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LETTER

# Effects of pesticides on exposure and susceptibility to parasites can be generalised to pesticide class and type in aquatic communities

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**Abstract**

Pesticide pollution can alter parasite transmission, but scientists are unaware if effects of pesticides on parasite exposure and host susceptibility (i.e. infection risk given exposure) can be generalised within a community context. Using replicated temperate pond communities, we evaluate effects of 12 pesticides, nested in four pesticide classes (chloroacetanilides, triazines, carbamates organophosphates) and two pesticide types (herbicides, insecticides) applied at standardised environmental concentrations on larval amphibian exposure and susceptibility to trematode parasites. Most of the variation in exposure and susceptibility occurred at the level of pesticide class and type, not individual compounds. The organophosphate class of insecticides increased snail abundance (first intermediate host) and thus trematode exposure by increasing mortality of snail predators (top–down mechanism). While a similar pattern in snail abundance and trematode exposure was observed with triazine herbicides, this effect was driven by increases in snail resources (periphytic algae, bottom–up mechanism). Additionally, herbicides indirectly increased host susceptibility and trematode infections by (1) increasing time spent in susceptible early developmental stages and (2) suppressing tadpole immunity. Understanding generalisable effects associated with contaminant class and type on transmission is critical in reducing complexities in predicting disease dynamics in at-risk host populations.

**Keywords**

Aquatic ecology, community ecology, disease transmission, ecotoxicology, pesticides.

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## INTRODUCTION

Anthropogenic activities can increase parasite transmission (Friggens & Beier 2010; Johnson *et al.* 2010), leading to increased risks to public health, wildlife populations and global economies (Binder 1999; Daszak *et al.* 2000; Morens *et al.* 2004). Hence, a key to predict the spread of parasites is to understand how anthropogenic activities alter parasite transmission within a community context (Pedersen & Fenton 2007; Johnson *et al.* 2008, 2015). Chemical pollution is an anthropogenic factor that can disrupt parasite transmission via direct or indirect effects on hosts and parasites (Rohr *et al.* 2008a; Rumschlag & Rohr 2018). For instance, contaminants can be directly toxic to hosts(Blakley *et al.* 1999; Rohr *et al.* 2008b) and parasites (Lafferty & Kuris 1999; Morley *et al.* 2003), or the effects of contaminants may be mediated indirectly by interactions with other species (Pedersen & Fenton 2007; Johnson *et al.* 2008, 2015). Thus, the net effect of pesticides on parasite transmission is determined by these direct and indirect effects (Rohr *et al.* 2008a). The influence of chemical pollution on parasite transmission is important for freshwater communities given widespread pesticide contamination in freshwater ecosystems (Dudgeon *et al.* 2006; Gilliom & Hamilton 2006; Stone *et al.* 2014). Each year in the United States, more than 350 individual pesticides(Baker & Stone 2015) are applied totalling upwards of 500 million kilograms of active ingredients (Atwood & Paisley-Jones 2017) and resulting in aquatic communities that are exposed to a diversity of pesticides (Thelin & Stone 2013; Baker & Stone 2015). While it is well-documented that responses of organisms to pesticides vary according to taxa and functional group (Blanar *et al.* 2009), a quantitative assessment of how generalisable the effects of individual pesticides are themselves has yet to be completed (Rohr *et al.* 2006). A central challenge to predicting the influence of pesticides on parasite transmission is to determine if individual pesticides have independent effects or if effects of individual pesticides share some consistencies. For instance, if effects of pesticides are generalisable to 'chemical class' (pesticides that share similar chemical structures) or 'type' (pesticides that target similar pests), an enormous amount of complexity could be reduced in predicting impacts of hundreds of pesticides on parasite transmission.

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Parasite transmission can be determined by two processes: 'exposure', defined as contact between hosts and parasites, and 'susceptibility', defined as infection risk given exposure. However, researchers often fail to partition transmission into these constituent components. For instance, many predictive disease models lump exposure and susceptibility into a single transmission term (McCallum *et al*. 2001), and experimental studies most commonly examine susceptibility or exposure, but not both (Hawley & Altizer 2011). Unfortunately, omission of exposure and susceptibility obscures a mechanistic understanding of the ecological and evolutionary drivers of parasite transmission, which is needed to accurately predict disease risk across space and time.

Our objectives were to (1) determine effects of pesticides on exposure and susceptibility of larval amphibian hosts to parasitic trematodes from the family Plagiorchidae within replicated experimental tri-trophic communities and (2) evaluate consistency of effects across pesticide types (herbicides, insecticides), classes (chloroacetanilides, carbamates, triazines, organophosphates) and individual pesticides (12 in total). Trematodes are flatworms that release larval cercariae from snail intermediate hosts; cercariae of Plagiorchidae trematodes swim through water and penetrate amphibians' superficial epithelium where they also encyst (Grabda-Kazubska 1976). First, we hypothesised that insecticides would reduce snail predators (including water bugs, larval salamanders and larval dragonflies) and their consumptive effects, thus increasing parasite exposure via an increase in snails and potentially the parasitic trematodes they harbour, a top–down mechanism (Fig. 1a). Second, we hypothesised that triazine herbicides would be directly toxic to phytoplankton, leading to an increase in periphyton, a food resource for snails; increased

