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ORIGINAL PAPER

Prior infections or defence priming: what determines the risk of trematode infections in amphipod hosts?

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Abstract

Inducible defences against parasites that are only activated when needed can mitigate the cost of immune or behavioural evasion of parasites. Priming of the immune system and activation of behavioural defences can follow exposure to cues associated with imminent infection risk. In contrast, prior infection can cause immune depression or leave the host with less energy to defend itself against further infections. We investigate the priming of anti-parasite defences and the effect of prior infections in the amphipod *Paracalliope fluviatilis*, the second intermediate host of the trematode *Coitocaecum parvum*. During experimental infections, amphipods that had been primed by exposure to chemical cues (from first intermediate snail hosts infected by *C. parvum*) of infection risk were not better at avoiding further infection than control amphipods. All amphipods showed the same swimming behaviour, whether or not they had been primed by chemical cues from infected snails, or whether or not they were in the presence of live infective stages. In contrast, regardless of whether or not they had been exposed to control water or chemical cues from infected snails, amphipods harbouring prior infections acquired in nature were significantly more likely to acquire new parasites under controlled conditions. These results suggest that the induction of defences via external cues associated with the threat of infection do not play a role in the amphipod's anti-parasite strategy. However, prior infections may predispose a host to acquire further parasites, with consequences for the distribution of parasites among host individuals and the regulation of the host population.

Keywords *Coitocaecum parvum* · Immune defence · Metacercariae · *Paracalliope fluviatilis* · Resistance · Swimming activity

Introduction

To reduce the fitness costs associated with parasitic infections, animals have evolved a range of anti-parasite defences, ranging from complex immune responses to simple behavioural avoidance of parasites (Hart 1994). However, these defences themselves are costly, whether energetically or, in the case of behavioural defences, in terms of lost time (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000). As a consequence, selection has favoured inducible defences, i.e. defences that are only activated if and when needed (Harvell 1990; Shudo and Iwasa 2001). For instance, humans visually perceiving signs of potential infection risk upregulate their immune system compared to control individuals that are shown similar images not associated with infection threat (Schaller et al. 2010; Stevenson et al. 2011). Priming of immune or behavioural defences by naïve individuals may occur through various kinds of external cues associated with an elevated risk of infection, either originating from conspecifics (Masri and Cremer 2014; Mothersill et al. 2015) or from the parasites themselves (Karvonen et al. 2004a; Rohr et al. 2009; Sharp et al. 2015).

Here, we investigate the potential priming of anti-parasite defences in the amphipod *Paracalliope fluviatilis*, which serves as the second intermediate host of the trematode *Coitocaecum parvum* (Opecoelidae). Trematodes have a complex life cycle, involving two or three transmission steps among different host species (Galaktionov and Dobrovolskij 2003). Typically, after multiplying asexually in a snail first intermediate host, infective stages known as cercariae leave the snail to seek and penetrate a second intermediate host, in which they encyst as metacercariae and await transmission via predation to their vertebrate definitive host. Second

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intermediate hosts of trematodes can be primed for increased defence upon perceiving cues indicative of imminent infection risk. For instance, fish and amphibians alter their behaviour to avoid infection after detecting chemical cues they associate with the presence of cercariae, or alarm cues released by conspecifics during infection by cercariae (Poulin et al. 1999; Karvonen et al. 2004a; James et al. 2008; Rohr et al. 2009). In addition, adult female treefrogs can even avoid laying their eggs in water bodies in which they detect chemical cues from either cercariae or just the snail species from which cercariae may emerge, thereby avoiding infection for their offspring (Kiesecker and Skelly 2000). However, it is unclear whether priming of anti-parasite defences based on perceived chemical cues of infection risk occurs in invertebrate intermediate hosts, such as amphipods.

