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Parasitism in a community context: trait-mediated interactions with competition and predation

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Abstract. Predation and competition can induce important density- and trait-mediated effects on species, with implications for community stability. However, interactions of these factors with parasitism remain understudied. Here we investigate interactions among competition, predation and parasitism by crossing tadpole density (*Bufo americanus*), presence of a caged predator (*Notophthalmus viridescens*), and *Echinostoma trivolvis* trematodes, experimentally partitioning their effects on tadpole exposure and susceptibility to infection. Predation did not affect *E. trivolvis* infection but accelerated tadpole development and growth, and decreased activity. The presence of *E. trivolvis* caused the opposite effects on these three responses and reduced tadpole survival. High conspecific density reduced tadpole survival, growth, and development, and increased tadpole activity. Effects of predation and parasitism on activity were only evident at low tadpole density. High-density mesocosms also had twice the number of *E. trivolvis* infections as low-density mesocosms, despite a lack of evidence for stress-induced immunomodulation. Instead, this effect was explained by high density delaying tadpole development, which increased both the duration of exposure to cercariae and susceptibility to infection, because tadpoles spent more time in highly susceptible early stages. These results highlight the importance of accounting for trait-mediated effects, host plasticity, and exposure vs. susceptibility in parasite ecology.

Key words: *American toad; amphibian; dilution effect; newt;* Planorbella trivolvis; *plasticity; snail.*

Introduction

Predation and competition have been the traditional foci of community ecologists (Wilbur 1997, Chase et al. 2002, Chesson and Kuang 2008), but recently the roles of parasites in community processes have gained increased attention as ecologists begin to appreciate the importance of parasites to complex food webs (Lafferty et al. 2008, Raffel et al. 2008). For instance, theoretical research has shown that predation and competition can influence rates of parasite infection by altering the population densities of hosts and vectors with varying degrees of competency (McCallum et al. 2001, Packer et al. 2003, Ostfeld and Holt 2004, Dobson et al. 2006, Keesing et al. 2006). Higher host densities can either increase density-dependent transmission (Ostfeld and Holt 2004) or reduce per capita exposure, when the source of infection is at least temporarily independent of host density (Raffel et al. 2008). Despite repeated calls for experimental quantification of the effects of predators and competitors of hosts on infection rates and host species composition in communities (Packer et al. 2003, Keesing et al. 2006), only one such study exists and effects were presumed to be density mediated (Borer et al. 2009). Predator- and competitor-induced changes in species' traits might also affect parasite transmission, but trait-mediated indirect effects of competitors and predators on host–parasite interactions remain largely unexplored (Raffel et al. 2008, Rohr et al. 2008*a*). Addressing these gaps in the literature will entail discriminating between effects mediated by changes in host density vs. traits, and by changes in exposure (how many infective stages attempt to enter a host) vs. susceptibility (ability to prevent or eliminate infection once exposure has occurred; e.g., Rohr et al. 2008*b*). For example, a predator might directly reduce host density, thus increasing per capita parasite exposure for the remaining hosts, but might also alter host behavior (e.g., feeding activity, movement) and development, thereby altering exposure to parasites.

While it is generally understood that organisms often face parasitism in conjunction with competition and predation (Hatcher et al. 2006), surprisingly few studies have experimentally assessed how hosts simultaneously manage these three factors (but see Parris and Beaudoin 2004). Parasitism is, in many ways, analogous to predation (Raffel et al. 2008). Trematode cercariae can induce behavioral antiparasite responses analogous to antipredator responses, including avoidance (Rohr et al. 2009) and seemingly adaptive changes in locomotor activity (Thiemann and Wassersug 2000*b*, Taylor et al. 2004, Koprivnikar et al. 2006, Rohr et al. 2009). Hence,

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responses to parasites (including behavior) are likely to trade off with responses to competition and predation, just as antipredator responses trade off with responses to competition and other predators (Relyea 2003, 2004, Hoverman and Relyea 2007). These tradeoffs likely have important but under-explored consequences for disease and community dynamics.