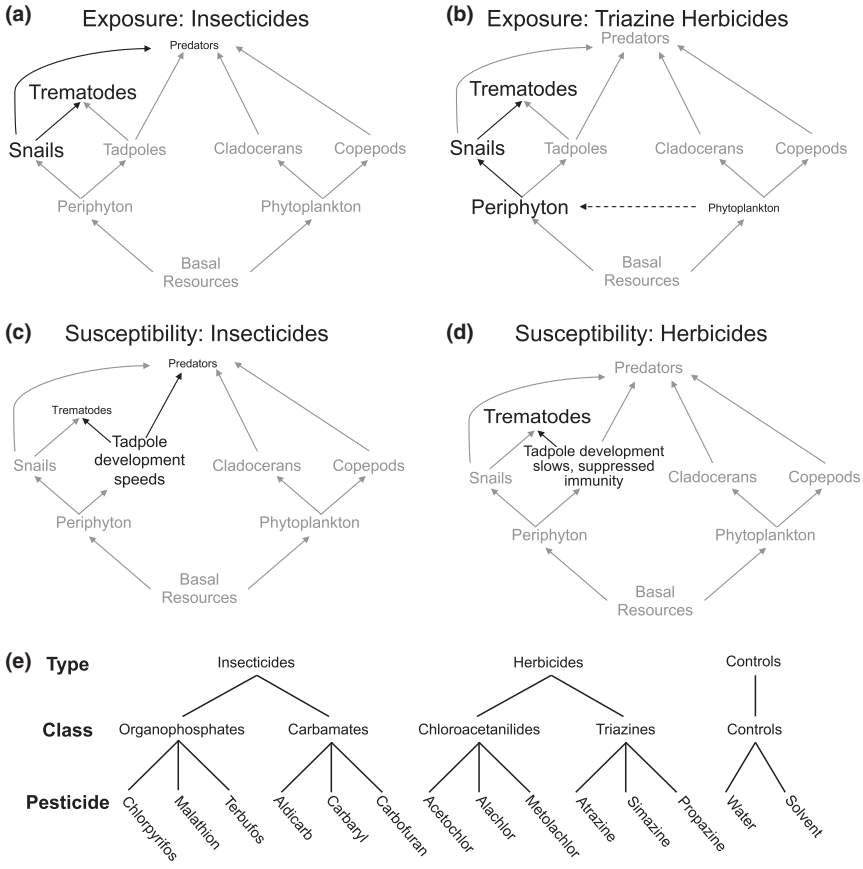



**Figure 1** Primary hypothesised effects of insecticide and herbicide exposure on community dynamics (a–d). Alternative hypotheses are not included. Pathways pertinent to a prediction are highlighted in black, while the rest of the pathways are in grey. The font size of the community member is large for predicted positive effects and small for predicted negative effects. Solid lines represent direct relationship. Dashed lines indicate indirect food web relationships. Not all possible indirect relationships are shown for viewing ease. *A. maculatum*, *B. flumineum* and *A. junius* are likely predators of tadpoles, snails and zooplankton in the current experiment. For parasite exposure, we hypothesise that (a) insecticides will reduce snail predators leading to an increase in snails and potentially the parasitic trematodes they harbour, and (b) triazine herbicides will be directly toxic to phytoplankton, leading to an increase in periphyton, a food resource for snails; increased periphyton will result in an increase in snails and potentially the trematodes they carry. For host susceptibility, we hypothesise that (c) insecticides will reduce predators of tadpoles, increasing the energy intake associated with increased tadpole foraging, leading to less time in susceptible early life stages and decreasing trematode infections, and (d) herbicide exposures will slow tadpole development, increasing time in early susceptible developmental stages (see text for additional details), and suppress tadpole immune responses. Together, these effects should lead to increased trematode infections. (e) experimental design depicting nested pesticide treatments within pesticide class types. Each pesticide treatment, along with water and solvent pesticide controls, was replicated four times at the mesocosm level.

periphyton, in turn, would result in increased trematode exposure to tadpoles by increasing snail abundance (Fig. 1b). This mechanism has been shown for the triazine herbicide atrazine (Rohr *et al*. 2008b; Halstead *et al*. 2018), but has not been tested on other representatives within the triazine class. Hence, triazine herbicides were expected to increase parasite exposure via bottom–up mechanisms.

Third, we hypothesised that impacts of insecticides and herbicides on host susceptibility would likely be determined by net effects of direct and indirect pathways on tadpole resource availability, developmental rate and immunity. Tadpole immunity improves with development; a diversity of innate and adaptive immune responses develop throughout the larval period (Rollins-Smith 1998, 2017; Robert & Ohta 2009). Thus, if insecticides reduce tadpole predators (including water bugs, larval salamanders and larval dragonflies), tadpole foraging would increase relative to controls (Fig. 1c). Whether increased tadpole foraging would result in less time in susceptible early life stages of tadpoles to parasite infection would depend on: (1) the magnitude of energetic costs of insecticide detoxification, (2) the strength of competition caused by high tadpole survival and (3) the magnitude of the cascading effect of insecticide exposure on per-capita periphyton availability (Relyea & Diecks 2008; Relyea 2009). Similarly, the net effect of herbicides on development might be a function of how much periphyton can be consumed by tadpoles in the presence of predators. Given that herbicides are generally designed not to be toxic to water bugs, larval salamanders and larval dragonflies, these predators might prevent tadpoles from taking advantage of increased periphyton. Thus, costs of detoxifying herbicides might slow tadpole development relative to tadpoles in insecticide tanks, increasing time in early trematode-susceptible developmental stages. Additionally, several herbicides are directly immunosuppressive (Mann *et al*. 2009; Rohr & McCoy 2010), which could further increase susceptibility and trematode infections (Fig. 1d). Alternatively, given that periphyton likely increases in association with herbicides, tadpoles might consume more food, leading to quicker development and less time spent in susceptible stages relative to controls.