There are good a priori reasons to expect selection to have favoured adaptive inducible defences based on perceived cues in amphipods. First, amphipods often experience huge fitness losses following infection by trematode metacercariae, including intensity-dependent increases in mortality and reductions in reproductive output (Thomas et al. 1995; Fredensborg et al. 2004; Bates et al. 2010; Rauque et al. 2011). Second, some defences against infection are costly in amphipods. Behavioural defences, such as increased activity and swimming away from cercariae, can be very effective (O. McPherson and R. Poulin, personal observations) and are not very costly. However, immunological defences against parasites, involving encapsulation and melanisation, are costly in arthropods (Moret and Schmid-Hempel 2000), including amphipods (Cornet et al. 2009a). The ability to mount such immune responses against trematodes is lost in amphipod populations never exposed to infection, presumably because they are costly to maintain (Bryan-Walker et al. 2007). Therefore, natural selection should favour efficient defences against trematodes, but because of their cost, these should be inducible defences that are only induced via priming when needed.

Besides priming and upregulation of anti-parasite defences after perceiving external cues indicating a high infection risk, there is another way in which a host can have prior information about parasites, since it may already harbour parasites, with consequences for its ability to resist further infections. In vertebrates, acquired immunity often allows a host to better resist subsequent exposure to parasite species it already harbours (Litman et al. 2010). However, for some types of parasites, acquisition of one or a few parasites can alter the behaviour or odour profile of the host and make it more susceptible to acquire further parasites in future exposures (e.g. Norval et al. 1989; Poulin et al. 1991). Some invertebrates display acquired immunity that is simpler but functionally equivalent to that of vertebrates (Kurtz and Franz 2003). However, prior infection can also lead to immune depression, leaving the host more susceptible to acquire further parasites (Cornet et al. 2009b). Alternatively, individuals that already pay the cost of infection by a parasite may have less energy to defend themselves against further parasite attacks. Indeed, invertebrates that have acquired many trematode metacercariae during a first exposure may accumulate more during a second exposure than those that were more resistant to begin with (e.g. Leung et al. 2010). This is either due to intrinsic differences in susceptibility among individuals, parasite-induced immune depression, or prior infection predisposing a host to acquire further parasites later. Given the lack of evidence for acquired immunity against trematodes in our system, we feel that the energetic cost of prior infection is likely to increase the susceptibility of the host to further infections.

The present study tests how both prior infections and priming via olfactory cues associated with infection risk affect the avoidance behaviour and overall ability to resist infection by the trematode *C. parvum* in the amphipod *P. fluviatilis*. We hypothesise that (i) amphipods primed with chemical cues from the parasites will subsequently better resist cercarial attacks than control individuals and (ii) amphipods already harbouring metacercariae will be more susceptible to further infections, regardless of whether they are primed or not. We test these in terms of both actual acquisition of parasites and induced behavioural defences manifested as altered swimming patterns.

Material and methods

Animal collection and handling

Snails (*Potamopyrgus antipodarum*) and amphipods (*Paracalliope fluviatilis)* were collected in May–June 2017 from among macrophytes in Lake Waihola, Otago, New Zealand (46° 01′ 14.1″ S, 170° 05′ 05.8″ E), using a dip net. In the laboratory, snails were separated into wells of culture plates, with five individuals per well and approximately 2 ml of filtered lake water. Infected snails were identified by being incubated for at least 3 h at 20 °C under a light source, a procedure which induces cercarial emergence (Fredensborg et al. 2005). Snails were subsequently screened again individually to verify infection status. Infected and uninfected snails ($N = 200$ in each case) were maintained separately in aerated 10 L stock tanks filled with aged lake water and macrophytes (*Chara corallina*).

New amphipods were collected from the field prior to each series of trials. Only male amphipods were used to reduce inter-individual variability in susceptibility to infection ($N = 600$ for experimental infections; $N = 270$ for the behavioural study). Amphipods were first maintained in an aerated 2-L stock tank containing lake water and macrophytes for 24 h. Amphipods were then haphazardly separated into batches of 20–30 individuals in 1-L containers (one for each treatment

group—control, exposed to uninfected snail water, and exposed to infected snail water; see below). Conditions were similar to those of the stock tank (500-ml filtered lake water, aerated and containing macrophytes). Amphipods were acclimatised to these new containers for a further 24 h prior to the odour exposure.

