



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

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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 (non-viable transmission routes), 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 m², 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 over a third 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 us understand the spread or emergence of disease in both freshwater and terrestrial environments, since hairworms infect terrestrial arthropods to complete their life cycle.

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1. 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). Although the dilution effect may only apply to parasites in localised communities (Randolph and Dobson, 2012; Salkeld et al., 2013), empirical evidence has shown that infection risk does decrease with increased biodiversity in freshwater systems (Laguerre and Poulin, 2015b; 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 cycles of parasites that rely on intermediate or paratenic (transport) hosts for transmission to another host or environment (Keesing et al., 2006). Some organisms play

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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) in its tissues 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). 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, which could help predict the spread of infection or disease in an ecosystem.

Gordioid nematomorphs, or freshwater hairworms, may be one of the most common parasites in lotic ecosystems. Surveys have shown that dormant hairworm cysts 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). 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 transport 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). 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 (see Doherty et al., 2019), 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 2 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. Although these studies helped uncover the complex, yet hidden, dynamics of parasite transmission in a community of intermediate 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 m² of stream bed. Although this measure is rarely used to characterise parasite density (Laguerre and Poulin, 2015a), it nonetheless provides a comparable metric for both host and parasite. Here, we hypothesised that the presence of key species in aquatic host communities contributes either positively or negatively to the transmission of hairworms from water to land, depending on the category of host (paratenic or dead-end, respectively). Since hairworm larvae appear to encyst indiscriminately within hosts, we predicted that, if present, these hosts would have a higher abundance of parasites than other taxa and act as important population sinks. We also tested if there were any seasonal peaks in hairworm abundance across host taxa. 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.

2. Materials and methods

2.1. 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 (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 m section of each stream. Samples were taken from both streams within 24 h, at each start date 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 s. Anything collected downstream in the fine mesh net ($500 \mu\text{m}$) of the Surber sampler was immediately stored in 75% ethanol until further processing in the laboratory. Additionally, a 1 m 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. This would allow us to estimate the potential arrival period of new hairworm larvae in the stream. For this, we counted the total number of individual adults and any mating knots present.

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. Due to 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 (van Achterberg, 2009). They were installed perpendicularly to the stream (all traps were placed on the same side of 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 their 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 7 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.

2.2. 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 h. 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 (Doherty et al., 2019). For some larger individuals, tissues had to be minced with tweezers and a fine scalpel prior to flattening them. Due to 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 (Type A, B, or C; see Section 3.2 for details). 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 LLC, California, United States of America). These measurements, together with the morphology of each morphotype (Fig. 1), were used to identify hairworms to species level (see Section 3).

2.3. 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 (Fig. 1), 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 (Fig. 2).

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 (Fig. 3). Note that, for any given sample, a maximum of 60 individuals per taxon were examined for hairworm cysts or larvae, to properly estimate hairworm abundance. 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.

2.4. Data analysis

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Firstly, to test whether hairworm abundance varied statistically between host taxa or sampling date within each stream, we used

