Natural enemy ecology: comparing the effects of predation risk, infection risk and disease on host behaviour

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Summary

1. Growing interest in unifying the field of natural enemy ecology has revealed similarities between predation and parasitism. In parallel with predation, parasite infection – and even the threat of infection – can alter host traits and indirectly affect community interactions. Nonetheless, few studies have considered multiple mechanisms of natural enemy-induced behavioural alteration in parallel (e.g. effects before and after enemy contact) or the factors that drive variation in behavioural responses.

2. We first evaluated how the threat of infection by a virulent trematode (*Ribeiroia ondatrae*) compared to the well studied risk of predation in triggering inducible defences in amphibian hosts, prior to direct contact with either enemy. We then evaluated five separate factors that influenced the magnitude of parasite-induced behavioural changes after successful transmission.

3. In both the laboratory and an outdoor mesocosm experiment, we found no evidence that tadpoles of two species (*Pseudacris regilla* and *Anaxyrus boreas*) altered their activity levels in response to chemical cues from uninfected host snails, trematode-infected snails, or from conspecifics actively becoming infected. In contrast, tadpoles sharply reduced their activity in response to lethal predation risks posed by caged dragonfly larvae.

4. After infection, however, *Ribeiroia* caused strong decreases in host activity and escape distance that correlated positively with infection intensity and negatively with host size and developmental stage. Five days after infection with a one-time pulse exposure, hosts recovered to near-normal activity levels. Hosts exposed to a chronic daily exposure of equal intensity, however, continued to decrease activity. Unlike *Ribeiroia*, two less virulent trematodes had no detectable effects on host behaviour.

5. Our results highlight key distinctions between predation and parasitism. The contrasting effects prior to enemy contact may stem from the fact that unlike predation, the consequences of macroparasite infection are intensity-dependent and unpredictable. In contrast, the strong changes in host behaviour after infection are more similar to non-consumptive predator effects in terms of their potential influences on host fitness and community interactions.

Key-words: alarm substance, behavioural alteration, ecology of fear, parasite avoidance, phenotypic plasticity, trematode cercariae

Introduction

Animals in nature exist in complex communities in which they must concurrently defend against multiple natural enemies, including parasites and predators. Recognition of this 'natural enemy ecology' has spurred growing interest in the ecological similarities of host/parasite and predator/prey interactions (Lafferty & Kuris 2002; Raffel, Martin & Rohr 2008; Kortet, Hedrick & Vainikka 2010). Both parasites and predators have potential to control the densities and alter the individual traits of hosts or prey (Raffel, Martin & Rohr 2008). Such effects of parasites and predators can occur at multiple phases of an interaction, including before, during, and after physical contact with an enemy, and can lead to qualitatively similar density- and

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trait-mediated indirect effects on other community members (Schmitz, Krivan & Ovadia 2004; Sih *et al.* 2012).

Despite the need to integrate research on predation and parasitism, ecologists have historically studied host/parasite and predator/prey interactions in isolation, rarely using the same context to evaluate the effects of both natural enemies. Most prior research on inducible defences against predators has focused on non-consumptive effects, in which the presence of a predator alters prey traits before direct contact and a traditional predator/prey interaction has occurred (Preisser, Bolnick & Benard 2005; Peckarsky *et al.* 2008). In contrast, most research on inducible defences against parasites has focused on the consequences of infection after contact between parasite and host (e.g. behavioural changes and immune responses; but see Moore 2002). This disparity in approaches likely stems from the fact that, aside from certain sublethal predators, a successful predation event typically involves death of the prey, such that most defensive strategies emphasize preventing a predator encounter. Indeed, the presence of non-fatal cues has been posited as a prerequisite for inducible defences to be favoured over constitutive defences (Harvell 1990). For most parasites, however, hosts have ample opportunities for defensive action during and after the initial infection event. During infection hosts can utilize behaviours that limit infection success (e.g. grooming; Clayton 1991) and after infection they can rely on immune responses to eliminate parasites (Frost 1999). Ultimately, linking our understanding of inducible defences across multiple types of trophic interactions will require considering how existing theory – including environmental cues, phenotypic trade-offs and plasticity – applies to both predation and parasitism.

A comprehensive understanding of the similarities and differences between predation and parasitism requires examining the effects of both natural enemies at multiple phases of the interaction (e.g. before and after parasite infection) and an exploration of factors that drive variation in responses. Relatively few studies have examined the ability of host species to modify their behaviour or other traits to reduce disease risk prior to parasite contact (Kiesecker *et al.* 1999; Behringer, Butler & Shields 2006; Fritzsche & Allan 2012). Consequently, few generalities have emerged with regard to how and when hosts will react to infection risk. Because behavioural modifications that mitigate risks from natural enemies are costly (Loose & Dawidowicz 1994; Downes 2001), the behavioural responses to threats from distinct natural enemies are predicted to vary with the severity of the risk imposed (McCarthy & Fisher 2000; Ferrari, Sih & Chivers 2009). Similarly, after an encounter has occurred, the consequences should vary with traits of the enemy (e.g. virulence), traits of the individual being attacked (e.g. host tolerance), and the dynamics of their interactions (e.g. timing of exposure and infection intensity) (Rohr, Raffel & Hall 2010; Johnson, Kellermanns & Bowerman 2011; Johnson *et al.* 2012). Comparisons between the effects of predators and parasites can therefore

enhance understanding of how these traits may lead to trade-offs in the responses of individuals.