Odour treatments

The three odour treatments included aged lake water (control), water conditioned with the smell of uninfected snails and water conditioned with the smell of snails infected with *C. parvum*. In what follows, these treatments will be referred to as control, uninfected snail and infected snail treatment. The two treatments which used snail conditioned water were prepared by placing 15 uninfected and 15 infected snails, respectively, into separate 1-L containers filled with 500 ml of filtered, aerated lake water and macrophytes; the control treatment included the same physical conditions but with snails absent. A high density of snails was used for the odour treatments to maximise the chances of amphipods responding to trematode infection risk. Snails were kept in these containers alongside the control container for 48 h under natural lighting and photoperiod, to allow chemical cues to concentrate in the water. Once daily for the next 2 days while the snails were still kept in their containers, 200 ml of water from each odour treatment was removed and filtered, then immediately added to amphipod containers (which had the same volume of water removed prior to addition of odour treatment to keep water level constant) and replaced with filtered lake water. Thus, amphipods all experienced 48 h in either control, uninfected snail water and infected snail water prior to being used in the experiments below.

Experimental infections

Coitocaecum parvum cercariae were obtained by placing 100 haphazardly chosen snails from the stock of infected snails in a Petri dish with 10 ml of filtered lake water. The snails were incubated at 20 °C for 30 min under constant light (Fredensborg et al. 2005). This usually yielded > 100 cercariae per incubation. Cercariae were transferred using a 20-μL micropipette to Eppendorf tubes (or to wells of a culture plate for the behavioural study; see below). After transfer, all cercariae were visually checked and damaged or inactive cercariae were discarded and replaced.

We used experimental infections to test whether prior infections and priming via olfactory cues associated with infection risk would influence subsequent parasite infection of amphipods. Firstly, two cercariae were transferred into each 0.6-ml Eppendorf tube along with 2.5 μL of filtered lake water and secondly, an amphipod (from the control, uninfected snail or infected snail treatment) was added to each tube using an extremely fine paint brush. The small volume of water used was enough to sustain amphipods but insufficient to allow them to swim freely in the tube, thus allowing the parasites to encounter amphipods and limiting host defences to grooming and beating of gills. Amphipods were left in the tube along with the two cercariae for 5 h at 18 °C, by which time unsuccessful cercariae die (Lagrue and Poulin 2007). Amphipods which survived these infections ($N = 420$) were transferred back to their treatment containers and maintained for a further 2 days before dissection, to give the successful cercariae time to burrow through the host cuticle and encyst within the body cavity. After this time, surviving amphipods ($N = 407$) were killed in 70% ethanol, rinsed with distilled water and dissected immediately. All parasites found within amphipods were recorded and measured, and the total length of each amphipod was recorded (from rostrum to telson) to the nearest millimetre.

Parasites recovered during dissection were categorised as either new infections (infections of *C. parvum* which occurred during the experimental infection) or prior infections (infections acquired in the field). Infections with other parasite species were found: metacecariae of the trematode *Maritrema poulini* were treated as prior infections, whereas the single amphipod found with a juvenile of the acanthocephalan *Acanthocephalus galaxii* was excluded from further analysis. *Coitocaecum parvum* metacercariae were classified as prior infections if they exceeded 0.33 mm in length, as it is unlikely they could reach this size a mere 2 days after infection (C. Lagrue, personal communication). *Coitocaecum parvum* metacercariae were classified as newly acquired if they were 0.1–0.23 mm in size. Amphipods harbouring *C. parvum* in the intermediate size range 0.24–0.32 mm were excluded from analysis as it was unclear whether these parasites were prior infections or acquired during the experimental infection. This resulted in the exclusion of 10 amphipods; therefore, with the exclusion of the single amphipod with an *A. galaxii* infection, the final sample size was 396 amphipods for which we had data on body size, number of prior infections and number of newly acquired metacercariae. We report the abundance of parasites (number of new metacercariae per host) for amphipods of the different treatments.