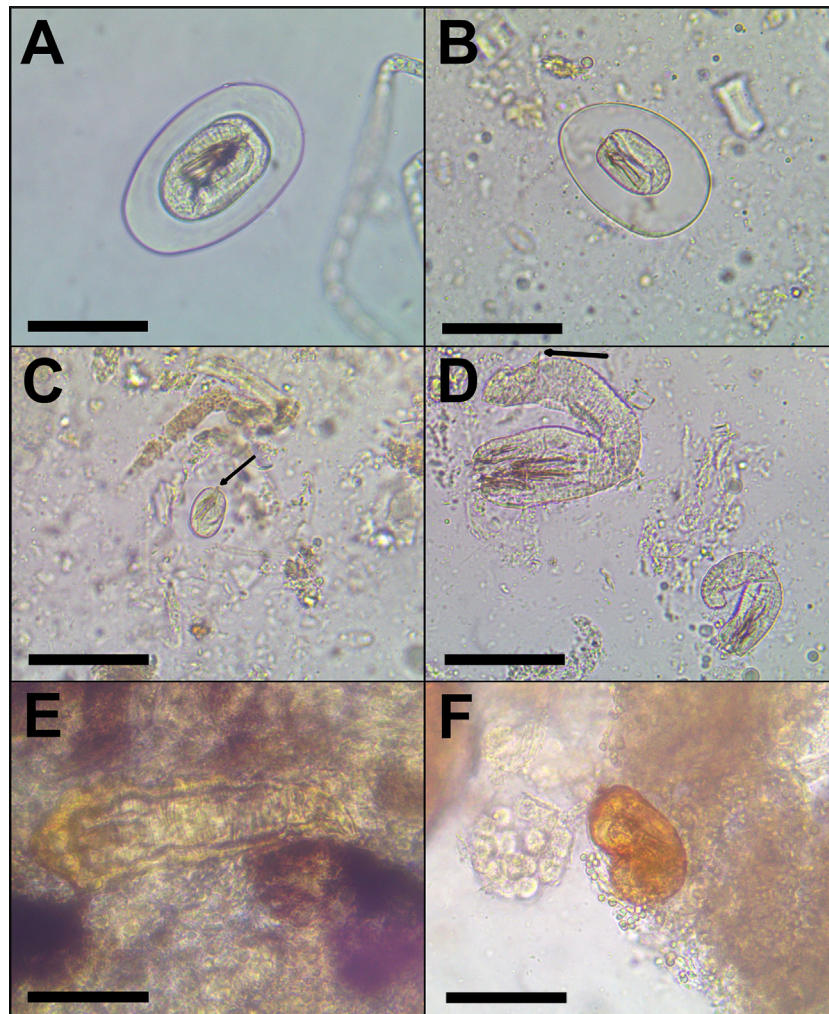


Fig. 1. Hairworm (phylum Nematomorpha) cysts and larvae observed in multiple aquatic hosts collected from two subalpine streams in Otago, New Zealand. Host tissues were flattened prior to images being taken with a C-Mount microscope camera at 100 $\times$ magnification. (A) Non-melanised Type A cyst 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 μ m). Note: Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

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 m² across all three morphotypes (Fig. 2). 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 *Deleatidium 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). We selected this distribution because it better accounted for overdispersion (dispersion parameter closer to 1) than the Poisson distribution. To assess model fit, residual plots were verified for all models. 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).

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.