To test for interactive effects of competition, predation and parasitism, and to quantify the trait-mediated effects of competition and predation on host parasite interactions, we conducted a factorial experiment in which the density of American toad (*Bufo americanus*) tadpoles was crossed with the presence or absence of a caged newt (*Notophthalmus viridescens*) predator and the presence or absence of *Planorbella trivolvis* snails infected with *Echinostoma trivolvis*. This experiment was designed to tease apart effects on parasite exposure from effects on susceptibility to infection. Red-spotted newts are voracious predators of toad tadpoles, and echinostomes are common parasites of toads and other tadpoles in North America. *Echinostoma trivolvis* utilizes planorbid snails as first intermediate hosts, from which the motile cercaria stage emerges to find and infect the kidneys of a range of second intermediate hosts (e.g., snails, tadpoles, fish; Fried and Graczyk 2004). Definitive hosts, including mammals and water birds, complete the life cycle by ingesting an infected second intermediate host (Fried and Graczyk 2004).

Based on previous research, we generated several predictions for how predation, parasitism, and competition would affect tadpole survival, development, growth, and behavior. We predicted that predation would increase exposure to *E. trivolvis* by suppressing tadpole antiparasite behaviors, and that exposure to *E. trivolvis* would suppress antipredator behaviors, because the typical response to echinostome parasites is increased activity, opposite the antipredator response (Thiemann and Wassersug 2000*b*, Taylor et al. 2004, Koprivnikar et al. 2006, Rohr et al. 2009). Based on the analogy between predation and parasitism, we predicted that antiparasite responses would be stronger at low tadpole density, as has been shown consistently for antipredator responses (Relyea 2004). Based on previous studies, we predicted that *E. trivolvis* infection would decrease tadpole survival (Fried et al. 1997, Schotthoefer et al. 2003, Belden 2006, Holland et al. 2007), and we predicted that, like predators, parasites would decrease tadpole growth and development (Relyea 2007), though previous studies of *E. trivolvis* in tadpoles have not always found significant effects on these factors (Belden 2006, Koprivnikar et al. 2008, Orlofske et al. 2009).

Consistent with theory, we postulated that a two-fold increase in tadpole density would halve the per capita exposure rate to *E. trivolvis*. Both competition and predation typically delay tadpole development (Relyea 2007); hence, we predicted that the presence of competitors and a caged predator would increase exposure duration. However, late-stage tadpoles are typically more resistant to *E. trivolvis* infection (Schotthoefer et al. 2003, Holland et al. 2007), and thus delayed development might also increase tadpole susceptibility to infection because of stage-dependent susceptibility. Finally, we predicted that physiological stress in response to high density or predation might compromise immunity and increase host susceptibility to infection (Hayes 1997, Glennemeier and Denver 2002, Belden and Kiesecker 2005, Fraker et al. 2009).

Methods

Two adjacent experiments were run simultaneously (11 June to 20 July 2007), one to test for effects of predation and tadpole density on tadpole trematode infection rates (exposure experiment) and the other to isolate the effects of these variables on tadpole susceptibility to infection by experimentally controlling for trematode exposure (susceptibility experiment). Both were fully factorial randomized block experiments, with tadpole density (15 vs. 30 tadpoles per mesocosm) crossed with predation threat (presence or absence of caged newt) crossed with infection status (exposure experiment, presence of a snail infected or not infected with *E. trivolvis*; susceptibility experiment, five tadpoles exposed or not exposed to 30 trematode cercariae each). The selected tadpole densities are common in natural ponds (T. R. Raffel, *personal observation*). There were four spatial blocks in each experiment, each containing one replicate of each treatment combination, for a total of 32 mesocosms (30-L plastic tubs containing 19 L well water) per experiment. Detailed methods for animal collection and maintenance are described in Appendix A.

Caged predators (newts) were held in 1-L deli cups with fiberglass window screen covering the opening to allow passage of chemical cues and half were placed on the right and half on the left side of the mesocosm (in a stratified random design). Newts were fed 10 toad tadpoles (100 mg total) three times a week. Mesocosms without newts received empty cages, half on the left side and half on the right side. These cages were briefly lifted and replaced into each tank during newt feedings to equalize disturbance.

In the exposure experiment, designed to test for effects of predation and tadpole density on tadpole trematode infection rates, infected or uninfected snails were placed in cages that were identical to the newt cage and on the same side of the mesocosm. To ensure that the overall trematode exposure was not higher than that in nature and to control for individual variation in snail shedding rates, snails were placed at random in half the mesocosms and rotated every other day to the next mesocosm with the same infection treatment. Caged snails were fed ad libitum with boiled lettuce and dead snails were replaced with snails of the same infection status. Fewer than 10 snails died during the course of the experiment.