## MATERIALS AND METHODS

### Composition of communities and experimental design

To evaluate our experimental hypotheses and predictions, we conducted an experiment at Russell E. Larson Agricultural Research Center (Pennsylvania Furnace, PA, USA) using a randomised block design and replicated aquatic mesocosms. Mesocosms were 1100-L cattle tanks filled with 800 L water and covered with 60% shade cloth. Three weeks before application of pesticide treatments, each tank received 300 g of mixed hardwood leaves and inoculations of zooplankton, periphyton and phytoplankton that were homogenised mixtures from four local ponds. Just before pesticide application, each tank received four larval amphibian, two snail, one beetle, one water bug and one dragonfly species (3 *Ambystoma maculatum*, 20 *Hyla versicolor*, 20 *Lithobates palustris*, 20 *Lithobates clamitans*; 11 *Helisoma* (*Planorbella*) *trivolvis*, 10 *Physa gyrina*; 5 *Hydrochara* sp.; 2 *Belostoma flumineum*; 6 *Notonecta undulata*; 2 *Anax junius*, respectively). These species naturally coexist and were applied at densities found locally. *A. maculatum*, *B. flumineum* and *A. junius* are likely predators of tadpoles, snails and zooplankton in the current experiment.

We randomly assigned 14 treatments with four replicate mesocosms of each treatment, which resulted in a total of 56 mesocosms. Each treatment was a single pesticide or a control. There were two pesticide types (insecticides and herbicides), two classes within each pesticide type (triazine herbicide, chloroactenilide herbicide, carbamate insecticide, organophosphate insecticide) and three different pesticides in each of the four classes, as well as water and solvent controls (Fig. 1e). We applied a single dose of pesticides (technical grade) at estimated environmental concentrations (calculated using the US Environmental Protection Agencies GENEEC v.2 software), solvent (acetone, 0.0001%) or water to controls. The pesticides were obtained from ChemService (West Chester, PA, USA). Nominal concentrations of pesticides in $\mu g\ L^{-1}$ were: 64 chlorpyrifos, 101 malathion, 171 terbufos, 91 aldicarb, 219 carbaryl, 209 carbofuran, 123 acetochlor, 127 alachlor, 105 metolachlor, 102 atrazine, 202 simazine and 106 propazine. One hour after dosing, water samples were taken and shipped on ice to Mississippi State Chemical Laboratory to verify nominal concentrations. Measured concentrations of pesticides in $\mu g\ L^{-1}$ were: 60 chlorpyrifos, 105 malathion, 174 terbufos, 84 aldicarb, 203 carbaryl, 227 carbofuran, 139 acetochlor, 113 alachlor, 114 metolachlor, 117 atrazine, 180 simazine and 129 propazine.

During the experiment, we sampled for snail egg masses and hatchling by using two rectangles of plexiglas (465 $cm^2$) in each mesocosm, one that hung on the side and one that was on the bottom of the mesocosm. Because discriminating between snail species at early life stages is challenging, both snail species *P. gyrina* and *H. trivolis* were included in snail counts. Both *P. gyrina* and *H. trivolis* are intermediate hosts for plagiorchid trematodes. In addition, we sampled for periphyton using clay tiles (100 $cm^2$) that were oriented perpendicularly along the bottom of the mesocosm. At the end of the experiment, the tanks were drained, and the animals were removed, counted, euthanatised, fixed in formalin and transferred to 70% ethanol. We took water samples (10 cm below the water surface) for phytoplankton analyses and scraped periphyton from the periphyton samplers. We scored water clarity, a metric of light availability, on a scale from one (clear) to five (opaque) blind to treatment. Additionally, zooplankton samples were collected from the entire water column by placing a PVC pipe (10 cm diameter, 60 cm height) upright in the centre of each tank, capping the bottom and pouring the water sample through 20 μm Nitex mesh. Two zooplankton pipe samples were collected from each tank and the subsamples were combined and preserved in 70% ethanol. Zooplankton identity and abundance in each sample were determined by placing a 5 mL subsample in a zooplankton counting wheel (Wildlife Supply Company, Yulee, FL, USA) and examining it under a dissecting microscope. Chlorophyll-*a* concentrations from phytoplankton and periphyton were measured using standard fluorometric techniques (see below). The experiment ran for 4 weeks, from the end of June into July.

### Periphyton and phytoplankton analytical procedures

Periphyton scrubbed from tiles and water samples (10-mL) from each tank were filtered onto glass fibre filters (under low vacuum pressure, < 10 psi; Whatman EPM 2000, 0.3 μm, 47 mm) to estimate chlorophyll concentrations from periphyton and phytoplankton respectively. The chlorophyll concentration of each filter was determined using an organic extraction procedure (50 : 50 mixture of 90% Acetone to DMSO). Chlorophyll-*a* concentrations were measured using a standard fluorometric technique (Carrick *et al*. 1993). Coefficients of variation among duplicate subsamples were typically < 5%.