Amphibians provide a useful study system to examine parallels in how animals respond to distinct natural enemies. The inducible defences of amphibian larvae towards predators have been extensively documented and provide a useful point of comparison to threats from other classes of natural enemies. Many amphibian species react predictably to chemical cues signalling predation risk by altering their morphology and reducing activity levels (e.g. Van Buskirk & Relyea 1998; Relyea 2001). To date, however, few studies have examined how amphibians respond to infection risk prior to parasite contact. Cues from infected conspecifics (Kiesecker *et al.* 1999) and from infectious parasite stages (Kiesecker & Skelly 2000; Rohr *et al.* 2009) have been suggested to elicit behavioural responses in amphibian larvae, although the mechanisms are not entirely clear. More studies have demonstrated that amphibians will react to infectious stages on initial physical contact in an effort to dislodge parasites and reduce infection success. Free-swimming larval trematode parasites, for instance, elicit strong behavioural responses in amphibian hosts in the form of evasive tail movements and short bursts of activity (Taylor, Oseen & Wassersug 2004; Daly & Johnson 2011). The majority of studies have focused on the behavioural responses of amphibian larvae after successful parasite infection (e.g. Lefcort & Blaustein 1995; Parris, Reese & Storfer 2006; Venesky, Parris & Storfer 2009; Han, Searle & Blaustein 2011). In general, these prior experiments have aimed to determine whether infection has an effect on behaviour, rather than decomposing factors underlying variation in host responses.

Our goals were to: (i) compare the effects of predators and parasites on amphibian larvae prior to direct contact with either natural enemy; and (ii) evaluate the factors driving variation in host responses after infection, including both host and parasite characteristics. We combined outdoor mesocosm and laboratory experiments to compare the behavioural inducible defences of amphibian larvae in response to a lethal predator (*Anax junius* dragonfly larvae) and a highly virulent trematode (*Ribeiroia ondatrae*). In both study venues, we included treatments that allowed us to separate potential effects from snails (i.e. first intermediate hosts), trematodes themselves, and conspecific amphibians actively becoming infected. We followed up the pre-infection studies with laboratory experiments to test how parasite identity, infection intensity, time since exposure, exposure duration (pulse vs. chronic) and host developmental stage influenced host behavioural outcomes after parasite infection. We hypothesized that in response to infection risk, amphibian larvae would increase their activity levels to avoid free-swimming parasite stages (Taylor, Oseen & Wassersug 2004; Rohr *et al.* 2009). After successful infection, we predicted that trematode parasites would cause decreases in host activity and that host behaviours would vary with the virulence of the parasite, the timing and duration of exposure and the

developmental stage of hosts (Johnson, Kellermanns & Bowerman 2011).

Materials and methods

STUDY SYSTEM

Our experiments involved Pacific chorus frogs (*Pseudacris regilla*), western toads (*Anaxyrus boreas*), predatory green darner dragonfly larvae (*Anax junius*), rams horn snails (*Helisoma trivolvis*) and three species of trematode parasites (*Ribeiroia ondatrae*, *Echinostoma trivolvis* and *Alaria* sp. 2) (Fig. 1). Amphibian egg masses and invertebrates were collected from ponds in Mendocino County, California, USA. The trematodes have complex life cycles in which they reproduce asexually within rams horn snails, produce free-swimming larval stages (cercariae) that actively seek amphibian hosts, and then are trophically transmitted into predatory bird or mammal definitive hosts (Fried & Graczyk 1997). To acquire trematode parasites, we screened field-collected rams horn snails for infection using standardized methods (see Johnson & Hartson 2009). Table S1 (Supporting information) contains average body sizes of amphibians used in all experiments.

PRE-INFECTION BEHAVIOUR EXPERIMENTS

We conducted complementary laboratory- and outdoor mesocosm experiments to compare the responses of amphibian larvae to chemical cues signalling a threat from predation by *Anax* or infection by *Ribeiroia*. Both experiments consisted of the following five treatments that were each replicated five times: (i) controls without chemical cues from predators or parasites; (ii) exposure to chemical cues from an uninfected snail; (iii) exposure to chemical cues from a snail infected with *Ribeiroia*; (iv) exposure to chemical cues from an infected snail in the presence of a tadpole host; and (v)

exposure to chemical cues from a dragonfly larva that was fed tadpoles *ad libitum*. We included treatments with uninfected snails to test for possible effects of snail cues alone. The treatment containing an infected snail with a tadpole, combined with the treatment containing an infected snail alone, allowed us to distinguish possible effects of cues coming from trematodes versus cues from tadpoles becoming infected.

In the laboratory study, our experimental units consisted of plastic tubs (41 × 29 × 17 cm) containing 12 L of water. In the centre of each tub, we attached a cage enclosed in 35 µm Nitex mesh that was designed to allow passage of chemical cues into the water without predators or parasites contacting focal tadpoles within the tub (Fig. S1, Supporting information). Dissections of hosts at the end of the experiment ($n = 3$ per replicate) confirmed that cercariae were unable to pass through cages. Each experimental unit contained 15 randomly assigned *P. regilla* (see Appendix S1, Supporting information).