The susceptibility experiment was designed to isolate effects of density and predation on tadpole susceptibility

to infection, by experimentally controlling for trematode exposure. On 2 July, five tadpoles from each mesocosm were placed individually into 75 mL of artificial spring water (Cohen et al. 1980) and exposed to 30 *E. trivolvis* cercariae each for 24 h. All the cercariae were introduced to tadpoles within 30 minutes of emerging from infected snails. Placing tadpoles individually into a small volume of water limits behavioral avoidance of cercariae and thus isolates the effects of the treatments on the immunological defenses of the tadpoles. Based on prior experience, few cercariae fail to enter toad tadpoles under these conditions (Rohr et al. 2008*a*). After exposure to cercariae, these tadpoles were returned to their mesocosms for the remainder of the experiment to match how the tadpoles in the exposure experiment were treated, ensuring that the treatments could affect any potential clearance of the encysted trematodes (Holland 2009).

Tanks were checked daily and metamorphs and dead tadpoles were removed (when found; some dead animals decomposed too quickly to be recovered). Metamorphs, defined as having a tail <1 cm long, were euthanized by immersion in 1% benzocaine and all animals were preserved in 10% neutral buffered formalin. All toad specimens were massed following formalin fixation. Trematodes were enumerated for all toads from the susceptibility experiment and up to five randomly selected individuals per mesocosm from the exposure experiment, as described in Appendix A.

Activity levels of tadpoles in the exposure experiment were assessed on five dates (13, 25, and 26 June, 5 and 6 July), using the proportion of tadpoles moving in a 10 s period as a measure of activity. Six measurements were taken for each mesocosm on a given day and the average of these six observations was used as our response variable. Because the predator cages were translucent with black mesh covers, observers were blind to the presence or absence of a newt predator.

Blood smears were obtained from five randomly selected toads from each mesocosm in the susceptibility experiment on 2 July, the same day as the experimental trematode exposure of five different tadpoles from this experiment, to obtain leukocyte profiles. To determine whether changes in kidney development could account for changes in trematode resistance with development, developmental stage (Gosner 1960) and pronephros size were measured for the tadpoles from which blood was collected. Details of blood smear processing and kidney measurements are described in Appendix A.

Statistical analyses were conducted using R statistical software, version 2.8.1 (R Development Core Team 2006), as described in Appendix A.

Results

Exposure experiment

Tadpoles metamorphosed earlier at low conspecific density, in the presence of a caged predator, and in the absence of trematode exposure, with no significant interactions between these variables (Appendix B: Table B1, Fig. 1A). Toads metamorphosed with greater mass at low than high density, but there were no effects of the caged predator or trematode exposure on toad mass (Appendix B: Table B1, Fig. 1B). Tadpoles at high density or with trematode exposure had significantly higher mortality, but the caged predator had no detectable effect on mortality (Appendix B: Table B1, Fig. 1C). Activity increased in response to high density and trematode exposure, and decreased in response to a caged predator (Appendix B: Table B1, Fig. 1D). The predator and trematode exposure effects on activity depended on density (Appendix B: Table B1), with both effects being stronger in the low-density treatment (Fig. 1D). Analyzing the two density levels separately revealed significant effects of both predator and trematode exposure on activity at low density (predator, $F_{1,10} = 26.6$, $P < 0.001$; infection, $F_{1,10} = 16.9$, $P = 0.002$) but not at high density (predator, $F_{1,10} = 4.5$, $P = 0.060$; infection, $F_{1,10} = 0.3$, $P = 0.627$).

There were no predator or density effects on mean log trematode burden per individual in the infected mesocosms of the exposure experiment (predator, $F_{1,9} = 0.5$, $P = 0.491$; density, $F_{1,9} > 0.1$, $P = 0.810$; predator × density, $F_{1,9} < 0.1$, $P = 0.876$; Fig. 2A). However, the estimated number of total trematodes per mesocosm was significantly higher at high tadpole density ($\chi^2_1 = 62.1$, $P < 0.001$) and in the absence of a caged predator ($\chi^2_1 = 13.5$, $P < 0.001$; Fig. 2B). Tadpoles at high density or without predators also had longer exposure duration to trematodes due to delayed development (Figs. 1A, 2C). Controlling for exposure duration rendered the predator effect on total trematode infections nonsignificant ($F_{1,9} = 0.8$, $P = 0.400$). The magnitude of the density effect was reduced by controlling for trematode exposure duration but remained significant ($F_{1,9} = 5.6$, $P = 0.043$), with high-density mesocosms having approximately 1.5 times the infection rate as low-density mesocosms (39.6 vs. 26.0 infections per day, Fig. 2C).