### Trematode exposure and immune and infection quantification

Once a week for 3 weeks before the experiment began, we individually placed H. trivolvis snails under light and searched for shed cercariae in the water to identify infections with trematodes. Only snails that did not shed during these 3 weeks were added to the mesocosms. Twenty-eight snails shedding plagiorchid cercariae were individually placed in floating cages in every other mesocosm. These cages were rotated daily to homogenise amphibian exposure to trematodes. Snails were thoroughly rinsed before being moved to another tank and the cages were not rotated. If any infected snails died, they were replaced with another plagiorchid shedding snail. Given that snails can be infected with trematodes for 6–7 weeks before they begin shedding cercariae, it was possible that some of the snails we screened before the experiment began shedding during the experiment, affecting amphibian exposure to trematodes. Thus, at the end of the experiment, all the living adult snails in each tank were shed again, weighed and necropsied to determine their infection status at the end of the experiment.

We used the procedures described in our previous work for metacercarial and eosinophil quantification (Rohr *et al*. 2008, 2008). Briefly, five *L. clamitans* (green frog) tadpoles that survived until the end of the experiment were randomly selected from each mesocosm, weighed and their developmental Gosner stages(Gosner 1960) determined. For each tadpole, eosinophil cells (white blood cells that target worm infections) were counted per field of view from sectioned and stained livers. Then, each tadpole was cleared, stained and their larval trematodes were counted under a compound scope. We could not logistically conduct all the immune and parasite quantification on all four amphibian species in each mesocosm, and so we arbitrarily picked one as our focal species.

### Statistical analyses

We completed a series of permutational analyses of variance (PERMANOVA) using PRIMER and PERMANOVA+(Anderson 2001) to test for the effects of type, class and individual pesticide on endpoints and attribute the variance explained to each pesticide level of organisation, accounting for the appropriate nested structure of our experimental design (Fig. 1e). All univariate and multivariate PERMANOVA models were based on resemblance matrices of endpoints constructed using Euclidean distances and included the following random effects: type (insecticide, herbicide), class (chloroacetanilide, triazine, organophosphate, carbamate) nested within type and pesticide (12 in total) nested within class nested within type. PERMANOVA models did not include water and solvent controls because these control treatments were not nested hierarchically (Fig. 1e). Each PERMANOVA evaluated 9999 permutations using residuals under a reduced model, which yields the best power and the most accurate Type I error for multifactorial designs (Anderson & Legendre 1999; Anderson & ter Braak 2003).

More specifically, to address our predictions that insecticides and triazine herbicides would influence potential exposure to trematode, we first tested for the influence of pesticides on snail predators using PERMANOVA. In three separate univariate models, the responses were mass of surviving *A. maculatum*, proportion survival of *A. junius* or proportion survival of *B. flumineum*. Next, we used PERMANOVA to evaluate the effects of pesticides on snail abundance, which served as an indicator of possible trematode exposure because snails are the intermediate host of trematodes. In this model, the univariate response was number of snails from snail samplers. Then, we evaluated the impacts of pesticides on zooplankton communities; using PERMANOVA, the multivariate response was density of cladocerans and copepods. Finally, we used PERMANOVA to examine the influence of pesticides on algal communities; treating phytoplankton and periphyton (measured via chlorophyll-*a*), as the multivariate response. When a group of pesticides (class, or pesticides within class) explained a significant amount of the variance in the response, we used pair-wise multiple comparisons tests within PERMANOVA to evaluate differences within that group. We included water and solvent controls as treatments in these multiple comparisons tests. Because water and solvent controls never differed from each other in preliminary analyses, they were pooled as a single control group. In all PERMANOVAS, test statistics associated with Type III partial sums of squares were evaluated.

To compare the level of support for various direct and indirect pathways by which pesticides might affect amphibian susceptibility to trematode infections, we performed path analyses using the *lavaan* package in R. Path analysis is a statistical technique that can test multiple linked hypotheses through the analysis of multiple variance–covariance matrices in which variables can serve as both dependent and independent variables. We chose to include variables in the candidate path model using our *a priori* hypotheses on the impact of herbicides, but not insecticides, on host susceptibility. The unit of replication for the path analyses was the mesocosm. We evaluated the fit of the model to the data using Chi-square statistics, the comparative fit index (CFI, relative to a null 'independence' model, which constrains all covariances to zero), and the root mean square error of approximation (RMSEA) and tested for missing pathways by examining modification indices. Tadpole density was divided by 10 in the path analysis to get the variance estimate on a similar scale as the other variables in the model, which helps with model convergence (Shipley 2000). Path analyses were conducted only on mesocosms for which we had all the following measured

