mated females and parthenogenetic females (Hanelt et al., 2012) typically lay egg strings in streams and rivers; these egg strings are highly dense structures packed with eggs (Figure 1.2). In fact, they can produce enormous quantities of eggs. One species

was estimated to shed 1,350 eggs per millimetre of egg string, for a total of 200,000 to 8,000,000 eggs, according to the length of the individual female (Hanelt, 2009). Depending on the species, females can produce either pieces of egg string of variable length that drift into the current or attach long pieces of egg string to a substrate such as a submerged rock or stick (Szmygiel et al., 2014). The larvae within these eggs take anywhere from a few weeks (Hanelt and Janovy, 2004b) to several months (Müller, 1926) to hatch. Like all poikilotherms, their development time depends mainly on ambient temperature (Achiorno et al., 2008a). Once hatched, larvae are semi-sessile and cannot swim efficiently, therefore they tend to remain in the vicinity of egg strings or be swept away by the current (Bolek and Coggins, 2002; Bolek et al., 2013a). They all have, in their anterior section or pre-septum (Figure 1.3A), a complex set of mouthparts including three rings of cuticular hooks and an eversible proboscis (Müller et al., 2004; Szmygiel et al., 2014). Larvae appear to remain viable for up to a few weeks (Poinar and Doelman, 1974; Hanelt et al., 2005). However, apart from a few observations in the field, e.g., Bolek and Coggins (2002), little is known of larval hairworm development in nature. Moreover, practically nothing is known of the proportion of hairworm larvae that is lost to the stream or to predation, nor the number of larvae that make it to the next step in their life cycle.

1.3.3 From larva to cyst

Comparative studies indicate that hairworm larvae must be ingested by an aquatic host (Hanelt and Janovy, 2003; Hanelt and Janovy, 2004a). Once inside the host, they penetrate the gut wall using their specialised mouthparts (de Villalobos and Ronderos, 2003) to then form a cyst somewhere within the tissues of the body cavity by emptying the contents of their pseudo-intestine to form a clear, thick cyst wall around them (Figure 1.3B) (Poinar and Doelman, 1974; Hanelt et al., 2012). Hairworm cysts have been found within most groups of aquatic animals, including insects (Hanelt and Janovy, 2004a), snails (Hanelt et al., 2001), and even vertebrates such as fish (Torres et al., 2017) and tadpoles (Poinar, 2010), among others (Bolek et al., 2015). It is understood that only the aquatic hosts that eventually exit the water to spend time on land contribute to the life cycle of hairworms (see next section) (Schmidt-Rhaesa, 2013), i.e., aquatic insect larvae such as mayflies and stoneflies. Cysts are capable of surviving the metamorphosis of these various insect hosts (Hanelt and Janovy, 2004a; Meguro et al., 2020) and it is possible to

observe multiple hairworm species encysted within the same individual (Chiu et al., 2016). These insect hosts effectively transport hairworms from water to land, thus acting as true paratenic (transport) hosts (Bolek et al., 2015). The animals that never exit water, e.g., aquatic snails, therefore act as dead-end hosts and probably represent population sinks for hairworms (Hanelt et al., 2001; Thieltges et al., 2008).

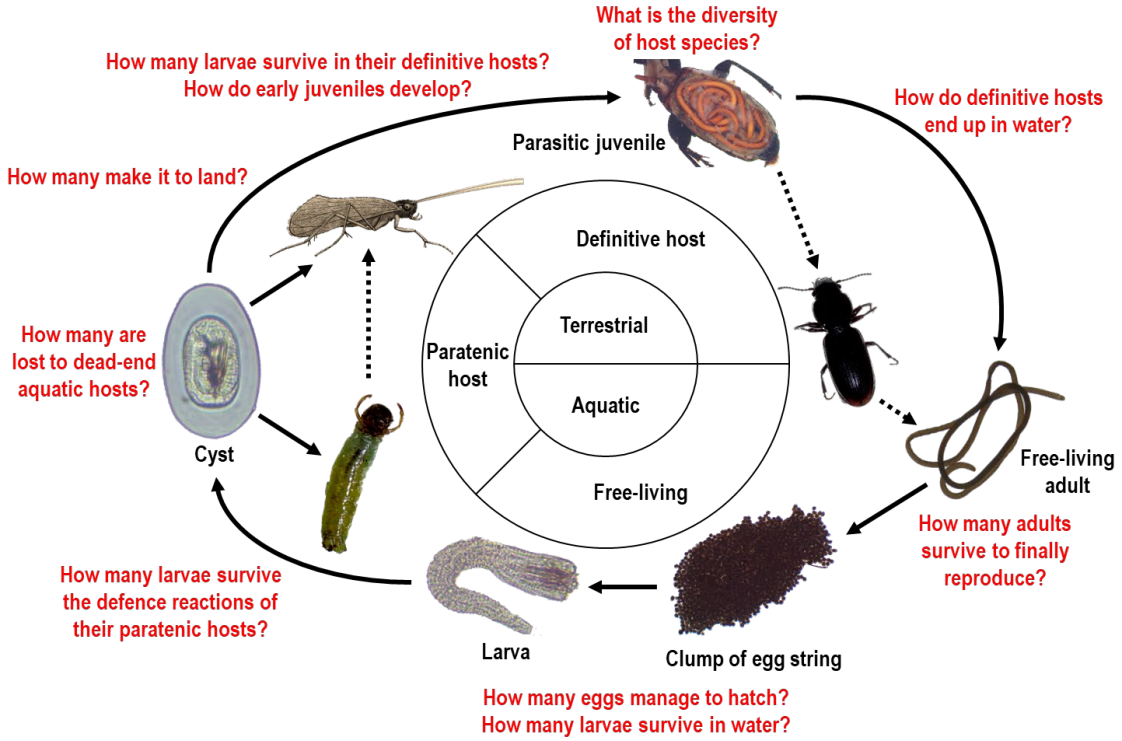


Figure 1.2 Life cycle of freshwater hairworms (phylum Nematomorpha) of the genus *Gordius* in New Zealand. Larvae hatch from short segments of egg string produced by free-living females. They are then consumed by paratenic hosts (here, the caddisfly *Olinga jeanae*), which metamorphose and transport dormant hairworm cysts to land, where they are consumed by definitive hosts (here, the ground beetle *Mecodema* sp.). When mature, hairworms somehow cause their terrestrial hosts to enter water, where they can exit as free-living adults to mate and reproduce. Broad questions (in red) on the ecology and host infection dynamics of hairworms remain unresolved. Figure inspired by Bolek et al. (2015).

Interestingly, cysts are extremely hardy; it has been shown that cysts of some species of *Paragordius* can survive at -80 °C for several months and still continue their life cycle afterwards (Bolek et al., 2013b). The authors of previous studies concluded that hairworms do not develop within their cyst and are dormant during this stage (Schmidt-Rhaesa, 2013; Bolek et al., 2015), indicating that aquatic insect larvae serve only to transport cysts to land as paratenic hosts (Hanelt and Janovy, 2004a). However, these paratenic hosts can mount internal defence reactions against both hairworm larvae and

cysts through melanotic encapsulation, a common form of immunity in insects (Gillespie et al., 1997; Nakhleh et al., 2017). Experimentally, some insect larvae appear to react to the presence of hairworm larvae by rapidly depositing melanin after the parasites penetrate their body cavity (Poinar and Doelman, 1974). This reaction also appears to vary with respect to host instar (de Villalobos and Ronderos, 2003; de Villalobos et al., 2006) and host species (Hanelt and Janovy, 2003). In nature, melanised cysts have been found in variable proportions throughout a number of insect orders (Poinar, 1991a; Chiu et al., 2016).

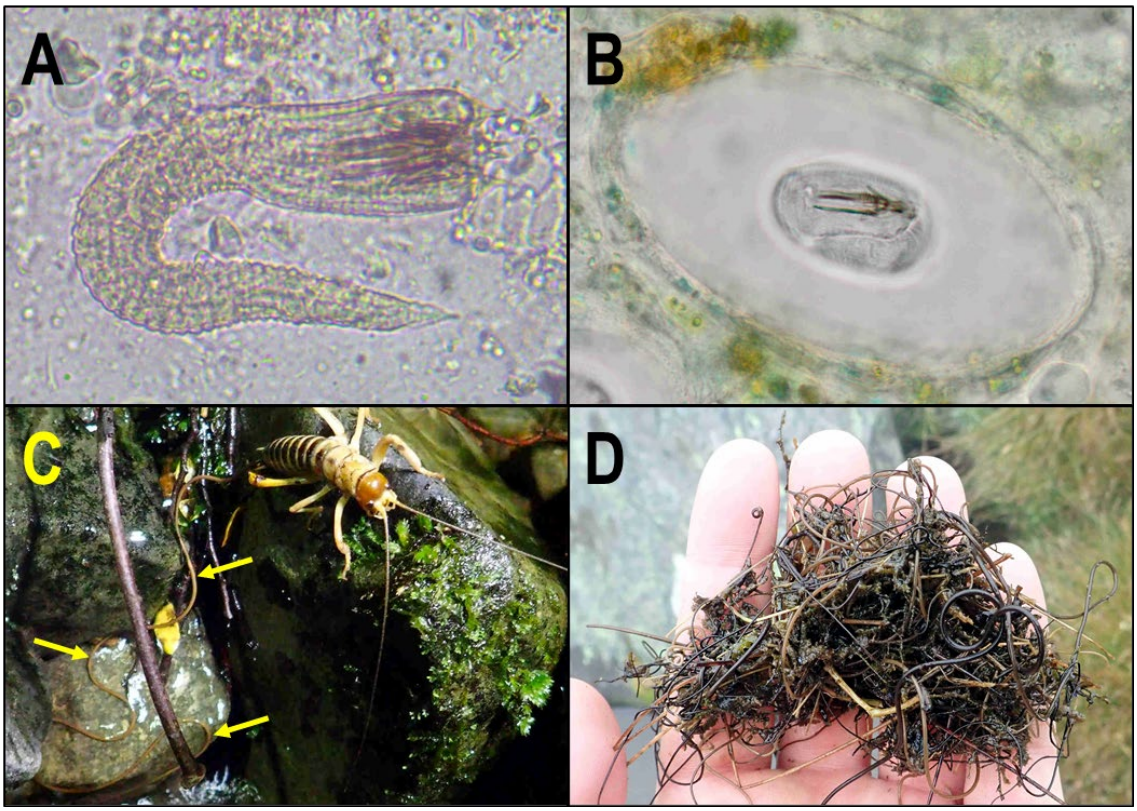


Figure 1.3 Some life stages of freshwater hairworms (phylum Nematomorpha) in New Zealand. (A) *Gordius* sp. larva inside the tissues of a paratenic host. (B) Encysted *Gordius* sp. larva within the tissues of its paratenic host (here, the caddisfly *Olinga jeanae*). (C) Adult *Gordius* sp. hairworm exiting its terrestrial host (here, a tree wētā from the genus *Hemideina*) in a montane forest stream; yellow arrows point the hairworm. (D) Mating knot with multiple species of hairworm mixed with some vegetation, found in a subalpine stream.

Although some authors have concluded that melanotic encapsulation is lethal towards hairworm larvae (Hanelt and Janovy, 2003), it is unknown whether larvae within melanised cysts can eventually excyst to continue their life cycle (see next section). Conversely, apart from some observations on experimentally infected hosts, e.g., Poinar

and Doelman (1974), it is unknown whether natural hairworm infections impact the development or survivability of aquatic hosts. As mentioned, dead-end hosts such as aquatic snails, which are usually present where hairworm cysts are reported (Hanelt et al., 2001; Harkins et al., 2016), may represent important population sinks for hairworms within aquatic habitats. Like the hairworm larvae that are potentially lost in aquatic environments (previous section), it is currently unknown what proportion of hairworm cysts are lost in dead-end hosts.

1.3.4 From cyst to juvenile

The aquatic insect larvae that metamorphose into terrestrial adults and transport hairworm cysts to land can eventually be preyed or scavenged upon by a number of predaceous or omnivorous arthropods (Inoue, 1962; Hanelt and Janovy, 2004a). Once again, hairworms need to be ingested, this time by a terrestrial arthropod, where they can exit from their cyst and penetrate the intestinal wall (as they did in their paratenic host) to wind up in another body cavity (Schmidt-Rhaesa, 2013). How hairworm larvae reactivate within their cyst remains unknown, but it has been suggested that the digestive enzymes of the terrestrial host are possibly detected by larvae (Poinar, 2010). What happens immediately after the larvae finds its way into the body cavity of its terrestrial host remains to be discovered (Figure 1.2). It is unknown how larvae change morphologically into juveniles during the initial stages of growth, when they are microscopic in size. However, what does take place is an immense amount of growth. For example, a hairworm larva measuring approximately 100 µm in length can easily grow to an adult well over 10 cm in length, a difference of around three orders of magnitude. During development, these juvenile hairworms apparently absorb nutrients through their relatively simple cuticle (Schmidt-Rhaesa, 2005), within the haemocoel of their host. In the laboratory, hairworm growth inside definitive terrestrial hosts can take anywhere from a few weeks (Hanelt and Janovy, 2004b) to several months (Bolek et al., 2013a), depending on the species. Besides this, practically nothing is known of the development of juvenile hairworms in nature, nor whether definitive host immunity plays a role in decreasing the chances that hairworm larvae successfully develop into juveniles.

1.3.5 From juvenile to adult

In the wild, hairworm juveniles are usually found alone within their host (Hanelt et al., 2005), although multiple infections have been reported (Valvassori et al., 1988; Schmidt-Rhaesa et al., 2005). Moreover, field observations suggest that host specificity may be quite high for some species, and quite low for others (Bolek et al., 2015), but data are lacking. Schmidt-Rhaesa (2013) collated the known host records for hairworms worldwide and found that most species infect mainly ground beetles, orthopterans, mantids, or cockroaches, but also other arthropods such as millipedes, centipedes, or spiders. The author also found that prevalence, i.e., the proportion of infected individuals within a sample or population, was relatively low across studies, typically less than 5% of the arthropods examined. During juvenile development, hairworms can impact host development, if the latter is immature when it becomes infected. For instance, the gonads and fat body appear to be the main organs affected by hairworm growth in insects (Studier et al., 1991; Anaya and Bolek, 2021), which could lead to a reduction in egg or sperm production, or even intersexuality (Roy, 2003). Other studies found no differences in size or developmental asymmetry between infected and non-infected insect hosts (Poulin, 1995b; Thomas et al., 1998a). When juvenile hairworms near maturity, they shed their cuticle to produce an adult one (Schmidt-Rhaesa, 2005). This complex structure consists of a thick internal layer of large fibres and a thinner external layer with or without structures called areoles, which are used for proper species identification and descriptions (Schmidt-Rhaesa, 2013; Bolek et al., 2015). Once hairworms reach maturity, there remains one crucial step in completing their life cycle: they need to reproduce. Therein lies the crux of hairworm host manipulation (Figure 1.2), making these parasites arguably one of the most well-known examples of this phenomenon.

1.3.6 Host manipulation by hairworms

The name horsehair worm, or hairworm for short, originates from the myth that these long, slender worms were actually the hairs of a horse that fell into the water and magically sprung to life (Leidy, 1850). In reality, the horse is a terrestrial arthropod that typically avoids large bodies of water, i.e., streams and rivers, and the horse hair is a nematomorph trapped within it. What is clear is that hairworms need to return to water to reproduce, but how such a life cycle evolved remains a mystery (Schmidt-Rhaesa, 2013).

Early observations suggested that hosts either approach water for the hairworm to exit (McCook, 1884) or that hosts enter water, where the hairworm can effectively swim out (Jolivet, 1944). More recently, field and laboratory experiments support the idea that infected individuals are more likely to enter water than uninfected ones (Thomas et al., 2002). Here, the authors suggested that hosts may display erratic behaviours that sooner or later bring them to water. Later studies revealed that juvenile hairworms are capable of inducing erratic movements in their hosts, but that only mature hairworms may induce an additional change in behaviour that causes the host to enter water once near it (Sanchez et al., 2008). Another study found that infected individuals may direct their movements toward light, suggesting that the behavioural changes in hosts are likely complex and multidimensional (Ponton et al., 2011). Most recently, it was shown that horizontally polarised light can increase the likelihood of infected hosts entering water (Obayashi et al., 2021). In the wild, orthopteran hosts were found to be 20 times more likely to enter a stream than their uninfected conspecifics, thus becoming an important source of food for local fish (Sato et al., 2011). In turn, this flow of energy, driven by host manipulation from land to water, was found to alter local ecosystem functioning during peak hairworm activity (Sato et al., 2012).

How hairworms accomplish such remarkable changes in host behaviour remains poorly understood. Differences in levels of neurotransmitters and neuromodulators have been detected between infected and uninfected insects (Thomas et al., 2003). Studies have also shown differences in protein expression linked to neurotransmitter activity, circadian rhythm, and neurogenesis (Biron et al., 2005a; Biron et al., 2006). While these differences may impact host behaviour, all the aforementioned studies were done on naturally infected hosts, which limits our ability to draw sound conclusions on the nature of host manipulation by hairworms. Unless it were possible to pinpoint to a hairworm gene (or set of genes) responsible for the changes in host behaviour, this phenomenon can only currently be explained with conjuncture and correlation. With this in mind, many recent reviews and articles on host manipulation have misrepresented and even exaggerated the behavioural changes of hosts infected with hairworms, e.g., Knight (2013), van Houte et al. (2013), and Libersat et al. (2018), by claiming that hosts are suicidal and actively seek water. It is unclear how such misinformation impacts our understanding of host

manipulation, knowing that hairworms are not necessarily lethal for their definitive terrestrial host (Anaya and Bolek, 2021). Studies on the behaviour of experimentally infected hosts, accompanied by an in-depth analysis on the gene expression of both hairworm and host during parasite development, would provide a strong line of evidence on the nature of hairworm host manipulation. Regardless, hairworms have to somehow end up in water (Figure 1.3C), in order to mate and complete their life cycle.

1.3.7 From adult to egg

When a host enters water, the mature hairworm is already ready to exit. Typically, hairworms stick their head out through an open wound they created on the posterior end of their host, by penetrating the soft integument (Hanelt and Janovy, 2004b; Schmidt-Rhaesa, 2005). Once submerged, a hairworm can completely exit its host within a minute (Bolek et al., 2013a). This rapid egression likely reduces the risk of hairworms being ingested along with their terrestrial host; the latter becomes an easy meal for aquatic predators once fallen into water (Sato, 2011). However, observations suggest that hairworms can escape these aquatic predators after ingestion of the host, for example by swimming back out of the mouth or gill opening of fish (Ponton et al., 2006). Depending on the species, free-living adults will eventually bury within the sediment or wrap themselves around a submerged stick or leaf (Bolek and Coggins, 2002). In these conditions, adults can live anywhere from two to eight weeks, although longevity data are limited to a few species (Bolek and Coggins, 2002; Hanelt, 2009). Hairworms can either mate in small numbers or form aggregations called mating knots (Figure 1.3D) (Cochran et al., 2004), also known as Gordian knots (from Greek mythology). It is unknown whether females mate with one or several males, or whether males compete with each other (Schmidt-Rhaesa, 2013). In the end, those hairworms that successfully reproduce have managed to survive potential adversity in the water as larvae, within their paratenic hosts as cysts, and within their terrestrial hosts as juveniles, to finally produce another generation of hairworms after returning to water. The odds of surviving through each life stage are unknown (Figure 1.2), but they are likely to be very low, making it extremely unlikely that an individual hairworm completes it life cycle. This may be why hairworms are adapted to produce such a great number of offspring (Hanelt, 2009;

Schmidt-Rhaesa, 2013), as an evolutionary response to the struggle to survive through such a unique mode of life, which has ultimately resulted in a game of numbers.

1.3.8 Hairworms in New Zealand

Six species of freshwater hairworms from four genera have been reported in New Zealand, five of which have been formerly described (Yadav et al., 2018). They are, as follows: *Euchordodes nigromaculatus*, *Gordionus maori*, *Gordius dimorphus*, *Gordius paranensis*, *Gordius* sp., and *Parachordodes diblastus*. Most of these are restricted to forested and subalpine areas, likely due to the agricultural practices that have modified large areas of landscape at lower elevations (Tobias et al., 2017). As a result, adult hairworms have been collected mainly from lowland forest rivers and subalpine streams (Poinar, 1991a; Thomas et al., 1999). Currently, a total of 15 definitive host species records exists, which includes two ground beetles, 11 orthopterans, and two cockroaches (Poinar, 2001; Schmidt-Rhaesa, 2013), although a few of these cannot be confirmed. Hairworm cysts have also been found in a number of paratenic and dead-end aquatic hosts, including caddisflies, stoneflies, mayflies, and even two species of fish (Blair, 1983; Poinar, 1991a; Winterbourn, 2005; Winterbourn and Pohe, 2017). A recent study suggested that cryptic species of hairworms may exist in some populations (Tobias et al., 2017), which has also been shown to be the case in other parts of the world (Hanelt et al., 2015). However, apart from these limited studies, in addition to some studies on free-living adults (Poulin, 1996; Thomas et al., 1999), little is known of the distribution, diversity, and host-parasite interactions of New Zealand hairworms.

1.4 Thesis development

As my doctoral project progressed, the structure and development of my thesis went through a few iterations. Initially, my main objective was to experimentally infect artificial hosts to create a model system useful for studying the development of hairworms *in vivo* and the behavioural changes of terrestrial hosts throughout infection. I attempted to infect both hosts from the wild (two populations of the cave wētā *Pleioplectron simplex*) and laboratory-reared hosts (the black field cricket *Teleogryllus commodus* and the American cockroach *Periplaneta americana*), using different techniques borrowed

from the literature (Hanelt and Janovy, 2004b; Bolek et al., 2013b; Bolek et al., 2015). Eventually, these trials proved to be unsuccessful (for a complete account, see Appendix A). Each trial lasted anywhere from one to three months; all in all, these trials took up most of the first two and a half years of my PhD. When I was about to start another infection trial in late March 2020, the nationwide lockdown took place, effectively halting my progress during this time. During the lockdown, I decided to dive into the literature on host manipulation to see whether the vocabulary that researchers and journalists use to describe this phenomenon can somehow drive its narrative, potentially impacting future research. This eventually became the basis for Chapter 2. In the early months of my initial infection trials, I had been looking for hairworm cysts in naturally infected aquatic insects to feed to definitive hosts. This is when I realised that cysts can be melanised by their paratenic hosts to varying degrees, thus adding a level of complexity to the cryptic interactions between hairworms and their paratenic hosts (Chapter 3). After over two years of attempting to infect definitive hosts, I finally decided to redirect my attention to field-based studies. For this, I collected infection data in the aquatic macroinvertebrate communities of two subalpine streams to quantify the distribution of hairworms in potential paratenic hosts versus dead-end ones (Chapter 4). Previously to this, during the later infection trials, I tested the impacts of hairworm cysts on the development time of late-instar caddisfly larvae in a controlled observational study, to see whether hairworms affect the life history of their paratenic hosts, potentially accelerating the transition of hairworms from water to land (Chapter 5). Finally, a field study on the infection patterns of two communities of terrestrial arthropods was conducted to explore the behaviour of definitive hosts in a natural setting and uncover new hairworm-host records for New Zealand (Chapter 6).

1.5 The aims of this thesis

Throughout this introductory chapter, I called attention to some gaps in the knowledge of hairworms (Figure 1.2). In response to this, the overarching theme of this thesis is to fill some of these gaps and elucidate the hidden interactions between hairworms in both their aquatic and terrestrial hosts. First comes a commentary on the language used in both the popular media and the scientific literature, which may ultimately impact our

understanding of host manipulation as an adaptive phenomenon in parasites (Chapter 2). Then, from the perspective of the life cycle of hairworms, larvae not only have to survive the potentially lethal defence reactions of their paratenic hosts (Chapter 3), but have to end up in the correct host in order to pursue their development on land (Chapter 4). In turn, hairworms may impact the development of their paratenic hosts, accelerating their transition to a terrestrial environment, thus increasing the odds of infecting a definitive host (Chapter 5). Lastly, new definitive host records are provided for New Zealand hairworms, with a study on the behaviour of infected terrestrial insects in the wild (Chapter 6).

1.6 Thesis outline

This thesis includes five core chapters, which were written as manuscripts intended for publication in peer-reviewed journals. Therefore, some relatively small sections are repeated between chapters. I am the first or sole author of all these manuscripts. All of the core data chapters were designed by me, with critical and practical input from my supervisor. I conducted most of the work, including fieldwork, laboratory work, data analysis, and writing. This was all possible with the help from my colleagues and the input from my co-authors (Xuhong Chai, Antoine Filion, and Robert Poulin). The outline below provides the current status for each core chapter.

Chapter 2. When fiction becomes fact: exaggerating host manipulation by parasites

Published as Doherty, J.-F. 2020. *Proceedings of the Royal Society B: Biological Sciences* 287(1936): 20201081.

Chapter 3. Varying levels of melanotic encapsulation of gordiid hairworm cysts (Nematomorpha) by aquatic insect larvae: seasonal and host effects

Published as Doherty, J.-F., Chai, X., and Poulin, R. 2019. *Journal of Invertebrate Pathology* 168: 107258.

Chapter 4. Come with me if you want to live: sympatric parasites follow different transmission routes through aquatic communities

Under review in *International Journal for Parasitology* as Doherty, J.-F. and Poulin, R.

Chapter 5. Going full circle: impact of hairworm infection on aquatic insect development may accelerate its return to land

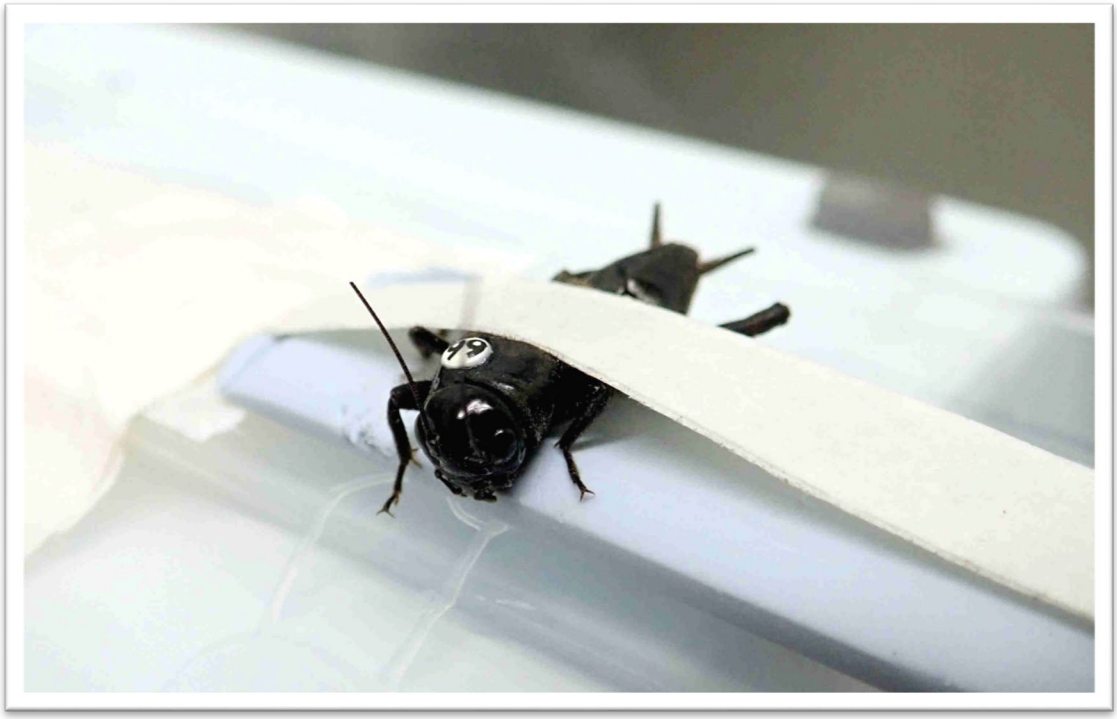
Under review in *Parasitology Research* as Doherty, J.-F. and Poulin, R.

Chapter 6. Infection patterns and new definitive host records for New Zealand gordiid hairworms (phylum Nematomorpha)

In preparation for submission to *Parasitology International* as Doherty, J.-F., Filion, A., and Poulin, R.

Chapter 2

When fiction becomes fact: exaggerating host manipulation by parasites



A live male black field cricket (*Teleogryllus commodus*) being tagged with a numbered bee tag for one of the experimental infection trials for hairworms.

2.1 Abstract

In an era where some find fake news around every corner, the use of sensationalism has inevitably found its way into the scientific literature. This is especially the case for host manipulation by parasites, a phenomenon in which a parasite causes remarkable change in the appearance or behaviour of its host. This concept, which has deservedly garnered popular interest throughout the world in recent years, is nearly 50 years old. In the past two decades, the use of scientific metaphors, including anthropomorphisms and science fiction, to describe host manipulation has become more and more prevalent. It is possible that the repeated use of such catchy, yet misleading words in both the popular media and the scientific literature could unintentionally hamper our understanding of the complexity and extent of host manipulation, ultimately shaping its narrative in part or in full. In this commentary, the impacts of exaggerating host manipulation are brought to light by examining trends in the use of embellishing words. By looking at key examples of exaggerated claims from widely reported host-parasite systems found in the recent scientific literature, it would appear that some of the fiction surrounding host manipulation has since become fact.

2.2 From harmless to harmful

A parasite, etymologically speaking, is a person that eats at the table of another. In biological terms, it is an organism that acquires nutrients at the expense of a host (Oxford English Dictionary online, 2020). Harmless though the origin of the word is, the mere thought it evokes today of an unwelcomed creature invading the body can trigger feelings of disgust and even paranoia (Trabert, 1995; Curtis et al., 2011). Arguably, the pervasive nature of parasitic infection and disease throughout human history has inspired numerous works of fiction (Glassy, 2001), from the amoeboid aliens in the 1935 short story *Brain Leeches* (Mund, 1935) to the covetous characters in the award-winning 2019 film *Parasite* (Bong, 2019). But if parasitic invasion does not sound gruesome enough, it would seem that science fiction has in turn inspired parasites. Remarkably, certain parasite species have evolved the ability to alter the phenotype of their hosts in ways that favour the transmission to another host or to an environment suitable for reproduction (Moore, 2002; Poulin, 2010). This phenomenon, known as host manipulation, ranges from striking changes in host appearance, e.g., limb malformation in amphibians infected with the trematode *Ribeiroia ondatrae* (Johnson et al., 2002), to conspicuous alterations of host behaviour, e.g., the "death grip" in ants infected with the fungus *Ophiocordyceps unilateralis* (Andersen et al., 2009). Such parasite-driven modifications can have harmful, even lethal impacts on the host. Whether or not the phenotypic alterations in infected hosts are the result of adaptive parasitic manipulation or the by-products of infection has been debated for decades (Moore and Gotelli, 1990; Poulin, 2000a; Cézilly and Perrot-Minnot, 2005; Nickol, 2005; Poulin and Maure, 2015). As a concept, host manipulation is inherently complex and includes a plethora of biochemical interactions between hosts and parasites (Hébert and Aubin-Horth, 2014), making it difficult to understand and communicate to a wider audience. Such complexity may have encouraged researchers to introduce scientific metaphors about host manipulation into the literature. In this regard, the current piece strives to shine a light on what may yet be another contentious aspect of the phenomenon of host manipulation by parasites, which involves its very depiction in the popular media and the scientific literature.

2.3 How have the popular media and the scientific literature portrayed host manipulation?

Many online newspapers and magazines (referred to as popular media throughout the text) struggle to maintain readership in the age of digital journalism (Loosen and Schmidt, 2012; Franklin, 2014). The competition resulting from this is a known factor promoting sensationalism, which is a writing style that provokes interest at the expense of accuracy (Vettehen and Kleemans, 2018). This type of journalism typically includes exciting headlines and covers not only crime stories and scandals, but also "hard" news like science and technology (Kilgo et al., 2018). Therefore, unsurprisingly, the popular media outlets that have reported on host manipulation tend to focus on parasites that cause spectacular changes in the host, e.g., the "caterpillar tentacles" of snails infected with *Leucochloridium paradoxum* (Wesołowska and Wesołowski, 2014), or ones that infect humans, e.g., *Toxoplasma gondii* (Appendix B). Understandably, these stories typically include captivating words such as "mind control", "zombie", or "hijack" in their title and main text, along with catchy headlines that may attract readers (Figure 2.1A). While the use of such wording, which appears to be either anthropomorphic in nature, i.e., words that give humanlike characteristics, emotions, or intentions to non-human agents (Epley et al., 2007), or directly borrowed from science fiction, may increase readership and result in profit for media corporations, the words themselves are inherently vague and misleading. Obviously, parasites do not literally hijack the body of their hosts, i.e., there is no unlawful seizure of the host at play. Nor do they cause the resurrection of dead hosts with an insatiable appetite for brains. But if popular media have widely adopted the use of such captivating, yet ambiguous words to describe host manipulation, how has the subject been represented in the scientific literature?

A quick search in Web of Science for articles and review papers that include eye-catching words describing host manipulation in their title or abstract shows some revealing trends (eye-catching words refer to metaphors, including the anthropomorphisms and terms appropriated from science fiction that are widely used in popular media, see Figure 2.1A). Firstly, the usage of such words tends to appear in the scientific literature during the late 1990s and the 2000s, a period which coincides with the biological significance of host

manipulation being put under scrutiny (Poulin, 2000a) and a renewed interest around host manipulation in the mid-2000s (see Figure 1 in Poulin and Maure (2015)). Secondly, while some words such as "puppeteer" or "bodyguard" appear only to be used in a small number of papers per year, other words like "zombie" and "hijack" occur more often and are used as frequently or more every year (Figure 2.1B). Thirdly, the popular media appear to focus on a slightly different set of catchy words than what can be found in the scientific literature (Figure 2.1).