Susceptibility experiment

High tadpole density caused significantly higher trematode encystment rates ($F_{1,9} = 25.3$, $P < 0.001$), approximately twice that in the low-density treatment (Fig. 2D), but there was no effect of a caged predator ($F_{1,9} < 0.1$, $P = 0.761$). High-density mesocosms also had less developed tadpoles at the time of trematode exposure ($F_{1,25} = 40.6$, $P < 0.001$; Appendix C: Fig. C1A), but predator presence did not have a significant effect on development at this stage of the experiment ($F_{1,25} = 0.6$, $P = 0.441$; Appendix C: Fig. C1A). Mean developmental stage at the time of trematode exposure was a significant predictor of encystment rates in the absence of other predictors ($F_{1,14} = 5.8$, $P = 0.031$; Appendix C: Fig. C1B). Mean pronephros volume also decreased linearly with developmental stage ($F_{1,30} = 91.8$, $P < 0.001$; Appendix C: Fig. C1C) and was a significant positive predictor of echinostome encystment

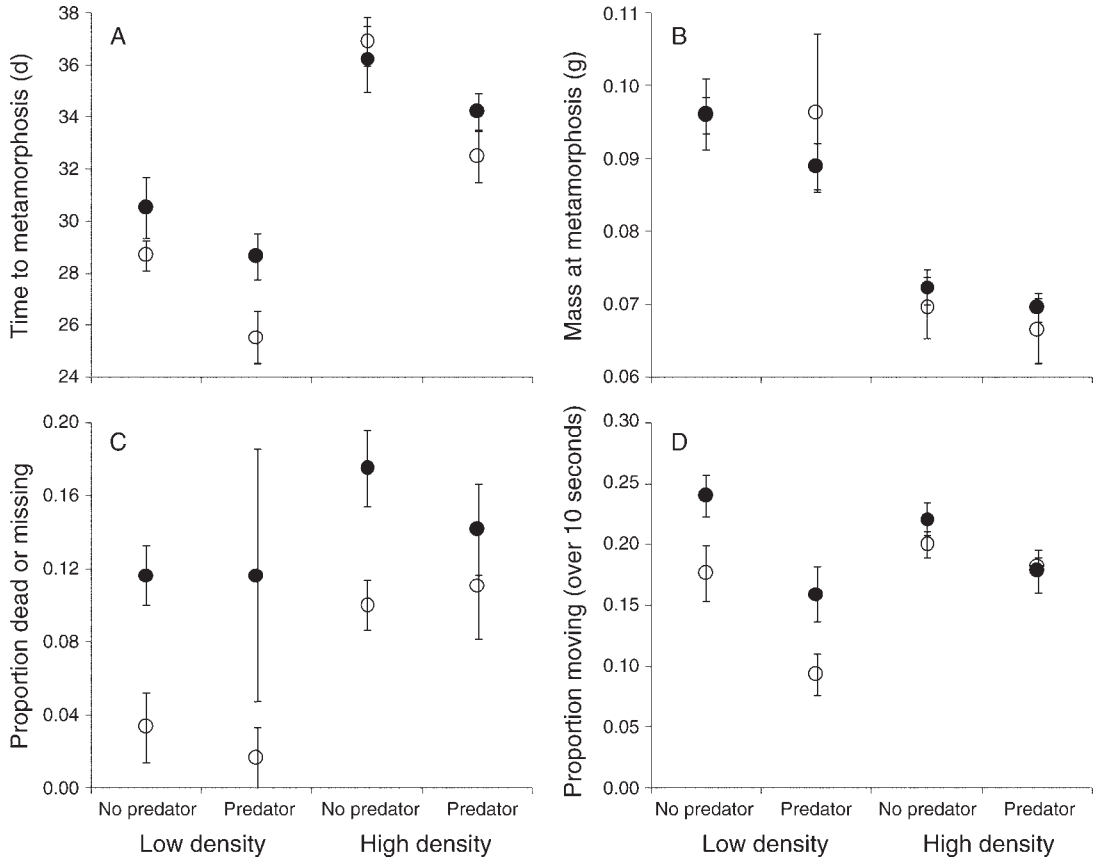

FIG. 1. Effects of density (15 vs. 30 tadpoles), the presence of a caged newt predator, and exposure to trematode infection (exposed, solid circles; unexposed, open circles) on (A) time to metamorphosis, (B) mass at metamorphosis, (C) mortality, and (D) activity levels of toad tadpoles (*Bufo americanus*) in the trematode exposure experiment. Error bars show ±SE.