variables: total tadpole density, *L. clamitans* tadpole stage, eosinophils from *L. clamitans* tadpole blood, mass of infected snails and *L. clamitans* tadpole trematode load. Only one mesocosm was excluded for a lack of data (i.e. 55 independent replicates and 13 free parameters in the model). We generated two defensible models: one is represented in Figure 3, the only difference with the second model is eosinophils predict trematode abundance in frogs instead of covary. RMSEA is derived from the Chi-square and can be positively biased; the degree of this bias is dependent on the sample size and degrees of freedom. If Chi-square is less than the degrees of freedom, RMSEA is set to zero. Despite the positive bias associated with low degrees of freedom and sample size, our RMSEA values are 0.000 and 0.021. Thus, we are confident in the fit of the model and the biological relevance. We did not perform path analyses on the influence of insecticides because PERMANOVA multiple-comparisons tests showed that the effects of insecticides generally did not differ from the controls for the susceptibility endpoints including: tadpole developmental Gosner stage, density of eosinophils and trematode infection load (Fig. S1). Organophosphate insecticides did have greater number of amphibians remaining at the end of the experiment, but this effect was likely due to the decrease in predator abundances (Fig. S1A).

## RESULTS

To evaluate hypotheses, we evaluated effects of 12 pesticides, nested in four pesticide classes (chloroacetanilides, triazines, carbamates, organophosphates), and two pesticides types (herbicides, insecticides) on larval *Lithobates clamitans* exposure and susceptibility to parasitic plagiorchid trematodes in replicated aquatic communities (Fig 1e). Pesticides were applied singly and at one time at standardised environmental concentrations (see Methods). In addition, our design included water and solvent controls. We randomly assigned these 14 treatments to replicated communities in mesocosms with four replicates of each treatment, resulting in a total of 56 mesocosms. Each community was composed of four larval amphibian, two snail, one beetle, one water bug, one backswimmer, and one dragonfly species. Additionally, communities included mixed hardwood leaves as a nutrient source and inoculations of local zooplankton, periphyton and phytoplankton.

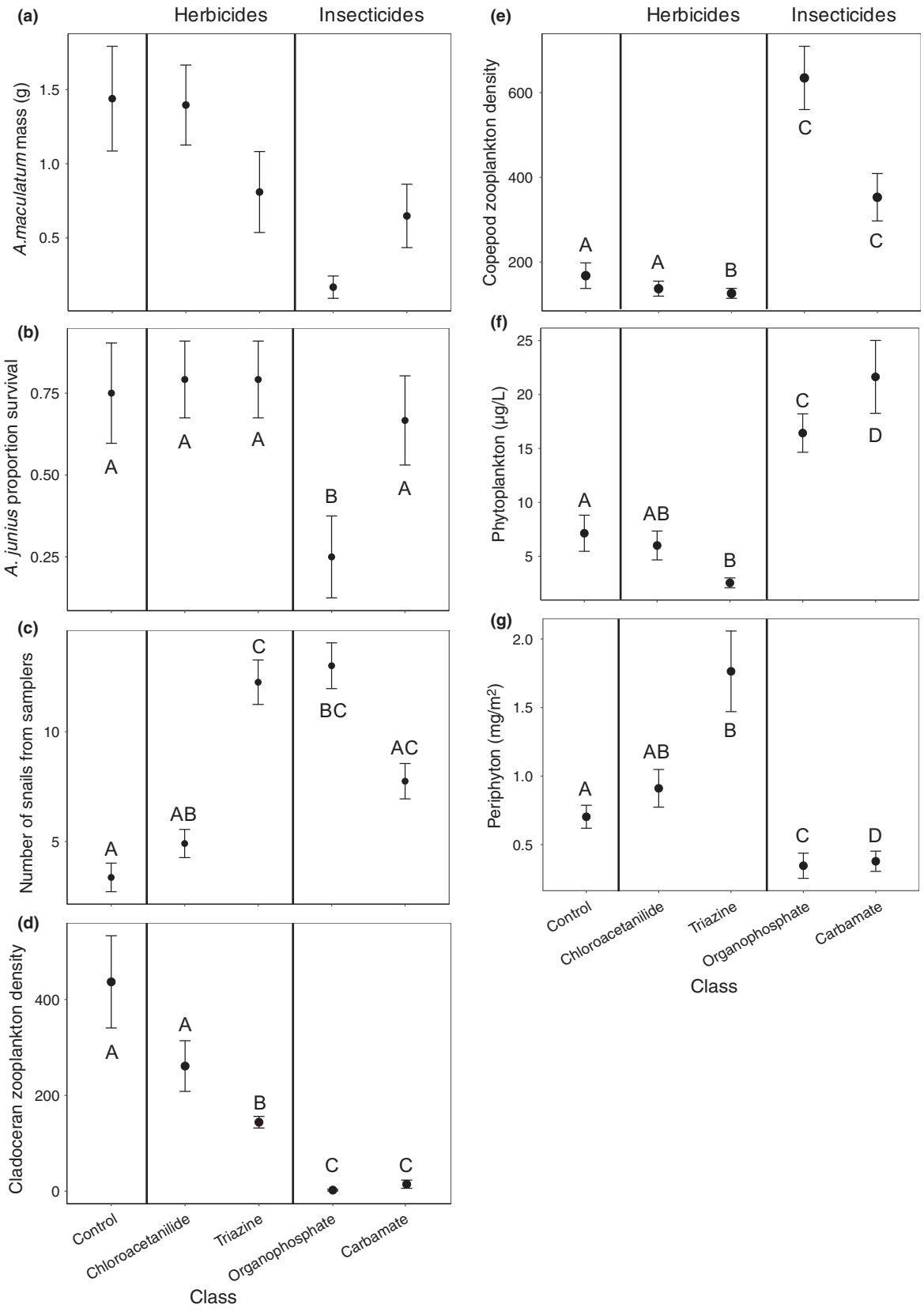
### Are there top–down effects of insecticides on potential exposure to trematodes?