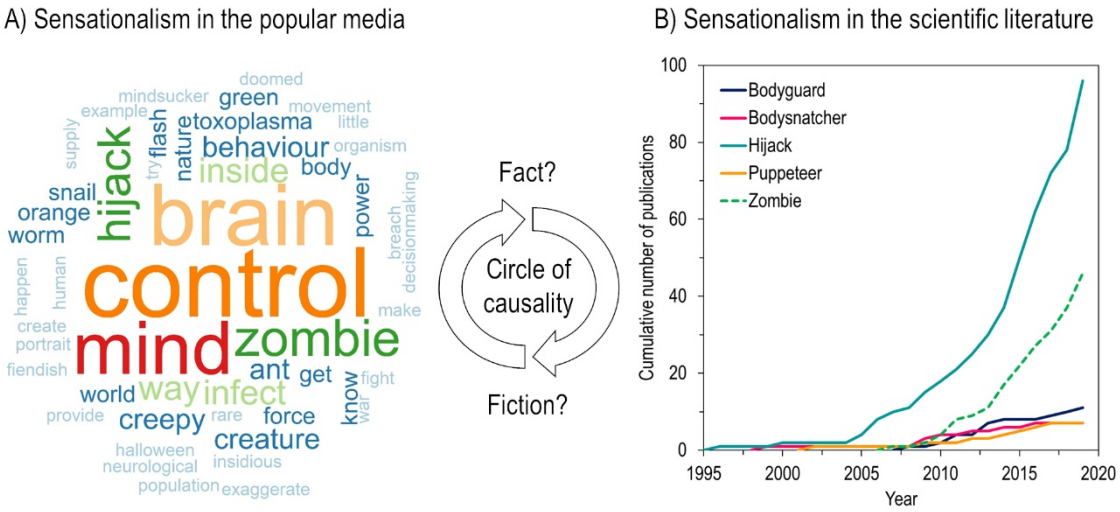


Figure 2.1 Trends in the use of catchy, yet misleading words describing host manipulation by parasites. The circle of causality in the centre highlights the ambiguous origin of the increasing popularity to portray host manipulation with exaggerated claims. (A) Word cloud of titles and headlines from randomly selected online newspaper and magazine articles (n = 25) covering host manipulation worldwide in the 2000s and 2010s (for methods, see Appendix B). The more frequently a word is used, the larger it appears in the word cloud. (B) Cumulative number of scientific articles and review papers searched in Web of Science (n = 167) that include at least one of a selection of catchy words depicting host manipulation (see legend) used either in the title or abstract (Appendix B). Bacteria and viruses were excluded from the search.

Interestingly, most of the popular media articles covering host manipulation, containing the catchy vocabulary described above, started to appear during the mid-2010s onward (Appendix B). Thus, one could advance that the usage of catchy, yet misleading words to describe host manipulation in the scientific literature pre-dates its adoption in popular media. This in itself does not indicate that the exaggerated claims and sensationalism surrounding host manipulation in the popular media are a direct consequence of what is published in the scientific literature. Perhaps the increased usage of a word like "zombie" in the scientific literature is fuelled by popular media, or vice versa, thus creating a circular chain of causality (Figure 2.1) (Rinaldi, 2012). Regardless, it does suggest that

both researchers and reporters, at the risk of being inaccurate, employ sensationalism or hype to perhaps attract more readers (Ransohoff and Ransohoff, 2001). Interestingly, hype is not uncommon in scientific writing; researchers regularly exaggerate their study systems in areas such as grant applications and institutional press releases (Caulfield, 2018). While this strategy may appear harmless at first glance, the misleading vocabulary that represents host manipulation in both the popular media and the scientific literature could unintentionally impact its narrative and our general perception of the topic.

2.4 Does the repeated use of catchy words impact the general narrative of host manipulation?

As previously mentioned, the vocabulary of host manipulation is rife with anthropomorphisms. Even the word "manipulation" strongly implies the humanlike ability to influence someone in a cunning and deceitful manner. Other words used to describe parasitic manipulation, such as "control", "usurp", "brainwash", or "bodysnatcher", are all anthropomorphic by definition. The use of humanlike descriptors is common across many disciplines (Epley et al., 2007; Davies, 2010); it helps readers to connect with a certain topic and perhaps better understand it, all while improving the capacity to retain information on said topic (Baker et al., 2018). Anthropomorphisms can even impact how people view nature, going as far as shaping their opinions on environmental protection (Tam et al., 2013). However, the general consensus drawn from research on science education strongly suggests that attributing humanlike qualities like intention to non-human subjects impedes the understanding of complex phenomena such as natural selection and climate change. People tend to use their generic background knowledge to integrate novel concepts, which can result in deep-rooted misconceptions about complex phenomena (Kallery and Psillos, 2004; Chi et al., 2012). Given that host manipulation is an intrinsically complex subject covering many biological interactions between two co-evolving species (Herbison et al., 2018), the abundant anthropomorphisms used to simplify and explain it may very well hinder its understanding by non-specialists and specialists alike. Although, attributing humanlike

descriptors to parasites may be just one of the factors impeding the factual interpretation of host manipulation.

All the anthropomorphisms and words borrowed from science fiction that are used to describe host manipulation form the essence of scientific metaphors (Figure 2.1), which basically means that they do not literally apply to biological systems (Pauwels, 2013). The power of such words becomes ever clearer when they are recognised as metaphors. Determining if a word such as "manipulation" is metaphorical can be done with three diagnostic criteria (Olson et al., 2019): expressiveness, paraphraseability, and silliness. Firstly, the expressiveness of a metaphor refers to its ability to evoke analogies that can be interpreted as predictions. For example, by assuming that a certain parasite manipulates its host, one can imagine that it is doing so to gain an increase in fitness. Predictions could thus be made regarding the changes in host phenotype that increase parasite fitness, which of course depends on the nature of the host-parasite association. Secondly, paraphraseability highlights the imprecise nature of a metaphor. For instance, a "trematode that manipulates its fish host" is not as precise as "fish infected with trematodes are more conspicuous to predators". Thirdly, the silliness of a metaphor is usually accentuated when it is applied literally. As stated above, it is highly unlikely that any parasite is literally capable of cunning or deceit, two defining characteristics of manipulation.

Recognising that host manipulation and its associated terms are metaphors is an important step in understanding the impact they have on our perception of the phenomenon. As defined by their expressiveness, metaphors are tools that help researchers think about their systems, which in turn helps generate novel predictions and explanations (Olson et al., 2019). Moreover, metaphors can help bring scientists from different perspectives together to think about the same phenomenon, simply because metaphors can be interpreted in many ways (Pauwels, 2013). However, researchers must be aware that when they use metaphors such as host manipulation and its many spinoffs, they may be highlighting certain aspects of their study systems while hiding other important ones (Kueffer and Larson, 2014). As mentioned above, metaphors are vague, which could result in numerous contradictory interpretations. In practice, anyone can decide what

"hijack" or "control" mean if they are used metaphorically. Because of their vagueness, metaphors are also difficult to quantify or measure. For example, it would be impossible to quantify the "zombification" of ants infected with *Ophiocordyceps*. Though, the real danger of metaphors lies in their reification, which occurs when they are treated as a real part of nature (Chew and Laubichler, 2003). Researchers must be cognisant of the potential dangers of scientific metaphors and understand that they can mask important information and dilute truth. Host manipulation as a metaphor may help predict how a certain parasite changes host phenotype. However, this metaphor may hide other aspects of host-parasite associations, such as the extent to which parasites actually cause phenotypic changes in their hosts. Ultimately, not recognising host manipulation and its associated terms as metaphors could lead to wrongful interpretations and generate misconceptions of the phenomenon.

Metaphor or not, whatever is published in the popular media and the scientific literature may have long-lasting impacts on human memory and belief systems. Known as the illusory truth effect (Begg et al., 1992), humans tend to remember erroneous statements, if they are repeated enough, and can even create false memories about them (Zaragoza and Mitchell, 1996). This tendency is stronger when the statement is presented using an official format such as a news article or a publicity slogan (Fazio et al., 2015). People are also known to incorporate information from works of fiction, e.g., novels and movies, into their general knowledge of the real world (Marsh et al., 2003). When presented with contradicting new information on a certain topic, people tend to accept the older repeated statements as truer, even when they are false (Unkelbach and Rom, 2017; Brashier et al., 2020). Therefore, repeated statements, be they true or false, can form the foundations of false memories that are gradually accepted as fact. For example, the recurrent sensationalism in news media is a known factor affecting the general perception of climate change in humans (Capstick et al., 2015). So, could the illusory truth effect impact the perception and understanding of host manipulation of both specialist and non-specialist readers? Well, combined with the dangers of using metaphors and the impact of anthropomorphisms on generating misconceptions about complex phenomena and hindering the ability for people to understand and interpret them, the abundant repetition of exaggerated and misleading claims about host manipulation in both the popular media

and the scientific literature may be hampering the ability to properly communicate fact over fiction (Figure 2.1). As such metaphors are vague and can hide certain truths, not only could this be steering the popular perception of host manipulation away from fact-based territory, it could very well have shaped the general scientific narrative of recent years.

2.5 Has the fictional narrative of host manipulation become fact?

If the hypothesis "the use of science fiction, anthropomorphisms, and catchy words to describe host manipulation by parasites in both the scientific literature and the popular media impacts its general narrative and popular perception" were true, one could make the following prediction: it should be possible to find claims in the popular media and the scientific literature that are either fully or partially unsubstantiated, or that negate the empirical evidence or lack thereof. Such claims could be made regardless of relative research effort, i.e., highly studied parasites versus ones that receive little attention. For example, the heavily studied *Toxoplasma gondii* (Tenter et al., 2000), a protist known for its negative health effects on humans and its wide-ranging behavioural impacts on warm-blooded animals (Berdoy et al., 2000; Flegr, 2007; House et al., 2011), supposedly manipulates small rodents to increase their chances of getting eaten by felids. In the life cycle of *T. gondii*, small rodents and felids represent the intermediate and final hosts, respectively (Dubey, 2009). To achieve transmission between hosts, *T. gondii* has been said to cause a "fatal feline attraction" in rodents by removing their aversion to cat urine through the expression of the disruptive dopamine-signalling *AaaH2* gene (Dass and Vyas, 2014; Vyas, 2015). This catchy narrative has been widely reported in the popular media (Appendix B) and in the scientific literature, even within recent years, e.g., see Knight (2013), Kaushik et al. (2014), and Hugues and Libersat (2019).

However, it was shown that experimental strains of *T. gondii* without the *AaaH2* gene cause the same behavioural patterns in rodents as did the wild strains (Afonso et al., 2017). More recently, it was shown that *T. gondii* cyst load in the brains of infected mice correlates with neuroinflammation, which could result in behaviours related to lower anxiety and an aspecific aversion to predators (Boillat et al., 2020). This evidence

supports the idea that the complex physiological impacts of *T. gondii* in warm-blooded animals are more than one-dimensional; they unlikely result in a simple dopamine-related attraction towards cat urine. Moreover, *T. gondii* may have experienced little to no selective pressures to adaptively manipulate small rodent hosts. In fact, there is no sound evidence that the behavioural changes in infected rodents increase the transmission of *T. gondii* to felids (Worth et al., 2013). The evidence surrounding the "fatal feline attraction" is inconsistent and contradictory at best (as it is for all the impacts of *T. gondii* on human and non-human behaviours (Johnson and Koshy, 2020)). The fatal attraction of infected rodents would appear to be a reasonable explanation because it fits with the narrative that *T. gondii* must change hosts to complete its life cycle. However, the predominant lineages throughout Europe and North America are essentially clonal and do not need to reproduce sexually in felid hosts (Sibley and Ajioka, 2008; Boothroyd, 2009). This begs the question: since its conception, has the anthropomorphic concept of fatal attraction in infected rodents driven the scientific narrative and impacted the general perception of the extent of host manipulation by *T. gondii*? Arguably, it has, because despite all the contradictory evidence challenging the claims of *T. gondii* host manipulation (Worth et al., 2013; Johnson and Koshy, 2020), it is possible to still find such misleading claims in recent works from both the popular media and the scientific literature (see above). Unless a complete scope of the literature is done to fully understand the extent of *T. gondii* host manipulation, it may be less exacting to adopt the popular narrative of the "fatal feline attraction" at face value. Although this metaphor is relatively simple to understand, its current usage may mask the true complexity of *T. gondii* host manipulation. It would appear that, despite evidence to the contrary, the "attractive" narrative of *T. gondii* host manipulation still holds a firm place in the scientific narrative.

2.6 The legend of the hairworm

With a parasite like *T. gondii*, which has been intensely researched around the world for decades (Tenter et al., 2000), it is admittedly difficult to assess the full impact of a few catchy words on the entire scientific and popular narrative of *T. gondii* host manipulation. However, the effects of sensationalism become ever clearer when looking at examples of host manipulation by parasites that have received far less scientific scrutiny. Take, for

example, gordiid hairworms (phylum Nematomorpha), which are parasites with a complex life cycle that live as dormant cysts within aquatic insect larvae and mature into long, slender worms typically within terrestrial scavenger-type insects (Hanelt et al., 2005; Bolek et al., 2015). Hairworms have a peculiar life cycle: the aquatic insect larvae that harbour cysts serve as paratenic hosts when they mature, reproduce, and die on land (Hanelt and Janovy, 2004a); the final hosts become infected by consuming dead paratenic hosts with cysts. Most hairworm species must exit their host to mate and lay their eggs in freshwater, although there are some exceptions to this (Hanelt et al., 2012; Anaya et al., 2019). Therefore, as they develop and mature within terrestrial insects, hairworms must somehow return to water to complete their life cycle. The hairworm could rely on chance and escape from the host when close to water (hairworms are prone to desiccation), but observations on infected hosts, dating back to the 19th century (McCook, 1884), suggest that the host brings the hairworm to water (Poinar, 1991b). Whether or not this unusual behaviour is the result of cooperation from the host or of host manipulation by hairworms, or a combination of both, is a question that still stands. Nonetheless, the popular media and the scientific literature have strung a far more sinister and fictitious narrative around hairworms.

According to popular media, hairworms have nearly absolute control over their insect hosts (Appendix B). They are apparently capable of coercing the host to commit suicide, forcing it to seek water at all costs. The host then supposedly drowns itself in a kamikaze-style death dive, unable to leave water under the mind-controlling powers of the hairworm (Figure 2.2A). Unfortunately, fantastical though this narrative is, not one of these extraordinary claims is currently supported by any data. There is currently no empirical evidence supporting the hypothesis that infected hosts actively seek water or that hosts voluntarily jump into water when close to it (Thomas et al., 2002). Even though these ideas are testable, the sheer lack of evidence would make them appear like adaptationist just-so stories (Olson and Arroyo-Santos, 2015). In fact, the few studies that have compared infected to uninfected hosts (Thomas et al., 2002; Thomas et al., 2003; Biron et al., 2005a; Biron et al., 2005b; Biron et al., 2005c; Biron et al., 2006; Sanchez et al., 2008; Ponton et al., 2011) have found perhaps a more subtle behavioural alteration possibly induced by hairworms. What has been suggested is that infected hosts tend to

move around more, perhaps erratically, than uninfected hosts (Sanchez et al., 2008), which has received some empirical support (Ponton et al., 2011) (Figure 2.2B). Also, the behavioural changes in infected hosts appear to be circadian in nature and only mature hairworms are capable of inducing behavioural change (Thomas et al., 2002; Biron et al., 2005b; Biron et al., 2006).

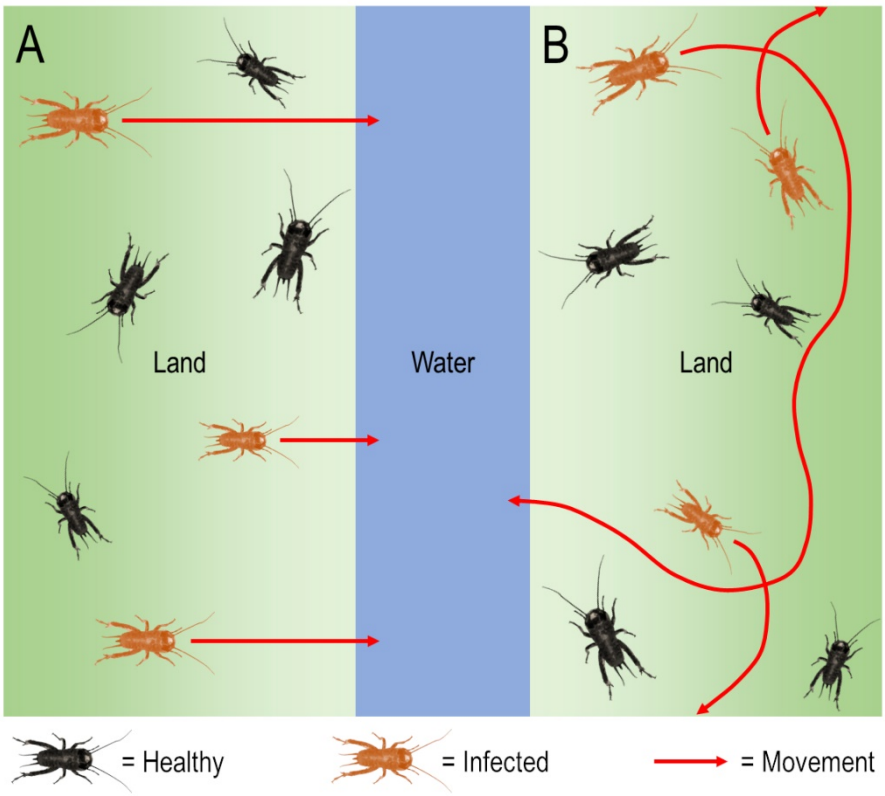


Figure 2.2 Behavioural depictions of terrestrial insects infected with hairworms (phylum Nematomorpha). (A) Behaviour of infected insects depicted in the popular media (online newspaper and magazine articles) and the scientific literature. According to this narrative, hairworms coerce their hosts to seek water, then force them to commit suicide by jumping into water and drowning. (B) Based on the available evidence, infected insects appear to be more active and to move around erratically, which may or may not lead them to water. Very little is currently known on the proximate mechanisms of hairworm host manipulation that are behind this more subtle change in behaviour.

Histological and biochemical studies have shown differences in neurogenesis and in levels of polyamines, monoamines, and amino acids between infected and uninfected hosts, which may impact host behaviour (Thomas et al., 2003). Proteomic studies also showed differential expression of proteins between infected and uninfected hosts that relate to neurogenesis, the circadian rhythm, and neurotransmitter activities in insects (Biron et al., 2005b; Biron et al., 2006). However, despite these promising indications, all the above studies compared only field-caught individuals; no observations were

performed on experimentally infected hosts. While this correlative and circumstantial evidence does offer some insight on the more subtle nature of behavioural changes in hosts infected with hairworms, it is impossible to draw sound conclusions of causation from naturally infected hosts that come with their own set of confounding and uncontrolled variables (Moore and Gotelli, 1990; Poulin, 1995a; Poulin and Maure, 2015). Moreover, some of the physiological differences between infected and uninfected hosts may simply result from the fortuitous side effects of infection or from the competition for nutrients between the hairworm and the host (Hanelt et al., 2005). Thus, if hairworms adaptively manipulate their hosts to increase the likelihood of entering water, the evidence for a proximate mechanism remains very weak. To date, the observations simply do not support the claims in the popular media that hairworms coerce their hosts to seek water and commit suicide by drowning in it.

This extraordinary narrative is not only found in the popular media. The anthropomorphic suicide of hosts infected with hairworms is a concept as old as the question of whether or not hairworms adaptively manipulate their terrestrial hosts (Thomas et al., 2002). In fact, the scientific narrative closely mirrors that of the phenomenal manipulative capabilities of hairworms depicted in popular media (Biron et al., 2006; Sanchez et al., 2008; Lefèvre et al., 2009a; Poulin, 2010; Ponton et al., 2011; Knight, 2013; van Houte et al., 2013; Libersat et al., 2018) (Figure 2.2A). While the use of such metaphors may seem quirky and harmless, and may be intended to help readers better connect with the subject, the repeated use of these anthropomorphisms and other misleading statements could ultimately impact the general understanding of the complex phenomenon that is host manipulation. Of course, it should seem obvious that infected insects do not literally commit suicide (see Poulin (1992)), nor do hairworms come close to coercing their hosts to seek water. The case of misleading readers could also be made for other parasites that seemingly manipulate their host and have been researched even less, e.g., the nematode *Myrmeconema neotropicum* that appears to induce fruit mimicry in ants (Yanoviak et al., 2008). However, the use of such false narratives as hallmarks of host manipulation is still found throughout review papers and articles of the past few years. Non-specialist and specialist readers, who may not verify these exaggerated claims at the source, could build a fictional representation of the extent of host manipulation. One could thus argue that,

over time, the metaphorical nature of host manipulation and its many spinoffs, repeated in popular media and endorsed by the scientific literature, may very well become indisputable fact.

2.7 Concluding remarks and future perspectives

Adaptive host manipulation is inherently difficult to study because separating accidental by-products of infection from trait-mediated manipulation, which occurs between a parasite and a host that may have co-evolved for millions of years, is intricately complex (Thomas et al., 2005; Poulin, 2010; Cézilly et al., 2013). Consequentially, there has been a strong focus on the same old model organisms (Poulin and Maure, 2015). Despite these challenges, this opinion piece is in no way a critique of the quality of research on host manipulation developed in the past few decades. However, for the sake of argument, if the scientific literature continues to exaggerate the claims of host manipulation by parasites without check, it is possible that, for example, specialists and non-specialists alike will start to unequivocally believe that hairworms are capable of creating suicidal insect hosts, or that *T. gondii* causes rodents to be attracted to cat urine. The fact is, these complex host-parasite systems need far more research effort to arrive at any sound conclusion on the nature of host manipulation. A holistic undertaking, incorporating many different scientific viewpoints, from the proximate (genomics and transcriptomics) to the ultimate (behavioural and evolutionary studies), would greatly enhance our ability to tease apart the intricate molecular interactions between host and parasite and better understand the true adaptive nature of host manipulation (Olson and Arroyo-Santos, 2015; Herbison et al., 2019b; Selbach et al., 2019). But all things considered, no piece of scientific writing, from peer-reviewed publications to grant applications and institutional press releases, is completely free from certain embellishment (including the current commentary). Metaphors have merit and researchers should use them to better understand complex phenomena and make insightful predictions. However, it is imperative that researchers understand the potential dangers associated with metaphors and strive to use objective descriptions to communicate their findings as accurately as possible. If not, the use of anthropomorphisms and science fiction, fuelled by sensationalism and repeated abundantly by the popular media, could ultimately hamper our ability to convey a factual

narrative and to correctly interpret and understand the complexities and scope of host manipulation by parasites.

Chapter 3

Varying levels of melanotic encapsulation of gordiid hairworm cysts (Nematomorpha) by aquatic insect larvae: seasonal and host effects

First field location of the project where macroinvertebrates collected from the stream were examined for hairworm cysts. Grasmere Stream, Cass Field Station, Canterbury, New Zealand.

3.1 Abstract

The defence reactions of insects to parasitic invaders are both varied and complex. Melanisation of pathogens is often an important step in insect immunity and can play a key role in isolating parasites. Within samples collected from a subalpine stream in New Zealand during two consecutive seasons (winter and spring), we observed and categorised different levels of melanotic encapsulation by aquatic insect larvae to dormant *Gordius* sp. hairworm (phylum Nematomorpha) cysts, a relatively obscure group of parasites. Some of these insect species act as paratenic hosts in the complex life cycle of hairworms. Based on these new observations, we calculated the melanisation response for an abundant species of caddisfly larvae (*Olinga* sp.) using the proportion of non-melanised cysts per individual host. We tested the hypothesis that season and total number of cysts in an infected host impact its melanisation response. Also, we explored the effect of host body size on the total number of cysts it carries. We found that the total number of cysts does not affect the melanisation response of the host. Season did have an impact on the melanisation response in *Olinga* sp., with lower levels observed in the spring. Additionally, larger caddisfly larvae harboured more cysts than smaller ones. Since little is known about the cryptic interactions between hairworms and their paratenic hosts, this new information adds some complexity to this poorly understood group of parasites.

3.2 Introduction

Parasites with complex life cycles have to overcome multiple challenges during their transmission from one host to the next (Thieltges et al., 2013; Poulin and Lagrue, 2015). In the case of trophic transmission, the capacity of a parasite to survive the internal defence reaction of its intermediate host may increase the odds of a successful transmission. Conversely, the internal defence reaction of intermediate hosts can considerably hinder the life cycle of parasites by effectively blocking their transmission (Buckling and Read, 2001; Fox et al., 2013). In particular, insect hosts have evolved rapid immune responses to parasites capable of breaching their outer defence barriers, e.g., the cuticle and endothelia (Schmid-Hempel, 2005). Moreover, the innate immune reactions of insects toward parasitic infections are complex and can result in multiple chemical and cellular responses (Gillespie et al., 1997). One of these responses involves melanotic encapsulation or melanisation, a humoral response resulting from the activation of phenoloxidase in reaction to injury, i.e., the proPO system (Brivio et al., 1996; Bidla et al., 2005; Nakhleh et al., 2017). This humoral response is different from cellular encapsulation, which occurs when foreign objects are encapsulated by haemocytes and does not necessarily involve melanogenesis. Here, we explore the melanisation of freshwater macroinvertebrates, specifically insect larvae, in response to natural parasite infections during two consecutive seasons in New Zealand.

Freshwater hairworms or gordiids (Nematomorpha: Gordiida) are a relatively obscure group of parasites with a complex life cycle that includes five recognised life stages and multiple invertebrate hosts (Hanelt et al., 2005). Adult gordiids, found mainly within scavenger or predatory terrestrial insects, e.g., cave wētā, praying mantids, and cockroaches, are recognised as manipulative parasites capable of inducing their definitive host to enter water so they can exit the host, mate, and lay their eggs (Thomas et al., 2002; Ponton et al., 2011). The larvae that hatch from these eggs do not swim efficiently and become part of the benthos, where they are consumed by a myriad of aquatic animals, mostly invertebrates but also including vertebrates, e.g., Torres et al. (2017). With the use of specialised mouthparts, gordiid larvae can move through and encyst indiscriminately within the tissues of these aquatic hosts (Hanelt and Janovy, 2003). Some aquatic hosts, such as aquatic snails or fish, never exit water and act as “dead-end” hosts, which could

represent population sinks for gordiids (Hanelt et al., 2001). Aquatic insect larvae, e.g., mayflies, midges, and stoneflies, are presumably the true paratenic hosts responsible for transporting cysts to dry land after they metamorphose, where they are eventually consumed by definitive hosts (Hanelt and Janovy, 2004a). Few studies have looked at the cryptic interactions between gordiid larvae or cysts and their paratenic hosts (Poinar and Doelman, 1974; Hanelt and Janovy, 2003). Therefore, relatively little is known on the effects of gordiid infections in aquatic macroinvertebrates.

The melanisation of gordiid larvae or cysts has been observed in certain species of paratenic insect hosts. Poinar and Doelman (1974) noted that heavy cyst loads can increase host mortality rates in laboratory-induced infections. However, it is unknown how gordiid cysts impact host survivability in nature. Most observations on melanisation of hairworms by paratenic insect hosts were done on gordiid larvae. Early reports found that chironomid *Chironomus* larvae react to the intrusion of gordiid *Chordodes japonensis* larvae by covering them in a special chitinous "cyst wall" (Inoue, 1960). Poinar and Doelman (1974) also noted that late-instar culicid *Culex pipiens* larvae react quickly to penetration of the larvae of the gordiid *Neochordodes occidentalis* by depositing melanin; gordiids encysted normally in early-instar *C. pipiens* larvae. This host instar-dependent reaction was also observed in other dipterans infected with *Paragordius varius* larvae (de Villalobos and Ronderos, 2003; de Villalobos et al., 2006). Lethal haemocyte encapsulation and melanisation was observed in larval *Culex tarsalis* infected with three common gordiid species, although these reactions were independent of host instar (Hanelt and Janovy, 2003). Melanised gordiid cysts were observed in species of immature Trichoptera, Plecoptera, Ephemeroptera, and Megaloptera in New Zealand (Poinar, 1991a). It would appear that melanotic encapsulation against gordiids varies greatly between paratenic host species in terms of host development and its lethality toward different gordiid life stages, i.e., larvae and cysts. However, it remains unknown if melanotic encapsulation can prevent the excysting of dormant gordiid larvae within their definitive host. Melanisation may be an important factor in the internal defence reaction against cysts and is known to be toxic for other parasites (Volz et al., 2006), but this complex biochemical cascade is only a part of insect immunity toward pathogens (Gillespie et al., 1997; Nakhleh et al., 2017).

Observations of gordiid cyst infections suggest that prevalence levels change according to season and insect host taxon. In Japan, differences of infection prevalence were observed in five insect taxa across all seasons (Yamashita et al., 2017). Chironomid larvae collected from a stream in northern Taiwan were found to harbour cysts almost year-round, with a peak of infection prevalence occurring in mid-September (Chiu et al., 2016). In this study, it was noted that some of the cysts were darker in appearance (up to half in one of three identified cyst morphotypes), presumably caused by the melanisation of the cysts by their dipteran hosts. Humoral encapsulation of gordiids by paratenic hosts is known to vary according to host taxon and development stage (see above), but it is not known whether melanisation is affected by seasonal changes. Additionally, seasonality and host taxon may not be the only factors impacting hairworm infection levels and the melanisation in paratenic hosts. Host body size is known to affect parasite prevalence and intensity, considering that a bigger host is more likely to harbour more parasites (Poulin, 2013; Yule and Burns, 2015). Also, parasite load can increase energy costs and weaken the immune system of a host (Sheldon and Verhulst, 1996; Hicks et al., 2018). Therefore, it is possible that host size plays a role in the internal defence reaction against hairworm cysts.

In the current study, we present new observations of melanotic encapsulation in different invertebrate taxa of aquatic hosts infected with *Gordius* sp. hairworms and categorise them according to level of intensity in order to calculate a general host melanisation response. With this new information, we investigated the relationship between the melanisation response, sampling season (winter and spring), and total cyst load, i.e., the intensity of hairworm infections, for larvae of one insect taxon, *Olinga* sp. caddisfly, that was abundant in both seasonal samples. We hypothesised that a greater parasitic load in a host impacts its capacity to defend itself, resulting in a higher proportion of hairworm cysts that elicit no visible melanisation response. We also explored the relationship between the size of *Olinga* sp., the total number of hairworm cysts it harbours, and its melanisation response. In this case, we hypothesised that host body size affects both parasitic load and melanisation response and predicted that larger hosts harbour more parasites, and thus are less capable of defending themselves against parasites, i.e., a lower

melanisation response. We also present data on seasonal changes in the prevalence of hairworm infections in all sampled aquatic macroinvertebrate host taxa.

3.3 Material and methods

3.3.1 Sampling natural hairworm infections in aquatic hosts

Samples were collected on 18 June 2018 (winter) and 2 October 2018 (spring) from Grasmere Stream near Cass Field Station (43°02'7"S 171°45'29"E), in the Canterbury region of New Zealand (Figure 3.1). In a 40-metre section of the stream, fine mesh dip nets were lightly dragged across the aquatic vegetation to collect macroinvertebrates; bottom fauna was collected downstream in the nets by kicking upstream rocks. These samples were kept alive in small containers of aerated river water until counted in the laboratory, where they were identified to genus or species level (Winterbourn et al., 2006). Afterwards, each macroinvertebrate was flattened between a microscope slide and cover glass for examination under the microscope at 100× magnification, in order to count hairworm cysts or larvae. For aquatic snails, tissues were separated from the shell using fine tweezers before flattening. Caddisfly larvae were also removed from their protective cases. In order to maintain osmotic pressure around cysts, a drop of Ringer's solution was added to the slide preparation (Barbosa et al., 2015). Hairworm larvae were identified to genus level, based on their folding pattern and morphological characteristics (Szmygiel et al., 2014).

3.3.2 Categorising melanotic encapsulation

Hairworm cysts were visually categorised into three levels of melanisation using the colouration caused by humoral encapsulation as an indicator of intensity (Figure 3.2A). A cyst without colouration nor the presence of haemocytes surrounding it was recorded as non-melanised; dormant hairworm larvae were clearly visible through the cyst wall (Figure 3.2B). Cysts with melanin deposits forming a characteristic "barrel-shaped" pattern were considered partially melanised; colour varied from light to dark amber and larvae were still visible through the cyst wall (Figure 3.2A and 3.2C). The third group showed no discernible melanisation pattern and cysts were completely covered in melanin deposits, resulting in an opaque amber colour. These cysts were categorised as fully

melanised and larvae were difficult to observe unless a very bright light was used (Figure 3.2D).

Figure 3.1 Antoine Filion (left) and I looking for aquatic macroinvertebrates freshly collected from Grasmere Stream at Cass Field Station, Canterbury, New Zealand. The top right panel shows a map of South Island, with the sampling location identified with a red pin. Photo taken by Eirik H. Henriksen.

3.3.3 Melanisation response in caddisfly larvae

To explore the relationship between the melanisation response in a paratenic host, the sampling season (winter and spring), and the total cyst load (number of hairworm cysts per infected individual), we selected larval *Olinga* sp. (Trichoptera: Conoesucidae) as a representative group for two reasons: (1) this species was the most abundant of all insect taxa sampled on both dates; (2) they had variable levels of melanisation corresponding to all three categories described above. The melanisation response was calculated as the proportion of non-melanised cysts per total number of cysts in each infected caddisfly. Therefore, the proportion of non-melanised cysts is inversely proportional to the

melanisation response of the host. Because caddisfly larvae are soft-bodied and it is difficult to measure their body length accurately, the protective cases of *Olinga* sp. sampled in June were measured to the nearest 0.5 mm with a microscope reticule as a proxy for host body size.

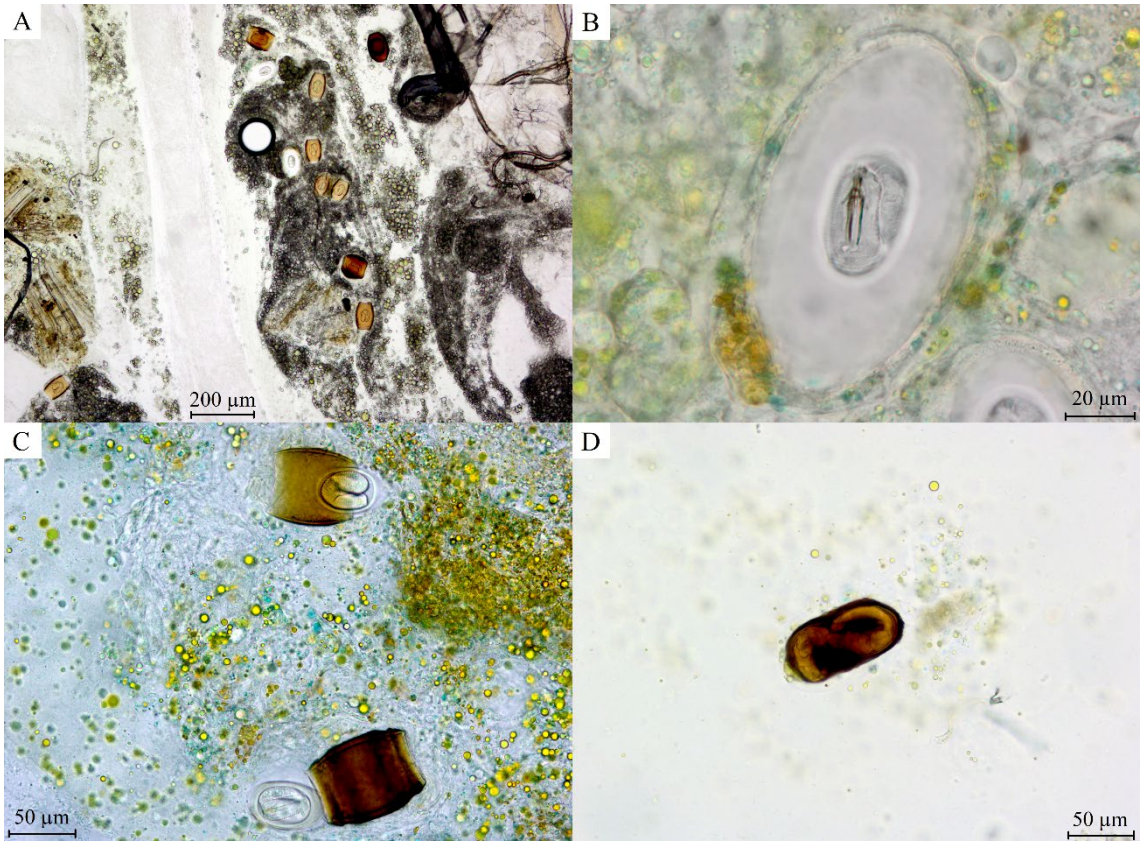


Figure 3.2 *Gordius* sp. hairworm (phylum Nematomorpha) cysts found in larvae of *Olinga* sp. sampled from Grasmere Stream, Cass Field Station in Canterbury, New Zealand on 18 June 2018. Host tissues were flattened and pictures taken with a C-Mount microscope camera. (A) Group of cysts with different levels of melanotic encapsulation located around the digestive tract (situated left of group) at 40× magnification. (B) Non-melanised cyst, denoted by a clear cyst wall around a dormant folded hairworm larva (centered) at 400× magnification. (C) Two partially melanised cysts, with the non-melanised portion separating from the melanised portion (top cyst) and completely separated (bottom cyst) at 100× magnification. (D) Fully melanised cyst with an unfolded larva apparently trapped within (centered) at 100× magnification.