Amphipod behaviour

This experiment tested whether amphipods show chemosensory recognition of chemical cues indicating the risk of infection by quantifying their behaviour (distance moved and time spent moving) after exposure to the chemical cues of parasites (i.e. water conditioned by snails infected with *C. parvum*), and in the presence or absence of a *C. parvum* cercaria. For this experiment, a total of 90 amphipods were primed with each odour treatment, in three batches of 30 individuals per treatment staggered over 3 days ($N = 270$). A

subset of surviving amphipods were then exposed to either one *C. parvum* cercaria with lake water or lake water alone, which created six treatment groups: control water, parasite absent ($N = 35$); control water, parasite present ($N = 34$); uninfected snail water, parasite absent ($N = 36$); uninfected snail water, parasite present ($N = 36$); infected snail water, parasite absent ($N = 37$); and infected snail water, parasite present ($N = 37$) (total $N = 195$, after exclusion of individuals with prior infections; see below). Amphipods from each group were transferred individually from their odour treatment containers to wells of a 96-well culture plate using a pipette, and given 30 min to acclimatise. The rims of the wells were individually marked to identify each treatment. The wells were filled up to the rim with filtered lake water. Half of the wells contained one *C. parvum* cercaria each, and half contained only filtered water. Amphipods were subsequently filmed for 5 min with a camera mounted on a dissecting microscope (Olympus SZ61) and recorded using the computer screen recording program Bandicam (Bandicam, Seoul, Korea). Six amphipods were filmed simultaneously, one per treatment group, to minimise the possible influence of nested trial effects. Illumination came from the base light of the microscope and was constant for all trials.

EthoVision XT (Noldus Information Technology) was subsequently used to assess swimming behaviour of amphipods when alone and when exposed to a parasite. The program tracks moving objects based on changes in pixel colour from each video frame to the next (see Noldus et al. 2001). The two specific behaviours measured were general measures of activity; total distance moved (tracking movement of the animal's centre point, measured in mm) and time spent moving (more than 20% of the animal has moved within the sample period of 0.05 s); both were calculated for each individual over 5 min using EthoVision XT. Note that the water level in the wells constrained the amphipods to movement in only two dimensions. Amphipods were then measured and dissected the same day. Amphipods with prior parasite infections (acquired in their natural habitat) were very few ($N = 14$) and were therefore excluded from further analysis.

Statistical analyses

Statistical analyses were carried out using the software package R 3.4.1 (R Core Team 2017). For the experimental infection experiment, the abundance of new *C. parvum* metacercariae per host was analysed using a cumulative link mixed model (clmm2) using the R package *ordinal* (Christensen 2015). The main goal was to test the effect of both prior infections and exposure to odour treatment on subsequent acquisition of parasites, while accounting for any effect of host size. Treatment, number of prior infections and size of amphipod were included as fixed factors in the model. The interaction between prior infections and treatment was included in the original model but excluded from the final model as it was not significant. During the experiment, amphipods were processed in batches because time constraints limited how many could be handled per day; therefore, batch, the order of batch for infection and date of collection were included as random factors to account for any variance due to these factors. A one-way analysis of variance (ANOVA) was conducted to determine whether treatment experienced by an amphipod influenced the average size of its newly acquired *C. parvum* metacercariae.

For the behavioural study, swimming behaviours of amphipods were analysed in R 3.4.1 with generalised linear mixed-effects models (GLMM) using the package *lme4* (Bates et al. 2015). Total distance moved and time spent moving were used as response variables, and amphipod size was included as a fixed effect. The main goal was to test whether there was a relationship between being primed to the presence of parasites through chemical cues (from water conditioned with *C. parvum* infected snails) and the resulting behaviour of amphipods; therefore, odour treatment (control, uninfected snail, infected snail) and presence/absence of a live cercaria were included as fixed factors. Acclimatisation time varied between 27 and 38 min and was therefore included as a random factor. Date of use was also included as a random factor as all amphipods were collected on the same day, but the experiment was conducted over three consecutive days and some amphipods experienced extra maintenance time prior to being tested.