(one-tailed test: $F_{1,14} = 3.3$, $P = 0.045$; Appendix C: Fig. C1D), accounting for 80.0% of the variation explained by developmental stage. The effects of developmental stage on standardized (adjusted by mean and standard deviation) pronephros volume and echinostome load were of similar magnitude (pronephros volume = −0.198 ± 0.176; encystment = −0.293 ± 0.120 [coefficient ± 95% CI]). There was no significant relationship between echinostome encystment and any of the leukocyte counts (all $P > 0.1$). Although tadpole density influenced some blood parameters (e.g., basophils), these changes were more consistent with effects of delayed development than of stress-induced immune suppression, and neither tadpole density nor predation influenced leukocyte counts in a way that could account for changes in echinostome encystment (Appendix D: Fig. D1).

DISCUSSION

Contrary to predictions, there was no effect of either competition or the threat of predation on the number of encysted trematodes per tadpole in the exposure experiment (Fig. 2A), despite strong effects of conspecific density and predation on tadpole activity levels and time to metamorphosis. This was unexpected, because tadpoles living at a higher population density should have a correspondingly lower per capita exposure to cercariae. However, total numbers of metacercariae per mesocosm were approximately twice as high in the high-density as in the low-density mesocosms (Fig. 2B), despite the total number of cercariae per mesocosm being the same (assuming that cercaria shedding from snails was independent of tadpole density). This effect was probably not due to space limitation in the tadpoles' kidneys, because previous studies have found no evidence of reduced encystment rates at higher *E. trivolvis* burdens, except in very early-stage tadpoles (Schotthoefer et al. 2003, Holland et al. 2007). The effect of tadpole density on total numbers of metacercariae was partially explained by a greater duration of exposure to cercariae at high density, due to delayed development, but there was still a significant effect of tadpole density after accounting for exposure duration (Fig. 2C). The remainder of the density effect could be accounted for by the effect of tadpole density on susceptibility to infection, indicated by a higher proportion of *E. trivolvis* cercariae encysting in tadpoles at high density in the susceptibility experiment (Fig. 2D).