3.3.4 Statistical analyses

All statistical analyses were performed using R version 3.6.0 (R core team 2019). To test the hypothesis that melanisation response varies according to season and total cyst load in *Olinga* sp., we used a beta regression model with the *betareg* package (Cribari-Neto and Zeileis, 2010). This model was selected because melanisation response is a proportion and can only assume values between 0 and 1 inclusively (Ferrari and Cribari-Neto, 2004).

Values assuming exactly 0 and 1 were transformed according to Smithson and Verkuilen (2006). Fixed effects were season (winter or spring) and total cyst load (number of cysts per infected caddisfly). Another beta regression was performed to evaluate the effect of host body size and total cyst load on the melanisation response, for *Olinga* sp. collected in June (the only month for which body size data were available). For both analyses, total cyst load was log-transformed. Model selection was done with the AICc and residuals were checked with the "standardised weighted residual 2" for beta regressions suggested by Espinheira et al. (2008). Also, we used a generalised linear model with a gamma distribution with the *lme4* package (Bates et al., 2015) to test the hypothesis that host body size positively affects total cyst load. This model was selected because only individuals harbouring cysts were considered, therefore zero counts were excluded from the dataset. No grouping factors were included in the models.

3.4 Results

3.4.1 Distribution of hairworm cysts in aquatic macroinvertebrates

The results presented in this section are summarised in Table 3.1. A total of 307 aquatic macroinvertebrates were examined for hairworm cysts in June (six taxa); 683 were processed in October (15 taxa). Individuals of only five taxa contained either melanised cysts or larvae. Interestingly, some of the encysted larvae were observed either adjacent to or breaking free from the partially melanised part of their cyst (Figure 3.2C). However, they remained inactive inside visibly smaller cysts with thinner walls. No haemocyte activity was observed around cysts from all three levels of melanisation. Based on the larval folding pattern, all hairworm cysts were identified to the genus *Gordius*.

The caddisfly *Olinga* sp. was most abundant, representing 31.5% of both samples. They also harboured 87.6% of the 1,466 hairworm cysts counted in the laboratory. In this caddisfly species, cysts were usually located in the haemocoel around the gastrointestinal tract (Figure 3.2A).

Table 3.1 Prevalence and intensity (number of cysts per infected host ± SE) of *Gordius* sp. hairworm (phylum Nematomorpha) cyst infections in aquatic macroinvertebrates captured by dip net from Grasmere Stream, Cass Field Station in Canterbury, New Zealand on 18 June 2018 and 2 October 2018.

Class	**Order***	**Family**	**Genus or species**	**June 2018**			**October 2018**		
				Sample size (n)	Infected (prevalence)	Number of cysts per infected individual ± SE	Sample size (n)	Infected (prevalence)	Number of cysts per infected individual ± SE
Insecta	Trichoptera	Conoesucidae	*Olinga* sp.**	177	96 (54.2%)	12.0 ± 0.9	135	19 (14.1%)	6.8 ± 1.8
			Pycnocentria sp.**	0	-	-	38	16 (42.1%)	7.6 ± 1.6
			Pycnocentrodes sp.	0	-	-	1	1 (100%)	2.0 ± 0.0
		Hydrobiosidae	*Hydrobiosis* sp.	0	-	-	6	0 (0%)	-
		Hydropsychidae	*Orthopsyche* sp.	0	-	-	7	0 (0%)	-
	Ephemeroptera	Coloburiscidae	*Coloburiscus* sp.***	13	0 (0%)	-	116	11 (9.5%)	1.7 ± 0.5
		Leptophlebiidae	*Deleatidium* sp.**	0	-	-	49	10 (20.4%)	2.2 ± 0.7
		Nesameletidae	*Nesameletus* sp.	0	-	-	1	0 (0%)	-
	Plecoptera	Gripopterygidae	*Zelandoperla* sp.	7	2 (28.6%)	1.5 ± 0.7	1	0 (0%)	-
	Diptera	Chironomidae	NA	0	-	-	10	0 (0%)	-
	Coleoptera	Elmidae	*Hydora* sp.	0	-	-	9	0 (0%)	-
	Megaloptera	Corydalidae	*Archichauliodes diversus****	2	0 (0%)	-	25	4 (16.0%)	2.8 ± 1.0
Gastropoda	Hygrophila	Physidae	*Physella acuta*	76	1 (1.3%)	2.0 ± 0.0	90	1 (1.1%)	1.0 ± 0.0
	Littorinimorpha	Tateidae	*Potamopyrgus antipodarum*	32	0 (0%)	-	189	0 (0%)	-
Clitellata	Haplotaxida	Lumbricidae	*Eiseniella* sp.	0	-	-	6	1 (16.7%)	1.0 ± 0.0

*Clades were used for both gastropod species, due to paraphyly in traditional orders.
**Taxa with melanised and non-melanised cysts.
***Taxa with melanised larvae.

3.4.2 Melanisation response in caddisfly larvae

The effects of season and total cyst load on melanisation response in *Olinga* sp. were tested and the beta regression model with the lowest AICc included both explanatory variables. For this model, the proportion of non-melanised cysts nearly doubled in October (Figure 3.3A), meaning caddisfly larvae had a significantly lower melanisation response in the spring. The total cyst load did not have any overall effect on the melanisation response (Figure 3.3B). In testing the effects of host body size and total cyst load on melanisation response with the June dataset, none of the models were more parsimonious than the null model according to their AICc, meaning that both variables had no significant effect.

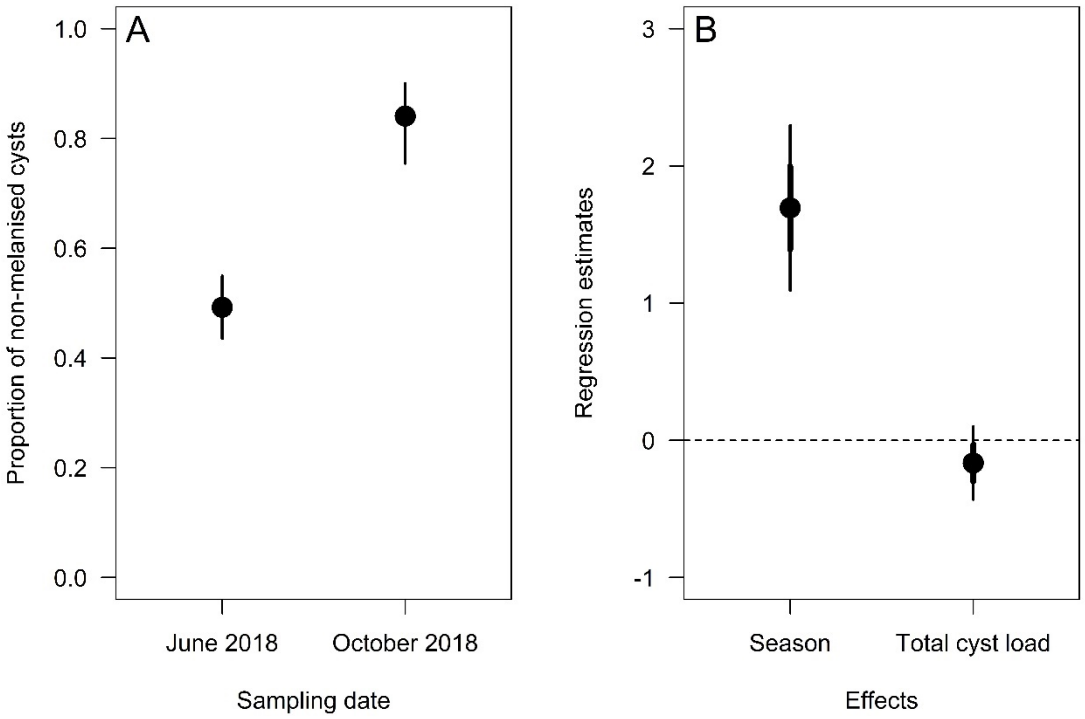


Figure 3.3 (A) Estimated proportions (± 95% confidence intervals) of non-melanised *Gordius* sp. hairworm (phylum Nematomorpha) cysts in *Olinga* sp. caddisfly larvae between sampling dates based on beta regression modelling. Samples were collected on 18 June 2018 and 2 October 2018. (B) Beta regression estimates for the fixed effects in the selected model with 95% error bars. A regression estimate different from 0 (including error bars) indicates a significant effect.

3.4.3 Host body size and total cyst load

For *Olinga* sp. collected in June, case length was measured as a proxy for body size and was tested as an effect on total cyst load. As seen in Figure 3.4, host body size had a

positive effect on total cyst load (effect size = -0.014; SE = 0.003), with larger caddisfly larvae harbouring more cysts on average than smaller ones.

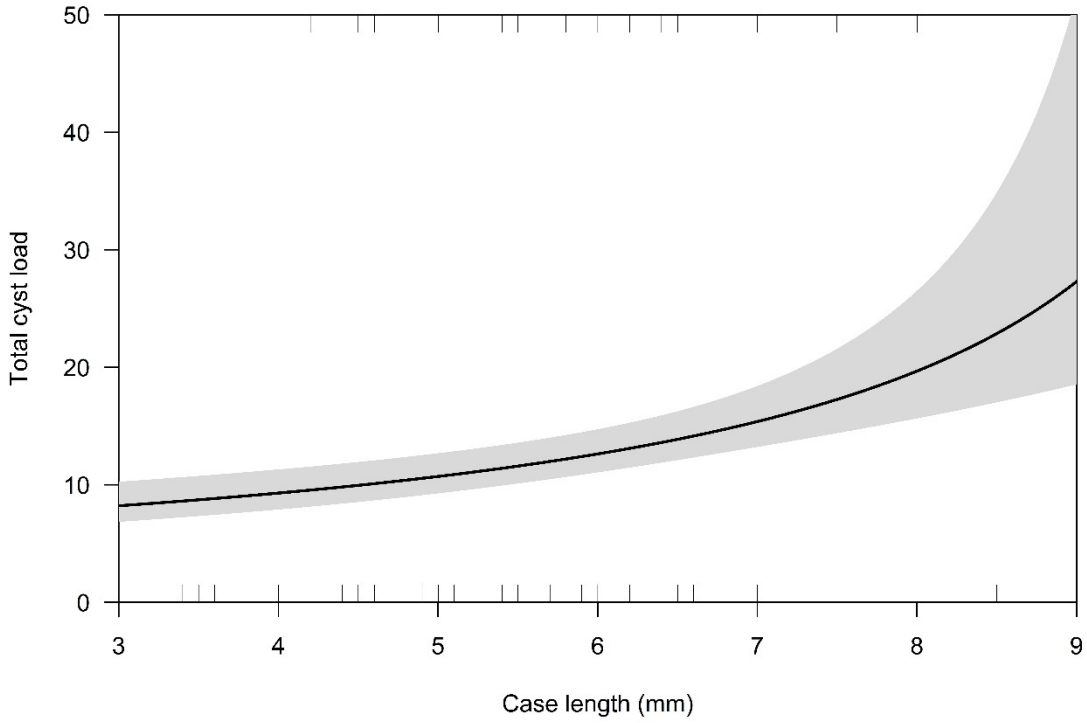


Figure 3.4 Predicted effect of case length (proxy for host body size) of caddisfly larvae *Olinga* sp. on total *Gordius* sp. hairworm (phylum Nematomorpha) cyst load. Dashed lines on the top and bottom are rug representations of the data. The grey area represents the 95% confidence band of the fitted gamma regression model.

3.5 Discussion

In this study, we report new observations on varying melanisation levels of hairworm cysts in aquatic insect larvae. An interesting finding is the characteristic pattern formed by melanin deposits on the surface of cysts, which was observed in three taxa from two distinct insect orders, Trichoptera and Ephemeroptera. Partially melanised cysts all had the same "barrel-shaped" pattern, which could be due to surface conformation. Because photographs of hairworm cysts are usually two-dimensional (caused by the flattening of host tissues for microscope slide preparation), they do not inform us on their three-dimensional shape. Ultrastructural photographs of hairworm cysts could provide information about their natural shape, which may help explain why melanin appears to

form initially around the midsection. Another interesting observation is the apparent interaction between the melanin deposits and the outer cyst wall. Partially melanised cysts flattened under the cover glass were sometimes separated from the non-melanised portion of the cyst. This may be an artifact from the slide preparation because hairworm cysts could have separated from the partial melanin layer due to the physical pressure used to flatten host tissues, which may have forced the inner layers of the cyst wall to separate from the outer layers. This would support previous observations that the hairworm cyst wall is a multi-layered structure (Poinar and Doelman, 1974; Poinar, 2010).

However, the separation of partially melanised cysts raises questions about the lethality of melanisation on encysted hairworms. Reports of immunity to hairworms in paratenic insect hosts are scarce and focus mainly on the melanisation of larval hairworms (Poinar, 1969; de Villalobos et al., 2006; Poinar, 2010). One report by Inoue (1962) found that melanised hairworm cysts in chironomid larvae fed to definitive mantid hosts resulted in a lower abundance of juvenile hairworms than that obtained when using non-melanised cysts, suggesting that melanisation hinders the transmission of hairworms. But it is unknown whether these cysts were fully or partially melanised. Poinar (1991a) also reported melanised hairworm cysts in several insect orders in New Zealand, but no distinction was made regarding the level of melanisation. Therefore, it remains unknown whether a hairworm larva can excyst from a partially melanised cyst once inside its definitive host. Feeding partially melanised cysts to definitive hosts could help determine the impact of paratenic host immunity on the life cycle of hairworms.

It appears, from the two seasonal samples collect here, that the diversity and abundance of macroinvertebrates in Grasmere Stream increased in October. This may be caused by an increase in general activity due to warmer springtime temperatures. However, this could also be a sampling artefact, since certain groups were completely absent from the June collection, which could be partly explained by minor differences in the specific areas sampled from the stream section on the two collection dates. The groups harbouring the highest number of cysts per infected individual (Trichoptera and Ephemeroptera) are part of the benthos macrofauna and thus are most likely to consume hairworm larvae and act as true paratenic hosts (Hanelt and Janovy, 2003). Interestingly, the megalopteran

Archichauliodes diversus also harboured cysts, but this species is predatory and probably ingested them by consuming primary paratenic hosts (Poinar, 1991a). In October, the prevalence was higher in likely paratenic host taxa, except for *Olinga* sp. and *Zelandoperla* sp., which may be due to a "dilution effect" caused by an increase in host density and diversity in the spring, meaning more potential hosts consuming hairworm larvae between June and October (Keesing et al., 2006). The prevalence was lower in *Olinga* sp. collected in October, which could indicate that interspecific competition increased and hairworm larvae were less available for consumption by immature caddisflies between collection dates.

As the prevalence and intensity of hairworm cysts decreased in *Olinga* sp. between seasons, so did the melanisation response. Temporal differences could play a role in the proportion of non-melanised cysts found in immature caddisflies. It was reported that species of immature *Olinga* can be found all year with several generations overlapping in the same stream (Cowley, 1978; Burrell and Ledger, 2003). Therefore, if new generations of *Olinga* sp. appeared before or after the June sampling date, larvae could have consumed hairworm larvae much closer to the October sampling date, thus impacting the proportion of non-melanised cysts. However, it is unknown how rapidly melanotic encapsulation occurs in naturally infected hosts. Moreover, current studies, which mostly focus on model organisms, e.g., *Drosophila* and mosquitos, show that melanogenesis may only occur in certain host tissues (González-Santoyo and Córdoba-Aguilar, 2012). Thus, we cannot fully explain if the patterns observed here reflect the seasonality of hairworm infection or the melanin response of the host. Instead, our findings indicate that the temporal and generational dynamics between paratenic hosts and hairworm cysts require further investigation. Regular sampling throughout an extended period could help explain the complex interactions between aquatic macroinvertebrates and the hairworm cysts they harbour.

We also investigated the effect of host body size on total cyst load. The cases of *Olinga* sp. sampled in June were measured as a proxy for body size and the results support our hypothesis that a larger host harbours more cysts on average than a smaller one. As a host feeds and grows, it is likely to have consumed more hairworm larvae and accumulated

more cysts. Of course, as these results pertain to only one host species, they do not inform us about the effect of body size in other paratenic host taxa, but it is reasonable to speculate that it plays a role regardless of host taxon. In fact, the positive relation between host size and parasite intensity has been reported in a varied number of host-parasite systems (Poulin, 2000b; Yule and Burns, 2015).

3.6 Conclusions

The new observations presented here on the different levels of hairworm cyst melanisation in aquatic hosts adds a new layer of complexity to the dynamics between hairworms and their paratenic hosts. We showed that melanotic encapsulation varies and it is possible to visually categorise different levels in order to calculate a melanisation response that is inversely proportional to the relative quantity of non-melanised cysts in a host. This melanisation response varied significantly between winter and spring for one species of caddisfly, which may be attributed to some temporal and generational dynamics of the host between both sampling dates. The total cyst load did not have a significant impact on the melanisation response for this species. However, this cyst load was affected by host body size; larger caddisfly larvae harboured more cysts on average than smaller ones. These new results add to our limited knowledge of the cryptic interactions between hairworms and their paratenic hosts and suggest further study to tackle some mysteries of this relatively understudied group of parasites.

Chapter 4

Come with me if you want to live: sympatric parasites follow different transmission routes through aquatic communities

Hairworms tangled with some vegetation in the stream of the Rock and Pillar Conservation Area sampling location.

4.1 Abstract

Community composition, including the relative density of each host species, plays a vital role in the transmission of parasites or disease in freshwater ecosystems. Whereas some host species can effectively transmit parasites, others can act as dead ends, accumulating large numbers of parasites throughout their life, thus becoming important sinks for parasite populations. Although population sinks have been identified in certain host-parasite systems, robust field estimates of the proportions of parasites that are lost to these hosts are lacking. Here, we quantified the distribution of encysted larval hairworms (phylum Nematomorpha), common parasites in lotic ecosystems, in two subalpine stream communities of New Zealand. With parasite and host population densities calculated per m^2, we identified which host species most likely contributed to the transmission of three sympatric hairworm morphotypes identified in both streams, and which species acted as population sinks. We also tested for seasonal patterns and peaks in the abundance of each morphotype in the two communities over the sampling season. Finally, we tested whether hosts emerging from the streams had comparable abundances of hairworm morphotypes throughout the sampling period. For each morphotype, different key sets of host species harboured more hairworms on average (abundance) than others, depending on the stream. For one morphotype in particular, two species of hosts were found to be important population sinks that inhibited a considerable proportion of these parasites from completing their life cycle. We also observed a clear peak in abundance for another hairworm morphotype during summer. Our data suggest that hosts emerging from the streams matched their aquatic counterparts with respect to hairworm abundance, indicating no infection-dependent reduction in emergence success. Our findings suggest that, depending on relative community composition, sympatric parasites follow different host transmission pathways, some of which lead to dead ends that potentially impact overall infection dynamics. In turn, this information can help understand the spread or emergence of disease in both freshwater and terrestrial environments, since hairworms infect terrestrial arthropods to complete their life cycle.

4.2 Introduction

Living in a freshwater environment comes with its own challenges, setting aside those already brought on by anthropogenic change (Ormerod et al., 2010; Jackson et al., 2016). These challenges can be especially taxing on the survival of parasitic organisms, which not only have to face potentially adverse abiotic conditions (Pietrock and Marcogliese, 2003) and avoid predation (Johnson et al., 2010), but also have to successfully infect the right host in order to complete their life cycle. An important force opposing the spread of parasites or disease is the dilution effect, through which an increase in species diversity in a given environment can reduce the risk of infection through mechanisms such as within-species transmission and density-dependent transmission (Keesing et al., 2006; Civitello et al., 2015). Though the dilution effect may only apply for parasites in localised communities (Randolph and Dobson, 2012; Salkeld et al., 2013), empirical evidence has shown that infection risk does decrease with an increase of biodiversity in freshwater systems (Lagrue and Poulin, 2015a; Rohr et al., 2015).

While biodiversity can decrease the odds of parasites encountering a suitable host, it can also increase their odds of ending up in the wrong one. An inappropriate vector or a suboptimal host could effectively halt the life cycle of parasites that rely on intermediate or paratenic hosts for transmission to another host or environment (Keesing et al., 2006). Some organisms play little to no role in advancing the life cycle of parasites and constitute "dead-end" hosts (Thieltges et al., 2008). These hosts can effectively accumulate large numbers of parasites throughout their life and act as sinks in a parasite population. For instance, the trematode *Curtuteria australis* is highly unlikely to complete its life cycle when consumed by the bivalve *Macomona liliana*, which can accumulate metacercariae (dormant trematode cysts) throughout its life. This is because the bivalve burrows too deeply into the sediment to be consumed by the trematode's definitive shorebird host (Leung and Poulin, 2008). Thus, the metacercariae remain trapped within the tissues of the deep-burrowing bivalve. Apart from dead-end hosts, some organisms are just physiologically incompatible with certain parasites, further decreasing the odds of a successful transmission (Keesing et al., 2006). By quantifying the distribution of parasites throughout an entire community of viable host species versus dead-end ones, it may be possible to estimate the probability that an individual parasite successfully completes its

life cycle or not. In turn, such data could help predict the spread of infection or disease in an ecosystem.

Gordiid nematomorphs, also known as freshwater hairworms, may be one of the most common parasites in lotic ecosystems. Surveys have shown that hairworm cysts, the dormant stage of these parasites, can be found within the tissues of multiple taxa of aquatic macroinvertebrates from most streams and rivers (Hanelt et al., 2001; Harkins et al., 2016). Adult hairworms are conspicuously long and have been recorded in many freshwater habitats around the world. Moreover, internet search metadata gathered by the general population reveal that their geographical range may be far greater than what is currently known to science (Doherty et al., 2021). Their common occurrence in streams has even prompted some researchers to use them as toxicity bioindicators to test for the impacts of chemical compounds in freshwater ecosystems (Achiorno et al., 2008b; Achiorno et al., 2018). Once mated in water, females can lay millions of eggs that are either deposited onto a substrate, such as a submerged rock or stick, or released directly into the current (Bolek et al., 2015). Thus, with the potential for millions of hairworm larvae hatching locally each year, their common presence in lotic environments makes hairworms an ideal study system to explore hidden infection dynamics in a community of host species.

Hairworm larvae do not swim efficiently and therefore must be consumed by an aquatic organism to form a dormant cyst within its tissues or body cavity. Larvae encyst in practically any animal that consumes them: insects, crustaceans, and even vertebrates (Hanelt and Janovy, 2003; Torres et al., 2017). This low host specificity observed in larval hairworms may increase the odds of completing their life cycle, since infecting multiple species of paratenic hosts may increase the number of transmission routes. However, infected animals only serve as true paratenic hosts if, at some point later in their life cycle, they leave water to spend time on land. After leaving their cyst, hairworms can only develop and mature within terrestrial arthropods (mostly scavenger or predatory insects), so paratenic hosts must exit water to eventually be consumed by a definitive host. The aquatic insect larvae present in streams and rivers are presumably the true paratenic hosts of hairworms, for most of these eventually mature into terrestrial flying adults that can be

eventually consumed by definitive hosts, e.g., ephemeropterans and plecopterans (Hanelt and Janovy, 2004a). Animals harbouring cysts that never exit the water can act as dead-end hosts, representing population sinks for hairworms, e.g., aquatic gastropods (Hanelt et al., 2001). In fact, through the accumulation of hairworms in their tissues and a lack of a defence reaction towards cysts, gastropods have been used experimentally to infect definitive hosts and replicate the hairworm life cycle artificially in the laboratory (Hanelt and Janovy, 2004b). It is unknown whether cysts are usually confined to the host that first consumes them, however, observations on the hairworm *Paragordius varius* suggest that larvae can pass through one animal to then encyst within another, following predation of the former by the latter (de Villalobos et al., 2003). Hosts can mount defence reactions toward cysts, such as melanotic encapsulation, a common form of insect immunity (Nakhleh et al., 2017). However, unless a cyst is completely covered in melanin (Chapter 3), it is unclear whether paratenic host immunity plays a role in regulating hairworm transmission.

Seasonal studies of aquatic macroinvertebrates have shown that cysts of sympatric hairworm species can occur within the same paratenic host (Chiu et al., 2016). Moreover, their presence in these hosts peaked only two months after the reproductive season of hairworms. Another study found that hairworm cyst infection was highest in only a subset of aquatic species collected (Yamashita et al., 2017), which could have been attributed to host-specific feeding behaviours and habitat use. In this study, it was also possible to observe seasonal peaks in the mean number of hairworm cysts per host. A more detailed study on the life history of hairworms found that cysts of the genus *Gordionus* can follow diverse host transmission pathways from water to land (Meguro et al., 2020). The authors observed that insect host species emerged at different periods, suggesting that the temporal window for hairworm transmission from water to land extended to a large portion of the season. Though these studies helped uncover the complex, yet hidden dynamics of parasite transmission in a community of freshwater hosts, it is currently unknown how many hairworm larvae are consumed by viable paratenic hosts and how many are lost in population sinks such as dead-end hosts.

In the current study, our main goal was to investigate the spatial and temporal distribution of hairworm cysts throughout the invertebrate communities of two subalpine streams in New Zealand. Through an exhaustive examination of all taxa collected from both streams across three seasons, we were able to quantify the proportions of three sympatric hairworm species occupying either potential paratenic hosts or dead-end ones. To do so, we used two measures, hairworm abundance (mean number of parasites per host) and host density, to calculate the density of hairworms per m^2 of stream bed. Although this measure is rarely used to characterise parasite density (Lagrue and Poulin, 2015b), it nonetheless provides a comparable metric for both host and parasite. Since almost nothing is known of the proportion of hairworms lost in dead-end aquatic hosts, we hypothesised that, if present, these hosts would accumulate large numbers of parasites and act as important population sinks. Additionally, during our sampling season, we tested whether the abundance of hairworms within aquatic insect hosts correlated with that in conspecific insects emerging from the streams using a passive insect trapping method. Our goal here was to test if the proportion of viable hairworms in the stream was mirrored by what is potentially available for definitive hosts on land. In sum, our study highlights the impacts that relative host community composition can have on parasite or disease transmission pathways in a freshwater environment.

4.3 Material and methods

4.3.1 Field methods

Stream samples were collected on a monthly basis from late October 2020 to early May 2021 (for a total of seven sampling dates) at two locations: Rock and Pillar Conservation Area (45°26'03"S 170°04'32"E; Stream A) and Kopuwai Conservation Area (45°20'43"S 169°11'55"E; Stream B) in Otago, New Zealand (Figure 4.1A) (Department of Conservation Authorisation Number 68065-RES). These streams were selected because adult hairworms from multiple species have been found in both (Tobias et al., 2017; Yadav et al., 2018). Additionally, they are subalpine in elevation (approximately 1,320 and 1,580 m in altitude, respectively) and relatively similar with respect to general width, velocity, and surrounding vegetation cover. On each sampling date, eight Surber samples were haphazardly taken from pools and riffles along the same 150-metre section of each

stream. Samples were taken from both streams within 24 hours, at the beginning of the Malaise trapping period (see below). The Surber sampler had a sampling frame of 0.3 × 0.3 m, i.e., it sampled 0.09 m^2 of the stream bed. We always collected from downstream to upstream, to avoid disturbing the fauna of subsequent samples. The stream bed was mostly made up of loose sediment and small to large rocks; little to no vegetation was present. A sample consisted of agitating the stream bed with a wooden stake for 60 seconds. Anything collected downstream in the fine mesh net (500 µm) of the Surber sampler was immediately stored in 75% ethanol until further processing in the laboratory. Additionally, a one-metre section immediately upstream from each Surber sample was visually searched for adult hairworms, in order to record their presence in the streams across sampling dates. For this, we counted the total number of individual adults and any mating knots present.

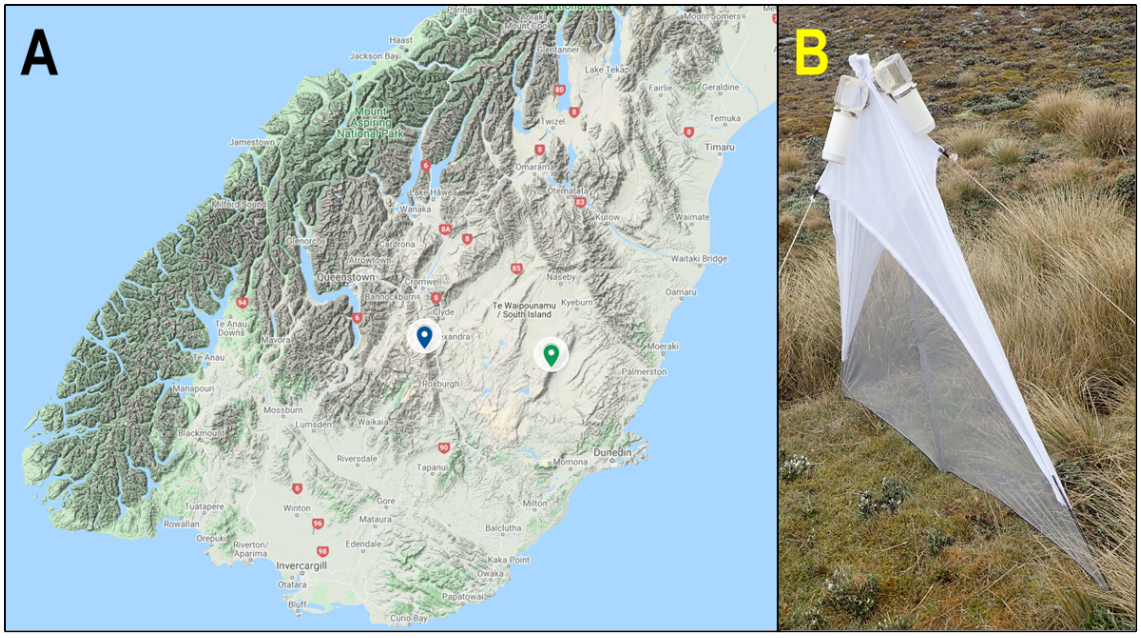


Figure 4.1 (A) Lower half of South Island with sampling locations identified with a green pin (Stream A) and a blue pin (Stream B). (B) Malaise trap deployed near Stream B (masked by the tussock).

To capture adult insects that had potentially emerged from the two streams described above, we used Malaise traps during the peak activity months of summer. Because of the subalpine climate, sampling was possible between late November 2020 and early March 2021 (for a total of four sampling periods). The Malaise traps used here were bilateral with a triangular wall of 1.5 m^2 and a triangular roof (Figure 4.1B) (van Achterberg,

2009). They were installed perpendicularly to the stream, with the centre pole at 5 m from the edge of the stream. They were also bidirectional, i.e., both sides of the trap each had its own lateral collector. This design enabled us to determine the general flight direction of the insect (either flying upstream or downstream). Malaise traps were kept open for seven days on a monthly basis. During these periods, we used 150 mL of propylene glycol in the collectors to preserve captured insects. Two traps per stream were installed during the first sampling period, alongside both ends of the 150-m section of the streams identified for Surber sampling (see above). During subsequent sampling periods, three Malaise traps were installed every 50 m alongside the same section of both streams. These traps were reinstalled in the same locations each month. After every sampling period, we filtered the insects from the collectors with cloth and immediately stored them in 75% ethanol until further processing in the laboratory.

4.3.2 Laboratory methods

In the laboratory, we first separated macroinvertebrates in the samples from any debris present, e.g., vegetation and small rocks. For this, Surber samples were inspected after being placed on a white plastic tray with water and shaken to evenly distribute individuals. Because chironomid larvae were usually abundant and relatively difficult to find among the debris, only a quarter of the tray was inspected for this taxon. We then stored the extracted macroinvertebrates in 50% ethanol, to slowly rehydrate tissues, which facilitated cyst counting. All aquatic insects caught in the Surber sampler (larvae and adults) were identified to the lowest taxonomic level possible following Winterbourn et al. (2006) (and references therein). Due to poor taxonomic resolution or to morphological features too small to properly distinguish under a dissecting microscope, some insects were assigned to a family and then separated into morphospecies or species groups based on their appearance, e.g., tabanid and chironomid larvae. Other macroinvertebrates were identified to genus level or lower when possible. For insects caught in the Malaise traps, we identified the taxa with an aquatic life stage to the lowest taxonomic level possible or divided them into species groups within a family using multiple literature sources (McLellan, 1993; Ward, 1995; Kialka and Ruta, 2017). The remaining insects (with no aquatic life stage) were separated by order and were not examined for hairworm cysts. Prior to counting cysts, macroinvertebrates were removed from the ethanol solution and

further rehydrated in tap water at room temperature for 24 hours. To count hairworms under the compound microscope, we followed methods previously described, which consist of identifying hairworm cysts or larvae that are either non-melanised or melanised by flattening host tissues between a microscope slide and cover glass (see section 3.3). For some larger individuals, tissues had to be minced with tweezers and a fine scalpel prior to flattening them. Because of the possibility of hairworm species complexes (Hanelt et al., 2015; Tobias et al., 2017), cysts or larvae were grouped into morphotypes based on their general size and appearance. The dimensions (length and width) of folded larvae within cysts were measured for a subset of individuals from each morphotype using a C-Mount digital microscope camera and its accompanying software (United Scope, California, United States of America).