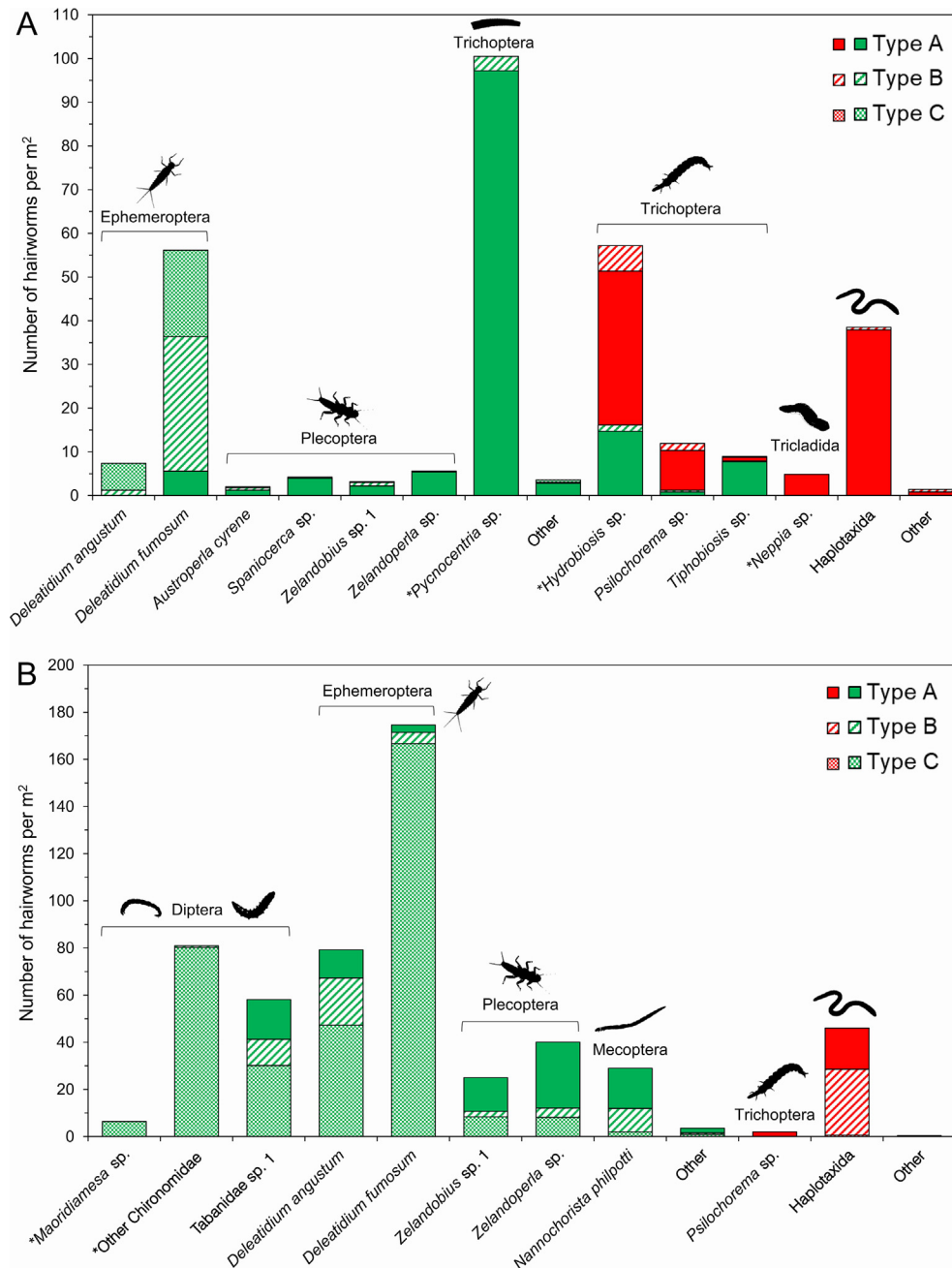


Fig. 2. Density of hairworm (phylum Nematomorpha) cysts and larvae per aquatic host taxon per m² of stream bed (in Otago, New Zealand), for three hairworm morphotypes (Type A, B, and C). Viable hairworms found in potential paratenic hosts are coloured in green, whereas non-viable hairworms found in dead-end 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. Note: Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

2.5. Data accessibility

Full datasets are available from Mendeley Data (<https://doi.org/10.17632/55jgy7zjt.1>).

3. Results

3.1. 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 chi-

ronomid 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 m² was over 12 times higher in Stream A than in Stream B and both communities differed in their relative taxonomic composition (Fig. 4). 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