We tested for effects of individual pesticides and pesticide classes and types on three predators of snails in our freshwater communities, *Ambystoma maculatum* salamander larvae (Petranka 1998), *Anax junius* dragonfly larvae (Turner & Chislock 2007) and *Belostoma flumineum* giant water bug adults (Chase 2003). Most of the explained variance in snail predator abundance and snail abundance, the source of trematode exposure, was attributed to pesticide class (Table 1). The organophosphate, but not carbamate, class of insecticides, reduced the

**Table 1** PERMANOVA models evaluating the effects of pesticides on endpoints influencing amphibian exposure to parasitic trematodes, including predators of snails and tadpoles (mass of *Ambystoma maculatum*, survival of *Anax junius,* survival of *Belostoma flumineum*), number of snails capable of carrying parasitic trematodes, cladoceran and copepod zooplankton communities and phytoplankton and periphyton algal communities. PERMANOVA tests on univariate models yield *F* statistics, while multivariate models yield pseudo *F* statistics. $P < 0.05$ are bolded. Variance is the estimated size of the component of variation. Variance terms of zero originally were negative values which indicate that the contributions of these terms to variance were zero. The largest variance values, excluding residual variance, for each test are bolded

| Endpoint and Source of Variation | d.f. | *F*/Pseudo *F* | *P* | Variance |
|---|---|---|---|---|
| Mass of *Ambytoma maculatum* | | | | |
| Type | 1 | 3.357 | 0.339 | **0.169** |
| Class(Type) | 2 | 1.933 | 0.206 | 0.069 |
| Pesticide(Class(Type)) | 8 | 1.684 | 0.136 | 0.091 |
| Residual | 36 | | | 0.530 |
| Proportion surviving *Anax junius* | | | | |
| Type | 1 | 2.560 | 0.329 | 0.034 |
| Class(Type) | 2 | 5.560 | **0.011** | **0.036** |
| Pesticide(Class(Type)) | 8 | 0.730 | 0.666 | 0.000 |
| Residual | 36 | | | 0.128 |
| Proportion surviving *Belostoma flumineum* | | | | |
| Type | 1 | 3.769 | 0.309 | **0.008** |
| Class(Type) | 2 | 1.000 | 0.428 | 0.000 |
| Pesticide(Class(Type)) | 8 | 1.258 | 0.294 | 0.003 |
| Residual | 36 | | | 0.054 |
| Number snails per mesocosm | | | | |
| Type | 1 | 0.158 | 0.665 | 0.000 |
| Class(Type) | 2 | 4.800 | **0.046** | **16.01** |
| Pesticide(Class(Type)) | 8 | 0.410 | 0.921 | 0.000 |
| Residual | 36 | | | 123.9 |
| Cladoceran and copepod densities | | | | |
| Type | 1 | 7.233 | 0.337 | **72715** |
| Class(Type) | 2 | 22.66 | **< 0.001** | 22304 |
| Pesticide(Class(Type)) | 8 | 0.295 | 0.996 | 0.000 |
| Residual | 36 | | | 41892 |
| Phytoplankton and periphyton densities | | | | |
| Type | 1 | 21.95 | 0.339 | **104.3** |
| Class(Type) | 2 | 5.147 | **0.024** | 8.023 |
| Pesticide(Class(Type)) | 8 | 0.415 | 0.917 | 0.000 |
| Residual | 36 | | | 56.01 |

**Figure 2** The influence of pesticides on response variables hypothesised to affect amphibian exposure to parasitic trematodes. Organophosphate insecticides, but not carbamate insecticides, have a negative effect on snail predators including (a) *Ambystoma maculatum* mass and (b) *Anax junius* survival, (c) which is associated with an increase in the number of eggs and hatchling snails and potentially the trematodes they harbour. (c) Insecticides decrease cladoceran zooplankton, which are efficient grazers of suspended phytoplankton algae. (d) Decreased cladocerans are associated with (e) increases in copepods, (f) increases in suspended phytoplankton algae and (g) decreases in attached periphyton algae because phytoplankton shades periphyton. Triazine herbicides reduce phytoplankton leading to an increase in periphyton, a food resource for snails; this increased periphyton presumably fuelled the increase in snails and potentially the trematodes they carry. Points represent treatment means across mesocosms. Bars are standard errors with the exception of binomial standard errors for *A. junix* survival and Poisson standard errors for number of snails. Points sharing the same letter are not different according to pairwise comparisons tests.




survival of *A. junius* (Fig. 2b), exerting a top–down effect that likely resulted in increased snail abundance measured by snail hatchlings and eggs (Table 1, Fig. 2c). Although there was no significant effect of pesticides on *A. maculatum* mass (Fig. 2a), the patterns were similar to *A. junius*, with organophosphates causing greater reductions in salamander mass than

carbamates (Fig. 2a). While almost none of the *B. flumineum* survived the entire experiment (Table 1), it is likely that early in the experiment, *B. flumineum* survival was reduced in the presence of insecticides similar to laboratory tests (Halstead *et al*. 2015).