Results

Experimental infections

Of the 396 amphipods used, 38 (9.6%) had parasites acquired in the field prior to the infection experiment. Most amphipods found with prior infections (28 out of 38) were infected with a single parasite. Seven amphipods hosted two parasites, two had three parasites and a single amphipod had four parasites. Prior infections included both *Coitocaecum parvum* metacercariae (found in both normal and progenetic life stages) and *Maritrema poulini* metacercariae. Most amphipods with prior infections harboured a single non-progenetic *C. parvum*. A single progenetic *C. parvum* was found in five amphipods. There were three cases of two *M. poulini* individuals infecting a single amphipod, four cases of a progenetic and non-progenetic *C. parvum* co-infecting the same host, and one amphipod had two *M. poulini* and two non-progenetic *C. parvum* parasites.

Each amphipod was exposed to two *C. parvum* cercariae during the experiment and could, therefore, end up with zero, one or two new parasites. The total number of amphipods that

acquired new infections was 207 (52.3%), with 154 of these having one metacercaria and 53 having two metacercariae. The size of newly acquired *C. parvum* metacercariae ranged from 0.10 to 0.23 mm. The mean size (±SE) of new metacercariae per treatment group was 0.162 mm ± 0.0007 for the control, 0.163 mm ± 0.0006 for the uninfected snail treatment and 0.163 mm ± 0.0009 for the infected snail treatment. There was no significant difference in metacercarial sizes among the different treatments (one-way ANOVA: $F_{2,210} = 0.30$, $p = 0.738$).

The cumulative mixed-effects model showed that treatment and amphipod size had no significant effect on parasite abundance (treatment: $R^2 = 0.00098$, $p = 0.82$, size: $R^2 = 0.0031$, $p = 0.26$) (Table 1). However, the number of parasites acquired during the experiment was significantly affected by the presence of prior infections, though the effect size was quite small ($R^2 = 0.04$, $p = 0.000007$; see Fig. 1). On average amphipods with prior infections acquired 1.08 ± 0.5 new *C. parvum* metacercariae, whereas amphipods free from prior infection acquired 0.61 ± 0.5 new parasites. Amphipods with prior infections that were exposed to either snail treatment (uninfected or infected snail treatments) acquired slightly more new parasites than those exposed to the control treatment or amphipods without prior infections (Fig. 1). None of the random factors explained a significant proportion of the variance in parasite abundance between amphipods (Table 1).

Amphipod behaviour

There were differences among the movement patterns of individual amphipods (see Fig. 2). The mean distance moved over the 5 min period was 327 mm ± 17.2 across all amphipods. However, the linear mixed-effects model found no overall effect of odour treatment on total distance moved (Table 2, Fig. 3). There was also no difference in distance moved between amphipods exposed directly to a live cercaria and those not exposed to a cercaria (331 mm ± 24.6 compared to 323 mm ± 24.1; Table 2, Fig. 3). None of the random factors explained a significant proportion of the variance in distance moved between amphipods (Table 2).

Similarly, there was no effect of odour treatment on time spent moving, and no effect of the presence of a live cercaria either (Table 2, Fig. 3). The mean time spent in motion across amphipods from all treatments over the 5 min period was 10.7 s ± 0.5. Again, none of the random factors explained a significant proportion of the variance in time spent in motion between amphipods (Table 2).

Discussion

Because defences against parasites are costly, inducible defences that are only activated if and when needed have evolved in many organisms (Harvell 1990; Shudo and Iwasa 2001). Indeed, upregulation of the immune system and activation of behavioural defences are common in animals primed by exposure to various cues associated with imminent infection risk (Karvonen et al. 2004a; Rohr et al. 2009; Schaller et al. 2010; Stevenson et al. 2011; Masri and Cremer 2014; Mothersill et al. 2015; Sharp et al. 2015). We tested the hypothesis that priming of anti-parasite defences in the amphipod *Paracalliope fluviatilis*, by exposure to chemical cues from its trematode parasite *Coitocaecum parvum*, would boost its ability to resist subsequent infection. We found no support for this hypothesis: whether amphipods were exposed to plain water, water conditioned by uninfected snails or water conditioned by infected snails, they were equally susceptible to infection during standard exposure to infective cercariae.