4.3.3 Data visualisation

Firstly, to visualise the distribution of hairworms across aquatic macroinvertebrates, we pooled all sampling dates per stream and calculated the average number of hairworm cysts or larvae per individual of each host taxon (this includes morphospecies and species groups), i.e., hairworm abundance. Because multiple hairworm morphotypes were observed in both streams (Figure 4.2), abundance was calculated per morphotype. Hairworm abundance was then multiplied by the density of each host taxon per m^2 of stream bed, which was calculated by dividing the total number of individuals collected from all samples by the surface area covered from total Surber sampling effort (5.04 m^2). For chironomid larvae, we multiplied by four the number of individuals counted per sample, since they were subsampled in the laboratory (see above). This gave us, for all sampling dates pooled, the average number of hairworm cysts or larvae per m^2 per hairworm morphotype per host taxon (Figure 4.3).

Secondly, to visualise any temporal patterns in the abundance of hairworms across sampling dates, we first identified the host taxa for which infection by at least one hairworm morphotype occurred (cyst or larva), i.e., each hairworm morphotype had its own set of host taxa per stream. By pooling host sets separately, we calculated the hairworm abundance across sampling dates per hairworm morphotype per stream (Figure 4.4). Note that, for any given sample, a maximum of 60 individuals per taxon were

examined for hairworm cysts or larvae. For taxa that were subsampled in the Surber samples, we made sure to have a representative distribution of body size by categorising individuals into three general size classes, i.e., small, medium, and large. These subsampled individuals were then used to calculate a size-corrected, taxon-specific hairworm abundance for that sample. However, subsampling only occurred in five of the most numerous taxa.

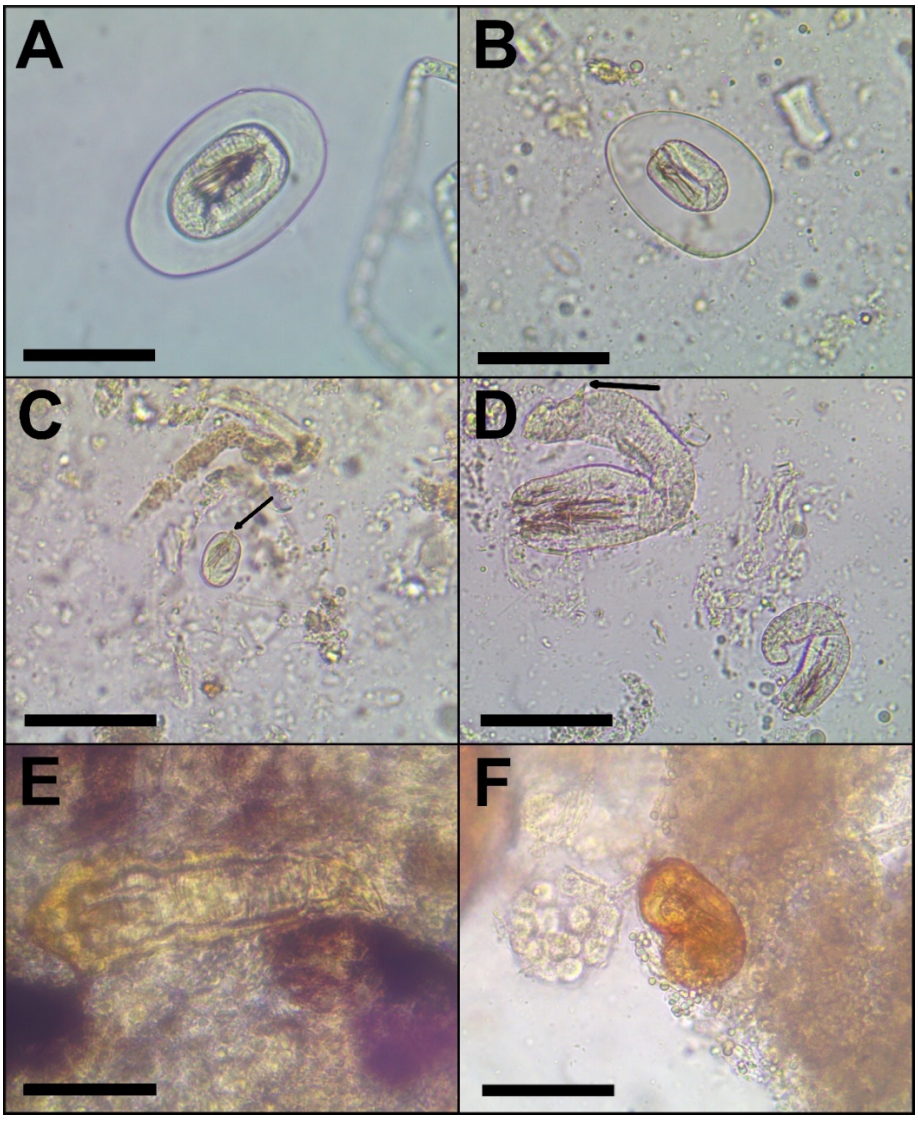


Figure 4.2 Hairworm (phylum Nematomorpha) cysts and larvae observed in multiple aquatic hosts collected from two subalpine streams in New Zealand. Host tissues were flattened prior to photos being taken with a C-Mount microscope camera at 100× magnification. (A) Non-melanised Type A cyst found in the trichopteran *Pycnocentria* sp. (B) Non-melanised Type B cyst found in *Pycnocentria* sp. (C) Non-melanised Type C cyst (centered) found in the ephemeropteran *Deleatidium fumosum*; the arrow points to spines protruding on the preseptum. (D) Non-melanised Type A (left) and Type B (right) larvae found in the predatory trichopteran *Hydrobiosis* sp., most likely excysted; the arrow points to the pointed tip of the postseptum (E) Melanised Type A larva found in *Hydrobiosis* sp. (F) Melanised Type B larva found in the predatory trichopteran *Psilochorema* sp. All images are to the same scale (scale bar = 100 µm).

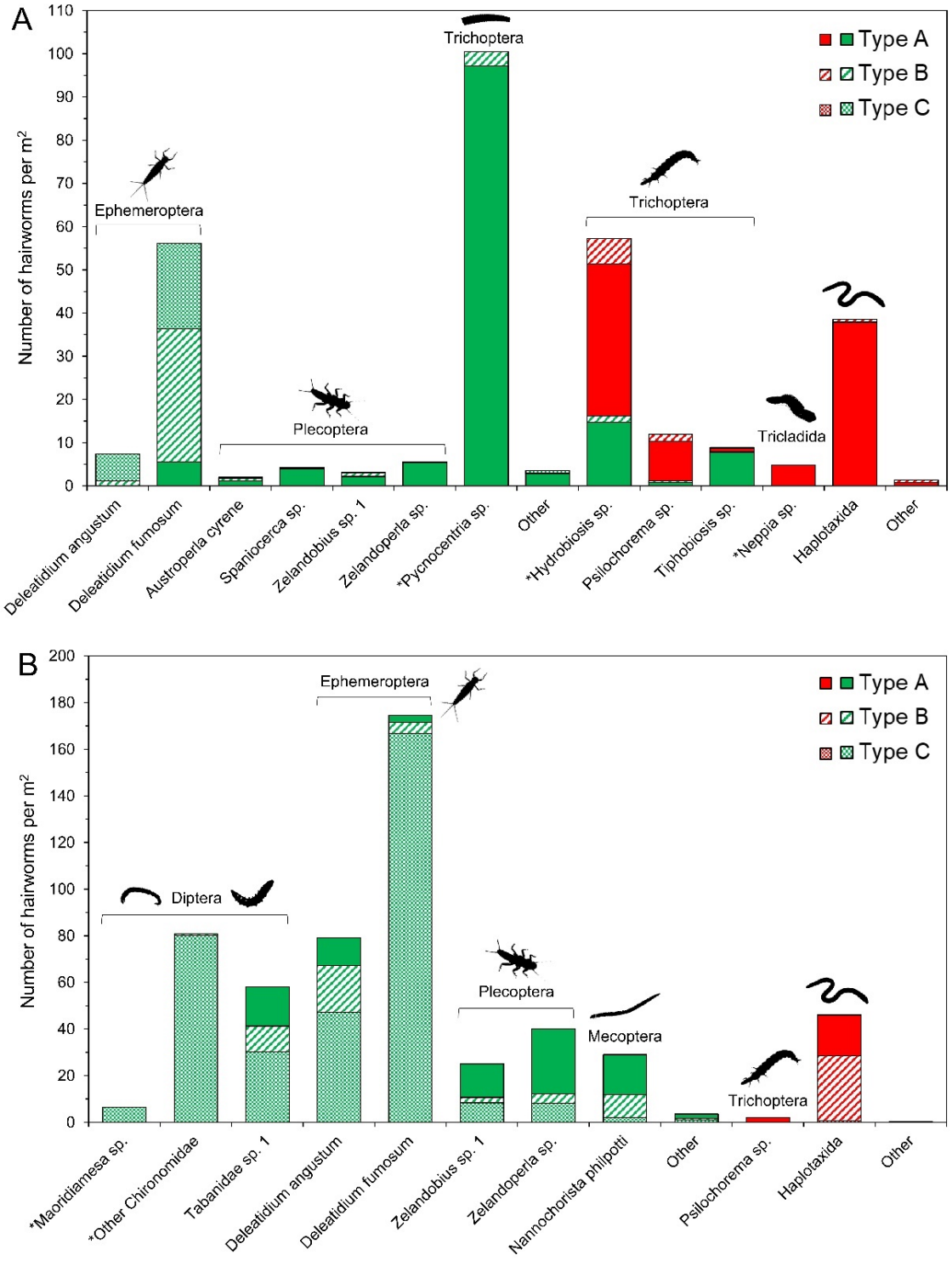


Figure 4.3 Density of hairworm (phylum Nematomorpha) cysts and larvae per aquatic host taxon per m^2 of stream bed, for three hairworm morphotypes (Type A, B, or C). Viable hairworms found in potential paratenic hosts are coloured in green, whereas non-viable hairworms found in dead-end hosts are coloured in red. (A) Host taxa collected in Stream A. (B) Host taxa collected in Stream B. An asterisk next to a host taxon indicates that the estimate of hairworm density is based on a subsample of hosts.

4.3.4 Data analysis

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021). Firstly, to test whether hairworm abundance varied statistically between host taxa or sampling date within each stream, we used generalised linear models (GLM), one per hairworm morphotype. More than half of the host taxa identified had fewer than 10 individuals in total, making it impractical to compare their hairworm abundance statistically. Therefore, we included only the five host taxa with the highest density of hairworms per m^2 across all three morphotypes (Figure 4.3). Because host community composition and hairworm densities varied greatly between both streams, different groups of hosts were tested for each stream. In Stream A, only two species of ephemeropterans (*Deleatidium angustum* and *D. fumosum*) had Type C hairworms and were numerous enough to compare statistically, thus only two host taxa were analysed for this morphotype in Stream A. Since the response variable was the total number of hairworms (cysts and larvae combined) counted per individual, a negative binomial distribution was implemented into the model using the "glm.nb" function in the package *MASS* (Venables and Ripley, 2002), to account for overdispersion of the data. One GLM was produced per hairworm morphotype per stream, for a total of six regression models. Two fixed categorical factors were used as predictors: host taxon (five levels, or two levels for Type C hairworms in Stream A) and sampling date (seven levels). For these six models, pairwise comparisons between different host taxa and sampling dates were performed using the "glht" function in the package *multcomp* (Hothorn et al., 2008).

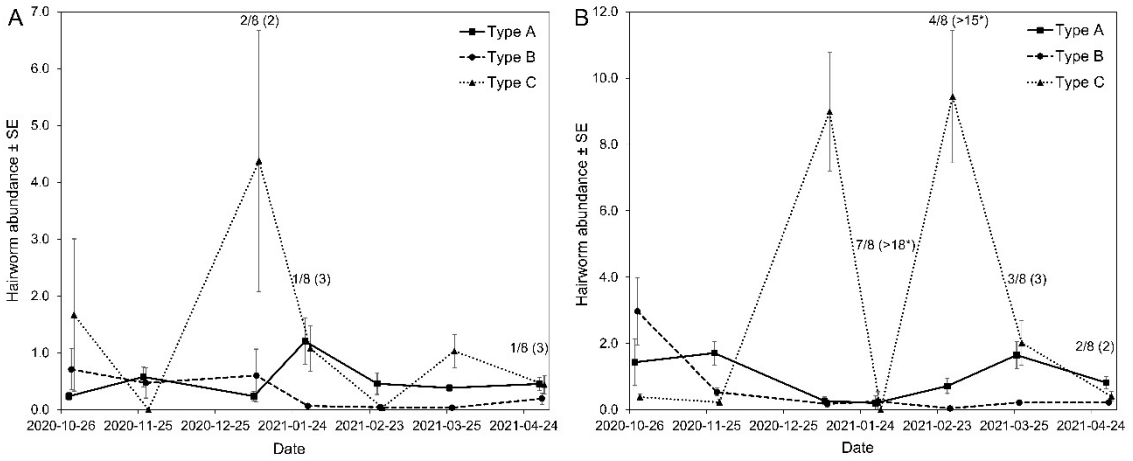


Figure 4.4 Mean number of hairworm (phylum Nematomorpha) cysts and larvae per individual aquatic host per morphotype per sampling date for (A) Stream A and (B) Stream B. The numbers above sampling dates indicate the proportion of Surber sampling sites where adult hairworms were observed and the total

number found across sampling sites in parentheses. An asterisk next to the number in parentheses indicates that hairworm mating knots were also found, thus the total number observed across sampling sites is a minimum.

A further three GLMs with negative binomial distributions were performed to test for differences in hairworm abundances between larval and adult stages of the plecopteran *Zelandobius* sp., which was the only host taxon occurring frequently enough in both Surber and Malaise trap samples to allow for statistical comparison. Again, each hairworm morphotype was tested separately and the response variable was the number of hairworms counted per individual, with sampling dates pooled. We used two fixed categorical factors as predictors: *Zelandobius* sp. life stage (larva or adult) and stream (A or B). Here, we only included host larvae that were classified as large, since these were more likely to be closer to maturation than smaller larvae.

4.4 Results

4.4.1 Surber sampling general results

A total of 10,674 macroinvertebrates were collected with the Surber sampler, an estimate based on the sum of the product of chironomid subsamples added to all the other taxa which were fully accounted for. Over 74% of individuals collected were chironomid larvae. Overlapping generations of individual taxa were clearly present in all samples, since we usually observed a homogeneous mix of body sizes or stadia among individuals of a given taxon. Across sampling dates, the density of macroinvertebrates per m^2 was over 12 times higher in Stream A than in Stream B and both communities differed in their relative taxonomic composition (Figure 4.5). Out of the 48 taxa identified (including morphospecies and species groups), 22 were present in both streams. Notable differences were ostracods, amphipods, and muscid larvae collected from Stream A that were absent in samples from Stream B. In contrast, we collected scirtid and mecopteran larvae in Stream B, but not in Stream A. From all samples, we flattened 2,269 macroinvertebrates and counted a total of 3,414 hairworm cysts and larvae. It was possible to distinguish between three hairworm morphotypes, based on their appearance and size (Table 4.1). We refer to them here as Type A, Type B, and Type C (Figure 4.2). All three were present in both streams and were found in multiple host taxa (Table 4.1). Type A hairworms were

found in more taxa than both other morphotypes. The prevalence and abundance of these morphotypes varied widely across host taxa, sampling dates, and streams (Tables 1 to 6 in Appendix C). Also, we observed adult hairworms in both streams, but found more across sampling dates in Stream B than in Stream A; mating knots were only observed in Stream B (Figure 4.4).

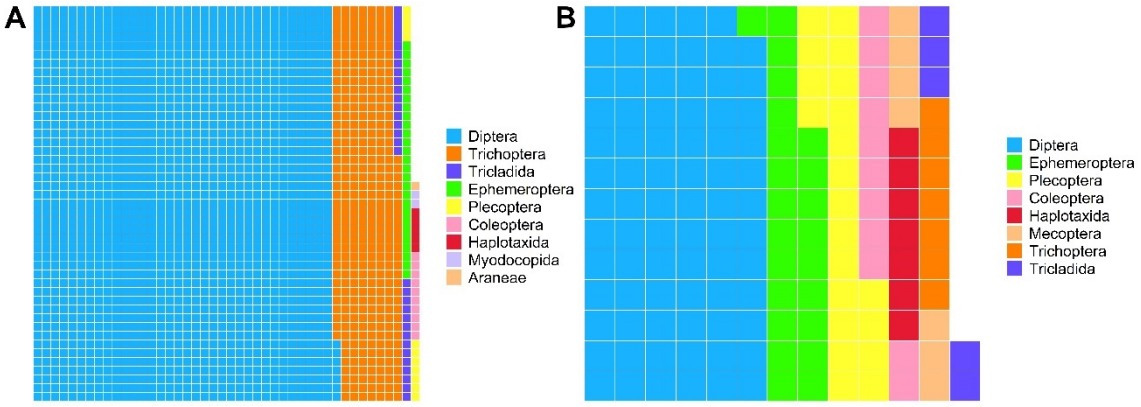


Figure 4.5 Waffle chart displaying the average number of aquatic macroinvertebrates per m^2 of stream bed, ranked by order for (A) Stream A and (B) Stream B. One square represents one individual on average per m^2. The colour for each order is identical for both streams, except for Araneae, Mecoptera, and Myodocopida, each of which occurred in one stream only.

Table 4.1 Hairworm morphotypes (phylum Nematomorpha) found in various aquatic macroinvertebrates collected from two subalpine streams in New Zealand, with measurements of folded larvae within cysts.

Hairworm morphotype	Total number of cysts and larvae		Host taxa identified (total collected)			Encysted larval dimensions	
	Stream A	Stream B	Stream A	Stream B	Shared taxa	Mean length ± SE (*n*)	Mean width ± SE (*n*)
Type A	642	572	21 (37)	15 (33)	7	102.3 ± 2.0 µm (7)	63.5 ± 0.7 µm (7)
Type B	206	413	12 (37)	13 (33)	6	63.9 ± 0.6 µm (5)	41.8 ± 1.2 µm (5)
Type C	137	1,444	6 (37)	10 (33)	3	45.0 ± 0.6 µm (6)	26.7 ± 0.7 µm (6)

4.4.2 Hairworm larvae and melanised cysts

From all the hairworms found in aquatic hosts, we only identified 11 (0.3%) as melanised cysts. Since it is unknown whether melanotic encapsulation kills hairworms, we decided to exclude this small number of cysts from statistical analyses. We also counted 138 (4%) unencysted hairworm larvae that were non-melanised or melanised (Figure 4.2D to 4.2F). Of these, over 90% were found in three species of uncased predatory hydrobiosid trichopterans, namely *Hydrobiosis* sp., *Psilochorema* sp., and *Tiphobiosis* sp.; the remaining 13 larvae were found sporadically among other host taxa. We found no broken cysts near the larvae counted within these trichopterans, therefore we assumed that they

had come out of their cyst prior to collection. Upon closer inspection, we did not observe any contents within their pseudo-intestine (Figure 4.2D), which further supported our assumption that these larvae had already produced a cyst and were thus excysted. We also identified the head capsules of other insects in the gut of these predatory hosts, confirming that these insects could have consumed hosts that were harbouring cysts. Therefore, these hairworm larvae were likely non-viable and were counted in the proportion of hairworms that could not complete their life cycle.

4.4.3 Viable and non-viable hairworms

From the Surber samples, we distinguished between hairworms that had the possibility to continue their life cycle and those that did not (Figure 4.3), which we categorised here as viable and non-viable, respectively. Viable hairworms consisted of non-melanised cysts that were found in a host with an eventual terrestrial life stage, i.e., potential paratenic hosts. Non-viable hairworms consisted of cysts found in strictly aquatic macroinvertebrates and the non-melanised and melanised excysted larvae that were found mainly inside predatory trichopterans, i.e., dead-end hosts. Across hairworm morphotypes, 583 (17%) were counted as non-viable. Type A hairworms were the most common among the non-viable at 71%, with over 34% of this morphotype found in dead-end hosts (Figure 4.3).

4.4.4 Statistical results

Stream A

From our regression analyses, haplotaxid worms (dead-end oligochaete hosts) had a higher abundance of Type A hairworms than the four other taxa tested (Table 4.2 and Table 7 in Appendix C). Although the trichopteran *Pycnocentria* sp. did not have a higher abundance of Type A hairworms, their relatively high density accounted for the large number of hairworms per m^2 found in this taxon (Figure 4.3). Like haplotaxids, the trichopteran *Hydrobiosis* sp. also had a higher abundance of Type A hairworms than *Pycnocentria* sp., yet most of these hairworms were counted as non-viable. We did not observe any statistical difference between sampling dates for this hairworm morphotype. For Type B hairworms, the ephemeropteran *D. fumosum* had a higher abundance than most other taxa tested, except for *Hydrobiosis* sp. (Table 4.2 and Table 8 in Appendix C).

Pycnocentria sp. had a lower abundance of Type B hairworms than most other taxa tested, except for haplotaxids. Type B hairworm abundance was lower in March 2021 than in October 2020, November 2020, and January 2021, without any obvious peak throughout sampling dates. For Type C hairworms, no difference in abundance was detected between the two taxa tested, *D. angustum* and *D. fumosum* (Table 4.2). The peak abundance of Type C hairworms observed during January 2021 in Figure 4.4 was only higher than those of March and May 2021 (Table 9 in Appendix C).

Stream B

Among the taxa tested, Type A hairworm abundance did not vary statistically, except for between the two ephemeropterans *D. angustum* and *D. fumosum*, with abundance being higher in the former (Table 4.3 and Table 10 in Appendix C). Although a slight peak in Type A hairworm abundance is observed in November 2020 (Figure 4.4), it was only different from those in January and February 2021. For Type B hairworms, the chironomid species group clearly had a lower abundance than all four other taxa (Table 4.3). Apart from that, no difference was observed. In Figure 4.4, the peak in abundance of Type B hairworms in October 2020 was statistically higher than most other months, yet no clear peak in abundance was observed for this morphotype (Table 4.3 and Table 11 in Appendix C). For Type C hairworms, the two ephemeropterans *D. angustum* and *D. fumosum* had higher abundances than most other taxa tested, except for between *D. angustum* and a morphospecies of tabanid (Table 4.3 and Table 12 in Appendix C). Even though Type C hairworm abundance did not differ between both ephemeropterans, *D. fumosum* was more abundant in the stream, which accounted for its greater contribution to the number of Type C hairworms per m^2 (Figure 4.3). The chironomid species group appears to have contributed substantially to the density of Type C hairworms in Stream B (Figure 4.3), but since only 101 cysts were found in 19 individuals during the month of January 2021, their relatively high density in the stream could have accounted for this (Figure 4.5). Interestingly, the appearance of Type C cysts in chironomids coincided with the peaks of abundance observed in Figure 4.4. The peaks of Type C hairworm abundance observed in January and February 2021 were both higher than all other sampling dates, except for the one in late January, in which very few individuals were captured and could thus not be properly compared statistically (Table 12 in Appendix C).

Table 4.2 Results of generalised linear modelling for the abundance (average number per host) of hairworm (phylum Nematomorpha) cysts and larvae among host taxa and sampling dates for each hairworm morphotype in Stream A (factors that impact hairworm abundance are in bold). Note that dates are relative to the first sampling date (2020-10-30) and that host taxa are relative to *Deleatidium fumosum* for Types A and B and *D. angustum* for Type C.

Hairworm morphotype	Response variable	Fixed factors	Coefficient estimate	Standard error	*z*-value	*p*-value
Type A	Number of hairworms per host	Intercept	-1.6731	0.4405	-3.7980	< 0.001
		Date, 2020-11-28	0.4946	0.4475	1.1050	0.2690
		Date, 2021-01-10	-0.0446	0.4938	-0.0900	0.9280
		Date, 2021-01-30	**1.0558**	**0.4576**	**2.3070**	**0.0211**
		Date, 2021-02-27	0.0914	0.4595	0.1990	0.8424
		Date, 2021-03-27	0.3507	0.4119	0.8510	0.3946
		Date, 2021-05-01	0.3384	0.4032	0.8390	0.4013
		Host, Haplotaxida	**3.5490**	**0.5701**	**6.2250**	**< 0.001**
		Host, *Hydrobiosis* sp.	**1.7318**	**0.4585**	**3.7770**	**< 0.001**
		Host, *Psilochorema* sp.	0.6348	0.3963	1.6020	0.1092
		Host, *Pycnocentria* sp.	0.2618	0.3290	0.7960	0.4261
Type B	Number of hairworms per host	Intercept	1.2647	0.6467	1.9560	0.0505
		Date, 2020-11-28	-0.2233	0.7871	-0.2840	0.7767
		Date, 2021-01-10	0.3510	0.7933	0.4420	0.6581
		Date, 2021-01-30	-1.5208	1.0551	-1.4410	0.1495
		Date, 2021-02-27	**-3.1159**	**0.9574**	**-3.2550**	**0.0011**
		Date, 2021-03-27	**-3.8213**	**0.9532**	**-4.0090**	**< 0.001**
		Date, 2021-05-01	**-1.3809**	**0.6706**	**-2.0590**	**0.0395**
		Host, Haplotaxida	**-3.0121**	**0.9806**	**-3.0720**	**0.0021**
		Host, *Hydrobiosis* sp.	-0.9351	0.6868	-1.3610	0.1734
		Host, *Psilochorema* sp.	**-2.6381**	**0.6184**	**-4.2660**	**< 0.001**
		Host, *Pycnocentria* sp.	**-4.9322**	**0.6709**	**-7.3520**	**< 0.001**
Type C	Number of hairworms per host	Intercept	0.0380	1.0010	0.0380	0.9697
		Date, 2020-11-28	-37.8900	3.00E+07	0.0000	1.0000
		Date, 2021-01-10	1.3330	0.9744	1.3680	0.1714
		Date, 2021-01-30	-0.7933	0.9899	-0.8010	0.4229
		Date, 2021-02-27	-37.9200	1.37E+07	0.0000	1.0000
		Date, 2021-03-27	-0.8391	0.7921	-1.0590	0.2894
		Date, 2021-05-01	-1.4860	0.7603	-1.9540	0.0507
		Host, *Deleatidium fumosum*	1.0120	0.7196	1.4060	0.1597

Table 4.3 Results of generalised linear modelling for the abundance (average number per host) of hairworm (phylum Nematomorpha) cysts and larvae among host taxa and sampling dates for each hairworm morphotype in Stream B (factors that impact hairworm abundance are in bold). Note that dates are relative to the first sampling date (2020-10-29) and that host taxa are relative to the chironomid species group.

Hairworm morphotype	**Response variable**	**Fixed factors**	**Coefficient estimate**	**Standard error**	***z*-value**	***p*-value**
Type A	Number of hairworms per host	Intercept	-35.9900	8.51E+06	0.0000	1.0000
		Date, 2020-11-29	**1.0520**	**0.4512**	**2.3330**	**0.0197**
		Date, 2021-01-11	**-2.0060**	**0.7871**	**-2.5480**	**0.0108**
		Date, 2021-01-31	-1.0500	6.77E+07	0.0000	1.0000
		Date, 2021-02-28	-0.4852	0.5305	-0.9150	0.3604
		Date, 2021-03-27	-0.3235	0.5690	-0.5680	0.5697
		Date, 2021-05-01	-0.4021	0.5470	-0.7350	0.4622
		Host, *Deleatidium angustum*	36.5400	8.51E+06	0.0000	1.0000
		Host, *Deleatidium fumosum*	34.9000	8.51E+06	0.0000	1.0000
		Host, Haplotaxida	36.0300	8.51E+06	0.0000	1.0000
		Host, Tabanidae sp. 1	36.0000	8.51E+06	0.0000	1.0000
Type B	Number of hairworms per host	Intercept	-1.9210	1.0570	-1.8180	0.0691
		Date, 2020-11-29	**-1.9970**	**0.4704**	**-4.2460**	**< 0.001**
		Date, 2021-01-11	**-2.3570**	**0.5790**	**-4.0720**	**< 0.001**
		Date, 2021-01-31	-35.1200	6.71E+07	0.0000	1.0000
		Date, 2021-02-28	-37.9100	9.29E+06	0.0000	1.0000
		Date, 2021-03-27	-3.7230	0.7800	-4.7730	1.82E-06
		Date, 2021-05-01	-3.3310	0.6282	-5.3030	1.14E-07
		Host, *Deleatidium angustum*	**3.6950**	**1.1170**	**3.3080**	**< 0.001**
		Host, *Deleatidium fumosum*	**3.4560**	**1.1190**	**3.0900**	**0.0020**
		Host, Haplotaxida	**3.6540**	**1.1090**	**3.2930**	**0.0010**
		Host, Tabanidae sp. 1	**3.1690**	**1.0910**	**2.9050**	**0.0037**
Type C	Number of hairworms per host	Intercept	-2.2120	0.4360	-5.0740	< 0.001
		Date, 2020-11-29	-0.7428	0.4895	-1.5170	0.1292
		Date, 2021-01-11	**3.1740**	**0.4018**	**7.8990**	**< 0.001**
		Date, 2021-01-31	-34.8300	6.71E+07	0.0000	1.0000
		Date, 2021-02-28	**2.5430**	**0.3815**	**6.6660**	**< 0.001**
		Date, 2021-03-27	**1.3660**	**0.4264**	**3.2040**	**0.0014**
		Date, 2021-05-01	-0.2526	0.4400	-0.5740	0.5660
		Host, *Deleatidium angustum*	**2.0550**	**0.4103**	**5.0080**	**< 0.001**
		Host, *Deleatidium fumosum*	**2.5090**	**0.3511**	**7.1450**	**< 0.001**
		Host, Haplotaxida	-0.8284	0.6654	-1.2450	0.2132
		Host, Tabanidae sp. 1	**1.6880**	**0.3702**	**4.5590**	**< 0.001**

4.4.5 Malaise trapping

A total of 6,164 insects were captured in the Malaise traps, 72% of which were captured near Stream B (Tables 13 and 14 in Appendix C). Of the 2,067 individuals with an aquatic life stage, 1,100 were flattened under the microscope to look for hairworms. As with the Surber samples, the presence of chironomids was highest among the groups with an aquatic life stage at 85%. Only two taxa were found to harbour hairworm cysts, the plecopteran *Zelandobius* sp. and the chironomid species group. Of the 555 chironomids counted, one individual captured near Stream A had one Type A cyst, another individual captured near Stream B had nine Type C cysts. This second individual was captured during January, which coincided with the only chironomid larvae collected in Stream B that were infected with Type C cysts. For *Zelandobius* sp., the 22 individuals caught near both streams were all infected with one or more of the three hairworm morphotypes. The regression analysis comparing *Zelandobius* sp. adults and the large larvae collected from both streams showed no difference in the abundance of each hairworm morphotype (Table 4.4). However, we did find a higher abundance of Type B cysts in Stream A for this taxon across life stages.

Table 4.4 Results of generalised linear modelling for the abundance (average number per host) of hairworm (phylum Nematomorpha) cysts in the plecopteran *Zelandobius* sp. between different life stages and sampling sites for each morphotype (the factor impacting hairworm abundance is in bold). Note that host stage is relative to adults and that sampling site is relative to Stream B.

Hairworm morphotype	Response variable	Fixed factors	Coefficient estimate	Standard error	*z*-value	*p*-value
Type A	Number of hairworms per host	Intercept	0.9202	0.3483	2.6420	0.0083
		Host stage, Larva	-0.1712	0.4164	-0.4110	0.6809
		Site, Stream A	0.6856	0.4179	1.6410	0.1009
Type B	Number of hairworms per host	Intercept	-1.1115	0.5493	-2.0230	0.0430
		Host stage, Larva	-0.5144	0.6189	-0.8310	0.4060
		Site, Stream A	**1.9908**	**0.5913**	**3.3670**	**0.0008**
Type C	Number of hairworms per host	Intercept	-0.1753	0.7751	-0.2260	0.8210
		Host stage, Larva	-0.1459	0.9352	-0.1560	0.8760
		Site, Stream A	-0.1292	0.9617	-0.1340	0.8930

4.5 Discussion

Host availability impacts the transmission of parasites and disease in freshwater environments (Keesing et al., 2006; Lagrue and Poulin, 2015a). Investigating the infection dynamics among viable and non-viable hosts could help to better predict the spread or emergence of parasites. Here, we quantified the spatial and temporal distribution of hairworm cysts and larvae across two communities of freshwater macroinvertebrates in subalpine streams of New Zealand. In doing so, we discovered three hairworm morphotypes living in sympatry within a multitude of aquatic hosts. In both streams however, the abundance of hairworms for each morphotype across sampling dates was highest in different subsets of host taxa. Some taxa, like the chironomid species group and the trichopteran *Pycnocentria* sp., did not have many parasites per individual on average, but, due to their higher densities in the streams, harboured a relatively higher proportion of hairworms in the community. For other taxa, like haplotaxid oligochaetes, only a few dozen individuals harboured a considerable proportion of hairworms. Since these hosts are strictly aquatic, the hairworms they consumed cannot continue their life cycle. For instance, more than a third of Type A hairworm cysts were counted in haplotaxids from Stream A alone, representing an important sink for this hairworm population. Other groups, such as predatory trichopterans, also constituted an important barrier for hairworm transmission. Seasonal patterns across infected host taxa were clearest for Type C cysts, which showed peaks of abundance during austral summer. Temporal peaks of abundance have also been found in other host-hairworm systems (Chiu et al., 2016; Yamashita et al., 2017). However, the appearance of two peaks of abundance for this morphotype in Stream B is probably a sampling artifact; we collected fewer than 10 macroinvertebrates in total from that stream in late January, most likely because an invasive diatom had permeated large stretches of the stream during that period (personal observation). The abundances of the other two hairworm morphotypes did not show such a strong correlation with sampling date. Finally, the limited data on insects emerging from the streams suggest that the abundances of each hairworm morphotype per individual host reaching land appear to mirror those found in the streams.

The main limitation of this kind of study is the overrepresentation of some species relative to others due to the sampling methods employed. Collecting mobile species in a fixed

sampling frame can only capture part of the full community composition. However, we consider that the sampling effort conducted throughout this study was adequate to characterise the main species found in both local communities. For instance, even though chironomids were the dominant taxon in both streams, they were, in most part, non-hosts for hairworms. In taxa with only one or a few individuals collected, it was not possible to obtain an accurate representation of hairworm abundance. Nonetheless, these species were probably far less common in the streams and contributed relatively little to the transmission of hairworms. The density of hosts in aquatic systems drives parasite density (Lagrue and Poulin, 2015b; Sonnenholzner et al., 2011), which appears to also be the case here. An abundant host like the trichopteran *Pycnocentria* sp. is more likely to acquire cysts simply because it may encounter hairworm larvae more often than other, less abundant hosts. Another limitation is the possible persistence of hairworm cysts in their hosts for more than a year. It is currently unknown how long cysts can remain viable in aquatic hosts. Since overlapping generations of hosts were found in most samples, it is possible that older hosts were infected sometime in the previous year, which would be impossible to differentiate from more recent infections. This could partially mask the arrival of new cysts in the season, making it more difficult to identify the peak occurrence of each hairworm morphotype among sampling dates.