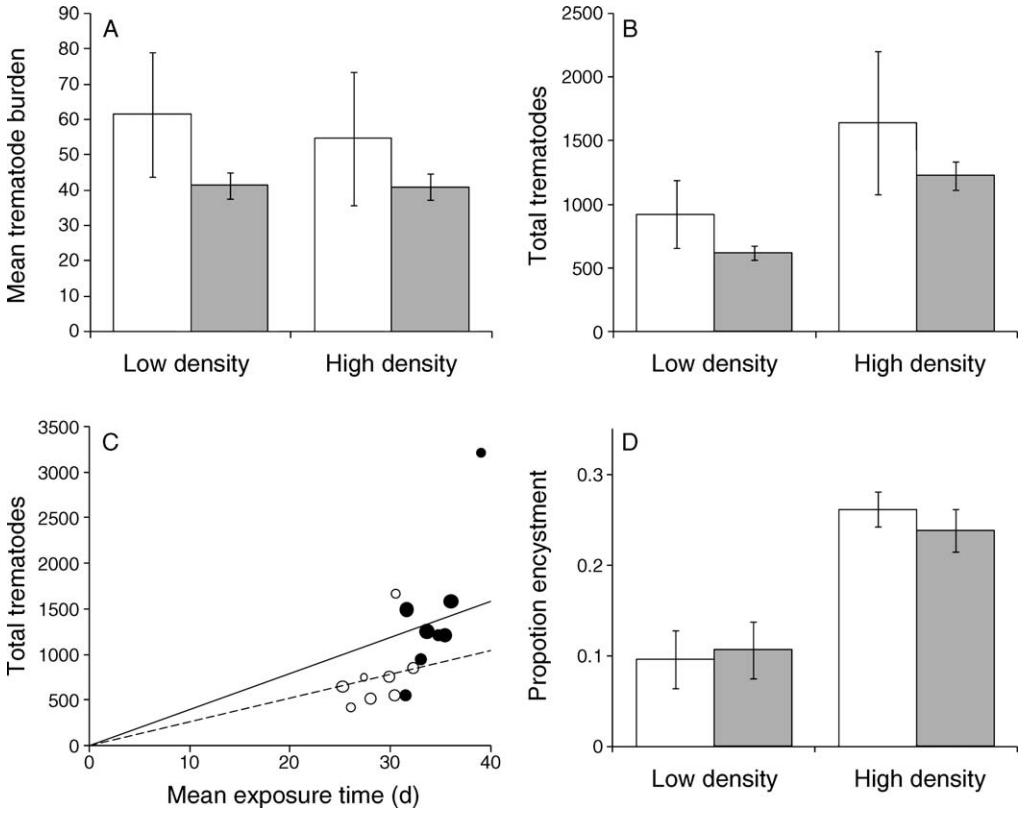

FIG. 2. Effects of density (15 vs. 30 tadpoles) and the presence (gray bars) or absence (white bars) of a caged newt predator on exposure and susceptibility to *Echinostoma trivolvis* infection. Panels show differences in (A) mean trematode burden (per individual) and (B) the estimated number of total trematodes (per mesocosm) in the exposure experiment. (C) The effect of tadpole density on total trematodes in the exposure experiment, controlling for the mean number of days tadpoles were exposed to infection in each mesocosm. Solid circles indicate high-density mesocosms, open circles indicate low-density mesocosms, circle sizes indicate weights (sample size used to estimate trematode burden; maximum = 5), and solid and dashed lines indicate model fits for high and low density, respectively. (D) The effects of tadpole density and a caged newt predator on the proportion of trematodes (out of 30) that successfully encysted per tadpole in the susceptibility experiment. Error bars show ±SE.

However, higher susceptibility to trematode infection at high density did not appear to be caused by stress-induced immune suppression. Tadpoles from the high-density tanks lacked changes in leukocytes that could account for increased susceptibility to infection, and neutrophil-to-lymphocyte ratios were higher in the high-density mesocosms, opposite the effect predicted by stress-induced immunomodulation (Appendix D; Davis et al. 2008). This result is consistent with previous studies, which have only found significant stress responses in tadpoles at much higher densities than used in this experiment (2–10 tadpoles/L; Hayes 1997, Glennemeier and Denver 2002, Belden et al. 2007).

A more parsimonious explanation for high susceptibility to *E. trivolvis* at high tadpole density is stage-dependent susceptibility. We observed an increase in resistance to echinostome infections with development (Appendix C: Fig. C1B), similar to previous studies across this range of tadpole developmental stages (Schotthoefer et al. 2003, Holland et al. 2007). Hence, by slowing tadpole development, higher densities increased the amount of time tadpoles spent at *E. trivolvis*-susceptible early Gosner stages. The factor causing an increase in resistance to echinostomes through development does not appear to have been an ontogenetic change in immunity, because none of the quantified immune parameters increased significantly through development (Appendix D). Another possible mechanism is loss of *E. trivolvis* habitat due to developmental changes in tadpole kidney morphology. As tadpoles approach metamorphosis, they absorb their pronephros, the most common location of *E. trivolvis* encystment within the kidney (Thiemann and Wassersug 2000*a*), leading to more encystment in the larger mesonephros (Schotthoefer et al. 2003). We found a positive correlation between decreasing pronephros size through development and susceptibility to echinostomes (Appendix C: Fig. C1C, D), explaining 80% of the effect of development on echinostome encystment.

The observed effects of competition, predation and parasitism on tadpole survival and development were generally consistent with theory and previous empirical studies. Tadpoles had higher survival at low density and in the absence of parasitism, consistent with most

previous studies of echinostomes in tadpoles (Fried et al. 1997, Schotthoefer et al. 2003, Belden 2006, Holland et al. 2007, Rohr et al. 2008*a*), though not all studies have found tadpole mortality due to infection (Koprivnikar et al. 2008, Orlofske et al. 2009). Also as predicted, high tadpole density led to delayed development and slower growth rates, and tadpoles responded to competition and predation by increasing and decreasing their activity levels, respectively. Toad tadpoles accelerated development in response to a newt predator, as predicted by theory (e.g., Werner 1986) but seldom supported by empirical work (Relyea 2007). Only two of 41 studies in a recent review found accelerated tadpole development in response to caged predators (Relyea 2007). However, these two studies were among the most similar to our experiment, including the only study in the review to use a newt predator (Kiesecker et al. 2002) and one of only six studies on toad tadpoles, the other five of which found no significant effect on development (Laurila et al. 1998, Relyea 2007).

