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Infection deflection: hosts control parasite location with behaviour to improve tolerance

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Anti-parasite behaviour can reduce parasitic infections, but little is known about how such behaviours affect infection location within the host’s body and whether parasite distribution ultimately affects tolerance of infection. To assess these questions, we exposed both anaesthetized (no behaviour) and non-anaesthetized *Hyla femoralis* tadpoles to plagiorchiid cercariae (larval trematodes), and quantified resistance, tolerance (relationship between mass change and infection intensity) and encystment location. Non-anaesthetized tadpoles had significantly more infections in the tail region than anaesthetized tadpoles, which had the majority of their infections in the head. This pattern indicates that parasites preferred to infect the head, but that hosts shunted infections to the tail when possible. Furthermore, there was a significant effect of encystment location on tolerance, with head-infected tadpoles having poorer tolerance to infection than tail-infected tadpoles. Variance partitioning suggests that, among infected tadpoles, behaviour contributed more to tolerance than resistance. These results suggest that, in addition to using behaviour to resist parasites, *H. femoralis* tadpoles also use behaviour to enhance infection tolerance by deflecting infections posteriorly, away from their vital sensory organs. These findings highlight the need to assess how widespread and important behaviour is to the tolerance of infections.

1. Introduction

Nearly every organism has at least one parasite, but hosts vary in their strategies for coping with infections. These strategies can be broadly characterized as belonging to one of two categories: (i) resistance, which entails preventing or reducing infections; and (ii) tolerance, which entails mitigating deleterious effects of infection as a function of infection intensity (e.g. weight loss). Although immunology is a frequent subject of resistance and tolerance research, these parasite-related coping strategies can also be mitigated behaviourally. Animals can avoid infected conspecifics [1] or microhabitats where infections could be contracted [2,3,4]. Furthermore, hosts might prevent or reduce infections with behaviours that physically remove parasites, such as grooming (i.e. removal) of ectoparasites [5,6].

Tadpoles are susceptible to infection by cercariae, a motile, free-living, aquatic larval stage of parasitic trematodes. Tadpoles are capable of behavioural resistance to these parasites, both avoiding cercariae [4] and exhibiting stereotyped anti-parasite behaviour, characterized by rapid swimming with many directional changes [7]. Both of these behaviours can reduce prevalence [8] and intensity of infection [9] relative to tadpoles that have been anaesthetized, suggesting that these behaviours are successful forms of resistance. Although a few taxa of trematodes specialize in infecting the inguinal region of tadpoles (e.g. *Echinostoma* spp., *Riberoia ondatrae* [7]), many are generalists in their distribution on the bodies of tadpoles and encyst subcutaneously on tadpole hosts. This poses an interesting scenario in which all infections by a generalist trematode might not be equal: infections near vital organs might be much...
more costly than infections far from vital organs, and therefore result in relatively poorer tolerance.

If the location of parasites on the host's body affects tolerance to infection, selective pressures might exist for hosts not only to avoid infection, but to prevent infection from occurring in the most expensive body locations. Using behaviours to prevent infections is often referred to as behavioural resistance; thus, using behaviour to minimize infections in the most costly body locations might naturally be referred to as behavioural tolerance. The relative strength of behavioural resistance versus tolerance could be measured by quantifying both the body location and cost of infections for hosts that can and cannot exhibit behaviours. For example, a host exhibiting behavioural resistance would only use behaviours to reduce infections and would not reduce the relative probability of infections in the most costly body locations. Assuming the same exposure to parasites, a host exhibiting only behavioural tolerance would have the same number of infections when they can and cannot exhibit behaviours, but would have fewer infections in the most costly body locations when exhibiting behaviours. Statistically, multiple regression models should be able to partition the independent contributions of behavioural resistance and tolerance to fitness. These models would have the number of parasites resisted (behavioural resistance) and the proportion of parasites in the least costly body location (behavioural tolerance) as predictors of a fitness proxy.

In this experiment, we exposed anaesthetized and non-anaesthetized tadpoles of *Hyla femoralis* to plagiorchiid, armatae cercariae (encystment generalists) and recorded encystment location, resistance and tolerance of infection (mass change) in each treatment. We tested two predictions based on the hypothesis that hosts should avoid infection in costly body regions: (i) infections in the head region of tadpoles, because of the proximity to vital sensory organs, should result in poorer tolerance than infections in the tail, which lacks vital organs and is lost during metamorphosis; and (ii) tadpoles capable of exhibiting behaviour should therefore attempt to minimize infections in the head. Conversely, we predicted that cercariae would predominantly infect the head of anaesthetized tadpoles, which might facilitate completion of the parasite's life cycle by making tadpoles vulnerable to predation by the parasite's definitive host.