The outdoor mesocosm experiment extended our results from the laboratory study by including a second species of amphibian, the western toad (*Anaxyrus boreas*), and by examining natural enemy interactions in a more realistic setting. The mesocosms consisted of covered 378 L livestock watering tanks containing sand, algae, zooplankton and 15 tadpoles each of *P. regilla* and *A. boreas* (see Preston, Henderson & Johnson 2012 and Appendix S1 for details on mesocosm methods). We placed two floating cages covered with 35 µm Nitex mesh into each mesocosm. In the treatments with dragonfly predators or infected snails housed with tadpoles, we maintained one tadpole of each amphibian species within each of the two cages in every mesocosm. This approach ensured that tadpoles were always receiving cues from conspecifics being preyed on or infected (see Appendix S1).

Our primary response variables included tadpole activity levels (laboratory and mesocosm) and position in the water column (laboratory only). The activity data were collected in the same manner in the laboratory and mesocosm experiments; on each day an
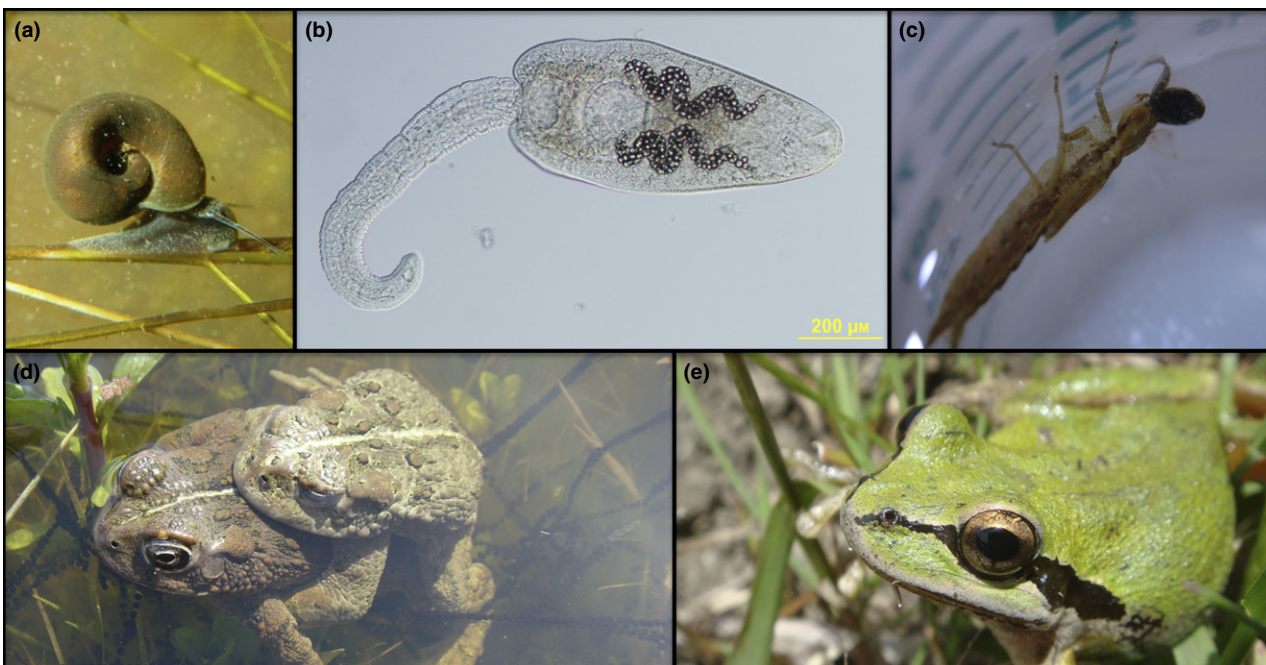

Fig. 1. Natural enemies and their hosts or prey. *Helisoma trivolvis* snail (a) that is the first intermediate host to the pathogenic trematode *Ribeiroia ondatrae* (b). Predatory *Anax junius* dragonfly larva consuming a chorus frog tadpole in the laboratory (c).Western toads (*Anaxyrus boreas*) (d) and a Pacific chorus frog (*Pseudacris regilla*) (e). Photos by Jeremy Monroe/Freshwaters Illustrated (a), Pieter Johnson (b) and Dan Preston (c, d, and e).

observer recorded the number of tadpoles that were moving within each replicate tub or mesocosm. The behavioural observations were repeated five times per replicate per day in the laboratory study and fifteen times per replicate per day in the mesocosm experiment (see Appendix S1). In both experiments, we recorded 2 days of behavioural data before the introduction of chemical cues and 8 days after the introduction of chemical cues. On the 7 days after chemical cue exposure in the laboratory study, we also recorded the number of tadpoles that were on the surface of the water (< 5 cm below the surface, again taking five repeated observation per replicate). At the end of both studies, we recorded the survival and wet mass of all individuals.

POST-INFECTION BEHAVIOUR EXPERIMENTS

We conducted four related laboratory experiments to examine how traits of trematode parasites and of *Pseudacris regilla* tadpoles influence host behavioural responses after successful transmission. Unlike the pre-infection studies, the post-infection experiments were conducted with individual tadpoles as replicates ($n = 10$ per treatment). These experiments were conducted with similar designs (excluding the escape distance experiment; see below). Tadpoles were infected and maintained within containers of 750 mL water and the primary response variable was tadpole activity. Each tadpole was observed 30 times per day to quantify activity (yes/no data based on whether each tadpole was active or inactive; see Appendix S1). At the conclusion of all four experiments, tadpoles were killed with an overdose of MS-222 and the number of successfully encysting *Ribeiroia* parasites was quantified using standard methods (Johnson & Hartson 2009; Johnson, Kellermanns & Bowerman 2011).