Organophosphate and carbamate insecticide exposures also led to a decrease in the abundance of cladoceran zooplankton (Table 1, Fig. 2d) and, likely in turn, an increase in competing copepod zooplankton (Table 1, Fig. 2e). Cladocerans are generally more efficient phytoplankton grazers than copepods given their ability to feed on a broader range of phytoplankton (Sommer & Sommer 2006). The virtual elimination of cladocerans likely led to a change in competition and a shift in algal communities, as mesocosms that received insecticides had greater amounts of suspended phytoplankton relative to controls (Fig. 2f). This greater amount of phytoplankton shaded periphyton, as tanks exposed to insecticide classes had decreased light availability compared to all other treatments (water clarity scores: control $2.1 \pm 0.23$, chloroacetanilide $1.8 \pm 0.25$, triazine $1.2 \pm 0.11$, organophosphate $4.3 \pm 0.22$, carbamate $4.7 \pm 0.14$ [mean $\pm$ standard error]). So, the treatments with greater phytoplankton also had less attached periphyton (Table 1, Fig. 2g).

#### Are there bottom–up effects of herbicides on potential exposure to trematodes?

We tested for effects of individual pesticides and pesticide classes and types on periphyton, the food resource for snails. Like the effects of insecticides on snail predators, most of the explained variance in periphyton and snail abundance was attributed to pesticide class (Table 1). The triazine, but not chloroacetanilide, herbicide class increased snail abundance (Fig. 2c) via bottom–up effects, and thus increased potential trematode exposure. Triazine herbicides reduced phytoplankton (Table 1, Fig. 2f), which allowed attached periphyton to flourish because of greater light availability (see water clarity scores reported above, Table 1, Fig. 2g).

#### What are the effects of pesticides on host susceptibility to trematode infections?

We predicted that insecticide-induced reductions in predators of tadpoles might increase tadpole foraging and reduce time spent during particularly susceptible early life stages of tadpole development (Fig. 1e). Whether increased tadpole foraging would result in less time in particularly susceptible early life stages of tadpole development would depend on (1) the magnitude of energetic costs of insecticide detoxification, (2) the strength of competition caused by high tadpole survival and (3) the magnitude of the cascading effect of insecticide exposure on per-capita periphyton availability (Relyea & Diecks 2008; Relyea 2009). While organophosphate insecticide exposure reduced predators (Fig. 2a–b, Table 1), which likely led to greater amphibian density relative to all other treatments (Fig. S1a), this effect did not speed or slow developmental rate, nor did it result in tadpoles with different eosinophil densities or trematode loads relative to controls (Fig. S1b–d). Thus, we simplified our path model (described below) to focus on effects of herbicides on tadpole development and susceptibility to infection. In support of this simplification, variance explained was always higher at pesticide type than pesticide class or individual compound in all variance partitioning analyses conducted on dependent variables that were included in our susceptibility analyses (see path model below; Table 1). Additionally, herbicide treatments were more often different from the controls compared to insecticide treatments (Fig. S1).

The net effect of herbicides on tadpole development is likely a function of how much periphyton can be consumed by tadpoles in the presence of predators. For instance, given that herbicides are generally not toxic to predators, predators might prevent tadpoles from taking advantage of increased periphyton. Thus, the costs of detoxifying herbicides should slow tadpole development relative to tadpoles in insecticide tanks. Additionally, we hypothesised that herbicides would be immunosuppressive, further increasing susceptibility and trematode infections (Fig. 1d). Alternatively, given that periphyton increased with herbicide exposure, tadpoles might consume more food, leading to an increase in developmental rate and less time spent in susceptible developmental stages. We used a path analysis to discriminate between direct and indirect pathways in our system. Our path model included effects of herbicides on tadpole density, tadpole developmental stage, tadpole eosinophil densities (immune cells that specialise in defence against trematodes (Rohr *et al*. 2008b; Kiesecker 2002)) and trematode infections per tadpole; effects of tadpole stage on eosinophil densities; effects of tadpole density on developmental stage; and effects of tadpole density, eosinophil densities and trematode exposure (mass of infected snails) on infections per tadpole (Fig. 3). Although our experiment rotated infected snails shedding cercariae among the tanks to homogenise exposure and we screened the snails that were allowed to roam freely in the tanks for parasite infections before the experiment began, some of the free roaming snails were shedding cercariae when they were rescreened at the end of the experiment. Snails can take 6–7 weeks to begin shedding cercariae after they are initially infected. While the mass of infected snails did not vary according to pesticide class (Fig. S1e), we controlled for this variation in exposure (mass of infected snails) in our path model when testing for effects on parasite susceptibility (Fig. 3) because the number of cercariae released from a snail often increases with snail size (Graham 2003).