2. Material and methods

The armatae cercariae used in this experiment were obtained from the trematode's first intermediate host, *Planorbella trivolvis* to plagiorchiid, armatae cercariae (encystment generalists) and recorded encystment location, resistance and tolerance of infection (mass change) in each treatment. We tested two predictions based on the hypothesis that hosts should avoid infection in costly body regions: (i) infections in the head region of tadpoles, because of the proximity to vital sensory organs, should result in poorer tolerance than infections in the tail, which lacks vital organs and is lost during metamorphosis; and (ii) tadpoles capable of exhibiting behaviour should therefore attempt to minimize infections in the head. Conversely, we predicted that cercariae would predominantly infect the head of anaesthetized tadpoles, which might facilitate completion of the parasite's life cycle by making tadpoles vulnerable to predation by the parasite's definitive host.

Tadpoles were divided into two anaesthesia treatments: 0.001 per cent benzocaine (anaesthesia) and ASW (control). A higher concentration of benzocaine (0.005%) than that used here has been demonstrated to have no side effects on measured tadpole immunological responses and does not immunosuppress tadpoles in a manner that affects encystment success by armatae cercariae [13]. Furthermore, in that experiment, benzocaine did not result in significantly different distribution of metacercariae on tadpoles' bodies than control tadpoles (see the electronic supplementary material, tables S1–S4 and figure S1). Immediately prior to parasite exposure, tadpoles were randomly assigned to an anaesthesia treatment and weighed, and then placed individually in plastic specimen cups with 30 ml of either 0.001 per cent benzocaine solution or ASW for 10 min (modified from [8]). This exposure is sufficient to induce immobility for a further 10 min, after which tadpoles fully recover mobility, including foraging behaviour. After anaesthesia or ASW exposure, tadpoles were then individually collected in a small net, rinsed with ASW and placed individually in a plastic cup with 30 ml of ASW and 0, 10, 15, 20 or 30 cercariae (n = 3 tadpoles per cercarial dose). Cercariae were collected directly from a specimen cup containing infected snails in ASW using a micropipette and dissecting microscope. Because cercariae at 1–6 h old are more infective than freshly shed or older cercariae [14], only those aged 1–6 h were used. Each tadpole was exposed to the assigned cercarial dosage for 10 min, rinsed with ASW to remove any attached cercariae that had not yet penetrated the tadpole's skin and transferred to a 1 l aquarium.

After parasite exposure, tadpoles were maintained in 1 l of ASW, fed frozen spinach ad libitum and monitored for mortality daily. Water was changed 5 days after exposure. Eleven days after cercarial exposure, tadpoles were weighed and euthanized in 0.1 per cent benzocaine solution. Tadpoles not surviving through this 11-day period were weighed and preserved immediately after death. To make encysted metacercariae visible, ethanol-preserved tadpoles were cleared according to Hanken & Wasserhaus [15]. In brief, 30 per cent hydrogen peroxide was added daily, in 1 per cent increments of the total ethanol volume, into vials containing tadpoles until all colour was bleached. Once colourless, specimens were transferred to a glycerol/KOH solution to clear until transparent. Metacercariae (the encysted form of cercariae) were counted and Gosner stage [16] assessed in cleared specimens at 100× magnification under a compound microscope. Cyst location was categorized as either in the 'head' (anterior to the eyes), 'body' (immediately behind the eyes to the base of the tail) or 'tail' (posterior to the base of the tail).

Ideally, measures of tolerance should be reliable proxies for lifetime fitness [17]; therefore, we measured mass change as a proxy for tolerance because mass of juvenile frogs at metamorphosis is predictive of adult frog fecundity [18,19]. The proportions of cercariae infecting individual tadpoles were arcsine square-root-transformed prior to analyses. We used analysis of variance (ANOVA) to test whether anaesthesia treatment affected the proportion of cercariae that successfully encysted, controlling for cercarial dose (continuous predictor) and Gosner developmental stage. Encystment locations (head, body and tail) were not independent because they were regions of the same host. Hence, a within-subjects ANOVA was used to test for differences in encystment location as a function of anaesthesia treatment, controlling for cercarial dose (continuous predictor) and Gosner developmental stage. Fisher's least significant difference (LSD) multiple comparison tests were conducted for post hoc analyses.