In the first experiment, we assessed the role of parasite identity in determining how infection alters behaviour by exposing tadpoles to 40 cercariae from one of three trematode species (*Ribeiroia ondatrae*, *Echinostoma trivolvis* or *Alaria* sp.), or to water without cercariae (see Appendix S1). We then quantified tadpole activity levels 1 day before and 1 day after infection at 14:00 h as described above. In the second experiment, we examined the effects of infection intensity and elapsed time since exposure on host behaviour. We exposed tadpoles to 0, 5, 10, 20, 30 or 40 *Ribeiroia* cercariae administered in a one-time pulse exposure, or 40 cercariae administered in a chronic exposure over 4 days (i.e. ten cercariae per day). This design allowed us to compare the effects of a pulse exposure, common in experimental designs, with a low-level chronic exposure that is more similar to how animals are exposed to parasites in nature. We then quantified activity levels for 4 days before infection and for 5 days after exposure in the pulse infection treatment. In the chronic exposure treatment we quantified activity for 4 days before infection and up until 1 day after the last exposure (all at 08:00 h).

In the third experiment, we examined how *Ribeiroia* infection intensity altered the escape distance of *Pseudacris regilla* tadpoles in response to a simulated predator. We exposed tadpoles to 0, 5, 10 or 20 *Ribeiroia* cercariae. One day after infection, tadpoles were placed into an aquatic track (1 m × 8 cm × 8 cm) and gently touched by a wooden stick, initiating a flight response. The starting and ending position of each tadpole was recorded and each individual was used in three trials with a 20 min rest period between runs (see Appendix S1). Lastly, in the fourth experiment we examined the effects of tadpole size and developmental stage on host behaviour by exposing 40 individually housed tadpoles that varied in Gosner stage (Gosner 1960) and snout-vent length (5–12 mm) to 25 *Ribeiroia* cercariae. We collected egg masses at different times to create the gradient of sizes. Tadpole activity levels were quantified 1 day before and 1 day after infection at 0:800 h as described above.

ANALYSES

For the pre-infection experiments, we used generalized linear mixed effects models (GLMMs) with a Poisson error distribution and a log link function to analyse data on the number of active tadpoles per observation in both the laboratory and mesocosm studies (Zuur *et al.* 2009). We focused our analysis only on the data from after the introduction of chemical cues and we included a fixed effect of treatment and random effects of observation date and of experimental unit (plastic tub or mesocosm) (see Appendix S1).

For the post-infection behaviour experiments, we utilized GLMMs with a binomial error distribution and a logit link function when the response variable was individual tadpole activity (yes/no) (Warton & Hui 2011). In all post-infection analyses, we specified individual tadpole host as a random effect. In the parasite identity experiment, we specified trematode species as a fixed effect and we focused our analysis on the data from 1 day after parasite exposure. In the experiment in which we varied parasite exposure intensity and monitored behaviour over time, we specified a GLMM with fixed effects of day, parasite dosage, and their interaction, and again focused on the data from after tadpoles were exposed to parasites. For the escape distance experiment we generated a model with a Gaussian error distribution that included a fixed effect of dosage to predict distance travelled of each tadpole (log transformed to improve normality). Because we ran three trials for each tadpole, we included a random effect of trial nested within tadpole. Lastly, in the experiment varying tadpole size and development stage, we used a model with a fixed effect of tadpole size (snout-vent length), a fixed effect of experimental period (before or after exposure) and an interaction between tadpole size and experimental period. All mixed effects models were run using the lme4 package in R (R Core Team 2013).

Results

PRE-INFECTION BEHAVIOUR EXPERIMENTS

Pseudacris regilla in the laboratory enclosures strongly decreased activity in response to dragonfly chemical cues during the first 5 days after the establishment of treatments (Fig. 2a; GLMM, $z = -7.548$, $P < 0.001$). There were also fewer tadpoles at the water surface within enclosures containing caged dragonflies relative to control treatments (GLMM, $z = -3.207$, $P = 0.001$). However, there were no significant effects of uninfected snails, infected snails, or infected snails + tadpoles on either *P. regilla* activity or the number of *P. regilla* at the water surface (all P values > 0.3). *Pseudacris regilla* survival and wet mass at the conclusion of the study did not differ among treatments.