Three obvious hairworm morphotypes were easily distinguishable, mainly by their size, but also by their appearance. In previous studies, five different species of adult hairworms were collected from Stream A (*Euchordodes nigromaculatus*, *Parachordodes diblastus*, *Gordius paranensis*, *Gordius* sp., and *Gordionus maori*) and Stream B (*E. nigromaculatus*, *P. diblastus*, *Gordius sp.*, *G. maori*, and an unidentified species) (Tobias et al., 2017; Yadav et al., 2018). Type A hairworms, based on the length of the postseptum relative to the preseptum, the posterior end finishing in a tip, and the shape of folded larvae within cysts, are most likely from the genus *Gordius* (Szmygiel et al., 2014). Due to a folding pattern similar to that seen in Type A larvae, and a lack of visible protruding spines on the preseptum, it is possible that Type B hairworms also belong to the genus *Gordius*. However, we cannot conclude with high certainty to which taxon this morphotype belongs. Type C hairworms were relatively smaller and had a postseptum length visibly equal to the length of their preseptum (personal observation). We also

observed spines protruding from the preseptum, which fits with descriptions of cysts from the genus *Chordodes* and *Parachordodes* (Poinar et al., 2004; Szmygiel et al., 2014). It is possible that Type C cysts comprise both *P. diblastus* and *E. nigromaculatus*, both of which were previously reported from these streams. If this is true, it was not possible to properly distinguish them under the microscope. Regardless, in order to properly assign a species rank to each morphotype, collecting a large number of cysts per morphotype to compare their DNA sequences would be necessary. However, identifying each morphotype to species level was not essential for the aims of this study.

In species-rich aquatic communities, sympatric parasites with similar transmission requirements may differ in their relative use of available host species and follow different transmission routes (e.g., Koehler & Poulin, 2010). Among the three hairworm morphotypes observed here, there were clear differences in either the host taxa with highest abundance or those that harboured the greatest proportion of cysts within the community. Another study also found that certain hosts have a higher abundance of cysts, which the authors attributed mainly to host-specific feeding behaviours (Yamashita et al., 2017). They also found that hairworm abundance differed between the pools and riffles of the stream. However, this was not accounted for in the current study. As our goal here was to characterise the overall hairworm abundance in both stream communities across microhabitats, our samples were collected randomly from pools and riffles to ensure an even representation. Based on our observations, we propose the following mechanism to explain the differences in abundance of morphotypes among host taxa, which involves the specific feeding behaviours of hosts and the egg-laying habits of hairworms. Type A hairworms, if indeed of the genus *Gordius*, hatch from short pieces of egg string produced by mated females (personal observation) (Szmygiel et al., 2014). These egg strings are released into the water column and drift into the current. Hatched larvae are therefore more likely to sink to the stream bed and accumulate in areas of low current velocity, e.g., the sediment of pools. *Pycnocentria* sp. larvae typically live in the slower parts of streams, where they feed on decaying organic matter (Cowley, 1978). Aquatic haplotaxids are usually found burrowing through the sediments of pools, feeding on an array of decaying organic matter (Brusca et al., 2016). These host-specific feeding habits, paired with the egg-laying habits of *Gordius*, could explain why these two hosts harboured the highest

proportion of Type A hairworms in the Stream A community. In contrast, Type C cysts, which may include both *E. nigromaculatus* and *P. diblastus*, probably hatch from egg strings attached to a submerged rock or stick, which is a characteristic egg-laying habit for species of the closely related genus *Chordodes* (Bleidorn et al., 2002; Szmygiel et al., 2014). The main hosts identified for Type C hairworms were the ephemeropterans *D. angustum* and *D. fumosum*, both of which are specialised to live on rocks and wood in low to moderate currents, where they feed on algae by scraping them off the substrate with specialised mouthparts (Towns and Peters, 1996). This feeding habit would place them at a higher risk of ingesting hairworm larvae that hatch within egg strings attached to these substrates.

The mechanism proposed above could account for the differences in hairworm morphotype abundance observed among host taxa. Another important finding was the loss of hairworms, particularly of Type A, in dead-end hosts. Apart from haplotaxids, which can accumulate cysts in large numbers, predatory trichopterans also played a more or less important role, depending on the stream. Interestingly, they apparently played a dual role in the life cycle of this hairworm morphotype: they both harboured cysts and excysted larvae. Hairworm cysts that are consumed along with their current host by such predators may excyst as they would in their terrestrial definitive host, to then end up dying in an incompatible aquatic one. These accidental excystments have also been observed in other aquatic predators such as the megalopteran *Archichauliodes diversus* (Poinar, 1991a; Chapter 3). Thus, in the case of this hairworm and its egg-laying habits, parasite transmission could be considerably inhibited if certain key dead-end hosts are present in the community. The other two morphotypes did not appear to be as hindered by dead-end hosts, which may also be attributed to the combination of their egg-laying habits and the specific feeding behaviours of hosts.

We were only able to recover two infected taxa emerging from the streams with the use of Malaise traps. Based on these, the abundance of each hairworm morphotype in plecopteran *Zelandobius* sp. adults corresponded with what was observed in both streams for the collected larvae that were closest to maturation. This result indicates that the host does not incur parasite-induced mortality, i.e., individuals with many cysts do not appear

to fail to emerge, therefore confirming that this plecopteran provides a successful route of transmission back to terrestrial environments. The fact that we did not trap any ephemeropterans and very few trichopterans may be due to a sampling artifact; traps were only opened for seven days every month due to restrictions of the collection permit. Moreover, the locations where traps were installed were sometimes exposed to harsh conditions of wind, rain, and hail (personal observation), reducing their efficacy. Nonetheless, we confirmed that, for at least one species of host, the temporal window of emergence lasted for the entire sampling season, a finding that matches previous observations (Meguro et al., 2020). If true for other host species, this large temporal window would provide ample opportunities for definitive hosts to consume paratenic hosts and ingest hairworms.

The main finding of this study is that sympatric parasites, each with the potential to infect many aquatic hosts, can follow distinct and specific transmission pathways through a freshwater community. However, if certain dead-end host species are present in the community, a considerable proportion of parasites in a population may end up never completing their life cycle. For hairworms, a common parasite in lotic ecosystems, these community-induced pressures could help explain the evolution of low host specificity in larval hairworms toward paratenic hosts and the extremely high fecundity observed in females. With the odds of survival stacked against each hairworm larva, only the ones that end up in the right host have a chance at furthering their development. Ultimately, understanding what proportion of parasites or disease agents in a freshwater community are lost in suboptimal host transmission pathways or dead-end hosts versus those that follow viable host transmission routes could help predict the spread or emergence of infectious disease.

Chapter 5

Going full circle: impact of hairworm infection on aquatic insect development may accelerate its return to land

Diversity of caddisfly larval cases collected from Grasmere Stream, Cass Field Station, Canterbury, New Zealand. The amber-coloured case on the left is produced by *Olinga jeanae*, the caddisfly with the highest abundance of hairworm cysts from this sampling location.

5.1 Abstract

Host manipulation by parasites can shape host behaviour, community structure, and the flow of energy through food webs. A well-known example of host manipulation comes from hairworms (phylum Nematomorpha), which somehow cause their terrestrial insect definitive hosts to enter water, a phenomenon that has received lots of attention in recent years. However, little focus has been directed towards the interactions between hairworms and their aquatic hosts and the return of dormant hairworms from water to land. Here, we ask whether hairworm cyst infections impact the life history of their aquatic hosts. By observing the development of last-instar *Olinga jeanae* (Trichoptera: Conoesucidae) caddisfly larvae naturally infected with *Gordius*-type hairworm cysts under controlled conditions, we found that higher numbers of cysts per infected caddisfly correlated with a decrease in time to pupation. These new observations suggest that the hairworm life cycle involves not only the striking host manipulation that brings the parasite from land to water, but also a more subtle one that could bring the parasite back from water to land.

5.2 Introduction

Host manipulation by parasites has received international recognition for the remarkable impacts it can have on hosts. From conspicuous changes in host appearance and behaviour (Andersen et al., 2009; Wesołowska and Wesołowski, 2014) to broad effects on host community structure (Thomas et al., 1998b; Lefèvre et al., 2009b) and energy flow within food webs (Kuris et al., 2008; Preston et al., 2013), parasitic manipulators can alter their environment in profound ways. One of the most striking examples of parasite-mediated energy flow occurs in the riparian zone of Japanese streams (Sato et al., 2011; Sato et al., 2012), where orthopterans infected with gordiid hairworms (phylum Nematomorpha) are twenty times more likely to enter water, thus becoming an important food source for trout. Hairworms mature within terrestrial arthropods, mainly scavenger-type insects, and somehow cause their definitive hosts to enter water in order to complete their life cycle (Bolek et al., 2015). Due to the somewhat obvious behavioural changes observed in definitive hosts, most studies on hairworm manipulation have focused on their transition from land to water (Thomas et al., 2002; Sanchez et al., 2008; Ponton et al., 2011). Even though hairworms may only subtly alter host behaviour in ways that increase their likelihood of entering water, this phenomenon has been widely sensationalised in both the popular media and the scientific literature (Chapter 2). However, far less attention has been given to the transition of hairworms from water to land. Here, we explore how hairworms could impact the life history of their aquatic hosts.

Most hairworm species enter water to reproduce (Bolek et al., 2015). After mating, females can lay several million eggs (Hanelt, 2009), from which larvae hatch and are consumed by practically any aquatic animal present: insects, crustaceans, and even vertebrates (Hanelt and Janovy, 2004a; Torres et al., 2017). Upon consumption, larvae use specialised mouthparts to move through host tissues, where they eventually form a cyst (Hanelt and Janovy, 2003). Aquatic macroinvertebrates that exit water contribute to the hairworm life cycle, as they can be consumed by the definitive terrestrial hosts. These paratenic hosts comprise most known groups of aquatic insect larvae that mature into terrestrial adults, e.g., mayflies, midges, and caddisflies (Bolek et al., 2015). Measuring approximately 60 to 100 μm in length, hairworm larvae are small, dormant, and do not grow within their cyst (Bolek et al., 2013b). Aquatic insects can mount defence reactions

against hairworm larvae and cysts through melanotic encapsulation, a common form of insect immunity (Poinar and Doelman, 1974; Chapter 3). However, apart from observations on host immunity, little is known of the impacts that hairworms have on their aquatic hosts.

Logically, the longer a hairworm spends as a cyst in its paratenic host, the likelier it is to die from host immunity, predation, or environmental perturbations, e.g., flooding or drought. Therefore, any change in host development time could affect the odds that a hairworm successfully completes its life cycle. Based on this rationale, we hypothesised that hairworm cysts, though apparently dormant, can accelerate the development of their paratenic host, either through direct or indirect mechanisms. This would expedite their return to land and improve their chances of completing their life cycle. For instance, larvae of the cat flea *Ctenocephalides felis* were found to develop faster when infected with the gregarine *Steinina ctenocephali* (Alarcón et al., 2017). This accelerated host development was suggested as an adaptive strategy of the parasite to increase its fitness when environmental conditions were favourable toward flea development.

In this study, we tested whether hairworm cyst infections impact the life history of aquatic hosts. To do so, we quantified the propensity to pupate in last-instar *Olinga jeanae* McFarlane (Trichoptera: Conoesucidae) caddisfly larvae under controlled conditions (caddisfly larvae must first pupate prior to emerging from water as flying adults). This is an abundant species in subalpine streams of New Zealand (Cowley, 1978; Ward and McKenzie, 1997) and is most likely one of the main paratenic hosts for *Gordius paranensis* found at the sampling site (Schmidt-Rhaesa et al., 2000; Chapter 3). We tested whether the infection status or the intensity of hairworm cyst infection (the number of cysts per infected individual) correlated with an increase in the odds that caddisfly larvae pupated during a laboratory-controlled observational study. If detected, such impacts would suggest that even hosts infected with dormant hairworms are not entirely free from parasite-induced life history changes.

5.3 Material and methods

5.3.1 Sampling last-instar caddisfly larvae

Caddisfly larvae were collected on 23 March 2020 from Grasmere Stream (43°01'55"S 171°45'28"E) in the Canterbury region of New Zealand (see Figure 3.1). In a 20-metre section of the stream, larvae were collected by dragging fine mesh dip nets across the vegetation. Samples were then transported back to the laboratory in small containers of aerated river water, where they were kept until processing. Based on caddisfly case morphology (Cowley, 1978), only *O. jeanae* larvae were collected from the sampling site. Live caddisfly pupae were removed from the samples and were used to measure pupal case width (the widest part of the case opening) and length using a microscope reticule (Figure 5.1). Twelve pupal case widths were measured to estimate a lower 95% confidence limit of 1.8 mm. This estimate was used as a minimum threshold size for the inclusion of larval *O. jeanae* in this study. Although head capsule width is a better indicator of larval development, we could not measure this on live caddisfly larvae that hide within their case when disturbed. Moreover, when *O. jeanae* larvae close their case to pupate, the width and length of their case decrease slightly in size (Figure 5.1). Therefore, by using pupal case width as a threshold, we assured that only last-instar larvae were included. These larvae were visibly longer and larger than earlier instars and were consistent in size with previous descriptions (Cowley, 1978; Ward and McKenzie, 1997). Caddisfly case width can be a better predictor of larval biomass than case length (Martins et al., 2014) and infected *O. jeanae* larvae tend to harbour more cysts with age (Chapter 3).

5.3.2 Observational study design

On 1 April 2020, samples were screened for larvae with a minimum case width of 1.8 mm. A total of 84 larvae fitting this criterion were then equally and randomly distributed into two 10-Litre clear plastic tanks (42 larvae each) filled with aerated river water, to avoid overcrowding. Air stones circulated the water, thus creating a current, which is preferable for caddisfly larvae. Tanks were kept at room temperature and water temperature was monitored in each tank with HOBO TidbiT v2 data loggers (Onset, Massachusetts, United States of America). Three small ceramic tiles were placed in each tank to allow larvae to move around and eventually use as a substrate on which to attach

their pupal cases. Larvae were fed *ad libitum* with frozen watercress (*Nasturtium* sp.) collected from the sampling site. This plant species was abundant in the stream and all the caddisflies used here were collected from that site. On a weekly basis, roughly two thirds of the water were replaced and food was added. At that time, the tanks were inspected for pupae, which are easily distinguished from larvae by their darker colour (Figure 5.1). These observations were stopped when approximately half of the larvae had pupated, which was on 2 September 2020. On that date, larvae and pupae were killed and stored in 75% ethanol until processed in the laboratory. Then, the intensity of hairworm cyst infection (total number of cysts per individual caddisfly) was determined by flattening individual host tissues between a microscope slide and cover glass (see section 3.3).

Figure 5.1 Lateral view of larval and pupal cases of the caddisfly *Olinga jeanae* (Trichoptera: Conoesucidae). Double-headed arrows indicate case width.

5.3.3 Statistical analyses

All statistical analyses were performed in R version 4.0.3 (R Core Team, 2021). Firstly, to test the prediction that infection status impacts the likelihood that caddisfly larvae pupate during the observation period, we used a generalised linear model (GLM) with the "glm" core function. Since the response variable can only assume one of two outcomes (larva or pupa), a logistic regression with a binomial distribution was implemented into

the model. Fixed effects were the infection status (infected or uninfected), the width of the larval or pupal case, and the tank in which the caddisfly was placed. A second binomial regression was used to test the prediction that the intensity of hairworm cyst infection impacts the odds that caddisfly larvae pupate before the study ended. Uninfected individuals were not included in this second model, as it focused on infected individuals only. Here, fixed effects were the total number of cysts per infected individual (intensity of infection), case width, and the rearing tank. The tank could be considered a random effect in both models, but since two tanks fall below the recommended minimum of five levels for a mixed-effects model to calculate a robust estimate of variance, we included it as a fixed effect in the GLM (Harrison et al., 2018). In both models, none of the data was transformed. To assess model fit, residuals were verified with a binned residual plot, as recommended for logistic regressions (Gelman and Hill, 2006).

5.4 Results

5.4.1 Hairworm cyst infection in *Olinga jeanae*

The study lasted a total of 154 days. In Tanks 1 and 2, 38 (90.5%) and 34 (80.9%) caddisflies survived and were either active larvae or pupae. The water temperature in both tanks varied within 0.5 °C from each other. The number of pupated larvae in these tanks were 20 (52.6%) and 17 (50.0%). A total of 52 (72.2%) caddisflies harboured at least one hairworm cyst and were thus included in the model testing for the intensity of infection. From these infected individuals, 26 (50.0%) pupated during the study. Larval case width varied between 1.8 and 2.0 mm. All hairworm cysts closely resembled each other in size and shape. Due to the possibility of a hairworm species complex (Hanelt et al., 2015; Tobias et al., 2017), cysts were identified as *Gordius*-type cysts based on their morphology (Szmygiel et al., 2014). The distribution of hairworm cyst intensity is presented in Figure 5.2A.

5.4.2 Host development and the intensity of hairworm cysts

All regression estimates from both models are presented in Table 5.1. In the first model testing the impact of infection on the propensity to pupate in larval caddisflies, none of the predictors had any effect. For the second model that included only infected

individuals, the intensity of hairworm cyst infection positively impacted the likelihood that *O. jeanae* larvae would pupate before the end of the study (Figure 5.2B); each additional hairworm cyst was associated with 43% higher odds of pupating (Figure 5.2C). In this model, the tank in which a caddisfly larva was placed and the width of its case had no impact on the likelihood of it pupating before observations stopped (Figure 5.2B).

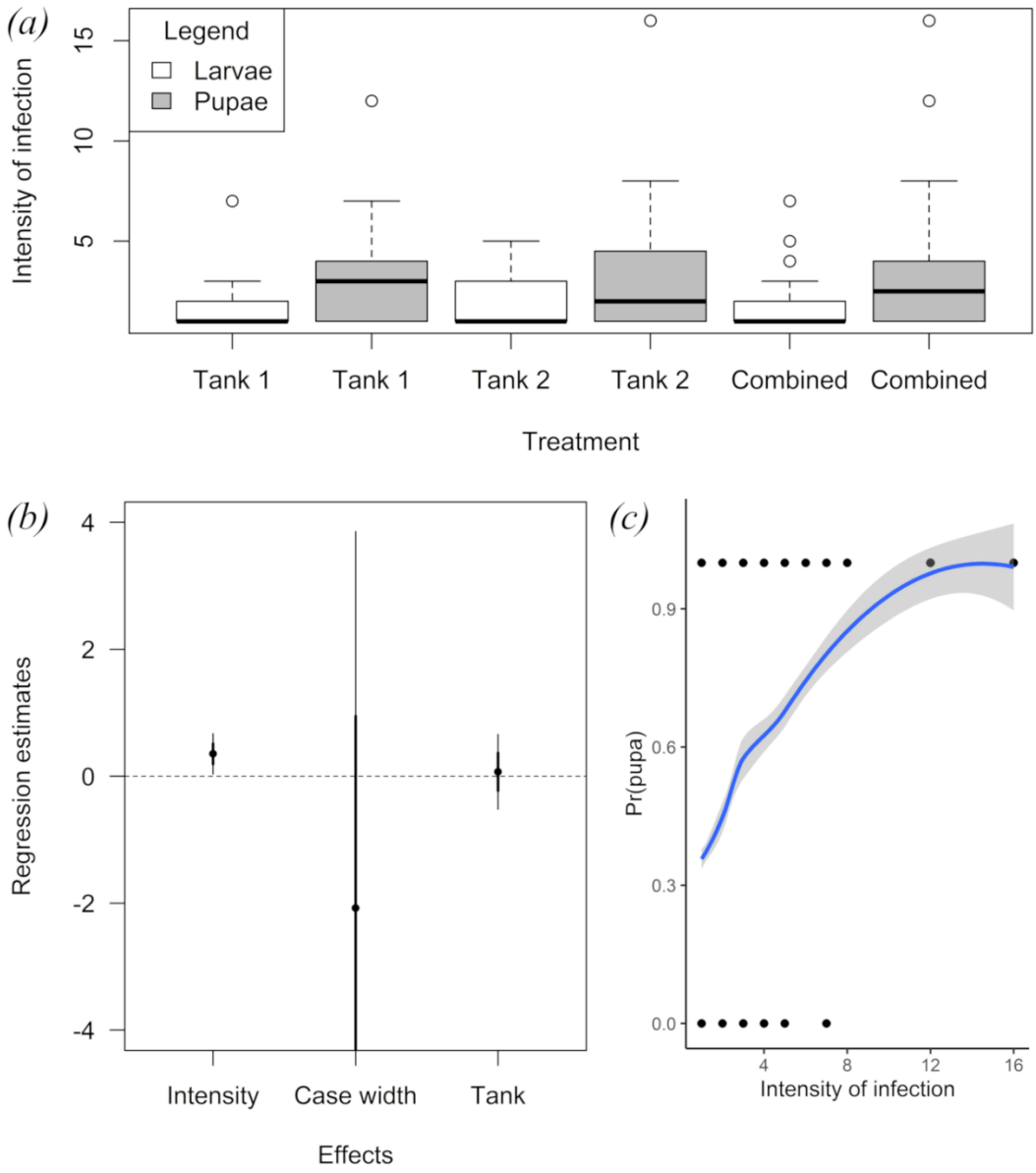


Figure 5.2 (A) Boxplot of the intensity of *Gordius*-type hairworm (Nematomorpha) cyst infection (number of cysts per infected individual) in larval and pupal stages of the caddisfly *Olinga jeanae* (Trichoptera: Conoesucidae). (B) Logistic regression estimates for the fixed effects of the model testing for intensity of infection, with 95% error bars. A regression estimate that is different from 0 (including error bars) indicates that the corresponding effect has an impact on the likelihood that a caddisfly will pupate before the end of the study. (C) Loess curve of the predicted effect of intensity of hairworm cyst infection on the probability that a caddisfly will pupate before the study ended (blue line). The grey area represents the 95% confidence band of the fitted logistic regression model.

Table 5.1 Regression estimates for the generalised linear models testing the impact of the infection status or the intensity of *Gordius*-type hairworm (Nematomorpha) cyst infection on the propensity to pupate in larval caddisfly *Olinga jeanae* (Trichoptera: Conoesucidae). The significant effect (in bold) has a regression estimate whose standard error range does not overlap zero and a *z*-score greater than 2.

Model	Effect variables	Regression estimate	Standard error	*z*-score
Infection status	Infection status	-0.1766	0.5416	0.744
	Case width	-0.1760	0.4909	0.720
	Tank	-0.1181	0.4760	0.804
Intensity* of infection	**Intensity of infection**	**0.3542**	**0.1619**	**2.187**
	Case width	-2.0785	3.0281	-0.686
	Tank	0.0701	0.2997	0.234

*Denotes the mean number of cysts per infected individual.

5.5 Discussion

This study, to our knowledge, is the first to report noticeable changes in the natural life history of aquatic hosts infected with dormant hairworm cysts. We show that, within a laboratory-controlled observational study lasting several months, last-instar caddisfly larvae harbouring more cysts were likelier to pupate earlier than ones harbouring less. Interestingly, in only a few cases have caddisfly larvae been reported harbouring cysts (Poinar, 1991a; Table 3 in Schmidt-Rhaesa, 2013; Chapter 3); most reported cases consist of caddisfly larvae infected with juvenile or adult hairworms (Schmidt-Rhaesa & Kristensen, 2006; Table 10 in Schmidt-Rhaesa, 2013). Although controlling for the age of naturally collected caddisfly larvae is difficult, the data suggest that larval caddisfly case width did not impact pupation rates. In both tanks, the prevalence of cysts in caddisfly larvae (74.3%) was near that of pupae (70.3%). Also, infection status alone did not appear to impact the odds of caddisflies pupating during the study (Table 5.1), which suggests that the presence of at least one hairworm cyst was not enough to precipitate their transformation into pupae. More likely, what matters is the intensity of infection, e.g., only pupae were observed harbouring eight or more cysts (Figure 5.2A). In other words, although only half of the infected caddisflies pupated during the study, those that did generally harboured more cysts.

Collecting naturally infected hosts and placing them in controlled conditions obviously has its limitations. It is unknown whether all the caddisfly larvae from the collection site

had an equal access to the same food sources prior to collection, or whether larvae fed at similar rates. In a closely related species, *Olinga feredayi* (McLachlan) (Trichoptera: Conoesucidae), it was shown that larvae need to consume more when exposed to a lower-quality food source (Burrell and Ledger, 2003). This can lead to a state of starvation, which could decrease the time to pupation (Truman and Riddiford, 2002). However, the caddisfly larvae used in this study were collected from an abundant patch of watercress, suggesting that individuals had access to the same food source. Moreover, larval case width varied within a very narrow range (1.8 to 2.0 mm), indicating that individuals were closer in age to each other than to other instars, even though practically nothing is known of the development times of *O. jeanae* instars. In addition, individuals of all life stages (excluding adults during winter) from overlapping generations can be found year-round in the same area (Cowley, 1978; Chapter 3). These uncontrolled variables make it challenging to quantify the real impacts of hairworm cyst load on aquatic host development. Ideally, to test this, caddisfly larvae would need to be reared in the laboratory and exposed to variable numbers of hairworm larvae. Unfortunately, it has not been possible to obtain enough mature hairworms from the sampling region to effectively rear them in the laboratory, obtain eggs, and expose caddisfly larvae to infective hairworm larvae.

Our observations suggest that, the more cysts a caddisfly larva harbours, the more likely it is to pupate earlier. Within this context, it is impossible to determine if pupal development or the emergence of adult caddisflies could also be impacted by hairworm infection. Adult caddisflies need to emerge from the stream in order for the hairworms they carry to be consumed by their final host. This is a critical component in the transition from aquatic host to the terrestrial one. Although little is known of the life history of *O. jaenea*, adults have been observed at the height of austral summer in January (Cowley, 1978). Whether adults with more hairworm cysts emerge earlier in the season is unknown. However, caddisflies that spend less time as larvae or pupae are less likely to die from predation. Fish and other predators, like the New Zealand dobsonfly *Archichauliodes diversus* collected from the same sampling site (Chapter 3), decrease the odds of successful hairworm transmission (Chapter 4). Although this study provides a narrow scope on the life cycles of both host and parasite, it suggests that selection favoured a

decrease in host development time, which would maximise the successful transition of hairworms from water to land.

Apart from predation, multiple abiotic stressors such as water temperature and pesticides can affect the development and survival of caddisfly larvae (Schulz and Liess, 1995; Mochizuki et al., 2006). However, practically nothing is known of the impact that parasites can have. Here, we present two plausible mechanistic explanations as two why the intensity of hairworm cysts increased the odds of caddisfly larvae pupating. Firstly, the accumulation of hairworm cysts over time may trigger a host response that accelerates the development process toward pupation. As stated above, hosts may have evolved to develop faster in response to repeated exposure to hairworm larvae, to avoid future infections. For instance, larvae of the cat flea *Ctenocephalides felis* experience accelerated development rates and emerge from pupae earlier when experimentally infected with the gregarine protist *Steinina ctenocephali* (Alarcón et al., 2017). Arguably, caddisfly larvae that pupate more rapidly are less likely to acquire additional hairworm larvae. The second plausible scenario that could explain the effects observed in this study, and perhaps the more interesting one, relates to the potential manipulation of aquatic host development by hairworms. Even though hairworms are dormant (after a brief active period) within paratenic hosts, they may have evolved to alter host development through unknown mechanisms. For example, microphallid trematodes that encyst as metacercariae within the heads of the amphipod *Gammarus insensibilis* can alter host behaviour and even reproductive output (Arnal et al., 2015; Gates et al., 2018). If hairworms were adapted to manipulate the development of aquatic insect larvae, they would likely have evolved mechanisms targeting important regulatory functions such as the juvenile hormone signalling pathway, a vital system that controls insect growth (Jindra et al., 2013). For instance, microsporidians of the genus *Nosema* cause excessive amounts of juvenile hormone that disrupt normal growth patterns in multiple insect hosts (Fisher and Sanborn, 1962). Still, more research is needed to explore the hidden interactions between hairworms and their paratenic hosts.

To conclude, we have shown that the presence of hairworms in naturally infected caddisfly larvae correlate with a decrease in time to pupation. Ideally, this study should

be replicated with experimentally infected hosts to determine if dormant hairworm cysts truly impact paratenic host development through direct or indirect mechanisms. Nonetheless, these observations provide novel insight into the infection dynamics between hairworms and their aquatic hosts. Interestingly, the intensity of hairworm cyst infections in aquatic macroinvertebrates fluctuates throughout the year (Chiu et al., 2016; Chapter 3). Higher intensities have been observed from our sampling site (personal observation), so that the effects on host development may be even stronger in other seasons. This study has further elucidated the complex, yet hidden interactions between hairworms and their paratenic hosts. It also reveals that the dramatic host manipulation that brings the parasite from land to water may be matched by a more subtle one that brings the parasite back from water to land.

Chapter 6

Infection patterns and new definitive host records for New Zealand gordiid hairworms (phylum Nematomorpha)

Terrestrial arthropods that fell into one side of a pitfall trap installed at the Kopuwai Conservation Area sampling location. The large orthopteran at the bottom is a ground wētā of the genus *Hemiandrus*.

6.1 Abstract

Some parasites modify the phenotype of their host in order to increase transmission to another host or to an environment suitable for reproduction. This phenomenon, known as host manipulation, is found across many parasite taxa. Freshwater hairworms are known for the behavioural changes they cause in their terrestrial arthropod hosts, increasing their likelihood of entering water to exit the host and reproduce. Understanding how infected arthropods move around in the natural environment could help uncover alterations in spatial distribution or movement induced by hairworms in their terrestrial definitive hosts. Moreover, few hairworm-host records exist for New Zealand, so any additional record could help elucidate their true host specificity. Here, we investigated whether infected terrestrial arthropods were more likely to approach streams in two subalpine communities of invertebrates, using a spatial grid of specialised pitfall traps. Although hairworm infection could not explain the movements of arthropod hosts near streams, we found several new host records for hairworms, including the first records for the recently described *Gordionus maori*. We also found some new host-parasite associations for mermithid nematodes. These records show that the host specificity of hairworms is quite low, suggesting that their diversity and distribution may be greater than what is currently known for New Zealand.

6.2 Introduction

Parasites need to infect a host, either to pursue development or to reproduce. The pressures that accompany this fundamental aspect of parasitic life cycles have resulted, across an evolutionary timescale, in a multitude of adaptive solutions (Poulin, 2011). In fact, certain lineages are capable, through direct or indirect mechanisms, of altering the phenotype of their current host to increase the odds of transmission to a subsequent host or to an environment suitable for reproduction (Poulin, 2010; Bhattarai et al., 2021). This phenomenon, known as host manipulation, has been reported across numerous host-parasite systems (Moore, 2002), although its true adaptive nature has been debated for decades (Poulin, 1995a; Cézilly and Perrot-Minnot, 2005; Thomas et al., 2010; Poulin and Maure, 2015). Phenotypic alterations can include any change in the appearance or behaviour of the host that favours the life cycle of the parasite. For instance, tadpoles infected with the trematode *Ribeiroia ondatrae* develop into frogs with malformed or additional limbs, which increases the odds of these amphibian hosts being eaten by the definitive avian host of the trematode (Johnson et al., 2002). Other remarkable examples include caterpillars that appear to "guard" over the parasitic wasps that recently left their body to pupate, thus offering some protection against natural enemies (Harvey et al., 2011).

These striking examples of host manipulation display the wide array of strategies that parasites employ to increase the likelihood of completing their life cycle. Studying host phenotypic alterations in a natural setting can provide strong evidence that host manipulation is indeed adaptive (Poulin, 1995a; Lagrue et al., 2007). For example, only from a long-term observational study in the field was it possible to quantify the natural effects of a host-manipulating fungal infection in ants (Loreto et al., 2014). In that study, the authors concluded that the parasite was akin to a chronic infection for ant colonies. Therefore, exploring the effects of host manipulation in natural conditions can help understand its true impact on both host and parasite fitness.

In the current study, we focus on the behavioural manipulation of terrestrial hosts by gordiid or freshwater hairworms (phylum Nematomorpha). These specialised parasites develop and mature within terrestrial arthropods (mainly scavenger or predatory insects)

that consume paratenic hosts infected with dormant cysts (Hanelt et al., 2005; Bolek et al., 2015). Paratenic hosts consist of aquatic insect larvae that emerge as terrestrial adults from streams and rivers, thus transporting hairworm cysts from water to land (Hanelt and Janovy, 2004a). When mature, hairworms need to exit from their terrestrial host in water to mate. This life cycle trait likely explains why hosts infected with mature hairworms appear to move around more erratically (Thomas et al., 2002; Sanchez et al., 2008; Ponton et al., 2011), thus increasing the odds of hosts (and hairworms) entering water. Although this phenomenon has been largely misrepresented in both the popular media and the scientific literature (Chapter 2), empirical evidence does suggest that hosts infected with hairworms are far more likely to enter water than uninfected conspecifics (Sato et al., 2011). However, how exactly the hairworm accomplishes this considerable change in host behaviour remains poorly understood (Thomas et al., 2003; Biron et al., 2005b; Chapter 2).

In New Zealand, six species of freshwater hairworms have been reported from four genera, five of which are currently described (see Yadav et al. (2018) and references therein). Definitive host records, the last one dating back to 2000, were collated by Poinar (2001) and Schmidt-Rhaesa (2013) and include three families of orthopterans (Acrididae, Anostostomatidae, and Rhaphidophoridae), ground beetles (Carabidae), and cockroaches (Blattodea). Notably, three hairworm species were observed in various endemic wētā hosts (Poinar, 1991a). The genetic diversity of New Zealand hairworms was studied across locations in South Island (Tobias et al., 2017), highlighting the possibility of cryptic species within the same population. In sum, although currently recognised hairworm species are well characterised, little is known of their definitive hosts across New Zealand, a country that includes a rich diversity of endemic insect species (Buckley et al., 2015), many of which having the potential to be infected by hairworms.

The aim of the current study was twofold. Firstly, we explored the spatial and temporal infection patterns of hairworms throughout two subalpine communities of terrestrial invertebrates in New Zealand. Here, we tested whether insects infected with mature hairworms were likelier to approach streams than to move away from streams, using a series of specialised pitfall traps set within a spatial grid, and if this pattern correlated

with sampling season. We predicted that, if hairworms induce erratic behaviours in their hosts prior to entering water, there would be no obvious pattern in the distribution of infected hosts captured within the spatial grid. Alternatively, if hairworms do induce a directed movement towards water, this would be reflected in the number of infected insects captured per trap. Secondly, we aimed to identify new hairworm-host associations with the invertebrates captured in the traps. Any new definitive host record would provide more information on the host specificity of hairworms, as well as elucidate the potential distribution of hairworms in New Zealand. This study provides novel insights into the hidden infection patterns of this poorly understood group of parasites.