More immediate cues, such as visual or physical (i.e. vibrations) cues, may also alert an amphipod of imminent infection risk; however, only chemical cues would allow advance priming of defences. It is unlikely that the negative result we observed was due to a priming stimulus of insufficient strength. The snail *Potamopyrgus antipodarum* occurs at average densities of several thousands per square metre in our study lake, but its distribution is patchy, and prevalences of *C. parvum* also vary among patches (Lagrue and Poulin 2015). The density of *C. parvum*-infected snails used to condition the water in the experiment was an order of magnitude greater than average prevalence in the field (see Lagrue and Poulin 2015); if chemical cues are used by amphipods to detect the threat of trematode infection, they should have been

Table 1 Results of the cumulative mixed-effects model with the number of new *Coitocaecum parvum* metacercariae per *Paracalliope fluviatilis* as the response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors

Fixed factors	Estimate	Std error	*Z*	*P*	Random factors	% variance
Treatment	0.03528	0.13155	0.27	0.7890	Batch	0.16
Prior infections	0.94999	0.24047	3.95	0.000007	Order	0.01
Amphipod size	− 0.30337	0.26695	− 1.14	0.2560	Date	0.04

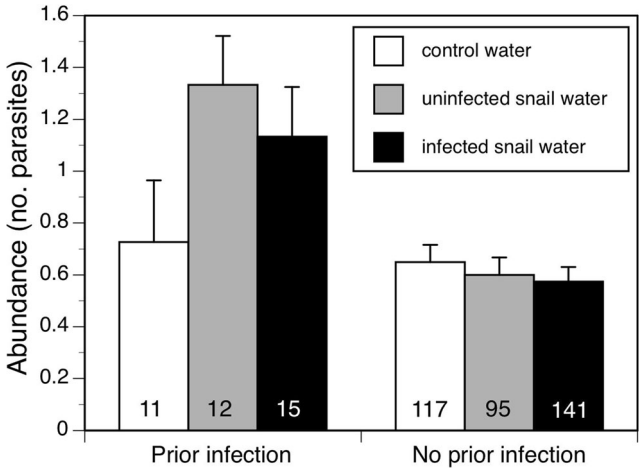

Fig. 1 Mean abundance (number of new metacercariae) ±SE of *Coitocaecum parvum* acquired during experimental exposure, across the three different treatments (control water, uninfected snail water, infected snail water) and shown separately based on whether or not they harboured prior infections. Sample sizes are shown on the bars

concentrated enough to be perceived. It is also unlikely that immune priming is not possible in invertebrates because of their simpler immune system, as it has been reported from other invertebrates, like molluscs. For example, snail first intermediate hosts of trematodes can be primed by prior exposure to substrate containing the parasite's eggs. Indeed, the same snail species used in our study, *Potamopyrgus antipodarum*, when exposed to the eggs of a different trematode species, rapidly increase their production of haemocytes relative to unexposed control snails (Osnas and Lively 2005, 2006). Given the relatively high prevalence of *C. parvum* in our study population (20–25%) and its negative impact on amphipod fitness (see Friesen et al. 2017), perhaps the defences of amphipods are always fully expressed and do not require priming.

We also did not find any effect of exposure to chemical cues from infected snails on the behaviour of amphipods, either in the presence or absence of a live *C. parvum* cercaria. This suggests that swimming activity is either unresponsive to infection threat or ineffective against it. Other movements that cannot be observed in our videos, such as grooming or gill beating, may have shown a response to priming with chemical cues from infected snails. Determining whether behavioural defences play a role against cercarial penetration would require anaesthesia of amphipods to compare infection rates between active and anaesthetised individuals exposed to a standard cercarial dose.