Supporting our results from the laboratory study, both *P. regilla* and *A. boreas* in outdoor mesocosms responded to caged dragonfly predators, but did not react to cues from uninfected snails, infected snails, or infected snails caged with tadpole hosts (Fig. 2b,c). Relative to control treatments, the presence of dragonflies reduced activity of both *P. regilla* (GLMM, $z = -3.900$, $P < 0.001$) and *A. boreas* (GLMM, $z = -6.183$, $P < 0.001$). There were no other significant effects of the other treatments on tadpole activity (all P values > 0.15). As with the laboratory study,
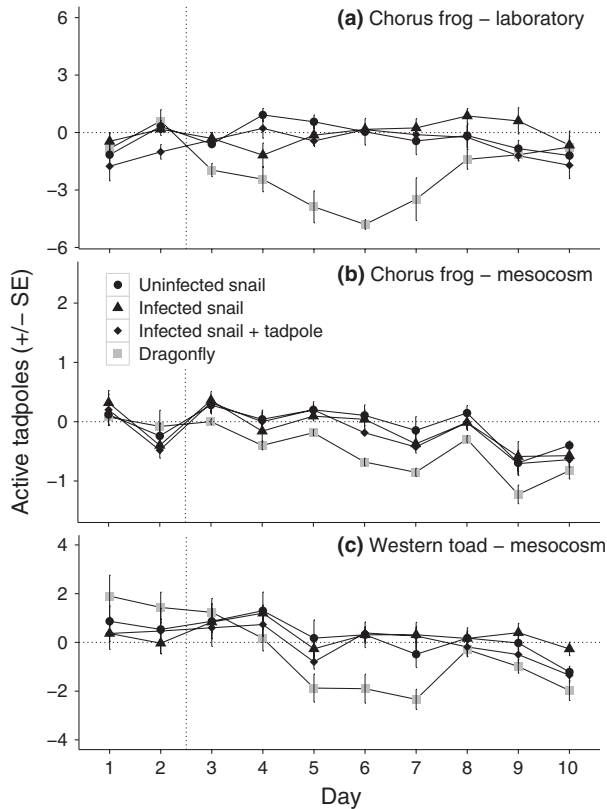
Fig. 2. The relative difference in the mean number of active Pacific chorus frog (*Pseudacris regilla*) and western toad (*Anaxyrus boreas*) tadpoles between control treatments and treatments exposed to cues from predatory dragonfly larvae, uninfected snails, trematode-infected snails and infected snails with amphibians in either the laboratory (a) or in an outdoor mesocosm experiment (b and c). Only tadpoles exposed to dragonfly cues (grey boxes) showed different activity levels from controls (dashed horizontal lines). The vertical dashed lines indicate the time at which chemical cues were introduced to all treatments.

Fig. 3. Box plots showing activity levels of *Pseudacris regilla* tadpoles before and one day after infection by one of three species of trematode parasites (*Echinostoma trivolvis*, *Alaria* sp 2. or *Ribeiroia ondatrae*). Time active on the *y*-axis represents the average proportion that individual tadpoles were active out of 30 observations (a). Activity levels of *Pseudacris regilla* tadpoles one and five days after a pulse exposure to *Ribeiroia ondatrae* (0–40 cercariae) or a chronic exposure of 10 cercariae administered nightly for four nights (the far right column labelled 'chronic') (b). In all box plots, the upper and lower hinges correspond to the first and third quartiles, the horizontal line shows the median, the whiskers extend to the highest and lowest values within 1·5 times the interquartile range, and outliers are shown as solid points.

behavioural changes were strongest in the days immediately following the establishment of treatments and then decreased over the duration of the study (Fig. 2b,c). Lastly, there were no differences in the wet mass or survival of either tadpole species between any of the treatments. Survival averaged 96% for *A. boreas* and 99% for *P. regilla* across all mesocosms.

POST-INFECTION BEHAVIOUR EXPERIMENTS

After successful transmission, *Ribeiroia* caused decreases in host activity levels that varied with characteristics of both the parasite exposure and the tadpole host. One day after infection, *Ribeiroia*-exposed tadpoles decreased activity by over five-fold relative to unexposed control tadpoles (Fig. 3; GLMM, $z = -5.898$, $P < 0.001$). In contrast, exposure to an identical dosage of *Echinostoma* or *Alaria* infectious stages had no effect on host activity 1 day after exposure (Fig. 3; GLMM, *Echinostoma*, $z = 0.946$, $P = 0.344$; *Alaria*, $z = 0.654$, $P = 0.513$).
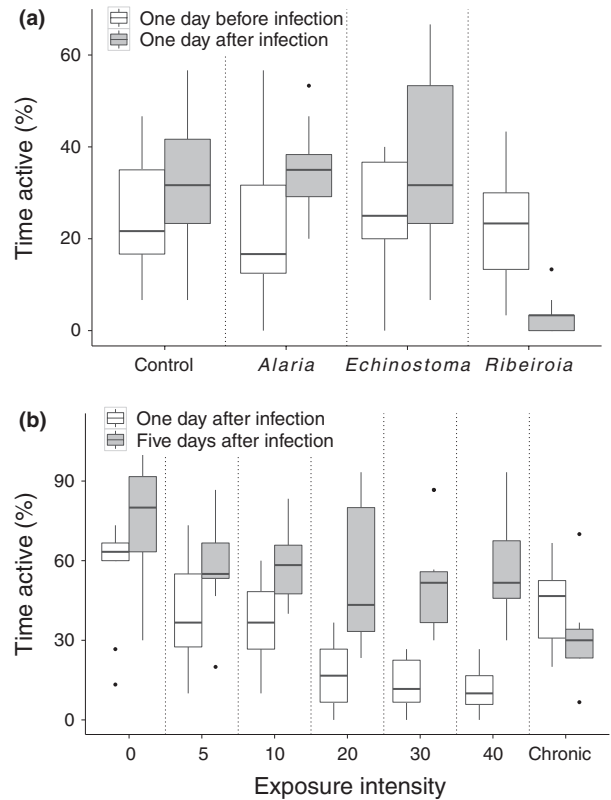
Both the magnitude of activity reduction and the time required for hosts to regain normal activity levels were influenced by the dosage of *Ribeiroia* administered (Fig. 3b). At high dosages, the magnitude of reduction in activity level depended on time-since-exposure, such that infection initially induced a strong reduction in activity that subsequently weakened over the next 4 days (Fig. 3b; GLMM, day x dosage, $z = 8.930$, $P < 0.001$). The initial effect of infection increased with parasite dosage; exposure to five or ten cercariae resulted in small reductions in host activity 1 day after exposure, whereas dosages of 20 or greater cercariae induced a threefold decrease in host activity. Five days after exposure, infected tadpoles were still 10–30% less active than controls, indicating that recovery of normal activity was not complete even at the lowest dosages (Fig. 3b). Importantly, the timing of host recovery after infection also depended on whether parasites were administered as a one-time pulse exposure or as a daily chronic exposure. On day five, tadpoles receiving a chronic

daily exposure of ten cercariae over 4 days were over 60% less active than unexposed controls and 40% less active than hosts receiving a pulse exposure of 40 parasites on day one (Fig. 3b; GLMM, pulse vs. chronic, $z = 2.062$, $P = 0.0392$).