6.3 Material and methods

6.3.1 Field methods

Terrestrial invertebrates were captured in pitfall traps installed near the streams of two locations: Rock and Pillar Conservation Area (45°26'03"S 170°04'32"E; Site A) and Kopuwai Conservation Area (45°20'43"S 169°11'55"E; Site B) in Otago, New Zealand (see Figure 4.1A) (Department of Conservation Authorisation Number 68065-RES). These sites were chosen because adult hairworms have been reported in the streams of both (Tobias et al., 2017; Yadav et al., 2018). Also, they are subalpine in elevation (approximately 1,320 and 1,580 m in altitude, respectively), had similar vegetation cover, and both streams were also relatively similar with respect to general width and velocity. For each site, a 100-m section of stream was selected and three transects were drawn perpendicularly from the stream every 50 m. Along the transects, we installed pitfall traps at 5, 25, and 45 m from the stream, for a total of nine traps per site set within a spatial grid of 3 × 3 (Figure 6.1).

We used X-shaped guidance barrier pitfall traps with 0.75-metre-long plastic barriers planted into the ground for a height of approximately 0.07 m above ground and an angle of 90° between barriers (Morrill et al., 1990) (Figure 6.1). We selected this design because it has been shown to substantially increase the overall effectiveness of pitfall traps with respect to total captures and relative taxonomic composition (Boetzl et al., 2018). For the container, we used a plastic cup (height × width: 0.115 × 0.09 m) filled with 250 mL of

propylene glycol to preserve invertebrates. Two out of the four sides of the X-shaped trap were blocked off with strong adhesive tape so that invertebrates could only fall into the container if walking either toward the stream or away from it (Figure 6.1). Each container was fitted with a plastic separator to properly identify from which side invertebrates fell into the trap. We covered the traps with metal roofs (0.25 × 0.25 m) to protect against flooding and placed rocks on top to stop the wind from dislodging them. Traps were installed for a seven-day period at the beginning of each month, from November 2020 to March 2021 (for a total of five sampling periods). These were reinstalled in the exact same locations every month. At the end of each period, traps were disassembled and the containers were sealed and transported back to the laboratory. There, we removed invertebrates from both sides of each trap and stored them separately in 75% ethanol until further processing.

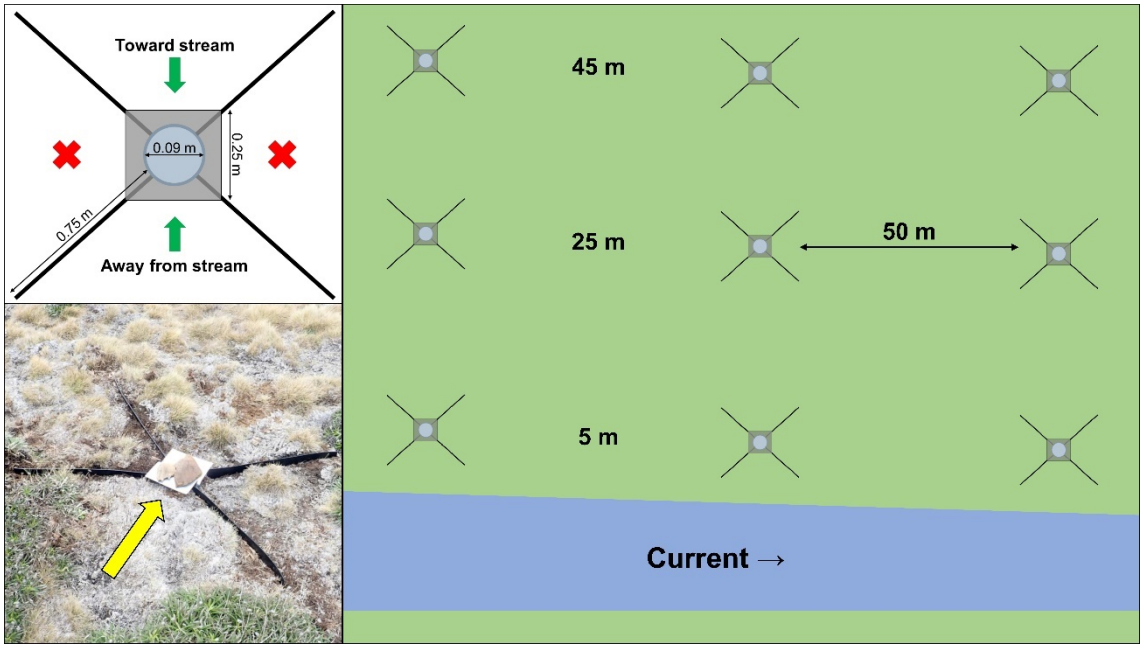


Figure 6.1 Field study design for investigating the spatial patterns of terrestrial insects infected with hairworms (phylum Nematomorpha) in two subalpine locations in Otago, New Zealand. The top left panel shows the schematic with dimensions (not proportional) of pitfall traps used in the study, with green arrows showing which sides invertebrates could fall into the container (blue circle) and red crosses showing the sides that were blocked off. The four black bars represent the plastic barriers and the grey square represents the metal roof, which is translucent here to see the container beneath. The bottom left panel shows a pitfall trap deployed in the field, with a yellow arrow pointing towards the stream. The right panel shows the spatial grid of nine pitfall traps set alongside the stream, with distances of traps from the stream and between transects.

6.3.2 Laboratory methods

We first identified all the scavenger and predatory arthropods captured in the traps that were likely to be infected with hairworms to the lowest taxonomic level possible using the following taxon-specific keys: Araneae (Vink, 2002; Paquin et al., 2010), Carabidae (Larochelle and Larivière, 2007), Dermaptera (Hudson, 1973), Orthoptera (Bigelow, 1967; Meads, 1990), and Scarabaeidae (Watt, 1984). For these, some individuals were assigned to a family and then separated into morphospecies based on their appearance, as some characteristics were difficult to confirm under the dissecting microscope, e.g., the pedipalp tarsi of some male spiders. All other invertebrates that were unlikely to host hairworms were separated by family, order, or class. To rehydrate tissues and facilitate dissections, we removed the samples from their ethanol solution and submerged them in tap water for at least 24 hours. Before dissection, we measured the width of head capsules of the invertebrates that had one using a microscope reticule. We also noted the sex of the individual when possible and looked for any external damage, e.g., a hole in the posterior end, indicating that a hairworm had egressed prior to collection. Afterwards, invertebrates were carefully opened up using fine tweezers and a scalpel or a pair of spring scissors with a 10-mm cutting edge. We started with the abdomen, since hairworms typically develop in that part of the host. If a worm was present and it was intact, we removed it to measure its length to the nearest mm by straightening it with tweezers over a ruler without stretching it, and its width at mid length using the microscope reticule. Regardless of taxon, all invertebrates captured in the pitfall traps were dissected to look for hairworms.

Mature hairworms, identified by their darkening cuticle, were initially assigned to a genus based on their external morphology and by comparing them to species previously reported in the streams (Tobias et al., 2017; Yadav et al., 2018; Zanca et al., 2020). Some individuals could not be identified by morphology alone, i.e., immature hairworms or mermithid nematodes. Therefore, we cut a small section (around 5 mm) from each worm to extract DNA using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then, we ran polymerase chain reactions (PCR) to amplify DNA using the nematode primers Nem18SF and Nem18SR targeting partial 18S ribosomal RNA, following the PCR conditions from Wood et al. (2013), and two sets of New Zealand hairworm primers from Tobias et al. (2017): NZHW_CO1_F and

NZHW_CO1_R targeting the mitochondrial CO1 gene; HW_Grp5_ITS_F and HW_Grp5_ITS_R targeting a partial region of ribosomal RNA. These two pairs of primers were used under the following PCR conditions: initial denaturing step at 94 °C for 4 minutes, followed by 40 cycles of 94 °C for 30 seconds, 48 °C for 30 seconds, and 72 °C for 60 seconds, and a final extension at 72 °C for 5 minutes. The PCR product was then visualised via gel electrophoresis on a 1.5% agarose gel. Amplified DNA was sequenced with Sanger sequencing provided by the University of Otago Genetic Analysis Services. These sequences were then matched with ones uploaded to NCBI using BLAST, which allowed us to confirm the species (or family) of each worm.

6.3.3 Data analysis

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021). Based on the arthropods captured per side per trap, we tested whether the probability of adult hairworm infection was higher in individuals that walked towards the stream versus those that walked away from it. We also tested whether this probability of infection differed between traps placed at increasing distances from the stream. Due to the small number of hairworms present in all of our samples (see results), we decided to use Bayesian multilevel modelling with the *brms* package (McNeish, 2016; Bürkner, 2017). Since infection status for an individual is strictly discrete with only two possible outcomes (infected or uninfected), we implemented a Bernoulli distribution into the models to account for this. Models were built with priors obtained from the "get_prior" function; the "adapt_delta" function was increased to 0.99 to lower the number of divergent transitions after warmup. Stacking weights were computed with the *loo* package (Vehtari et al., 2017) to select the model that best fitted the posterior distribution. The main predictors tested were the walking direction of individuals (two levels; toward or away from the stream, reference level = away) and the distance of the trap (three levels; 5, 25, and 45 m, reference level = 5). We also included as a random factor the family to which each individual arthropod belonged, to account for potential stochastic effects brought by the phylogeny of host taxa. Sampling sites, dates, and transects were all pooled together for this analysis, due to the very low number of adult hairworms collected in hosts (see below).

6.4 Results

6.4.1 General results

A total of 1,969 invertebrates were captured in the pitfall traps and were thus dissected to look for hairworms (1,163 from Site A and 806 from Site B); no vertebrate was captured. Out of the 41 taxa identified (including morphospecies and higher taxonomic groupings, see section 6.3.2), 24 were present in both sites. Notably, a few species of carabid and a species of dermapteran were captured only at Site A. In contrast, some coleopteran species of scarabaeid and scirtid were captured only in Site B. The total number of invertebrates captured per taxon per site varied across sampling dates (Tables 15 and 16 in Appendix C). From these, 21 worms were found inside 17 invertebrates (15 single infections, one double infection, and one quadruple infection). The BLAST results confirmed that 12 were hairworms and three were mermithid nematodes, of which the latter were found in spiders, i.e., one species of Lycosidae and a morphospecies of Amaurobiidae; six worms could not be identified with the DNA sequences obtained from PCRs (Table 6.1). For hairworms, eight mature individuals were found either inside the abdominal cavity of their host or egressing from them (Figure 6.2) (six from Site A and two from Site B). Apart from these, four worms were identified as immature hairworms that had not yet produced their adult cuticle (Table 6.1). Immature hairworms were not included in the statistical analysis, since they cannot leave their host until they mature. From the eight arthropod hosts harbouring mature hairworms, four were caught walking towards the stream and four were caught walking away from it, indicating that there was no trend in the direction of host movement. Four species of hairworm were identified (confirmed with BLAST results) and were found in four insect families across four orders and two families of spider (Table 6.1). The prevalence of each species per host taxa per site varied between 0.4% and 33.3% (the latter estimate was based only on three captured insects).

6.4.2 Statistical results

The taxonomic composition of insect host taxa walking either toward the stream or away from it, in interaction with the distance of pitfall traps relative to the stream, was somewhat consistent across sampling dates in both sites, except for the traps placed at 5 m in Site B, which captured fewer invertebrates in total (Figure 6.3). According to our

selection criteria for Bayesian multilevel modelling, the best model was the null model (stacking weight = 1.000); none of the factors tested here added any predictive value to the null model. Therefore, no effect sizes were observed in both direction of movement and distance from the stream.

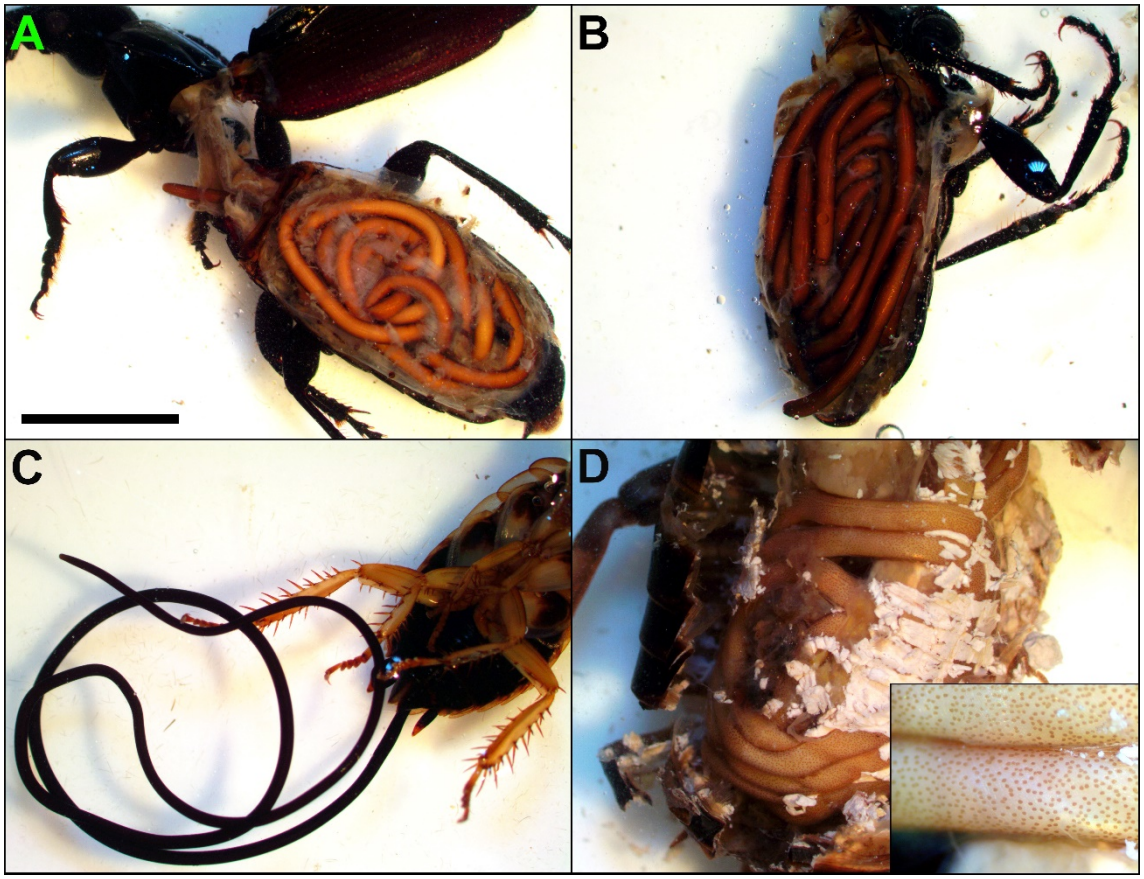


Figure 6.2 Hairworms (phylum Nematomorpha) found within various terrestrial insect hosts captured in pitfall traps in two subalpine locations in Otago, New Zealand. (A) *Gordius paranensis* found inside *Mecodema* sp. (Coleoptera: Carabidae). (B) *Gordius paranensis* found inside *Megadromus* sp. (Coleoptera: Carabidae). (C) *Euchordodes nigromaculatus* found egressing from *Celatoblatta quinquemaculata* (Blattodea: Blattidae). (D) *Parachordodes diblastus* found inside *Hemiandrus* sp. (Orthoptera: Anostostomatidae); the bottom right panel shows a closeup of the hairworm cuticle, which highlights the dark superareoles characteristic of this genus.

Table 6.1 Hairworms (phylum Nematomorpha) and mermithids (phylum Nematoda) found within various invertebrates caught in pitfall traps in two subalpine locations in Otago, New Zealand. Where worm identification was possible, the prevalence was calculated per host species per site.

Hairworm	Site	Identification	Age	Length (mm)	Width (mm)	Host species (family)	Prevalence (total captured)
1	A	*Gordionus maori*	adult	113	0.20	*Celatoblatta quinquemaculata* (Blattidae)	4.8% (21)
2	A	*Gordionus maori*	adult	104	0.13	*Megadromus* sp. (Carabidae)	3.7% (27)
3	A	*Gordionus maori*	adult	57	0.20	Labiidae morphospecies	33.3% (3)
4	A	*Gordius paranensis*	adult	78	0.33	*Holcaspis* sp. (Carabidae)	1.4% (72)
5	A	*Gordius paranensis*	adult	192	0.27	*Mecodema* sp. (Carabidae)	2.7% (37)
6	A	*Gordius paranensis*	adult	331	0.20	*Megadromus* sp. (Carabidae)	7.4% (27)
7	A	*Gordius paranensis*	juvenile	NA	0.20	*Megadromus* sp. (Carabidae)	7.4% (27)
8	A	*Gordius paranensis*	juvenile	NA	0.07	*Anoteropsis* sp. 1 (Lycosidae)	0.4% (229)
9	B	*Euchordodes nigromaculatus*	adult	92	0.13	*Celatoblatta quinquemaculata* (Blattidae)	0.6% (172)
10	B	*Parachordodes diblastus*	adult	300	0.40	*Hemiandrus* sp. (Anostostomatidae)	2.9% (34)
11	B	*Parachordodes diblastus*	juvenile	NA	NA	*Mecodema* sp. (Carabidae)	1.7% (58)
12	B	Nematomorpha sp.	juvenile	45	0.13	Amaurobiidae morphospecies 2***	2.6% (39)
Mermithid	**Site**	**Identification**	**Age**	**Length (mm)**	**Width (mm)**	**Host species (family)**	**Prevalence (total captured)**
1	B	Mermithidae sp. 1	NA	36	0.20	*Anoteropsis* sp. 1 (Lycosidae)**	1.1% (175)
2	B	Mermithidae sp. 1	NA	32	0.13	*Anoteropsis* sp. 1 (Lycosidae)**	1.1% (175)
3	B	Mermithidae sp. 2	NA	71	0.20	Amaurobiidae morphospecies 1***	3.3% (30)
Unidentified worms	**Site**	**Identification**	**Age**	**Length (mm)**	**Width (mm)**	**Host species (family)**	
1	A	unknown	NA	204	0.20	*Mecodema* sp. (Carabidae)	
2	A	unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)*	
3	A	unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)*	
4	A	unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)*	
5	A	unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)*	
6	A	unknown	NA	NA	0.07	*Anoteropsis* sp. 1 (Lycosidae)	

*Same individual host.
**Same individual host.
***These morphospecies were impossible to differentiate from species of the family Desidae.

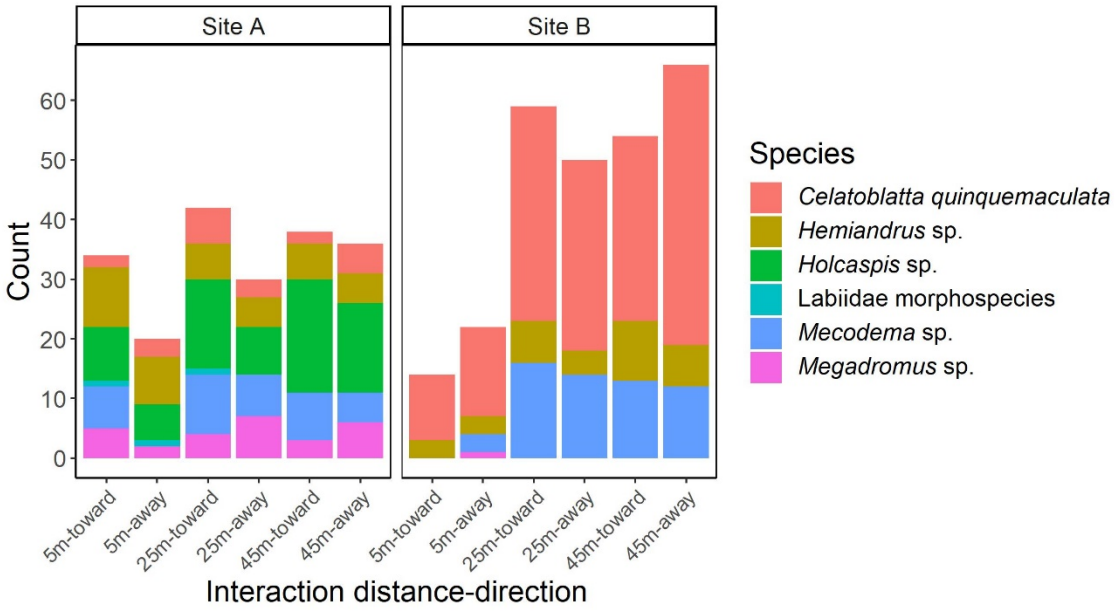


Figure 6.3 Stacked bar graphs of insects captured in pitfall traps in two sampling locations in Otago, New Zealand, with transects and sampling periods pooled together. The "distance" indicates how far the trap was from the stream, whereas "direction" indicates in which direction the insect was walking (toward or away from the stream). Only insect taxa that had at least one individual infected with a hairworm (phylum Nematomorpha) are shown here.

6.5 Discussion

Studying the effects of host manipulation in a natural context can help understand the true impact that parasites have on their hosts. The primary goal of the current study was to investigate the infection dynamics of gordiid hairworms in their definitive terrestrial hosts across two communities of subalpine invertebrates in New Zealand. Although we discovered multiple new hairworm-host associations in both sampling sites, it was not possible to observe whether mature hairworms affected host behaviour to increase their likelihood of entering water or not. This was due to the low number of infected insects captured throughout the sampling season, which can be explained by the overall low prevalence of hairworms observed here. In order to test such host behavioural patterns, we would need a sample size of mature hairworms with enough statistical power to properly quantify the odds that infected insects either approach streams in a directed movement towards water, or simply move around erratically in their environment, which ultimately increases their chances of encountering a stream and falling into it. Ideally, to remove interspecific differences of host manipulation between hairworms, this type of

study should only be done for one species of hairworm. Also, the spatial grid of pitfall traps used here were only active for seven days every month, due to restrictions of the sampling permit. Perhaps if traps were kept open throughout the sampling period and containers were replaced regularly, we would have captured enough infected individuals to properly test our hypotheses in a natural setting.

The four hairworm species collected at our sampling sites have all been previously reported from these locations (Tobias et al., 2017; Yadav et al., 2018). Here, the six insect species and two spider species infected with hairworms represent new host records in New Zealand. *Euchordodes nigromaculatus*, considered an endemic species of hairworm (Poinar, 1991a), has only been reported in three species of acridid and anostostomatid orthopterans that are known to inhabit subalpine habitats (Bigelow, 1967; Poulin, 1995b). Therefore, their occurrence in the alpine cockroach *Celatoblatta quinquemaculata*, which is common at higher altitudes in the Otago region (Worland et al., 2004), is unsurprising. Two species of Blattodea have previously been reported as hosts of unidentified hairworms in New Zealand (Zervos, 1989) and may represent an important group for these parasites (Poinar, 1999). In fact, the relatively high abundance of *C. quinquemaculata* captured in the traps at Site B could make this insect an important host for *E. nigromaculatus*. Although the acridid *Sigaus australis* is a known host species for *E. nigromaculatus* (Poulin, 1995b), none were found infected here. The endemic hairworm *Parachordodes diblastus* has only been observed in three wētā hosts, two species in the genus *Hemideina* and one *Hemiandrus* (Poinar, 1991a), therefore their presence in an undescribed species of the latter genus observed here is unsurprising. However, the ground beetle *Mecodema* sp. was identified as a new host order for *P. diblastus*.

No record exists for the recently described and endemic *Gordionus maori* (Yadav et al., 2018), so the fact that we found this species across three orders of insects, including the new host order Dermaptera for New Zealand, may signify that they have a relatively low host specificity. *Gordius paranensis*, which has also been reported in South America (Schmidt-Rhaesa et al., 2000), was the most numerous species collected in this study. Most individuals were found within ground beetles, which is not uncommon for this

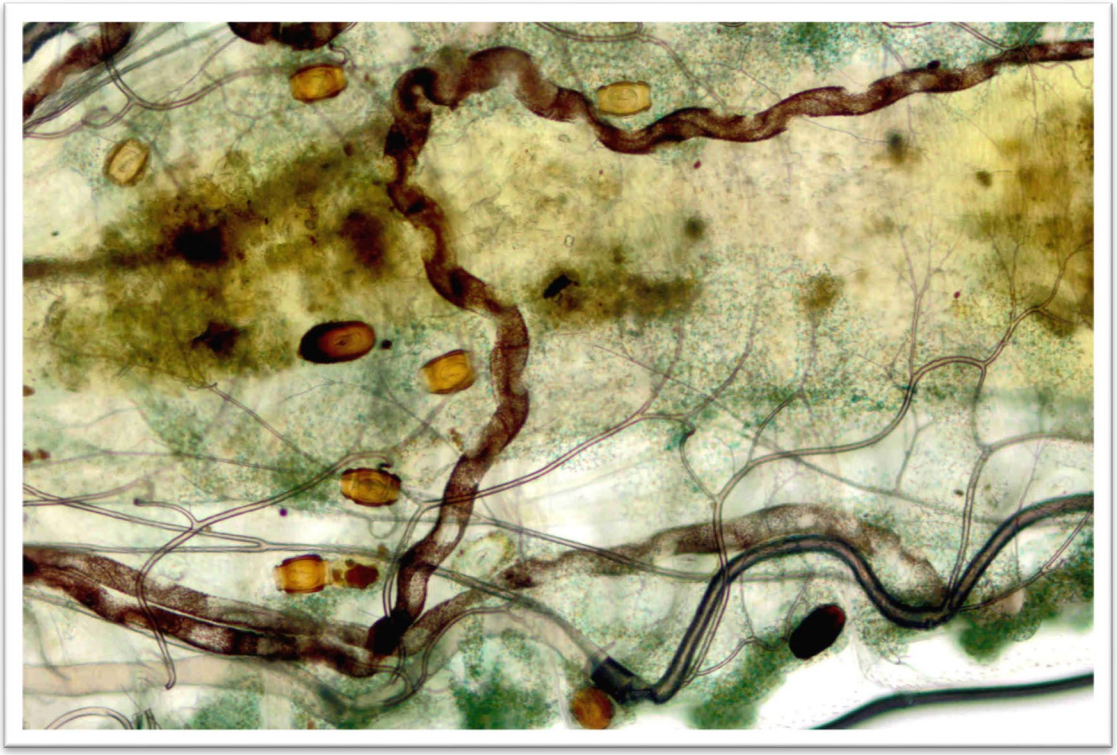
genus (Poinar et al., 2004). However, one juvenile was also found within a species of *Anoteropsis*, a type of wolf spider. Schmidt-Rhaesa (2013) collated records of hairworms in spiders, but still, few exist and doubts have been raised about their veracity (Poinar, 2000). The prevalence of *G. paranensis* was 0.4% in this species of arachnid and the hairworm was identified as a juvenile, therefore this may be a case of spurious infection in a suboptimal host. Another unidentified hairworm was found inside a morphospecies of spider in the family Amaurobiidae (or Desidae); this parasite was also a juvenile.

Two species of mermithid, confirmed with 18S sequences, were found within two families of spiders. There are some reports of mermithids in New Zealand, e.g., Yeates and Buckley (2009) and Presswell et al. (2015), and these arachnid hosts represent new records (Poinar and Early, 1990). Mermithids have been found in spiders in other parts of the world (Penney and Bennett, 2006; Meyer, 2014), which makes these new host-parasite associations unsurprising. With their potential negative impacts on invertebrate populations (Baker and Capinera, 1997), there is a need to better describe the diversity of mermithids in New Zealand. For instance, mermithids have been found in the Cromwell chafer beetle (Bronwen Presswell, personal communication), a critically endangered species that is limited to a small reserve in Central Otago. Therefore, characterising the diversity and host specificity of these parasites could help better assess the risk of infection in endangered populations.

In this study, we report new host-parasite associations for freshwater hairworms and mermithid nematodes, two parasite taxa that typically develop within terrestrial invertebrates. New Zealand hairworms do not appear to be very host-specific, infecting insects across multiple orders. This appears to be a general trait for this phylum (Schmidt-Rhaesa, 2013). Although it was not possible to confirm here, the behaviours of infected hosts may increase their odds of entering water, a crucial step in the life cycle of these parasites. For mermithids, we report new host records, highlighting the need for more research to better understand their distribution in New Zealand. Because of the unique diversity of animals in New Zealand and its varied topography, there is indeed the potential for further host-parasite associations of hairworms and mermithids to be discovered.

Chapter 7

General discussion



Gordius sp. hairworm cysts found in the body cavity (around the digestive tract) of an uncased *Olinga jeanae* caddisfly larva. The amber colour is caused by melanotic encapsulation from the host.

Parasites arguably represent the dominant form of life on this planet. Remarkably, their life history traits, including transmission modes and life cycles, have converged toward six general strategies (Poulin, 2011). In parallel, many parasite lineages have adapted solutions that increase the odds of successfully completing their life cycle, which involve changes in the phenotype of their hosts. This phenomenon, known as host manipulation, includes striking alterations in the behaviour or appearance of hosts that favour parasite transmission to another host or to an environment suitable for reproduction (Moore, 2002). Because of the cryptic nature of many parasitic life cycles, elucidating the hidden interactions between a parasite and its host requires a rigorous exploration of the study system at hand. With this in mind, the current thesis focused on a highly specialised parasite, the freshwater hairworm (phylum Nematomorpha), a poorly understood group of parasites that are well known for the behavioural changes they induce in their terrestrial arthropod hosts. Several broad research questions about the general ecology and host-parasite interactions of hairworms were brought up in the general introduction (Figure 1.2). This thesis provided answers to some of these questions (Figure 7.1).

Initially, the overall aim of this thesis was to provide an in-depth study of the behavioural changes in definitive hosts experimentally infected with hairworms in New Zealand. This type of research can provide strong lines of evidence that host changes are indeed the result of adaptive manipulation by a parasite (Poulin and Maure, 2015). While the experimental infection trials eventually proved to be unsuccessful (Appendix A), the work carried out in parallel provided some novel insights into host manipulation and the challenges hairworms face in their life cycle. Chapter 2 provided a commentary on the impacts of exaggerating what is currently known about host manipulation in both the popular media and the scientific literature, which may ultimately drive the narrative and future research on this complex phenomenon. In Chapter 3, new information on the defence reactions of aquatic paratenic hosts were presented, shedding some light on the potential obstacles that hairworms must overcome inside their aquatic hosts to continue their life cycle. In addition, it was shown that hairworms have to infect the correct paratenic hosts in the community to get to land, otherwise a considerable proportion could end up lost in dead-end hosts (Chapter 4). Despite these challenges that hairworms faced in the water, there was tantalising evidence that hairworms may alter the life history of

their paratenic hosts to accelerate their transmission to land (Chapter 5). In the end, hairworms must manage to infect an appropriate terrestrial host to grow and mature, to eventually cause the latter to enter water so they can reproduce. Only hosts in which hairworm development is possible will succumb to this parasitic manipulation, therefore, discovering new associations between terrestrial hosts and hairworms can help understand their host specificity and distribution in New Zealand (Chapter 6).

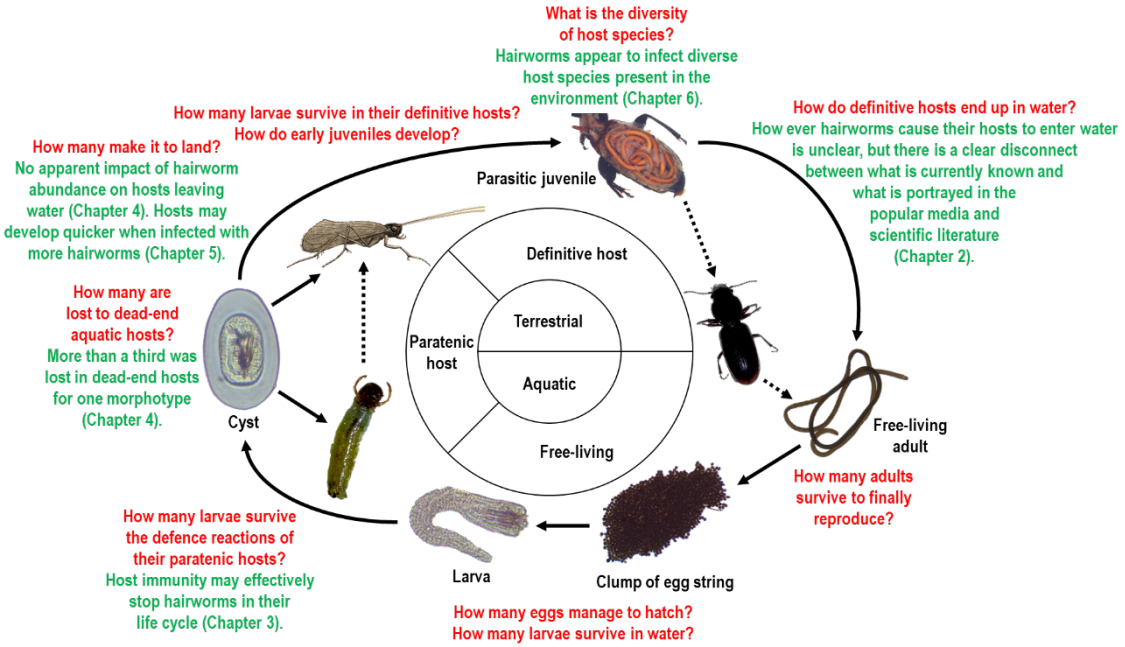


Figure 7.1 Life cycle of freshwater hairworms (phylum Nematomorpha) of the genus *Gordius* in New Zealand. Larvae hatch from short segments of egg string produced by free-living females. They are then consumed by paratenic hosts (here, the caddisfly *Olinga jeanae*), which metamorphose and transport dormant hairworms cysts to land, where they are consumed by definitive hosts (here, the ground beetle *Mecodema* sp.). When mature, hairworms somehow cause their terrestrial hosts to enter water, where they can exit as a free-living adult to mate and reproduce. Some broad questions (in red) on the ecology and host infection dynamics of hairworms are answered (in green) by the research conducted in this thesis. Figure inspired by Bolek et al. (2015).

Hairworms have a unique and complex life cycle, which includes two parasitic stages in an aquatic, then terrestrial host, and two free-living stages in both the earliest and latest phases of the life cycle (Figure 7.1). The odds that an individual hairworm can successfully complete its life cycle and reproduce are extremely low. In fact, there are really three main pressures that could have selected for such a high fecundity observed in females: the loss of hairworms in the environment, either to adverse abiotic conditions or predation (this includes eggs, free-living larvae, and adults, as well as the cysts that are lost on land), the loss of hairworms in dead-end or suboptimal hosts (this includes aquatic

dead-end hosts and the terrestrial hosts in which the development of the hairworm is impossible), and the destruction of infective stages by host immunity (this includes both paratenic and definitive hosts). All in all, these obstacles may account for a high probability of failure for hairworms to complete their life cycle (Schmidt-Rhaesa, 2013). While it may be especially challenging to quantify the proportion of free-living hairworms lost in the environment, mostly due to the small size of larvae and the monumental task of finding them, it is still possible to observe the distribution of hairworm cysts in a community of aquatic macroinvertebrates.