The second hypothesis we tested was that amphipods already harbouring metacercariae would be more susceptible to further infections, regardless of whether they were primed or not with chemical cues associated with parasites. Our results support this hypothesis. Indeed, the only pattern to emerge from our experiments was the higher susceptibility of previously infected amphipods to further infections by *C. parvum*, regardless of whether they had been primed by plain water or water treated with uninfected or infected snails. In our study, to increase the sample size, prior infections included both *C. parvum* metacercariae and *M. poulini* metacercariae, though only about a quarter of amphipods with prior infections harboured the latter. We feel it is reasonable to combine these because they both infect amphipods in the same manner (penetration of the cuticle), both have

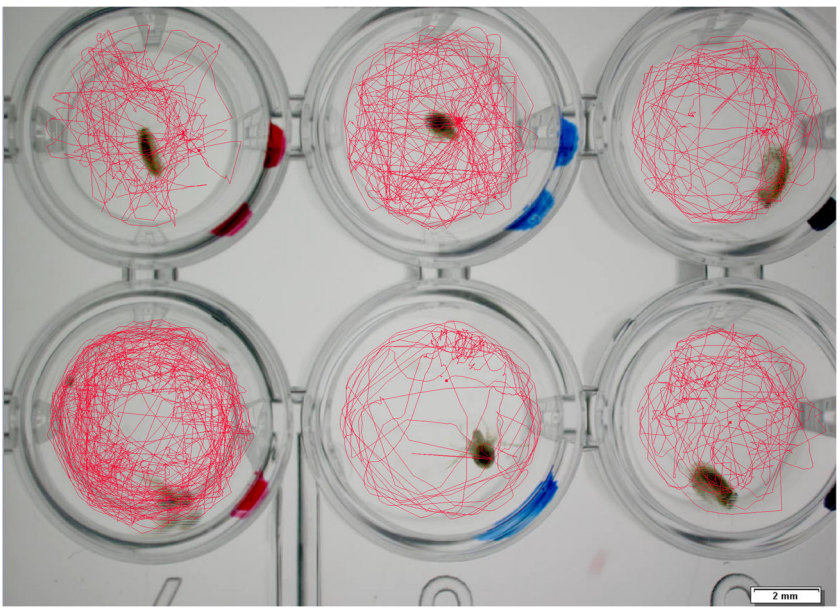

Fig. 2 Tracked paths showing the total distance travelled by six individual *Paracalliope fluviatilis* amphipods, one per well, over 5 min, as generated by EthoVision XT. These examples were chosen to show the full variance of movement of individual amphipods

Table 2 Results of the linear mixed-effects model with total distance moved and time spent moving by *Paracalliope fluviatilis* as response variables, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors

Fixed factors	Estimate	Std error	*T* value	*P*	Random factors	% variance
Distance moved						
Odour treatment	− 27.1730	19.7970	− 1.37	0.1669	Acclimatisation	0.03
Cercaria present	0.04750	0.11284	0.42	0.6670	Date of use	0.08
Amphipod size	0.07604	0.12809	0.94	0.1707		
Time spent moving						
Odour treatment	− 0.14961	0.65823	− 0.23	0.8176	Acclimatisation	1.03
Cercaria present	0.02596	1.05930	0.02	0.9889	Date of use	0.11
Amphipod size	− 0.32581	1.18577	− 0.28	0.7661		

similar sizes (at least for *M. poulini* versus non-progenetic *C. parvum*), neither of them affects amphipod swimming activity (Friesen et al. 2017), and the results look similar for both when considered separately.