In parallel with the effects on host activity, increasing exposure also correlated strongly with decreases in escape distance from a simulated predator (Fig. 4a). Exposure to 20 *Ribeiroia* cercariae caused an 80% decrease in escape distance relative to unexposed controls, whereas five cercariae caused only a 20% decrease (LME, dosage, $t = -6.201$, $P < 0.001$). Furthermore, dosage was a strong predictor of the number of successfully infecting parasites per host at the end of both the dosage experiment ($r^2 = 0.88$) and the escape distance experiment ($r^2 = 0.82$), giving us the same results whether we used dosage or infection intensity as the independent variable (see Appendix S1).

The magnitude of reductions in activity 1 day after infection was mediated by the size and stage of the tadpole hosts (Fig. 4b). Al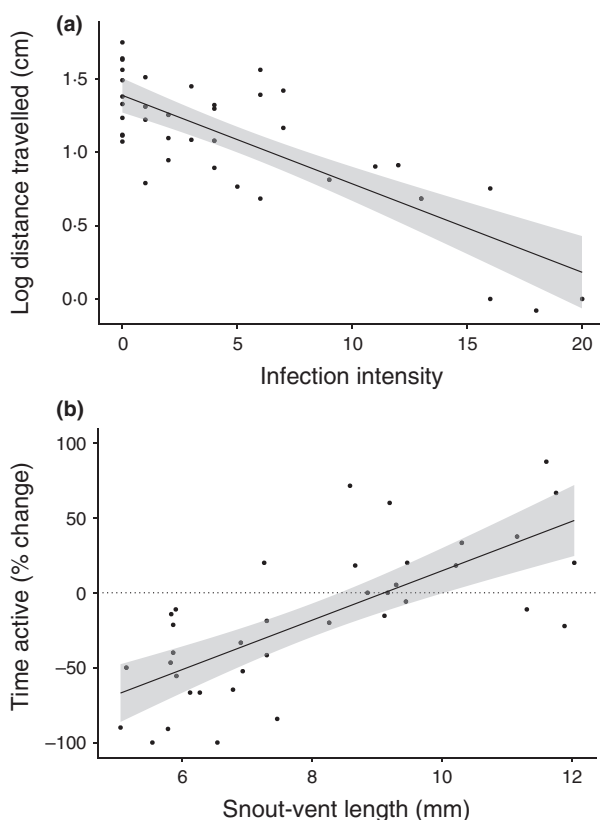though smaller hosts were slightly less active than larger individuals prior to infection, this effect became much stronger 1 day after infection, such that small individuals decreased activity by around 50% while larger individuals were unaffected by infection or increased in activity (GLMM, snout-vent length × experimental period, $z = -6.592$, $P < 0.001$). Similar results were obtained using developmental stage, rather than snout-vent length, as the predictor variable (GLMM, stage x experimental period, $z = -5.484$, $P < 0.001$). The effects of host size and developmental stage were not driven by differences in the number of encysting parasites, as there was no relationship between tadpole snout-vent length and *Ribeiroia* infection success 2 days after exposure ($F_{1,48} = 9.23$, $r^2 = 0.06$, $P = 0.126$).

Discussion

Parasites can alter the behaviour of their hosts at multiple phases of the host–parasite interaction, including before, during and after infection (Moore 2002). Here, we find that prior to parasite transmission, hosts did not respond to the threat of infection from a virulent parasite. Thus, in contrast to the well-documented inducible defences to predation risk (Relyea 2001), tadpoles did not exhibit phenotypic plasticity in response to infection risk. These results suggest that the mechanisms used to avoid or minimize threats from different classes of natural enemies have evolved along different trajectories within our study system; in contrast to defences used to minimize predation risk, amphibian hosts may have few options to minimize trematode infection prior to direct contact with infective stages. However, 9 h after successful transmission, infected hosts strongly reduced activity levels and escape distances from a simulated predator. The magnitudes of the changes in activity after infection were predictably influenced by multiple factors including parasite identity, infection intensity, time-since-exposure, exposure duration (pulse vs. chronic) and host developmental stage. These findings indicate that the behavioural consequences of infection, while variable in time and space, are predictable outcomes that depend on multiple traits of the parasite, the host, and the dynamics of their interaction.

A growing body of research has aimed to test for ecological similarities between predation and parasitism, including the roles of distinct natural enemies in the 'ecology of fear'. Our results add to this conceptual development in several key ways. As predicted, the presence of dragonflies induced the formation of behavioural defences in larval amphibians, which are known to reduce the risk of predation (Lawler 1989; Relyea 2001). In contrast, the same host species did not show behavioural changes in response to multiple types of chemical cues from parasites. Our experiments included treatments that tested for host responses from multiple ecologically relevant cues that could signal infection risk, including cues from infected host snails, infectious free-swimming parasite stages, and conspecific amphibian hosts actively becoming infected.