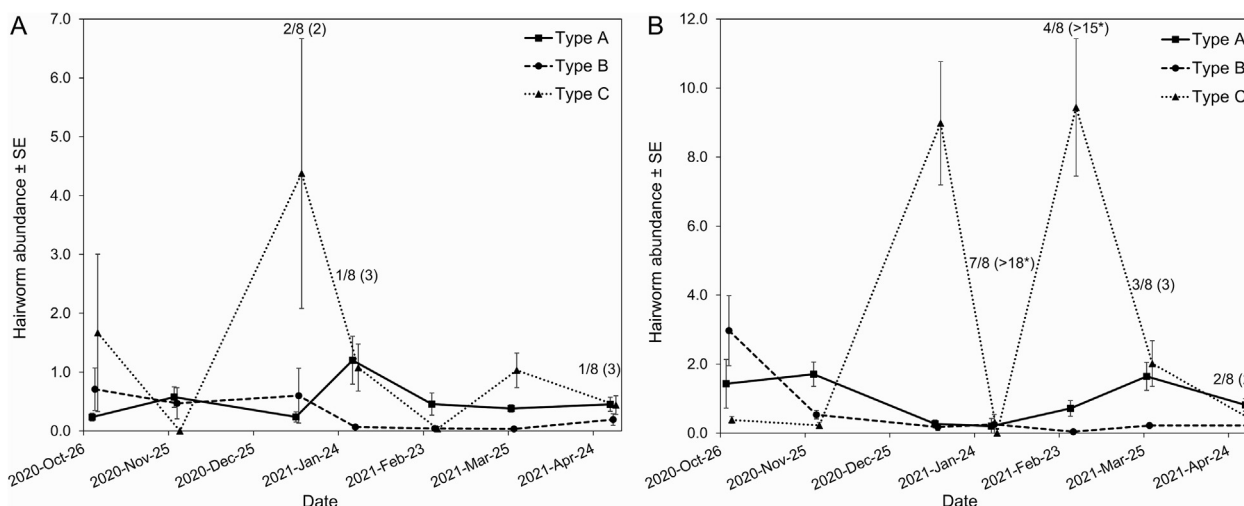


Fig. 3. Mean number of hairworm (phylum Nematomorpha) cysts and larvae per individual aquatic host per morphotype per sampling date for (A) Stream A and (B) Stream B (in Otago, New Zealand). The numbers above sampling dates indicate the proportion of Surber sampling sites where adult hairworms were observed 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. Note: Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

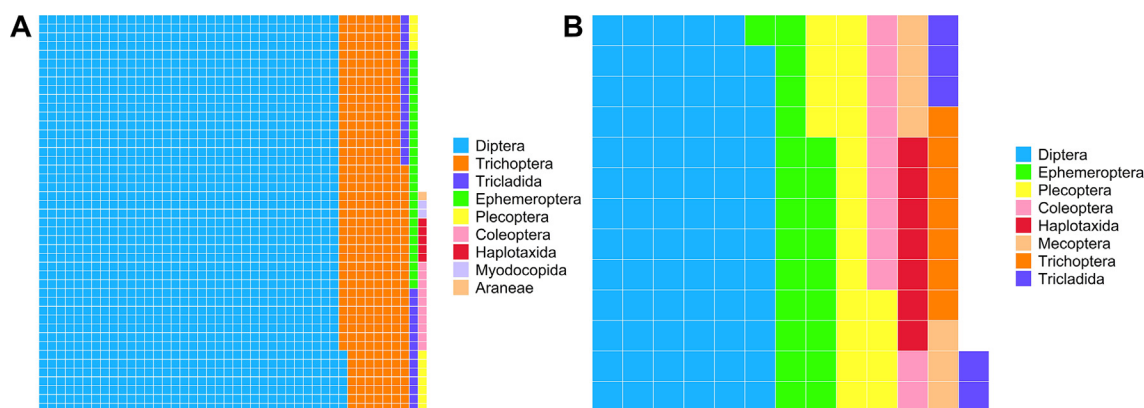


Fig. 4. Waffle chart displaying the average number of aquatic macroinvertebrates per m² of stream bed, ranked in order for (A) Stream A and (B) Stream B (in Otago, New Zealand). One square represents one individual on average per m². The colour for each order is identical for both streams, except for Araneae, Mecoptera, and Myodocopida, each of which occurred in one stream only.

and larvae. It was possible to distinguish between three hairworm morphotypes, based on their appearance and size (Table 1). We refer to them here as Type A, Type B, and Type C (Fig. 1). All three were present in both streams and were found in multiple host taxa (Table 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 (Supplementary Tables S1–S6). 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 (Fig. 3).

3.2. Tentative identification of hairworm morphotypes

Three obvious hairworm morphotypes were easily distinguishable, mainly by their size (Table 1), but also by their appearance (Fig. 1). In previous studies, five species of adult hairworms were collected from Stream A (*Euchordodes nigromaculatus*, *Parachordo-*

Table 1

Hairworm morphotypes (phylum Nematomorpha) found in various aquatic macroinvertebrates collected from two subalpine streams in Otago, 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)

Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

des 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 postseptum length 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, it is possible that Type B hairworms also belong to the genus *Gordius*, although 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 their preseptum length (personal observation). We also observed spines protruding from the preseptum, which fits with descriptions of *Chordodes* and *Parachordodes* cysts (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 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. Given that we were testing the general transmission patterns of parasites in host communities, identifying each morphotype to species level was not essential for the aims of this study.