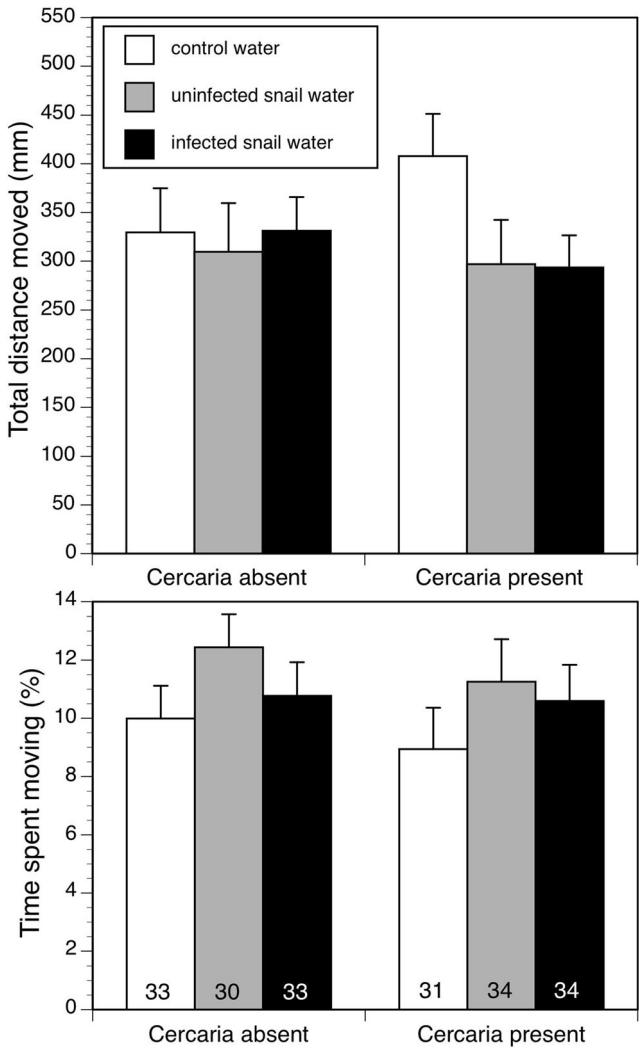

Fig. 3 Mean ± SE total distance moved (top) and percentage of time spent moving (bottom) by amphipods, across the three different treatments (control water, uninfected snail water, infected snail water) and shown separately based on whether or not a live *Coitocaecum parvum* cercaria was present during the 5-min test. Sample sizes (same for both graphs) are shown on the bars

The effect of prior infections clearly shows that infection history matters to the future susceptibility of amphipods to parasites. New infections are often not independent of old ones in various host-parasite systems. Acquired resistance to infection by trematode cercariae has been documented in fish hosts (Karvonen et al. 2004b), but not in invertebrates, where prior infections seem to pre-dispose the host to further infections (Leung et al. 2010). Whether due to immune depression (see Cornet et al. 2009b) or some other change in the host induced by prior infections, or due to some intrinsic (genetic) differences in susceptibility among amphipods, the greater acquisition of new parasites by previously infected amphipods can lead to the aggregation of parasites among hosts. This widespread pattern consists in the uneven and non-random distribution of parasites among hosts in natural populations, with a few individual hosts harbouring many parasites and most others harbouring very few or none (Shaw and Dobson 1995; Poulin 2013). This can enhance parasite-induced host mortality and contribute to host population regulation (Anderson and Gordon 1982). The phenomenon by which prior infections are associated with new ones thus has broader, population-level implications.

In conclusion, actual infection (i.e. prior infection), but not the perceived threat of new infection (i.e. smelling the parasite), influences susceptibility during exposure to infective cercariae in the amphipod *Paracalliope fluviatilis*. Given (i) the cost of infection, (ii) the advantages of inducible defences primed only upon detection of an immediate risk of infection, and (iii) the unnaturally high concentrations of uninfected and infected snail odours we used, it appears the amphipods are unable to chemically perceive the threat of cercarial infection despite the advantages this would provide. Chemical perception of predatory threats is very well-developed in small crustaceans (see Kats and Dill 1998; Hazlett 2010); therefore, it is surprising that this ability appears inefficient against fitness-reducing parasites. We suggest that the threat of trematode infection is consistently high enough in our amphipod population to have favoured defences that are not inducible but constantly active.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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