Fig. 4. The escape distance swam by *Pseudacris regilla* tadpoles in response to a simulated predator one day after exposure to 0–20 *Ribeiroia ondatrae* cercariae. The *y*-axis represents the log transformed mean of three trials per tadpole and the *y*-axis shows the numbers of successfully infecting parasites at the end of the experiment (a). The effects of tadpole size (snout-vent length) on the relative change in activity levels of *Pseaudacris regilla* tadpoles from one day before to one day after exposure to 25 *R. ondatrae* cercariae (b).

None of these cues elicited defences in tadpole hosts. Moreover, this result was consistent between a simplified laboratory experiment with one host species (*Pseudacris regilla*) and a more realistic outdoor mesocosm experiment with two host species (*Pseudacris regilla* and *Anaxyrus boreas*).

The lack of a response to infection risk contrasts with several previous studies involving amphibian hosts. Two studies have shown some ability of tadpoles to behaviourally react to threats from trematodes, potentially before parasite contact (Kiesecker & Skelly 2000; Rohr *et al.* 2009). In both studies, however, the exact mechanism of parasite avoidance was not entirely clear. In the more recent study, *Echinostoma trivolvis* cercariae were contained within 75 μm Nitex mesh to prevent direct contact with tadpoles (Rohr *et al.* 2009). However, *Ribeiroia ondatrae* cercariae, which can be twice as large as *Echinostoma trivolvis* cercariae (Preston *et al.* 2013), are able to pass through 63 μm Nitex mesh (D. Preston pers. obs) suggesting that parasites could have been directly contacting hosts in the prior study and necessitating the use of smaller mesh sizes (Lunde, Resh & Johnson 2012; Marino, Holland & Middlemis Maher 2013). More broadly, there is some evidence that certain hosts are able to detect waterborne cues signalling infection risk. Caribbean spiny lobsters, for example, avoid sharing dens with conspecifics that are infected with a lethal virus (Behringer, Butler & Shields 2006) and bullfrog tadpoles avoid conspecifics infected with a pathogenic yeast (Kiesecker *et al.* 1999). Whether such host responses are elicited by cues from the parasites or the infected conspecifics remains unclear. In our experiments, it is somewhat surprising that conspecifics becoming infected did not elicit host responses. *Ribeiroia* trematodes cause severe tissue damage and haemorrhaging (Johnson *et al.* 2004) and most species of tadpoles readily react to alarm cues from injured conspecifics (Chivers & Smith 1998; Schoeppner & Relyea 2005). We note, however, the possibility that tadpoles may have reacted to cues signalling *Ribeiroia* infection risk at night but not during daylight. Chemical cues from cercariae released at night might have weakened by the time behavioural observations were made each day, although cues from infected conspecifics were likely present continuously. Furthermore, host responses to cues other than chemicals (e.g. water vibrations from cercariae) should also be investigated in the future.

For the induction of inducible defences against natural enemies to enhance individual fitness, the potential benefits gained must outweigh the costs. Individuals expressing morphological and/or behavioural defences to specific natural enemies sometimes perform poorly at other vital functions including foraging, growth, seeking mates and responding to risks from disparate threats (Harvell 1990; Van Buskirk 2000; Relyea & Auld 2004). In the case of predation, these costs are commonly offset by the benefits of avoiding a predation event. This trade-off may be less predictable in the case of parasite infections, particularly involving macroparasites. While predator attacks are typically fatal for prey, the consequences of macroparasite infection are intensity dependent such that the ultimate risk depends on the number of infection events, rather than merely the presence of parasites. Furthermore, animal hosts are equipped with a variety of behavioural and physiological mechanisms to reduce or repair the consequences of infection after parasite contact. Macroparasite infection risk can be reduced through grooming behaviours or parasite avoidance strategies that are triggered by tactile cues coming directly from infective stages (Clayton 1991; Mooring, Blumstein & Stoner 2004; Taylor, Oseen & Wassersug 2004). Additionally, immune responses provide a further line of defence after successful infection. These responses to infection after parasite contact are costly (Lochmiller & Deerenberg 2000) and may make it inefficient for hosts to invest heavily in anti-parasite strategies both before and after infection.

In contrast to the lack of behavioural response to disease risk before infection, amphibian hosts exhibited strong reductions in activity levels after successful infection. These changes in behaviour, which were observed >9 h after parasite exposure, are distinct from the adaptive parasite avoidance behaviours tadpoles exhibit when they are first contacted by infectious cercariae (i.e. evasive tail movements and rapid bursts of swimming; Taylor, Oseen & Wassersug 2004; Daly & Johnson 2011). The host behaviours observed here occurred later in the host/parasite interaction and the magnitude of these effects varied with traits of the parasite and the host. Infection by *Ribeiroia* strongly reduced host activity (>80%) whereas two other trematodes (*Alaria* sp. 2 and *Echinostoma trivolvis*) administered at the same exposure intensity did not alter host behaviour relative to controls. These differences are most likely due to variation in virulence, some of which is associated with differences in the size and/or mode of entry of invading cercariae (Orlofske, Belden & Hopkins 2009; Rohr, Raffel & Sessions 2009; Johnson & Hoverman 2012; Preston *et al.* 2013). Unlike the two less virulent parasites, *Ribeiroia* cercariae use proteolytic enzymes to penetrate second intermediate hosts and cause haemorrhaging and tissue damage upon entry (Johnson *et al.* 2004). The behavioural outcomes of *Ribeiroia* infection also depended strongly on both the exposure dosage and the timing of infection. A dosage of 20–40 cercariae led to a threefold decrease in host activity 24 h after infection, whereas smaller dosages had minimal effects on host behaviour. Importantly, such effects were reversible after a one-time pulse exposure, but persisted over time during a daily chronic exposure. In nature, chronic low-level exposures are perhaps most realistic, suggesting that long term host behavioural changes may be common.