3.3. 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 (Fig. 1). Of these, over 90% were found in three species of uncased predatory hydrobiosid trichopterans (*Hydrobiosis* sp., *Psilochorema* sp., and *Tiphobiosis* sp.); the remaining 13 larvae were found sporadically within other host taxa. We found no broken cysts near the larvae in 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 (Fig. 1D), which further supported our assumption that these larvae had already produced a cyst and were thus excysted, since hairworm larvae empty the contents of their pseudo-intestine to produce their cyst (Bolek et al., 2015). 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.

3.4. Proportion of 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 (Fig. 2), 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, i.e., dead-end hosts, and the non-melanised and melanised excysted larvae that were found mainly inside predatory trichopterans (these insects appeared to play a dual role in hairworm transmission). 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 (Fig. 2).

3.5. Statistical results

3.5.1. 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 2 and Supplementary Table S7). 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 m² found in this taxon (Fig. 2). Similar to 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 2 and Supplementary Table S8). *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 2). The peak abundance of Type C hairworms observed during January 2021 in Fig. 3 was only higher than those of March and May 2021 (Supplementary Table S9).

3.5.2. 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 3 and Supplementary Table S10). Although a slight peak in Type A hairworm abundance is observed in November 2020 (Fig. 3), 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 3). Apart from that, no difference was observed. In Fig. 3, 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 3 and Supplementary Table S11). 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 3 and Supplementary Table S12). 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 m² (Fig. 2). The chironomid species group appears to have contributed substantially to the density of Type C hairworms in Stream B (Fig. 2), 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 (Fig. 4). Interestingly, the appearance of Type C cysts in chironomids coincided with the peaks of abundance observed in Fig. 3. 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 (Supplementary Table S12).

3.6. Malaise trapping

A total of 6,164 insects were captured in the Malaise traps, 72% of which were captured near Stream B (Supplementary Tables S13 and S14). Of the 2,067 individuals with an aquatic life stage, 1,100 were flattened under the microscope to look for hairworms. As

Table 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 (in Otago, New Zealand). Factors that impact hairworm abundance are in bold. 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 *Deleatidium 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–Nov–28	0.4946	0.4475	1.1050	0.2690
		Date, 2021–Jan–10	–0.0446	0.4938	–0.0900	0.9280
		Date, 2021–Jan–30	1.0558	0.4576	2.3070	0.0211
		Date, 2021–Feb–27	0.0914	0.4595	0.1990	0.8424
		Date, 2021–Mar–27	0.3507	0.4119	0.8510	0.3946
		Date, 2021–May–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, <i>Psilochorema</i> sp.	0.6348	0.3963	1.6020	0.1092
		Host, <i>Pycnocentria</i> 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–Nov–28	–0.2233	0.7871	–0.2840	0.7767
		Date, 2021–Jan–10	0.3510	0.7933	0.4420	0.6581
		Date, 2021–Jan–30	–1.5208	1.0551	–1.4410	0.1495
		Date, 2021–Feb–27	–3.1159	0.9574	–3.2550	0.0011
		Date, 2021–Mar–27	–3.8213	0.9532	–4.0090	<0.001
		Date, 2021–May–01	–1.3809	0.6706	–2.0590	0.0395
		Host, Haplotaxida	–3.0121	0.9806	–3.0720	0.0021
		Host, <i>Hydrobiosis</i> sp.	–0.9351	0.6868	–1.3610	0.1734
		Host, <i>Psilochorema</i> sp.	–2.6381	0.6184	–4.2660	<0.001
		Host, <i>Pycnocentria</i> 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–Nov–28	–37.8900	3.00E + 07	0.0000	1.0000
		Date, 2021–Jan–10	1.3330	0.9744	1.3680	0.1714
		Date, 2021–Jan–30	–0.7933	0.9899	–0.8010	0.4229
		Date, 2021–Feb–27	–37.9200	1.37E + 07	0.0000	1.0000
		Date, 2021–Mar–27	–0.8391	0.7921	–1.0590	0.2894
		Date, 2021–May–01	–1.4860	0.7603	–1.9540	0.0507
		Host, <i>Deleatidium fumosum</i>	1.0120	0.7196	1.4060	0.1597

Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

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). However, we did find a higher abundance of Type B cysts in Stream A for this taxon across life stages.