In aquatic environments, hairworm larvae have the potential to infect any animal that consumes them (Bolek et al., 2015). Here, it was possible to observe that, at least for one hairworm morphotype collected in the subalpine streams, over a third of individuals were lost within only a few species of dead-end hosts (Chapter 4). This considerable loss could be explained by the specific egg-laying habits of hairworms, in combination with the feeding habits of hosts. These mechanisms could also explain why hairworm morphotypes followed different host transmission routes in the community. Therefore, if certain key species are present, host community composition could drastically change the survival chances of hairworms in water. In addition to these losses, paratenic hosts, which primarily contribute to the life cycle of hairworms, may also decrease hairworm survival even further through non-specific internal defence reactions (Chapter 3). This host immunity, seen here as melanotic encapsulation, appears to vary between aquatic communities, i.e., it was generally much lower across macroinvertebrates sampled in Chapter 4 than those sampled in a different locality for Chapter 3. Also, it remains unknown whether partially melanised hairworm cysts are still viable. Perhaps this non-specific reaction to parasites, seen in relatively high proportions in some paratenic host taxa (Chapter 3), accounts for additional losses of hairworms. Nonetheless, if host immunity plays only a minor role in impeding the hairworm life cycle, dead-end hosts can represent important population sinks for these parasites. Potentially, future studies could look at how melanotic encapsulation impacts the rate of successful infections in definitive hosts. This would help uncover the true effects of paratenic host immunity on hairworm survival.

Hairworms that successfully infect their true paratenic hosts must eventually be transported to land by the latter, in order to be eaten by a definitive host. If hairworms affect the life history of hosts to maximise their chances of infecting definitive hosts, this may be reflected in the development of the former host. In Chapter 5, it was shown that infected late-instar caddisfly larvae pupated more rapidly if they had a higher intensity (number of parasites per infected individual) of hairworm cysts. While this observational study provided limited evidence of the actual impacts that hairworm infections have on paratenic hosts, it still suggests that development time may correlate inversely with the number of hairworms present in a host. This shorter time to pupation may accelerate the emergence of paratenic hosts on land, which in turn decreases the odds of hairworms being lost to adverse conditions in the water. To further support this line of evidence and test the impact of hairworm infections on paratenic host development, a long-term study in the laboratory with experimental infections would be ideal. However, as with infections of terrestrial definitive hosts, experimental infection of aquatic paratenic hosts could pose logistical challenges.

Although it was not possible to explain the movements of arthropod hosts near streams with the infection data acquired in Chapter 6, several new host records were found for at least four species of New Zealand hairworm. These records support the general conclusion from other authors that the host specificity of hairworms in terrestrial arthropods is usually quite low (Schmidt-Rhaesa, 2013; Bolek et al., 2015). Increasing the known number of hosts for hairworms could help better understand their distribution across New Zealand. There certainly is the potential for many insects and other arthropods to be parasitised with hairworms (Yadav et al., 2018), and there may be many more host-hairworm associations to uncover. However, given the low prevalence observed here in the two subalpine communities of terrestrial arthropods (Chapter 6) and in other studies outside of New Zealand (Schmidt-Rhaesa, 2013), finding new host records for hairworms may require substantial effort.

Previous studies on naturally infected hosts have shown that insects infected with hairworms are far more likely to enter water than uninfected conspecifics (Thomas et al.,

2002; Sato et al., 2011). To date, the proximate evidence of how hairworms cause their definitive hosts to enter water remains very weak (Chapter 2). Simply put, there is a need to experimentally infect insects in the laboratory to closely follow the development of hairworms and match these data to behavioural changes in the host. In parallel, a study on the gene expression of both hairworm and host could provide strong support that this parasite is actively manipulating host behaviour in order to increase its chances of entering water. Despite the lack of data, parasite manipulation of the host in this complex host-parasite system has been exaggerated in both the popular media and the scientific literature, with the use of metaphors and even analogies borrowed from science fiction (Chapter 2). While this may seem harmless at first glance, exaggerating our current understanding of host manipulation may impact its general narrative, which could lead astray the popular perception of this phenomenon. Ultimately, scientists and non-scientists inhabit the same environment, therefore the exchange of information should be as accurate as possible, to limit the spread of fiction.

To recapitulate, it should now be evident to the reader that parasites, in the broadest sense, are likely the most prevalent mode of life on Earth. Moreover, there are remarkable convergences in general life strategies, including life cycles, between the hundreds of parasitic lineages that exist. In parallel to the evolution of these life cycles, many parasites have evolved to alter host phenotype, which ultimately increases parasite transmission and fitness. In this thesis, I provide novel insights into the complex life cycle of a well-known, yet poorly understood, parasite that causes phenotypic changes in its definitive host, i.e., the hairworm. The research conducted throughout this thesis provided answers to some of the many gaps in the knowledge that currently exist for this highly specialised group. However, several research questions on the ecology and host-parasite interactions of hairworms remain (Figure 7.1). Indeed, there is still the potential for much to discover of these fascinating, and sometimes misrepresented, parasites.

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Appendix A

This appendix serves as a full account of all the infection trials that were done during the first two and a half years of my doctoral project, in the goal of experimentally infecting insects with a species of hairworm from the genus *Gordius* to study host behavioural changes throughout hairworm development. At the end of each trial, insects were carefully opened with fine tweezers to look for hairworms under a dissecting microscope. None of the trials resulted in any hairworm infections.

Account of the infection trials

To establish experimental hairworm infections, scouting was done in areas surrounding Dunedin during March 2018 to locate populations of cave wētā (Orthoptera: Rhaphidophoridae) possibly harbouring hairworms. Only two populations of the cave wētā *Pleioplectron simplex*, in Silver Peaks and Trotters Gorge, were found in sufficient numbers to collect and practice maintaining them under artificial conditions. These two populations were screened and no hairworms were detected, making them ideal candidates for experimental infections.

Hairworm collection in the field was done at Cass Field Station, near Arthur's Pass in Canterbury (see Figure 3.1). The first collection was in June 2018, when both cave wētā (also *P. simplex*) and stream macroinvertebrates were collected to improve rearing practices and assess the distribution of hairworm cysts in different aquatic hosts, respectively. The cave wētā were kept in the Animal Containment Facility at the Department of Zoology, in conditions similar to their natural environment, i.e., temperature fluctuating daily between 12 °C (night) and 16 °C (day), with a L12:D12 photoperiod. The samples of stream macroinvertebrates were processed in the laboratory and hairworm cysts were counted in the tissues, following the methods described in section 3.3. This allowed us to know the exact number of hairworms fed to each host. These were then frozen separately in microtubes at -20 °C for two weeks, after which they were fed to 48-hour starved cave wētā (approximately 40) isolated in individual plastic containers with water provided ad libitum. Hairworms in aquatic host tissues exposed to temperatures below zero have been shown to improve the experimental infection rates in definitive hosts (Bolek et al., 2013b). After ingesting cyst-infected

tissues, cave wētā were fed ground commercial cat food mixed with ground oats (50:50 weight) and kept alive for a month before dissections.

Two more field outings took place in December 2018 and January 2019 at Cass Field Station in an attempt to collect adult hairworms in the streams and have them reproduce in the laboratory. In December 2018, 17 adult hairworms were found, but all were male, so mating them was impossible. In January 2019, 81 hairworms of two species were collected. Of the species of interest (*Gordius* sp.), only five females were collected. These were each paired with several males in individual containers, at room temperature, filled with aerated water collected from the stream, in an attempt to have them mate. Only one of these females started producing eggs a few weeks later and these were kept in aerated water and observed every two weeks for hatching. After two months, none of the eggs hatched and no hairworm larvae were obtained from this sampling effort. Reproducing the entire life cycle of the hairworm in the laboratory therefore appeared unlikely.

The next attempt at experimentally infecting cave wētā involved feeding them fresh paratenic hosts directly, without counting cysts and freezing them. Stream macroinvertebrates were collected in May 2019 at Cass Field Station, but no hairworm cysts were found. Therefore, the same stream was re-sampled in June 2019 and macroinvertebrates were screened on site by setting up a laboratory at the field station, in an attempt to collect a sufficient number of cysts. The aquatic macroinvertebrates sampled here, namely the caddisfly *Olinga jeanae* that harboured a high abundance of hairworm cysts (see Chapter 3), were brough back to the laboratory and kept alive in tanks with aerated stream water until they were fed to hosts. A group of 20 *O. jeanae* larvae were examined for cysts to calculate a hairworm abundance, to estimate the number of hairworms fed to each experimental host.

For the next experimental infection trial, approximately 220 cave wētā were collected during two field trips to Silver Peaks, the first in July 2019 and the second in August 2019. In early September 2019, these cave wētā were tagged by gluing bee tags to their thorax in order to identify individual hosts during behavioural trials. Around mid-September 2019, almost 200 cave wētā suddenly died in the temperature control room.

This was likely attributed to a malfunction of the humidity control; water was pooling in the rooms and in the hallway of the Animal Containment Facility a few days after this incident. The cave wētā from Silver Peaks live in tunnels with a relative humidity near constant saturation (confirmed with data loggers). This would have made them especially vulnerable if the humidity in the room suddenly dropped for a few days.

Many cave wētā were collected from Silver Peaks for the experimental infection. Unfortunately, this cave wētā population appears to live in a very narrow range of abiotic conditions, which probably makes them quite vulnerable to sudden changes in temperature and humidity. In other words, I did not want to deplete a natural population of cave wētā that were apparently not ideal subjects under artificial conditions. Therefore, another field trip was conducted in early October 2019 to try and locate other cave wētā populations near Dunedin, but this was unsuccessful. It was decided that the cave wētā at Cass Field Station in Canterbury, where infected and uninfected hosts were found, would be collected for the experimental trials. This species has previously been kept for months in the temperature control room, which makes them ideal candidates, although collecting high numbers is difficult. Therefore, three field trips were made to Cass in October 2019, December 2019, and March 2020. Unfortunately, due likely to the generally cold weather experienced during that summer, cave wētā were found in very low numbers (less than a dozen were spotted during all three trips), making it impossible to collect these hosts for behavioural experiments.

Considering all of the above, we decided to use experimental hosts, i.e., insects reared under artificial conditions. American cockroaches (*Periplaneta americana*) that were reared from eggs since the beginning of the project and black field crickets (*Teleogryllus commodus*) ordered online were used for experimental infections. These insects were kept at a constant 25 °C, with the same photoperiod previously used for the cave wētā. During March 2020, preliminary behavioural trials were tested on uninfected crickets and the efficiency of the movement tracking software was assessed with the experimental arenas. Unfortunately, crickets started dying during the third week into these trials, probably due to ingesting the petroleum-based paste that was used to stop them from climbing the arena walls. The arenas were therefore redesigned to account for this, but this experiment had

to be cancelled due to the nationwide lockdown in late March 2020. Instead, smaller infection trials were done during this time with 20 individuals from both species. Here, insects were starved for 72 hours, then fed approximately 30 cysts each (based on the abundance of hairworms calculated from a subsample of *O. jeanae* larvae, see above) in freshly killed caddisfly larvae. These insects were kept under similar isolation conditions as the previous infection trial on cave wētā, and were dissected after two months.

A final infection trial was tested on 160 black field crickets, starting in June 2020. Insects were starved and isolated as was done in previous trials. This time, using *O. jeanae* larvae collected earlier that month at Cass Field Station (again, a subsample of 20 larvae were examined for hairworm cysts to calculate an abundance), 80 crickets were fed fresh caddisfly tissues and 80 were fed caddisfly tissues that had been decomposing for a week in an incubator at 25 °C (for each group, half were fed approximately 40 cysts each, the other half were fed approximately 80 cysts each). This was to test whether decomposing paratenic hosts had any impact on infection success in definitive hosts. This trial lasted three months before insects were dissected to look for hairworms.

To summarise this account, I attempted to infect four different types of hosts with hairworms (two natural populations of a species of cave wētā and two species of laboratory-reared insects). Tissues of natural paratenic hosts (*O. jeanae* larvae) harbouring hairworm cysts were fed to these insects either fresh, frozen, or after they had decomposed. Previous studies have been able to effectively domesticate the life cycle of hairworms of various species (Hanelt and Janovy, 2004b; Bolek et al., 2013a), however it proved to be unsuccessful here. Ideally, many mated female hairworms would have to be collected from the field in order to obtain enough free-living larvae to infect experimental paratenic hosts, such as aquatic snails used in the aforementioned studies. This would allow for a better control of the various life stages in this complex life cycle, which could improve the odds of domesticating hairworms in the laboratory.

Appendix B

B.1 Figure 2.1A

This section of the appendix includes all the online newspaper and magazine articles, from 2006 to 2019, covering host manipulation that were used to generate the word cloud in Figure 2.1A.

B.1.1 Methods

Online newspapers and magazine articles were searched in the Google search engine using the combinations of the word "parasite" with "manipulation", "mind", "hijack", or "zombie". Then, articles were randomly selected within the first five pages of results. A text file was created to include the titles and headlines from all the selected articles. This file was then used to generate a word cloud with packages *tm*, *wordcloud*, and *RColorBrewer* in R version 3.6.3 (R Core Team, 2020).

B.1.2 List of online newspaper and magazine articles

1 Mind Control by Parasites (2006)
Bill Christensen, Live Science
https://www.livescience.com/7019-mind-control-parasites.html

2 Crazy eyes and mind control – the power of parasites (2014)
Micaela Jemison, Smithsonian Insider
https://insider.si.edu/2014/11/crazy-eyes-mind-control-power-parasites/

3 Mindsuckers (2014)
Carl Zimmer, National Geographic
https://www.nationalgeographic.com/magazine/2014/11/mindsuckers/

4 There Are Hundreds of Examples of Mind-Controlling Parasites (2014)
Colin Schultz, Smithsonian Magazine
https://www.smithsonianmag.com/smart-news/there-are-hundreds-examples-mind-controlling-parasites-180950312/

5 These Parasite Bugs Can Control Their Hosts' Brains From Inside (2014)
Jesus Diaz, Gizmodo
https://www.gizmodo.com.au/2014/11/these-horrible-mindsucker-bugs-can-infect-and-control-their-hosts/

6 6 Of Nature's Most Horrifying Mind-Controlling Parasites (2015)
Tom Chivers, BuzzFeed
https://www.buzzfeed.com/tomchivers/parasites-that-control-your-brain

7 Engrossing Portraits of Parasites and the Creatures They Zombify (2015)
Alyssa Coppelman, Wired
https://www.wired.com/2015/12/anand-varma-mindsuckers/

8 Meet the Parasites That Control Human Brains (2015)
Ben Thomas, Discover Magazine
https://www.discovermagazine.com/planet-earth/meet-the-parasites-that-control-human-brains

9 Ten sinister parasites that control their hosts' minds (2015)
Lucy Jones, BBC Earth
http://www.bbc.com/earth/story/20150316-ten-parasites-that-control-minds

10 The Truth About The Mind-Controlling Parasite You Can Get From Your Cat (2015)
Chelsea Harvey, Business Insider Australia
https://www.businessinsider.com.au/toxoplasma-gondii-the-cat-brain-parasite-2015-1

11 How mind-controlling parasites can get inside your head (2016)
Alex Ford, The Conversation
https://theconversation.com/how-mind-controlling-parasites-can-get-inside-your-head-57131

12 You Know Those Parasites That Control Our Brains? (2016)
Bradley van Pardon, Scientific American
https://blogs.scientificamerican.com/guest-blog/you-know-those-parasites-that-control-our-brains/

13 Watch a brain-hijacking parasite sneak its way in (2016)
Belinda Smith, Cosmos Magazine
https://cosmosmagazine.com/biology/watch-brain-hijacking-parasite-sneak-its-way

14 Invasion of the Brain Snatchers (2017)
David Suzuki, The Nature of Things
https://www.cbc.ca/natureofthings/episodes/invasion-of-the-body-snatchers

15 Parasite turns mice into mindless cat-fighting zombies by hijacking immune cells (2017)
Bob McDonald, CBC Radio
https://www.cbc.ca/radio/quirks/parasite-turns-mice-into-mindless-cat-fighting-zombies-by-hijacking-immune-cells-1.4444512

16 Puppeteer parasite that creates zombie ants hijacks their bodies – not brains (2017)
Hannah Osborne, Newsweek
https://www.newsweek.com/parasite-zombie-ants-hijacks-bodies-not-brains-707816

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Mary Bates, National Geographic
https://www.nationalgeographic.com/news/2018/10/141031-zombies-parasites-animals-science-halloween/

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Samuel Osborne, The Independent
https://www.independent.co.uk/news/science/parasite-cat-faeces-mind-alter-humans-courage-fear-failure-toxoplasma-gondii-a8463436.html

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Ed Yong, The Atlantic
https://www.theatlantic.com/science/archive/2018/06/what-tapeworms-really-want/563189/

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Katie Pavid, Natural History Museum
https://www.nhm.ac.uk/discover/news/2018/june/the-brain-worm-that-turns-ants-into-zombies.html

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Conn Hastings, Frontiers Science News
https://blog.frontiersin.org/2018/05/15/psychology-parasites-insect-behavior/

22 Zombie insects: Four ways parasites 'hijack' the brains of their unsuspecting hosts (2018)
Danielle Edwards, National Post
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23 Creepy zombie snail flash green and orange as eyeball invading parasite force doomed creature to kill itself (2019)
Sean Keach, The Sun
https://www.thesun.co.uk/tech/9713906/creepy-zombie-snail-eyeball-parasite-flashing/

24 Inside the Forever War Against Parasites Trying To Control Our Brains (2019)
Joe Pappalardo, Popular Mechanics
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Vanessa Chalmers, Daily Mail Australia
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B.2 Figure 2.1B

Scientific papers that were used to create the graph in Figure 2.1B. For each group, the search terms used in Web of Science are provided in parentheses. Papers in each group are listed chronologically, according to their year of publication. Note that the following references have been formatted differently to those in the references section of the thesis.

B.2.1 Bodyguard

("*parasit*" AND ("bodyguard*" OR "body guard*" OR "body-guard*"))

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B.2.2 Bodysnatcher

("*parasit*" AND ("bodysnatch*" OR "body snatch*" OR "body-snatch*"))

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B.2.3 Hijack

("*parasit*" AND "hijack*" NOT ("bacteri*" OR "viru*" OR "viral*"))

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B.2.4 Puppeteer

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B.2.5 Zombie

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Appendix C

This appendix includes all of the supplementary tables for Chapter 4 (Tables 1 to 14) and Chapter 6 (Tables 15 and 16).

Table 1. Aquatic host taxa collected in Surber samples per sampling date for Stream A. The number of individuals collected is accompanied by the average number per individual of Type A hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic, "T" for terrestrial, and "B" for both).

Order	Family	Genus or species (adult type)	2020-10-30	2020-11-28	2021-01-10	2021-01-30	2021-02-27	2021-03-27	2021-05-01
Araneae	Pisauridae	*Dolomedes* sp. (B)	-	-	-	-	3	-	-
Haplotaxida	NA	NA (A)	4, 7.75 (75%)	6	7, 5.00 (29%)	-	-	3	4, 31.25 (75%)
Coleoptera	Elmidae	Morphospecies 1 (A)	8	8	12	3, 0.33 (33%)	-	1	-
		Morphospecies 2 (A)	-	-	-	1, 2.00 (100%)	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	-	-	1	-	-	-	18, 0.06 (6%)
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	376*	476*	364*	160*	464*	340*	104*
		spp. (T)	2112*	2684*	4*	80*	440*	-	68*
	Dixidae	*Nothodixa* sp. (T)	3	-	-	-	-	-	-
	Muscidae	Morphospecies 1 (T)	-	-	9	3	2	1	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
		Morphospecies 3 (T)	-	-	-	3, 0.33 (33%)	-	-	-
	Simuliidae	*Austrosimulium* sp. (T)	-	-	-	2	-	1	-
	Stratiomyidae	Morphospecies 1 (T)	1	2	2	-	-	-	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
	Tabanidae	Morphospecies 1 (T)	1	-	-	-	1	-	12, 0.08 (8%)
		Morphospecies 2 (T)	-	-	-	-	-	-	1
	Tipulidae	Morphospecies 1 (T)	2	7	2, 0.50 (50%)	4, 0.25 (25%)	1	8, 0.25 (13%)	2
		Morphospecies 3 (T)	-	1	1	1, 2.00 (100%)	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	-	1, 1.00 (100%)	3	1	4	1	8
		Deleatidium fumosum (T)	7, 0.86 (43%)	4, 0.50 (50%)	5, 0.80 (20%)	7	27	26, 0.42 (35%)	51, 0.11 (9%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	-	1
Plecoptera	Austroperlidae	*Austroperla cyrene* (T)	-	-	-	1, 6.00 (100%)	-	-	-
	Gripopterygidae	*Zelandobius* sp. 1 (T)	5	2, 3.50 (100%)	-	1	4	5	21, 0.19 (14%)
		Zelandoperla sp. (T)	-	-	-	2, 8.50 (100%)	6, 0.83 (17%)	1, 1.00 (100%)	6, 0.67 (33%)
	Notonemouridae	*Spaniocerca* sp. (T)	-	-	-	-	1, 20.00 (100%)	-	-
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	-	-	-	-	-	5, 0.60 (60%)	3, 0.33 (33%)
		Pycnocentria sp. (T)	113, 0.18 (14%)*	140, 0.25 (10%)*	67, 0.20 (14%)*	253, 0.80 (32%)*	179, 0.40 (22%)*	288, 0.47 (22%)*	293, 0.23 (15%)*
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	1	-	1	9	141, 0.06 (6%)*	17, 1.12 (12%)
		Psilochorema sp. (T)	18, 0.06 (6%)	10	4	2	4	59	56, 0.09 (9%)
		Tiphobiosis sp. (T)	2, 0.50 (50%)	-	-	12, 2.83 (8%)	12	32, 0.13 (9%)	22
	Leptoceridae	*Hudsonema alienum* (T)	-	-	-	-	-	1, 1.00 (100%)	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	1	-	-	-	-
		Pseudoeconesus sp. (T)	-	-	-	-	-	-	1
NA	Planorbidae	*Gyraulus* sp. (A)	-	-	1	-	-	-	-
Amphipoda	NA	NA (A)	-	-	-	-	-	1	-
Myodocopida	NA	NA (A)	4	2	-	-	-	2	1
Tricladida	Dugesiidae	*Neppia* sp. (A)	21	10	14	5	8	17	81, 0.32 (3%)*

*Estimate based on a subsample for that sampling date.

Table 2. Aquatic host taxa collected in Surber samples per sampling date for Stream A. The number of individuals collected is accompanied by the average number per individual of Type B hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic, "T" for terrestrial, and "B" for both).

Order	Family	Genus or species (adult type)	2020-10-30	2020-11-28	2021-01-10	2021-01-30	2021-02-27	2021-03-27	2021-05-01
Araneae	Pisauridae	*Dolomedes* sp. (B)	-	-	-	-	3	-	-
Haplotaxida	NA	NA (A)	4	6	7, 0.29 (29%)	-	-	3	4, 0.25 (25%)
Coleoptera	Elmidae	Morphospecies 1 (A)	8	8	12	3	-	1	-
		Morphospecies 2 (A)	-	-	-	1, 3.00 (100%)	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	-	-	1	-	-	-	18
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	376*	476*	364*	160*	464*	340*	104*
		spp. (T)	2112*	2684*	4*	80*	440*	-	68*
	Dixidae	*Nothodixa* sp. (T)	3	-	-	-	-	-	-
	Muscidae	Morphospecies 1 (T)	-	-	9	3	2	1	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
		Morphospecies 3 (T)	-	-	-	3	-	-	-
	Simuliidae	*Austrosimulium* sp. (T)	-	-	-	2	-	1	-
	Stratiomyidae	Morphospecies 1 (T)	1	2	2	-	-	-	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
	Tabanidae	Morphospecies 1 (T)	1	-	-	-	1	-	12
		Morphospecies 2 (T)	-	-	-	-	-	-	1
	Tipulidae	Morphospecies 1 (T)	2	7	2, 0.50 (50%)	4	1	8	2
		Morphospecies 3 (T)	-	1	1	1	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	-	1	3, 1.33 (66%)	1	4	1	8, 0.25 (25%)
		Deleatidium fumosum (T)	7, 7.57 (43%)	4, 9.25 (100%)	5, 6.40 (40%)	7	27, 0.10 (10%)	26, 0.12 (8%)	51, 0.61 (11%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	-	1
Plecoptera	Austroperlidae	*Austroperla cyrene* (T)	-	-	-	1, 3.00 (100%)	-	-	-
	Gripopterygidae	*Zelandobius* sp. 1 (T)	5, 0.20 (20%)	2, 0.50 (50%)	-	1	4, 0.25 (25%)	5, 0.20 (20%)	21
		Zelandoperla sp. (T)	-	-	-	2, 0.50 (50%)	6	1	6
	Notonemouridae	*Spaniocerca* sp. (T)	-	-	-	-	1, 1.00 (100%)	-	-
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	-	-	-	-	-	5	3
		Pycnocentria sp. (T)	113	140, 0.02 (2%)*	67, 0.04 (4%)*	253, 0.02 (2%)*	179, 0.02 (2%)*	288	293
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	1	-	1	9	141	17, 0.12 (6%)
		Psilochorema sp. (T)	18, 0.06 (6%)	10	4	2	4	59	56, 0.03 (3%)
		Tiphobiosis sp. (T)	2	-	-	12	12	32, 0.03 (3%)	22
	Leptoceridae	*Hudsonema alienum* (T)	-	-	-	-	-	1	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	1	-	-	-	-
		Pseudoeconesus sp. (T)	-	-	-	-	-	-	1
NA	Planorbidae	*Gyraulus* sp. (A)	-	-	1	-	-	-	-
Amphipoda	NA	NA (A)	-	-	-	-	-	1	-
Myodocopida	NA	NA (A)	4	2	-	-	-	2	1
Tricladida	Dugesiidae	*Neppia* sp. (A)	21	10	14	5	8	17	81

*Estimate based on a subsample for that sampling date.

Table 3. Aquatic host taxa collected in Surber samples per sampling date for Stream A. The number of individuals collected is accompanied by the average number per individual of Type C hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic, "T" for terrestrial, and "B" for both).

Order	Family	Genus or species (adult type)	2020-10-30	2020-11-28	2021-01-10	2021-01-30	2021-02-27	2021-03-27	2021-05-01
Araneae	Pisauridae	*Dolomedes* sp. (B)	-	-	-	-	3	-	-
Haplotaxida	NA	NA (A)	4	6	7	-	-	3	4
Coleoptera	Elmidae	Morphospecies 1 (A)	8	8	12	3	-	1	-
		Morphospecies 2 (A)	-	-	-	1	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	-	-	1	-	-	-	18
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	376*	476*	364*	160*	464*	340*	104*
		spp. (T)	2112*	2684*	4*	80*	440*	-	68*
	Dixidae	*Nothodixa* sp. (T)	3	-	-	-	-	-	-
	Muscidae	Morphospecies 1 (T)	-	-	9	3	2	1	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
		Morphospecies 3 (T)	-	-	-	3	-	-	-
	Simuliidae	*Austrosimulium* sp. (T)	-	-	-	2	-	1	-
	Stratiomyidae	Morphospecies 1 (T)	1	2	2	-	-	-	-
		Morphospecies 2 (T)	-	-	-	1	-	-	-
	Tabanidae	Morphospecies 1 (T)	1	-	-	-	1	-	12
		Morphospecies 2 (T)	-	-	-	-	-	-	1
	Tipulidae	Morphospecies 1 (T)	2	7	2	4	1	8	2
		Morphospecies 3 (T)	-	1	1	1	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	-	1	3, 10.33 (100%)	1	4	1	8
		Deleatidium fumosum (T)	7, 2.86 (29%)	4	5, 0.80 (40%)	7, 1.43 (71%)	27	26, 1.27 (50%)	51, 0.72 (28%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	-	1
Plecoptera	Austroperlidae	*Austroperla cyrene* (T)	-	-	-	1, 1.00 (100%)	-	-	-
	Gripopterygidae	*Zelandobius* sp. 1 (T)	5	2	-	1	4, 0.25 (25%)	5	21
		Zelandoperla sp. (T)	-	-	-	2	6	1	6
	Notonemouridae	*Spaniocerca* sp. (T)	-	-	-	-	1	-	-
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	-	-	-	-	-	5	3
		Pycnocentria sp. (T)	113	140	67	253	179	288	293
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	1	-	1	9	141	17
		Psilochorema sp. (T)	18	10	4	2	4	59	56
		Tiphobiosis sp. (T)	2	-	-	12	12	32	22
	Leptoceridae	*Hudsonema alienum* (T)	-	-	-	-	-	1	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	1	-	-	-	-
		Pseudoeconesus sp. (T)	-	-	-	-	-	-	1
NA	Planorbidae	*Gyraulus* sp. (A)	-	-	1	-	-	-	-
Amphipoda	NA	NA (A)	-	-	-	-	-	1	-
Myodocopida	NA	NA (A)	4	2	-	-	-	2	1
Tricladida	Dugesiidae	*Neppia* sp. (A)	21	10	14	5	8	17	81

*Estimate based on a subsample for that sampling date.

Table 4. Aquatic host taxa collected in Surber samples per sampling date for Stream B. The number of individuals collected is accompanied by the average number per individual of Type A hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic and "T" for terrestrial).

Order	Family	Genus or species (adult type)	2020-10-29	2020-11-29	2021-01-11	2021-01-31	2021-02-28	2021-03-27	2021-05-01
Haplotaxida	NA	NA (A)	15, 0.13 (7%)	12, 7.17 (25%)	4	-	1	1	3
Coleoptera	Elmidae	Morphospecies 1 (A)	-	1	1, 1.00 (100%)	-	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	1	-	-	-	-	-	-
	Ptilodactylidae	Morphospecies 1 (T)	-	-	1	-	-	-	-
	Scirtidae	Morphospecies 1 (T)	-	-	3	2	7	20, 0.10 (10%)	-
		Morphospecies 2 (T)	-	-	-	-	-	2	18, 0.06 (6%)
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	28*	-	8*	-	12*	-	-
		spp. (T)	20*	24*	104*	4*	36*	12*	24*
	Simuliidae	*Austrosimulium* sp. (T)	-	2	3	-	3	-	-
	Stratiomyidae	Morphospecies 2 (T)	-	1	-	-	-	-	-
	Tabanidae	Morphospecies 1 (T)	30, 0.79 (47%)	19, 2.00 (63%)	6, 0.50 (33%)	-	13, 0.62 (46%)	20, 0.70 (45%)	7, 1.00 (71%)
		Morphospecies 2 (T)	4	-	-	-	-	-	-
	Tipulidae	Morphospecies 1 (T)	-	-	-	-	3	-	-
		Morphospecies 2 (T)	3	3	1	1	1	8, 0.38 (25%)	2
		Morphospecies 4 (T)	-	1	-	-	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	13, 3.92 (31%)	6, 0.17 (17%)	5	-	8, 0.25 (25%)	1	6, 1.00 (67%)
		Deleatidium fumosum (T)	4, 0.25 (25%)	6	12	-	19, 0.32 (21%)	8, 0.38 (25%)	36, 0.23 (15%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	1	-
Mecoptera	Nannochoristidae	*Nannochorista philpotti* (T)	5	12, 3.17 (92%)	1, 3.00 (100%)	1, 1.00 (100%)	3, 3.33 (100%)	7, 3.57 (86%)	7, 1.29 (57%)
Plecoptera	Gripopterygidae	*Zelandobius* sp. 1 (T)	13, 1.00 (31%)	4, 1.50 (50%)	1, 1.00 (100%)	-	17, 0.18 (18%)	15, 1.13 (53%)	29, 1.10 (45%)
		Zelandobius sp. 2 (T)	2	-	-	-	-	-	-
		Zelandoperla sp. (T)	-	7, 1.86 (57%)	3, 0.33 (33%)	-	2, 10.00 (100%)	9, 9.33 (78%)	6, 3.83 (50%)
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	1	-	-	-	-	-	-
		Pycnocentria sp. (T)	-	-	-	-	-	1	10
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	-	-	-	1	-	1
		Psilochorema sp. (T)	1	1	-	-	2	1	-
		Tiphobiosis sp. (T)	-	-	-	-	-	1	2
	Leptoceridae	*Hudsonema amabile* (T)	-	-	-	-	1	1	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	2, 0.50 (50%)	1	-	-	2
	Philopotamidae	*Hydrobiosella mixta* (T)	-	1, 3.00 (100%)	-	-	-	-	-
		Hydrobiosella sp. (T)	-	1	-	-	-	-	-
	Philorheithridae	*Philorheithrus* sp. (T)	-	-	2	-	-	-	-
Tricladida	Dugesiidae	*Neppia* sp. (A)	2	1	2	-	4	4	10

*Estimate based on a subsample for that sampling date.

Table 5. Aquatic host taxa collected in Surber samples per sampling date for Stream B. The number of individuals collected is accompanied by the average number per individual of Type B hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic and "T" for terrestrial).

Order	Family	Genus or species (adult type)	2020-10-29	2020-11-29	2021-01-11	2021-01-31	2021-02-28	2021-03-27	2021-05-01
Haplotaxida	NA	NA (A)	15, 9.07 (27%)	12, 0.42 (8%)	4	-	1	1	3
Coleoptera	Elmidae	Morphospecies 1 (A)	-	1	1	-	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	1, 1.00 (100%)	-	-	-	-	-	-
	Ptilodactylidae	Morphospecies 1 (T)	-	-	1	-	-	-	-
	Scirtidae	Morphospecies 1 (T)	-	-	3	2	7	20, 0.05 (5%)	-
		Morphospecies 2 (T)	-	-	-	-	-	2	18
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	28*	-	8*	-	12*	-	-
		spp. (T)	20*	24*	104, 0.04 (4%)*	4*	36*	12*	24*
	Simuliidae	*Austrosimulium* sp. (T)	-	2	3	-	3	-	-
	Stratiomyidae	Morphospecies 2 (T)	-	1	-	-	-	-	-
	Tabanidae	Morphospecies 1 (T)	30, 1.95 (58%)	19, 0.58 (42%)	6, 1.00 (17%)	-	13	20	7, 0.29 (14%)
		Morphospecies 2 (T)	4, 0.25 (25%)	-	-	-	-	-	-
	Tipulidae	Morphospecies 1 (T)	-	-	-	-	3	-	-
		Morphospecies 2 (T)	3	3	1	1	1	8	2
		Morphospecies 4 (T)	-	1	-	-	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	13, 7.31 (62%)	6, 0.67 (50%)	5	-	8	1	6, 0.33 (33%)
		Deleatidium fumosum (T)	4, 2.25 (100%)	6, 1.00 (50%)	12, 0.25 (17%)	-	19	8, 0.38 (25%)	36, 0.11 (8%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	1	-
Mecoptera	Nannochoristidae	*Nannochorista philpotti* (T)	5, 7.80 (100%)	12, 0.33 (25%)	1	1, 1.00 (100%)	3	7, 0.29 (29%)	7, 0.57 (57%)
Plecoptera	Gripopterygidae	*Zelandobius* sp. 1 (T)	13, 0.15 (15%)	4, 0.50 (25%)	1	-	17, 0.12 (12%)	15, 0.07 (7%)	29, 0.17 (14%)
		Zelandobius sp. 2 (T)	2, 0.50 (50%)	-	-	-	-	-	-
		Zelandoperla sp. (T)	-	7, 0.71 (29%)	3	-	2, 0.50 (50%)	9, 1.22 (67%)	6, 0.50 (33%)
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	1	-	-	-	-	-	-
		Pycnocentria sp. (T)	-	-	-	-	-	1	10
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	-	-	-	1	-	1
		Psilochorema sp. (T)	1	1	-	-	2	1	-
		Tiphobiosis sp. (T)	-	-	-	-	-	1	2
	Leptoceridae	*Hudsonema amabile* (T)	-	-	-	-	1	1	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	2	1	-	-	2
	Philopotamidae	*Hydrobiosella mixta* (T)	-	1	-	-	-	-	-
		Hydrobiosella sp. (T)	-	1	-	-	-	-	-
	Philorheithridae	*Philorheithrus* sp. (T)	-	-	2	-	-	-	-
Tricladida	Dugesiidae	*Neppia* sp. (A)	2	1	2	-	4	4	10

*Estimate based on a subsample for that sampling date.