In addition to parasite identity and exposure dynamics, host traits influenced the behavioural outcome of infection. We found a strong correlation between host size and behavioural changes, where smaller hosts experienced the largest changes in activity level after infection. This result

is likely due to the fact that the area of tissue damage is larger relative to the tadpole's body in smaller individuals, although we note that tadpole immune systems also change considerably over the course of development, which can limit infection success. Our results are consistent with prior work that indicates host tolerance varies over the course of host growth and development (Sollid *et al.* 2003; Rohr, Raffel & Hall 2010). In a previous study, *Pseudacris regilla* tadpoles over the same range of developmental stages used in our experiment experienced an increase in survival from 55% in the smallest size class to 100% in the largest size class after *Ribeiroia* exposure (Johnson, Kellermanns & Bowerman 2011). This result parallels the size-dependent changes in behavioural effects observed here and in both studies these changes in activity and mortality can be attributed to differences in tolerance (the ability of a host to limit pathology) rather than resistance (the ability of a host to limit infection success) (Raberg, Graham & Read 2009). These findings indicate that environments that favour rapid host growth will narrow the window in which infection is likely to cause significant decreases in host activity.

The observed changes in behaviour likely have consequences for host and parasite fitness. In general, parasite-induced changes in host behaviour can be adaptive for the parasite, adaptive for the host, or simply side-effects associated with pathology (Poulin 2010). While we cannot rule out that the observed changes are somehow adaptive for the parasite, it seems more likely that they stem from side-effects of pathology and/or adaptive host responses. In the simplest explanation, injuries caused by the penetration of cercariae may lead to reduced muscle function, which impairs movement and reduces host activity. Alternatively, lethargy is consistent with adaptive host 'sickness behaviours' that allow hosts to divert resources towards fighting infection or repairing damage (e.g. immune responses; Hart 1988; Adelman & Martin 2009). While such responses are generally associated with microparasite infections (Martin, Weil & Nelson 2008; Llewellyn *et al.* 2011), it seems plausible that they could apply to our observed results with macroparasites. Furthermore, if the changes in host behaviour were adaptive for the parasite, they would be strongest when the parasites are infectious to the next host in the life cycle (>24 h after encysting in tadpoles). We found the opposite pattern, where the host behavioural changes were strongest before the parasite was infectious, supporting the idea that the observed changes are not a case of parasite manipulation.

Understanding the community level effects of changes in host behaviour – whether adaptive or otherwise – remains an important challenge in disease ecology (Lefèvre *et al.* 2009; Hawley & Altizer 2011). The increased lethargy observed in our study will likely have consequences for host competitive ability, predation risk and subsequent infection dynamics (Lefcort & Blaustein 1995). Many amphibian predators rely on visual cues, such that inactive tadpoles are less susceptible to predation (Lawler 1989).

However, our simulated predator experiment indicated that the escape distance of tadpoles is reduced with increasing infection intensity. For predators that actively pursue their prey, this could result in increased predation rates. Host behavioural modifications can also shape parasite transmission and subsequent host infections. Within our system, reduced activity levels make amphibian larvae more susceptible to trematode infection (Thiemann & Wassersug 2000; Szuroczki & Richardson 2012). In this instance, host lethargy may lead to feedbacks that enhance infection and contribute to parasite aggregation (Johnson & Hoverman 2014) and the skewed distribution of parasite infections in natural populations (i.e. 20% of hosts harbour 80% of the parasites), as well as the occurrence of superspreading individuals that contribute disproportionally to disease transmission (Lloyd-Smith *et al.* 2005; Paull *et al.* 2011). The net effect of reductions in activity levels on *Ribeiroia* transmission, however, is difficult to predict because *Ribeiroia* must be trophically transmitted from amphibians to its definitive hosts. If reductions in activity levels reduce predation rates by definitive hosts on infected tadpoles, they may reduce net transmission. Ultimately, the net effect of infection-induced behaviours on both predation rates and subsequent host infection will likely depend on the environmental context and the community composition in which the host-parasite interaction is embedded (Marino, Holland & Middlemis Maher 2013; Marino & Werner 2013; Orlofske *et al.* 2014).

Our results illustrate that despite some similarities, predation and parasitism can elicit disparate responses that depend on traits of the natural enemy and the individual being attacked. Interestingly, the changes in host activity after infection observed here show parallels to the behavioural changes often associated with non-consumptive predator effects (i.e. reduced activity). In this regard, the sublethal effects of predators on prey prior to direct contact may be most similar to the ecological consequences of parasite infection (rather than fear responses prior to parasite contact). These results underscore the need to consider similarities and differences between the ecology of distinct natural enemies at multiple phases of their interactions, which in turn will foster a more comprehensive understanding of natural enemy ecology.

Acknowledgements

Data accessibility

Data associated with this study can be found in the Dryad Digital Repository: doi: 10.5061/dryad.29470 Preston *et al.* (2014).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Methods details.

Table S1. Sizes of amphibian larvae used in experiments

Fig. S1. Laboratory enclosures used in the pre-infection experiments.

Fig. S2. Outdoor mesocosms used in the pre-infection experiments.