4. Discussion

Host availability impacts the transmission of parasites and disease in freshwater environments (Keesing et al., 2006; Lagrue and Poulin, 2015b). 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 such as 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 such as 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

Table 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 (in Otago, New Zealand). Factors that impact hairworm abundance are in bold. 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-Nov-29	1.0520	0.4512	2.3330	0.0197
		Date, 2021-Jan-11	–2.0060	0.7871	–2.5480	0.0108
		Date, 2021-Jan-31	–1.0500	6.77E + 07	0.0000	1.0000
		Date, 2021-Feb-28	–0.4852	0.5305	–0.9150	0.3604
		Date, 2021-Mar-27	–0.3235	0.5690	–0.5680	0.5697
		Date, 2021-May-01	–0.4021	0.5470	–0.7350	0.4622
		Host, <i>Deleatidium angustum</i>	36.5400	8.51E + 06	0.0000	1.0000
		Host, <i>Deleatidium fumosum</i>	34.9000	8.51E + 06	0.0000	1.0000
		Host, Haplotaixida	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-Nov-29	–1.9970	0.4704	–4.2460	<0.001
		Date, 2021-Jan-11	–2.3570	0.5790	–4.0720	<0.001
		Date, 2021-Jan-31	–35.1200	6.71E + 07	0.0000	1.0000
		Date, 2021-Feb-28	–37.9100	9.29E + 06	0.0000	1.0000
		Date, 2021-Mar-27	–3.7230	0.7800	–4.7730	1.82E-06
		Date, 2021-May-01	–3.3310	0.6282	–5.3030	1.14E-07
		Host, <i>Deleatidium angustum</i>	3.6950	1.1170	3.3080	<0.001
		Host, <i>Deleatidium fumosum</i>	3.4560	1.1190	3.0900	0.0020
		Host, Haplotaixida	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-Nov-29	–0.7428	0.4895	–1.5170	0.1292
		Date, 2021-Jan-11	3.1740	0.4018	7.8990	<0.001
		Date, 2021-Jan-31	–34.8300	6.71E + 07	0.0000	1.0000
		Date, 2021-Feb-28	2.5430	0.3815	6.6660	<0.001
		Date, 2021-Mar-27	1.3660	0.4264	3.2040	0.0014
		Date, 2021-May-01	–0.2526	0.4400	–0.5740	0.5660
		Host, <i>Deleatidium angustum</i>	2.0550	0.4103	5.0080	<0.001
		Host, <i>Deleatidium fumosum</i>	2.5090	0.3511	7.1450	<0.001
		Host, Haplotaixida	–0.8284	0.6654	–1.2450	0.2132
		Host, Tabanidae sp. 1	1.6880	0.3702	4.5590	<0.001

Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

Table 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 (Streams A and B, in Otago, New Zealand) 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

Types A, B, and C are hairworm morphotypes that each represent a species or species complex separated by morphological differences.

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 (Sonnenholzner et al., 2011; Lagrue and Poulin, 2015a), which appears to also be the case here. An abundant host such as 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 their 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. Finally, identifying hairworms with DNA analysis could help determine if the three morphotypes were composed of one or several species. This could change our interpretation of the results and help refine the host distribution of each hairworm species.

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 and 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) (Szymgiel 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; Szymgiel 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 together 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, 1991; Doherty et al., 2019). 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 7 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 intermediate hosts and ingest hairworms. Obviously, only the paratenic hosts that are consumed by definitive hosts actually contribute to the life cycle of hairworms; simply moving from an aquatic to a terrestrial environment does not guarantee transmission success for 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 of 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2021.11.009>.

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