Leung et al. [20] revealed a positive relationship between metacercarial intensity and trematode recruitment to a particular host body location in the cockle *Austrovenus stutchburyi*. If the same pattern occurred for the focal trematode and host in this study, then it could have implications for the effect of cercarial encystment location on host mass gain. Consequently, we tested for an association between metacercarial intensity (log +1) and the proportion of metacercariae in each body region (arcsine
square-root-transformed). The above analyses were performed in *Statistica* v. 9 [21]. For the tadpoles that were infected (because uninfected tadpoles cannot exhibit tolerance), we partitioned the variance in mass change that was unique to and shared among Gosner stage, number of cercariae resisted (resistance) and proportion of metacercariae in the tail (behavioural tolerance) using the ‘hier-part’ function in the ‘hier.part’ package of R. This allowed us to estimate what proportion of the host responses contributed to resistance versus tolerance. To evaluate whether each variable accounted for greater unique variation than expected by chance, we conducted a randomization test (1000 randomizations) using the ‘rand.hp’ function in the ‘hier.part’ package of R [22].

3. Results

Anaesthesia did not affect mass change in the absence of cercariae ($F_{1,9} = 0.017$, $p = 0.90$). Gosner stage and infection intensity were also unaffected by anaesthesia treatment ($F_{1,15} = 0.079$, $p = 0.78$ and $F_{1,15} = 1.85$, $p = 0.19$, respectively), but anaesthesia did increase infection prevalence ($F_{1,27} = 5.47$, $p = 0.027$). Encystment location did not affect Gosner stage at the end of the experiment ($F_{1,15} = 0.055$, $p = 0.91$), and Gosner stage was not a significant predictor of mass loss ($F_{1,15} = 0.013$, $p = 0.90$). Metacercarial intensity was not significantly associated with the proportion of metacercariae in any body region ($F_{1,17} < 0.391$, $p > 0.54$). Hence, given the range of encysted cercaria in this study, there was little evidence that other cercariae influenced the body region in which any individual cercariae chose to encyst.

Anaesthesia, however, significantly affected encystment location (encystment location x anaesthesia: $F_{2,32} = 4.328$, $p = 0.02$; head: $F_{1,17} = 4.53$, $p = 0.048$; body: $F_{1,17} = 0.067$, $p = 0.79$; tail: $F_{1,17} = 5.20$, $p = 0.035$). Anaesthetized tadpoles had significantly more metacercarial infections in the head than tail, whereas non-anaesthetized tadpoles had predominately tail infections; anaesthesia did not affect encystment in the mid-body region (figure 1b). Post hoc multiple comparison tests revealed that the proportion of metacercariae in the head when anaesthetized and the proportion in the tail when not anaesthetized were not significantly different from one another (LSD: $p < 0.05$), but were significantly different from all other treatments. Black circles denote anaesthetized and white circles denote control. Error bars denote standard error.

![Figure 1.](image)

**Figure 1.** Anaesthesia affected (a) prevalence of infection and (b) encystment location of trematode parasites. (a) Significantly more anaesthetized tadpoles were infected by plagiorchid cercariae than non-anaesthetized tadpoles. Black bar denotes anaesthetized and white bar denotes control. (b) Significantly more parasites encysted in the head of anaesthetized tadpoles but the tail of control (non-anaesthetized) tadpoles. Post hoc multiple comparison tests revealed that the proportion of metacercariae in the head when anaesthetized and the proportion in the tail when not anaesthetized were not significantly different from one another (LSD: $p < 0.05$), but were significantly different from all other treatments. Black circles denote anaesthetized and white circles denote control. Error bars denote standard error.

or, in other words, hosts were more tolerant to tail than head infections. Furthermore, consistent with our second hypothesis, we found that non-anaesthetized tadpoles were capable of preventing these costly infections in the head and had infections concentrated in their tail region. By contrast, parasite encystment location was concentrated in the head of anaesthetized tadpoles. Given the absence of an effect of anaesthesia on mass change in tadpoles not exposed to parasites, we can affirm that the effect of anaesthesia on mass change in parasite-exposed tadpoles was not mediated by chemical side effects of benzocaine exposure (see also [13]). Our results suggest that although anti-parasite behaviour in *H. femoralis* can improve resistance to parasites by reducing prevalence of infection (figure 1a) among infected tadpoles, behaviour also plays a crucial role in tolerance to infection, deflecting infections to the less costly tail region (figures 2b and 3). This behavioural adaptation is at odds with the apparent preference of parasites to infect hosts’ heads, which might constitute a red queen-type ‘arms race’ between host and parasite [23].