The presence of an echinostome-infected snail induced delayed tadpole development, as predicted based on the parasite-as-predator analogy (Raffel et al. 2008) and tadpole responses to predators in most empirical studies (Relyea 2007). However, the echinostome-infected snail had no significant effect on mass at metamorphosis. These two results together imply slower growth rates with parasitism, likely because antiparasite responses were costly or because echinostome infection caused direct sub-lethal effects on growth (Fried et al. 1997, Koprivnikar et al. 2008). This reduction of growth with echinostome exposure was probably not driven by selective mortality of larger tadpoles, because smaller tadpoles are generally less tolerant of echinostome infection (Schotthoefer et al. 2003, Holland et al. 2007), or by thinning due to parasite-induced mortality, which should reduce competition and accelerate growth (Peacor and Werner 2001). Note that, to our knowledge, this study is the first to report an effect of *E. trivolvis* on tadpole developmental rate.

Predation and parasitism induced opposite effects on tadpole activity, with reduced activity in response to the caged newt, and increased activity by a similar magnitude in response to echinostomes (Fig. 1D). Both results are consistent with previous studies and appear to be adaptive (Thiemann and Wassersug 2000*b*, Taylor et al. 2004, Koprivnikar et al. 2006, Rohr et al. 2009). As predicted, antipredator and antiparasite responses were stronger with low competition, consistent with previous studies of tadpole antipredator behavior (Relyea 2004). This result suggests that competition dominated the tadpole behavioral response, so that, at high density, tadpoles no longer respond to variation in predation or parasitism. Interestingly, the competition-induced increase in foraging activity was stronger with a predator present but weaker with parasitism, consistent with previous findings that the direction of such interactions depends on whether predator- and competitor-induction are in the same or opposite directions (Relyea 2004). These interactions suggest sophisticated amphibian plasticity in response to multiple factors, where responses are tuned to the level of threat and trade-offs among factors (Relyea 2004).

Although caged newt predators reduced time to metamorphosis, they did not reduce mass at metamorphosis, implying improved growth rates despite reduced activity with a caged predator. This counterintuitive effect was predicted by Peacor (2002), who showed that increased algal growth caused by reduced tadpole foraging could lead to increased tadpole growth rates if resource levels were limited by herbivory. Increased prey growth is commonly observed in response to the threat of predation (Bolnick and Preisser 2005, Relyea 2007). Alternative explanations for caged predators increasing prey growth include increased efficiency of nutrient processing by prey under threat of predation (Peacor 2002), and the addition of nutrients in the form of predator feces in only the predator mesocosms (Anholt et al. 2000).

Perhaps the clearest implication of this study is the need for a more thorough integration of trait-mediated effects into studies of infection dynamics. Our results show that effects of host density on parasites can themselves be largely trait-mediated, blurring the dichotomy between density- and trait-mediated effects in ecological studies. In this case, the indirect effect of density on tadpole resistance to infection (trait), apparently caused by delayed development at high density, entirely counteracted the direct effect of having lower per capita exposure at higher density. Without experiments explicitly designed to distinguish effects on exposure and susceptibility to infection, this important trait-mediated effect of competition would have been missed. Trait-mediated effects of competition, predation and parasitism might also be important in systems with multiple hosts, multiple parasites, or non-host competitors. Nonlinearities in functional responses and whether the trait-mediated effects of competition and predation on infections affect host populations remain to be tested (Bolker et al. 2003). Therefore, to develop a more thorough understanding of parasite and host community dynamics, we will need studies that quantify the functional responses of density- and trait-mediated effects on exposure and susceptibility to parasites and their impacts on population parameters.

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APPENDIX A

Detailed methods for animal maintenance, trematode enumeration, leukocyte and kidney volume quantification, and statistical analyses (*Ecological Archives* E091-128-A1).

APPENDIX B

Statistical results for effects of a caged predator, tadpole density, and trematode exposure on tadpole development, survival, and activity (*Ecological Archives* E091-128-A2).

APPENDIX C

Graphs showing effects of density and delayed tadpole development on susceptibility to *Echinostoma trivolvis* infection (*Ecological Archives* E091-128-A3).

APPENDIX D

Statistics and graphs describing effects of density, predation, and development on leukocyte parameters (*Ecological Archives* E091-128-A4).