Table 6. Aquatic host taxa collected in Surber samples per sampling date for Stream B. The number of individuals collected is accompanied by the average number per individual of Type C hairworm (phylum Nematomorpha) cysts and larvae with their prevalence (proportion of infected individuals) in parentheses. For each species, the adult habitat is provided in parentheses ("A" for aquatic and "T" for terrestrial).

Order	Family	Genus or species (adult type)	2020-10-29	2020-11-29	2021-01-11	2021-01-31	2021-02-28	2021-03-27	2021-05-01
Haplotaxida	NA	NA (A)	15, 0.20 (13%)	12	4	-	1	1	3
Coleoptera	Elmidae	Morphospecies 1 (A)	-	1	1	-	-	-	-
	Hydraenidae	*Orchymontia* sp. (A)	1	-	-	-	-	-	-
	Ptilodactylidae	Morphospecies 1 (T)	-	-	1	-	-	-	-
	Scirtidae	Morphospecies 1 (T)	-	-	3	2	7	20	-
		Morphospecies 2 (T)	-	-	-	-	-	2	18
Diptera	Chironomidae	*Maoridiamesa* sp. (T)	28*	-	8, 4.00 (100%)*	-	12*	-	-
		spp. (T)	5	24*	104, 3.88 (73%)*	4*	36*	12*	24*
	Simuliidae	*Austrosimulium* sp. (T)	-	2	3	-	3	-	-
	Stratiomyidae	Morphospecies 2 (T)	-	1	-	-	-	-	-
	Tabanidae	Morphospecies 1 (T)	30, 0.67 (37%)	19, 0.32 (21%)	6, 3.67 (67%)	-	13, 3.31 (85%)	20, 3.10 (35%)	7, 0.86 (29%)
		Morphospecies 2 (T)	4	-	-	-	-	-	-
	Tipulidae	Morphospecies 1 (T)	-	-	-	-	3	-	-
		Morphospecies 2 (T)	3, 0.33 (33%)	3	1, 1.00 (100%)	1	1	8, 0.38 (13%)	2
		Morphospecies 4 (T)	-	1	-	-	-	-	-
Ephemeroptera	Leptophlebiidae	*Deleatidium angustum* (T)	13, 0.46 (31%)	6, 0.17 (17%)	5, 16.80 (100%)	-	8, 16.75 (63%)	1, 8.00 (100%)	6, 0.83 (33%)
		Deleatidium fumosum (T)	4, 0.75 (75%)	6, 1.00 (50%)	12, 24.42 (92%)	-	19, 26.42 (84%)	8, 1.63 (50%)	36, 0.88 (23%)
	Nesameletidae	*Nesameletus austrinus* (T)	-	-	-	-	-	1	-
Mecoptera	Nannochoristidae	*Nannochorista philpotti* (T)	5, 0.80 (60%)	12, 0.08 (8%)	1	1	3, 1.67 (100%)	7	7
Plecoptera	Gripopterygidae	*Zelandobius* sp. 1 (T)	13	4	1	-	17, 1.24 (53%)	15, 1.27 (27%)	29, 0.07 (3%)
		Zelandobius sp. 2 (T)	2	-	-	-	-	-	-
		Zelandoperla sp. (T)	-	7	3	-	2, 1.50 (50%)	9, 4.22 (11%)	6
Trichoptera	Conoesucidae	*Pycnocentria evecta* (T)	1	-	-	-	-	-	-
		Pycnocentria sp. (T)	-	-	-	-	-	1	10
	Hydrobiosidae	*Hydrobiosis* sp. (T)	1	-	-	-	1	-	1
		Psilochorema sp. (T)	1	1	-	-	2	1	-
		Tiphobiosis sp. (T)	-	-	-	-	-	1	2
	Leptoceridae	*Hudsonema amabile* (T)	-	-	-	-	1	1	-
	Oeconesidae	*Oeconesus* sp. (T)	-	-	2	1	-	-	2
	Philopotamidae	*Hydrobiosella mixta* (T)	-	1	-	-	-	-	-
		Hydrobiosella sp. (T)	-	1	-	-	-	-	-
	Philorheithridae	*Philorheithrus* sp. (T)	-	-	2	-	-	-	-
Tricladida	Dugesiidae	*Neppia* sp. (A)	2	1	2	-	4	4	10

*Estimate based on a subsample for that sampling date.

Table 7. Pairwise comparisons for the fixed effects of sampling date and host taxon from the generalised linear model testing the average number of Type A hairworm (phylum Nematomorpha) cysts and larvae in Stream A (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	2020-11-28 - 2020-10-30 == 0	0.4946	0.4475	1.1050	0.9250
	2021-01-10 - 2020-10-30 == 0	-0.0446	0.4938	-0.0900	1.0000
	2021-01-30 - 2020-10-30 == 0	1.0558	0.4576	2.3070	0.2360
	2021-02-27 - 2020-10-30 == 0	0.0914	0.4595	0.1990	1.0000
	2021-03-27 - 2020-10-30 == 0	0.3507	0.4119	0.8510	0.9790
	2021-05-01 - 2020-10-30 == 0	0.3384	0.4032	0.8390	0.9800
	2021-01-10 - 2020-11-28 == 0	-0.5392	0.4730	-1.1400	0.9140
	2021-01-30 - 2020-11-28 == 0	0.5612	0.4359	1.2880	0.8550
	2021-02-27 - 2020-11-28 == 0	-0.4032	0.4407	-0.9150	0.9700
	2021-03-27 - 2020-11-28 == 0	-0.1439	0.3955	-0.3640	1.0000
	2021-05-01 - 2020-11-28 == 0	-0.1562	0.3875	-0.4030	1.0000
	2021-01-30 - 2021-01-10 == 0	1.1004	0.4836	2.2760	0.2520
	2021-02-27 - 2021-01-10 == 0	0.1360	0.4886	0.2780	1.0000
	2021-03-27 - 2021-01-10 == 0	0.3953	0.4494	0.8800	0.9750
	2021-05-01 - 2021-01-10 == 0	0.3830	0.4419	0.8670	0.9770
	2021-02-27 - 2021-01-30 == 0	-0.9644	0.4419	-2.1830	0.3000
	2021-03-27 - 2021-01-30 == 0	-0.7051	0.4039	-1.7460	0.5800
	2021-05-01 - 2021-01-30 == 0	-0.7174	0.3962	-1.8110	0.5360
	2021-03-27 - 2021-02-27 == 0	0.2593	0.3949	0.6570	0.9950
	2021-05-01 - 2021-02-27 == 0	0.2470	0.3853	0.6410	0.9950
	2021-05-01 - 2021-03-27 == 0	-0.0123	0.3247	-0.0380	1.0000
Host taxon	**Haplotaxida - *Deleatidium fumosum* == 0**	**3.5490**	**0.5701**	**6.2250**	**< 0.001**
	***Hydrobiosis* sp. - *Deleatidium fumosum* == 0**	**1.7318**	**0.4585**	**3.7770**	**0.0014**
	Psilochorema sp. - *Deleatidium fumosum* == 0	0.6348	0.3963	1.6020	0.4794
	Pycnocentria sp. - *Deleatidium fumosum* == 0	0.2618	0.3290	0.7960	0.9272
	***Hydrobiosis* sp. - Haplotaxida == 0**	**-1.8172**	**0.6182**	**-2.9400**	**0.0253**
	***Psilochorema* sp. - Haplotaxida == 0**	**-2.9142**	**0.5582**	**-5.2210**	**< 0.001**
	***Pycnocentria* sp. - Haplotaxida == 0**	**-3.2872**	**0.5041**	**-6.5210**	**< 0.001**
	Psilochorema sp. - *Hydrobiosis* sp. == 0	-1.0970	0.4565	-2.4030	0.1071
	***Pycnocentria* sp. - *Hydrobiosis* sp. == 0**	**-1.4699**	**0.4041**	**-3.6370**	**0.0023**
	Pycnocentria sp. - *Psilochorema* sp. == 0	-0.3730	0.3202	-1.1650	0.7592

Table 8. Pairwise comparisons for the fixed effects of sampling date and host taxon from the generalised linear model testing the average number of Type B hairworm (phylum Nematomorpha) cysts and larvae in Stream A (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	2020-11-28 - 2020-10-30 == 0	-0.2233	0.7871	-0.2840	1.0000
	2021-01-10 - 2020-10-30 == 0	0.3510	0.7933	0.4420	0.9994
	2021-01-30 - 2020-10-30 == 0	-1.5208	1.0551	-1.4410	0.7677
	2021-02-27 - 2020-10-30 == 0	**-3.1159**	**0.9574**	**-3.2550**	**0.0185**
	2021-03-27 - 2020-10-30 == 0	**-3.8213**	**0.9532**	**-4.0090**	**0.0012**
	2021-05-01 - 2020-10-30 == 0	-1.3809	0.6706	-2.0590	0.3630
	2021-01-10 - 2020-11-28 == 0	0.5743	0.8333	0.6890	0.9926
	2021-01-30 - 2020-11-28 == 0	-1.2975	1.1032	-1.1760	0.8969
	2021-02-27 - 2020-11-28 == 0	-2.8927	1.0171	-2.8440	0.0631
	2021-03-27 - 2020-11-28 == 0	**-3.5981**	**1.0142**	**-3.5480**	**0.0068**
	2021-05-01 - 2020-11-28 == 0	-1.1577	0.7551	-1.5330	0.7117
	2021-01-30 - 2021-01-10 == 0	-1.8718	1.1020	-1.6990	0.6026
	2021-02-27 - 2021-01-10 == 0	**-3.4670**	**1.0237**	**-3.3870**	**0.0120**
	2021-03-27 - 2021-01-10 == 0	**-4.1724**	**1.0236**	**-4.0760**	**< 0.001**
	2021-05-01 - 2021-01-10 == 0	-1.7320	0.7689	-2.2530	0.2559
	2021-02-27 - 2021-01-30 == 0	-1.5952	1.1645	-1.3700	0.8079
	2021-03-27 - 2021-01-30 == 0	-2.3006	1.1679	-1.9700	0.4193
	2021-05-01 - 2021-01-30 == 0	0.1399	0.9668	0.1450	1.0000
	2021-03-27 - 2021-02-27 == 0	-0.7054	1.0335	-0.6830	0.9930
	2021-05-01 - 2021-02-27 == 0	1.7350	0.8091	2.1440	0.3131
	2021-05-01 - 2021-03-27 == 0	**2.4404**	**0.8101**	**3.0130**	**0.0391**
Host taxon	**Haplotaxida - *Deleatidium fumosum* == 0**	**-3.0121**	**0.9806**	**-3.0720**	**0.0169**
	Hydrobiosis sp. - *Deleatidium fumosum* == 0	-0.9351	0.6868	-1.3610	0.6389
	***Psilochorema* sp. - *Deleatidium fumosum* == 0**	**-2.6381**	**0.6184**	**-4.2660**	**< 0.001**
	***Pycnocentria* sp. - *Deleatidium fumosum* == 0**	**-4.9322**	**0.6709**	**-7.3520**	**< 0.001**
	Hydrobiosis sp. - Haplotaxida == 0	2.0770	1.1353	1.8300	0.3427
	Psilochorema sp. - Haplotaxida == 0	0.3741	0.9983	0.3750	0.9955
	Pycnocentria sp. - Haplotaxida == 0	-1.9201	0.9695	-1.9810	0.2634
	Psilochorema sp. - *Hydrobiosis* sp. == 0	-1.7030	0.8275	-2.0580	0.2273
	***Pycnocentria* sp. - *Hydrobiosis* sp. == 0**	**-3.9971**	**0.8818**	**-4.5330**	**< 0.001**
	***Pycnocentria* sp. - *Psilochorema* sp. == 0**	**-2.2941**	**0.7066**	**-3.2470**	**0.0095**

Table 9. Pairwise comparisons for the fixed effect of sampling date from the generalised linear model testing the average number of Type C hairworm (phylum Nematomorpha) cysts and larvae in Stream A (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	2020-11-28 - 2020-10-30 == 0	-37.8900	3.00E+07	0.0000	1.0000
	2021-01-10 - 2020-10-30 == 0	1.3330	0.9744	1.3680	0.7568
	2021-01-30 - 2020-10-30 == 0	-0.7933	0.9899	-0.8010	0.9766
	2021-02-27 - 2020-10-30 == 0	-37.9200	1.37E+07	0.0000	1.0000
	2021-03-27 - 2020-10-30 == 0	-0.8391	0.7921	-1.0590	0.9121
	2021-05-01 - 2020-10-30 == 0	-1.4860	0.7603	-1.9540	0.3565
	2021-01-10 - 2020-11-28 == 0	39.2200	3.00E+07	0.0000	1.0000
	2021-01-30 - 2020-11-28 == 0	37.1000	3.00E+07	0.0000	1.0000
	2021-02-27 - 2020-11-28 == 0	-0.0337	3.30E+07	0.0000	1.0000
	2021-03-27 - 2020-11-28 == 0	37.0500	3.00E+07	0.0000	1.0000
	2021-05-01 - 2020-11-28 == 0	36.4100	3.00E+07	0.0000	1.0000
	2021-01-30 - 2021-01-10 == 0	-2.1260	0.9628	-2.2080	0.2188
	2021-02-27 - 2021-01-10 == 0	-39.2600	1.37E+07	0.0000	1.0000
	2021-03-27 - 2021-01-10 == 0	**-2.1720**	**0.7747**	**-2.8040**	**0.0498**
	2021-05-01 - 2021-01-10 == 0	**-2.8190**	**0.7239**	**-3.8940**	**0.0011**
	2021-02-27 - 2021-01-30 == 0	-37.1300	1.37E+07	0.0000	1.0000
	2021-03-27 - 2021-01-30 == 0	-0.0458	0.7988	-0.0570	1.0000
	2021-05-01 - 2021-01-30 == 0	-0.6924	0.7627	-0.9080	0.9568
	2021-03-27 - 2021-02-27 == 0	37.0900	1.37E+07	0.0000	1.0000
	2021-05-01 - 2021-02-27 == 0	36.4400	1.37E+07	0.0000	1.0000
	2021-05-01 - 2021-03-27 == 0	-0.6466	0.4859	-1.3310	0.7799

Table 10. Pairwise comparisons for the fixed effects of sampling date and host taxon from the generalised linear model testing the average number of Type A hairworm (phylum Nematomorpha) cysts and larvae in Stream B (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	2020-11-29 - 2020-10-29 == 0	1.0530	0.4512	2.3330	0.1901
	2021-01-11 - 2020-10-29 == 0	-2.0060	0.7871	-2.5480	0.1150
	2021-01-31 - 2020-10-29 == 0	-1.0490	6.77E+07	0.0000	1.0000
	2021-02-28 - 2020-10-29 == 0	-0.4852	0.5305	-0.9150	0.9618
	2021-03-27 - 2020-10-29 == 0	-0.3235	0.5690	-0.5680	0.9968
	2021-05-01 - 2020-10-29 == 0	-0.4021	0.5470	-0.7350	0.9873
	2021-01-11 - 2020-11-29 == 0	**-3.0580**	**0.7912**	**-3.8650**	**0.0016**
	2021-01-31 - 2020-11-29 == 0	-2.1020	6.77E+07	0.0000	1.0000
	2021-02-28 - 2020-11-29 == 0	**-1.5380**	**0.5314**	**-2.8940**	**0.0455**
	2021-03-27 - 2020-11-29 == 0	-1.3760	0.5622	-2.4470	0.1466
	2021-05-01 - 2020-11-29 == 0	-1.4550	0.5454	-2.6670	0.0850
	2021-01-31 - 2021-01-11 == 0	0.9562	6.77E+07	0.0000	1.0000
	2021-02-28 - 2021-01-11 == 0	1.5200	0.8250	1.8430	0.4644
	2021-03-27 - 2021-01-11 == 0	1.6820	0.8592	1.9580	0.3887
	2021-05-01 - 2021-01-11 == 0	1.6030	0.8311	1.9290	0.4069
	2021-02-28 - 2021-01-31 == 0	0.5643	6.77E+07	0.0000	1.0000
	2021-03-27 - 2021-01-31 == 0	0.7260	6.77E+07	0.0000	1.0000
	2021-05-01 - 2021-01-31 == 0	0.6473	6.77E+07	0.0000	1.0000
	2021-03-27 - 2021-02-28 == 0	0.1617	0.6121	0.2640	1.0000
	2021-05-01 - 2021-02-28 == 0	0.0831	0.5737	0.1450	1.0000
	2021-05-01 - 2021-03-27 == 0	-0.0786	0.6287	-0.1250	1.0000
Host taxon	*Deleatidium angustum* - Chironomidae spp. == 0	36.5400	8.51E+06	0.0000	1.0000
	Deleatidium fumosum - Chironomidae spp. == 0	34.9000	8.51E+06	0.0000	1.0000
	Haplotaxida - Chironomidae spp. == 0	36.0300	8.51E+06	0.0000	1.0000
	Tabanidae sp. 1 - Chironomidae spp. == 0	36.0000	8.51E+06	0.0000	1.0000
	***Deleatidium fumosum* - *Deleatidium angustum* == 0**	**-1.6450**	**0.5000**	**-3.2890**	**0.0065**
	Haplotaxida - *Deleatidium angustum* == 0	-0.5122	0.5154	-0.9940	0.8308
	Tabanidae sp. 1 - *Deleatidium angustum* == 0	-0.5414	0.4507	-1.2010	0.7102
	Haplotaxida - *Deleatidium fumosum* == 0	1.1320	0.5449	2.0780	0.1904
	Tabanidae sp. 1 - *Deleatidium fumosum* == 0	1.1030	0.4507	2.4480	0.0810
	Tabanidae sp. 1 - Haplotaxida == 0	-0.0292	0.4677	-0.0620	1.0000

Table 11. Pairwise comparisons for the fixed effects of sampling date and host taxon from the generalised linear model testing the average number of Type B hairworm (phylum Nematomorpha) cysts and larvae in Stream B (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	**2020-11-29 - 2020-10-29 == 0**	**-1.9970**	**0.4704**	**-4.2460**	**< 0.001**
	2021-01-11 - 2020-10-29 == 0	**-2.3570**	**0.5790**	**-4.0720**	**< 0.001**
	2021-01-31 - 2020-10-29 == 0	-35.1200	6.71E+07	0.0000	1.0000
	2021-02-28 - 2020-10-29 == 0	-37.9100	9.29E+06	0.0000	1.0000
	2021-03-27 - 2020-10-29 == 0	**-3.7230**	**0.7800**	**-4.7730**	**< 0.001**
	2021-05-01 - 2020-10-29 == 0	**-3.3310**	**0.6282**	**-5.3030**	**< 0.001**
	2021-01-11 - 2020-11-29 == 0	-0.3600	0.6194	-0.5810	0.9960
	2021-01-31 - 2020-11-29 == 0	-33.1200	6.71E+07	0.0000	1.0000
	2021-02-28 - 2020-11-29 == 0	-35.9200	9.29E+06	0.0000	1.0000
	2021-03-27 - 2020-11-29 == 0	-1.7250	0.8039	-2.1460	0.2470
	2021-05-01 - 2020-11-29 == 0	-1.3340	0.6594	-2.0230	0.3130
	2021-01-31 - 2021-01-11 == 0	-32.7600	6.71E+07	0.0000	1.0000
	2021-02-28 - 2021-01-11 == 0	-35.5600	9.29E+06	0.0000	1.0000
	2021-03-27 - 2021-01-11 == 0	-1.3650	0.8628	-1.5830	0.6090
	2021-05-01 - 2021-01-11 == 0	-0.9740	0.6904	-1.4110	0.7270
	2021-02-28 - 2021-01-31 == 0	-2.7920	6.78E+07	0.0000	1.0000
	2021-03-27 - 2021-01-31 == 0	31.4000	6.71E+07	0.0000	1.0000
	2021-05-01 - 2021-01-31 == 0	31.7900	6.71E+07	0.0000	1.0000
	2021-03-27 - 2021-02-28 == 0	34.1900	9.29E+06	0.0000	1.0000
	2021-05-01 - 2021-02-28 == 0	34.5800	9.29E+06	0.0000	1.0000
	2021-05-01 - 2021-03-27 == 0	0.3914	0.8817	0.4440	0.9990
Host taxon	***Deleatidium angustum* - Chironomidae spp. == 0**	**3.6949**	**1.1170**	**3.3080**	**0.0075**
	***Deleatidium fumosum* - Chironomidae spp. == 0**	**3.4563**	**1.1185**	**3.0900**	**0.0153**
	Haplotaxida - Chironomidae spp. == 0	**3.6536**	**1.1094**	**3.2930**	**0.0080**
	Tabanidae sp. 1 - Chironomidae spp. == 0	**3.1686**	**1.0906**	**2.9050**	**0.0272**
	Deleatidium fumosum - *Deleatidium angustum* == 0	-0.2386	0.6022	-0.3960	0.9944
	Haplotaxida - *Deleatidium angustum* == 0	-0.0413	0.5751	-0.0720	1.0000
	Tabanidae sp. 1 - *Deleatidium angustum* == 0	-0.5263	0.5369	-0.9800	0.8558
	Haplotaxida - *Deleatidium fumosum* == 0	0.1973	0.5972	0.3300	0.9972
	Tabanidae sp. 1 - *Deleatidium fumosum* == 0	-0.2877	0.5457	-0.5270	0.9834
	Tabanidae sp. 1 - Haplotaxida == 0	-0.4849	0.5082	-0.9540	0.8674

Table 12. Pairwise comparisons for the fixed effects of sampling date and host taxon from the generalised linear model testing the average number of Type C hairworm (phylum Nematomorpha) cysts and larvae in Stream B (differences detected between levels are in bold).

Fixed factors	Pairwise comparisons	Estimate	Standard error	*z*-value	*p*-value
Sampling date	2020-11-29 - 2020-10-29 == 0	-0.7428	0.4895	-1.5170	0.6931
	2021-01-11 - 2020-10-29 == 0	**3.1740**	**0.4018**	**7.8990**	**< 0.001**
	2021-01-31 - 2020-10-29 == 0	-34.8300	6.71E+07	0.0000	1.0000
	2021-02-28 - 2020-10-29 == 0	**2.5430**	**0.3815**	**6.6660**	**< 0.001**
	2021-03-27 - 2020-10-29 == 0	**1.3660**	**0.4264**	**3.2040**	**0.0179**
	2021-05-01 - 2020-10-29 == 0	-0.2526	0.4400	-0.5740	0.9968
	2021-01-11 - 2020-11-29 == 0	**3.9170**	**0.4664**	**8.3980**	**< 0.001**
	2021-01-31 - 2020-11-29 == 0	-34.0900	6.71E+07	0.0000	1.0000
	2021-02-28 - 2020-11-29 == 0	**3.2860**	**0.4477**	**7.3400**	**< 0.001**
	2021-03-27 - 2020-11-29 == 0	**2.1090**	**0.4826**	**4.3700**	**< 0.001**
	2021-05-01 - 2020-11-29 == 0	0.4902	0.4956	0.9890	0.9456
	2021-01-31 - 2021-01-11 == 0	-38.0100	6.71E+07	0.0000	1.0000
	2021-02-28 - 2021-01-11 == 0	-0.6305	0.3251	-1.9390	0.4054
	2021-03-27 - 2021-01-11 == 0	**-1.8080**	**0.3954**	**-4.5720**	**< 0.001**
	2021-05-01 - 2021-01-11 == 0	**-3.4260**	**0.3917**	**-8.7470**	**< 0.001**
	2021-02-28 - 2021-01-31 == 0	37.3700	6.71E+07	0.0000	1.0000
	2021-03-27 - 2021-01-31 == 0	36.2000	6.71E+07	0.0000	1.0000
	2021-05-01 - 2021-01-31 == 0	34.5800	6.71E+07	0.0000	1.0000
	2021-03-27 - 2021-02-28 == 0	**-1.1770**	**0.3716**	**-3.1690**	**0.0200**
	2021-05-01 - 2021-02-28 == 0	**-2.7960**	**0.3682**	**-7.5940**	**< 0.001**
	2021-05-01 - 2021-03-27 == 0	-1.6190	0.4279	-3.7830	0.0023
Host taxon	***Deleatidium angustum* - Chironomidae spp. == 0**	**2.0552**	**0.4103**	**5.0080**	**< 0.001**
	***Deleatidium fumosum* - Chironomidae spp. == 0**	**2.5088**	**0.3511**	**7.1450**	**< 0.001**
	Haplotaxida - Chironomidae spp. == 0	-0.8284	0.6654	-1.2450	0.7106
	Tabanidae sp. 1 - Chironomidae spp. == 0	**1.6881**	**0.3702**	**4.5590**	**< 0.001**
	Deleatidium fumosum - *Deleatidium angustum* == 0	0.4537	0.3491	1.3000	0.6762
	Haplotaxida - *Deleatidium angustum* == 0	**-2.8836**	**0.6733**	**-4.2820**	**< 0.001**
	Tabanidae sp. 1 - *Deleatidium angustum* == 0	-0.3671	0.3525	-1.0410	0.8261
	Haplotaxida - *Deleatidium fumosum* == 0	**-3.3373**	**0.6484**	**-5.1470**	**< 0.001**
	Tabanidae sp. 1 - *Deleatidium fumosum* == 0	**-0.8208**	**0.2929**	**-2.8020**	**0.0369**
	Tabanidae sp. 1 - Haplotaxida == 0	**2.5165**	**0.6501**	**3.8710**	**< 0.001**

Table 13. Insects caught in the Malaise traps per sampling period for Stream A. Note that each date corresponds to the end of the seven-day trapping period.

Order	**Family**	**Genus or species**	**2020-12-05**		**2021-01-10**		**2021-02-06**		**2021-03-06**	
			Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Araneae	NA	NA	-	-	-	2	-	-	-	-
Coleoptera	Scirtidae	*Contacyphon* spp.	-	-	2	-	4	1	1	-
	Other Coleoptera	NA	3	3	23	6	28	37	3	3
Diptera	Chironomidae	Morphospecies 1	2	-	4	21	21	7	1	5
		spp.	-	-	4	23	92	107	36	33
	Simuliidae	*Austrosimulium* spp.	-	1	5	-	1	-	5	2
	Tabanidae	spp.	-	-	-	-	-	1	-	-
	Tipulidae	spp.	-	-	1	-	2	2	2	1
	Other Diptera	NA	11	9	47	23	132	27	73	63
Hemiptera	NA	NA	-	1	12	8	42	64	35	44
Hymenoptera	NA	NA	1	-	24	6	83	77	18	31
Lepidoptera	NA	NA	3	8	12	11	184	107	14	7
Neuroptera	NA	NA	-	-	2	-	2	1	-	1
Orthoptera	NA	NA	1	-	8	1	11	6	3	1
Plecoptera	Gripopterygidae	*Zelandobius* sp.	1	-	5	2	2	-	2	-
Trichoptera	Conoesucidae	*Pycnocentria* sp.	-	-	-	-	2	1	-	-

Table 14. Insects caught in the Malaise traps per sampling period for Stream B. Note that each date corresponds to the end of the seven-day trapping period.

Order	**Family**	**Genus or species**	**2020-12-06**		**2021-01-11**		**2021-02-07**		**2021-03-07**	
			Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Blattodea	NA	NA	-	-	-	-	1	-	-	-
Coleoptera	Scirtidae	*Contacyphon* spp.	-	-	21	17	41	36	2	9
	Other Coleoptera	NA	-	-	3	1	30	6	2	-
Diptera	Chironomidae	Morphospecies 1	116	95	57	14	69	100	17	3
		spp.	69	85	39	18	233	298	89	110
	Simuliidae	*Austrosimulium* spp.	4	16	4	3	-	-	3	-
	Tabanidae	spp.	-	-	-	-	-	1	-	-
	Tipulidae	spp.	3	2	2	2	63	12	1	-
	Other Diptera	NA	125	99	164	105	444	316	94	24
Hemiptera	NA	NA	15	6	132	35	445	212	36	19
Hymenoptera	NA	NA	5	3	21	20	159	113	13	11
Lepidoptera	NA	NA	6	8	12	4	49	16	11	2
Neuroptera	NA	NA	-	-	2	-	2	-	1	-
Orthoptera	NA	NA	-	-	-	-	3	-	-	-
Plecoptera	Gripopterygidae	*Zelandobius* sp.	-	2	2	5	-	1	-	-
Trichoptera	Conoesucidae	*Pycnocentria* sp.	-	-	-	-	-	-	1	-

Table 15. Invertebrates caught in the pitfall traps per sampling period for Site A. The distance and direction of traps, as well as all three transects, are pooled for each period. Note that each date corresponds to the end of the seven-day trapping period.

Class	Order	Family	Genus or species	2020-11-05	2020-12-05	2021-01-10	2021-02-06	2021-03-06
Arachnida	Araneae	Amaurobiidae*	Morphospecies 1	1	2	3	10	6
			Morphospecies 2	12	55	5	43	6
		Gnaphosidae	NA	4	5	-	-	-
		Lycosidae	*Anoteropsis* sp. 1	64	64	15	62	24
			Anoteropsis sp. 2	5	7	-	-	-
		NA	spp.	-	-	1	-	-
		Salticidae	Morphospecies 1	-	-	-	1	-
		Stiphidiidae	Morphospecies 1	-	-	-	1	1
	NA	NA	spp.	-	-	1	6	-
	Opiliones	NA	spp.	-	-	2	1	-
Chilopoda	NA	NA	spp.	1	1	1	-	1
Clitellata	Haplotaxida	Lumbricidae	spp.	1	-	5	-	-
Diplopoda	NA	NA	spp.	4	3	-	1	-
Insecta	Blattodea	Blattidae	*Celatoblatta quinquemaculata*	3	6	3	7	2
	Coleoptera	Carabidae	*Cicindela* sp.	1	-	-	-	-
			Holcaspis sp.	33	14	12	8	5
			Mecodema sp.	18	2	8	5	4
			Megadromus sp.	14	3	4	2	4
			Morphospecies 1	-	1	-	-	-
			Notagonum sp.	1	2	-	1	-
		Chrysomelidae	spp.	-	-	-	1	-
		Coccinellidae	spp.	3	3	-	-	-
		Curculionidae	spp.	49	72	64	47	7
		Elateridae	Morphospecies 1	4	-	1	-	-
		NA	spp.	-	-	1	-	-
	Dermaptera	Labiidae	Morphospecies 1	-	2	1	-	-
	Diptera	NA	spp.	-	8	2	3	-
	Hemiptera	NA	spp.	1	2	1	1	-
	Hymenoptera	NA	spp.	-	-	1	4	-
	Lepidoptera	NA	spp.	-	1	-	1	-
	Orthoptera	Acrididae	*Sigaus australis*	34	21	2	128	58
		Anostostomatidae	*Hemiandrus* sp.	6	3	3	7	21
Malacostraca	Amphipoda	Talitridae	Morphospecies 1	2	1	4	1	4

*Species in this family are impossible to differentiate from species of the family Desidae.

Table 16. Invertebrates caught in the pitfall traps per sampling period for Site B. The distance and direction of traps, as well as all three transects, are pooled for each period. Note that each date corresponds to the end of the seven-day trapping period.

Class	Order	Family	Genus or species	2020-11-06	2020-12-06	2021-01-11	2021-02-07	2021-03-07
Arachnida	Araneae	Amaurobiidae*	Morphospecies 1	8	3	6	9	4
			Morphospecies 2	4	4	4	19	8
		Lycosidae	*Allotrochosina schauinslandi*	1	-	-	-	-
			Anoteropsis sp. 1	79	27	12	31	26
			Anoteropsis sp. 2	6	1	-	-	-
		NA	spp.	1	2	-	18	-
		Stiphidiidae	Morphospecies 1	-	-	-	3	4
	NA	NA	spp.	-	-	-	1	-
	Opiliones	NA	spp.	3	4	20	6	5
Chilopoda	NA	NA	spp.	2	2	-	1	3
Clitellata	Haplotaxida	Lumbricidae	spp.	-	-	-	1	-
Diplopoda	NA	NA	spp.	-	-	1	-	-
Entognatha	Entomobryomorpha	NA	Morphospecies 1	-	1	-	-	1
Insecta	Blattodea	Blattidae	*Celatoblatta quinquemaculata*	13	18	57	61	23
	Coleoptera	Carabidae	*Mecodema* sp.	32	4	12	6	4
			Megadromus sp.	-	-	-	-	1
			Notagonum sp.	8	4	-	2	1
		Chrysomelidae	spp.	1	3	2	1	-
		Curculionidae	spp.	6	3	4	3	-
		NA	spp.	1	-	2	-	-
		Scarabaeidae	*Aphodius* sp.	-	1	-	-	-
			Scythrodes sp.	3	1	2	1	1
		Scirtidae	spp.	1	-	5	1	-
	Diptera	NA	spp.	-	40	33	42	7
		Tipulidae	spp.	-	-	2	-	1
	Hemiptera	NA	spp.	1	-	2	1	-
	Hymenoptera	NA	spp.	-	-	2	5	1
	Lepidoptera	NA	spp.	-	-	5	4	1
	Neuroptera	NA	Morphospecies 1	-	-	1	-	-
	Orthoptera	Acrididae	*Sigaus australis*	-	-	-	1	2
		Anostostomatidae	*Hemiandrus* sp.	2	2	19	5	6
	Plecoptera	NA	spp.	-	1	-	1	-

*Species in this family are impossible to differentiate from species of the family Desidae.