There are several non-exclusive mechanisms by which head infections might have compromised tolerance of *H. femoralis* tadpoles. A decline in feeding activity could result if parasite encystment interfered with musculature or mouthparts [24], altered chemosensory detection of food, or comprised a stressor sufficient to reduce feeding behaviour.

4. Discussion

Consistent with our first hypothesis, we found that head infections were more detrimental to hosts than tail infections;
parasite’s definitive host, water snakes. In addition, any
mised, making them more vulnerable to predation by the
infections, predator avoidance by tadpoles might be compro-
tain locations of a moving host might be more difficult to
limited lifespan. Similarly, Taylor et al. [26] explained that
hosts. First, if the head is easier to penetrate than the tail,
apparent preference of cercariae for the head of tadpole
might facilitate parasite transmission, thus explaining the
ance associated with head infections relative to tail infections
material, tables S1–S4 and figure S1). The reduction of toler-
on-anaesthetized tadpoles (see the electronic supplementary
resulting in more head than tail infections, an analysis of tad-
sia with benzocaine might locally immunocompromise hosts,
organs [26], or parasite proximity to the brain could have
more expensive due to inflammatory damage of nearby
[25]. Furthermore, an immune response in the head might be
more expensive due to inflammatory damage of nearby
or parasitism proximity to the brain could have affected metabolism or host behaviour [27].

Although we cannot rule out the possibility that anaesthesia with benzocaine might locally immunocompromise hosts, resulting in more head than tail infections, an analysis of tadpoles that were anaesthetized, allowed to recover mobility and immediately infected [13] indicates that metacercariae do not encyst significantly more often in any body region of benzocaine-anaesthetized tadpoles when compared with non-anaesthetized tadpoles (see the electronic supplementary material, tables S1–S4 and figure S1). The reduction of tolerance associated with head infections relative to tail infections might facilitate parasite transmission, thus explaining the apparent preference of cercariae for the head of tadpole hosts. First, if the head is easier to penetrate than the tail, cercariae might prefer the head simply because they have a limited lifespan. Similarly, Taylor et al. [7] suggested that certain locations of a moving host might be more difficult to infect than others. Second, head infections could alter host traits that facilitate transmission to the definitive host. For instance, if olfaction or activity levels are modified by head infections, predator avoidance by tadpoles might be compromised, making them more vulnerable to predation by the parasite’s definitive host, water snakes. In addition, any

interference of head infections with foraging could result in
a decline in body condition that might make the tadpole slow to respond to predation attempts. Conversely, by shunting infections to the tail, tadpoles could avoid the compromising effects of infection, improving their likelihood of avoiding predation, as well as their post-metamorphosis fitness. Future work will address by which mechanisms trematode infections reduce fitness in tadpole hosts and whether susceptibility to predation is affected by parasite encystment location.

To our knowledge, this is the first description of behavioural tolerance to a parasite. Nevertheless, this phenomenon might be widespread. That is, many hosts that cannot entirely avoid infections might shunt parasites to parts of their body that minimize damage or might preferentially remove parasites from parts of their body where the parasites are most costly. This pattern was observed in our own results, with behaviour decreasing prevalence, but not intensity of infection, and simultaneously increasing tolerance to infection. Asymmetry in location of infections among hosts is not uncommon, especially among motile parasites such as trematodes and ectoparasites [28,29]. Although some of this asymmetry might be parasite-mediated, how host behaviour or parasite preference influences this asymmetry is unclear [30]. For example, bonobos direct most of their allogrooming to the face of conspecifics [31], perhaps because ectoparasitic infections of the eyes, nose or ears are more costly than on the body (though this has apparently not been quantified). Consequently, grooming, like the anti-cercarial behaviours of H. femoralis, might serve to both resist and tolerate infections. We certainly need a better understanding of where on the body certain parasites cause the greatest harm, and how widespread and important behaviour is to both resistance and tolerance of infections. Moreover, given that species vary in their resistance and tolerance to infection [32,33,26], future studies should use variance partitioning to investigate what portion of observed resistance and tolerance is comprised by behaviour.

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References