In accordance with our predictions, the path model suggests that herbicides affect trematode infections in two ways. First, herbicides were associated with reduced developmental stages of tadpoles (Fig. 4b), forcing tadpoles to spend more time at susceptible early life stages that are associated with lower eosinophil densities (Fig. 4d). Second, herbicide exposure directly reduced eosinophil densities, even while controlling the effects of herbicides on developmental stage (Figs 3 and 4c). As predicted, eosinophil densities and mass of infected snails were associated with trematode load in tadpoles (Figs 3 and 4d–e). When controlling these pathways, neither herbicide nor tadpole densities had residual associations with trematode loads per tadpoles (Fig. 3). Overall, the data fit the path model well (Chi-square $P$-value with 5 degrees of freedom = 0.528, Comparative Fit Index = 1.000, RMSEA = 0.000, RMSEA

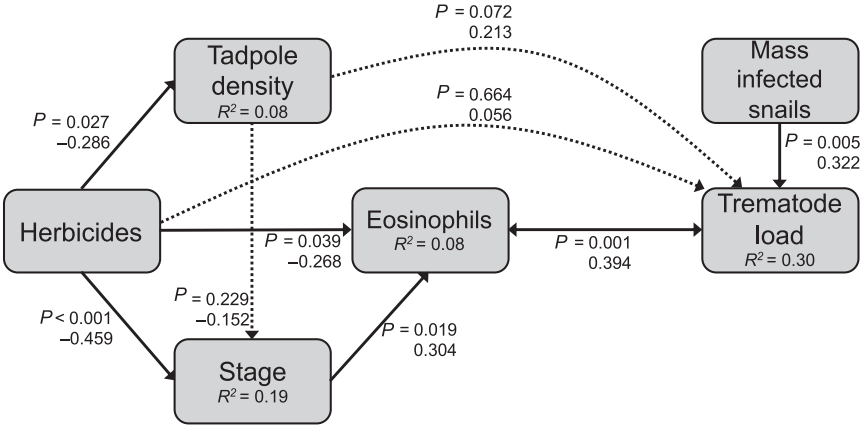



**Figure 3** Path model showing the relationships among herbicides, total amphibian density, tadpole developmental Gosner stage, eosinophil density in tadpole blood, and trematode loads in tadpoles, controlling the mass of trematode-infected snails per mesocosm. Insecticides never differed from controls, but herbicides did, and so herbicide was coded as a dummy variable. The model suggests that herbicides affect trematode infections in two ways, by reducing developmental stage, thus increasing the amount of time at infection-susceptible early life stages, and by reducing eosinophils even when controlling for effects on stage. Double-headed arrows represent covariances, single-headed arrows represent regression relationships, solid arrows are significant paths and dashed arrows are non-significant paths. Next to each path is the probability value and standardised coefficient. The data fit the model well (Chi square *P*-value = 0.528, Comparative Fit Index = 1.000, RMSEA = 0.000, RMSEA *P*-value = 0.603), and investigation of the modification indices did not suggest that any paths were missing.

*P*-value = 0.603), and investigation of the modification indices did not suggest any important paths were missing.

## DISCUSSION

Parasite transmission is driven by variation in parasite exposure and host susceptibility to infection, yet what remains largely unknown is how the relative importance of exposure vs. susceptibility determines the disease patterns in a community context (Hawley & Altizer 2011). Using replicated aquatic experimental communities, we evaluated effects of 12 pesticides, nested in four pesticide classes and two pesticide types on larval amphibian exposure and susceptibility to trematodes. Our results demonstrate that: (1) effects of pesticides on exposure and susceptibility can be generalised to pesticide class and pesticide type, respectively, (2) the impact of pesticides on parasite exposure is driven by indirect top–down and bottom–up effects that vary with pesticide class and (3) the influence of pesticides on host susceptibility to trematode infections is increased by herbicides via effects on rate of host development and host immune response.

First, we found that effects of pesticides on exposure and susceptibility can be generalised to pesticide class and pesticide type. Pesticide class and type explain the greatest amount of variance in exposure and susceptibility respectively. A significant challenge in risk assessment is the complexity associated in evaluating thousands of potential chemical contaminants that could affect aquatic communities. Our results support the idea that ecological complexity can be reduced because pesticides of similar classes, modes of action and types produce similar community-level effects when compared across standardized chemical concentrations (Rohr *et al.* 2006). Reducing complexity by considering similarity of toxic effects of chemical classes, modes of action and use types on individual organisms is an approach taken by Quantitative Structure–Activity Relationships (QSAR) models (Mazzatorta *et al.* 2005; Knaak *et al.* 2008). We suggest that this QSAR approach could be extended to effects on communities.

Second, our results show that differences in pesticide class drive food web-mediated effects that shape parasite exposure. Through a top–down mechanism, organophosphate insecticides reduced predators which increased the abundance of snails, the first intermediate host of the parasite. Through a bottom–up mechanism, triazine herbicides shifted algal communities to attached periphyton, an important resource for snails, which also increased snail populations. While we suggest that the increase in periphyton was driven by less competition for light as has been shown in other studies (Relyea & Diecks 2008; Relyea 2009; Hua & Relyea 2014), less competition with phytoplankton for other resources, such as phosphorous or nitrogen, cannot be ruled out. We likely did not observe the same bottom–up effects of chloroacetanilide herbicides because they generally have much shorter half-lives than the triazine herbicides (soil half-lives of 14–26 days vs. 110–146 days respectively [Pesticide Acton Network Pesticide Database]). Previous research shows that increases in intermediate snail hosts can occur via top–down mechanisms with chlorpyrifos, an organophosphate insecticide (Halstead *et al.* 2018), and bottom–up mechanisms with atrazine, a triazine herbicide (Rohr *et al.* 2008b; Halstead *et al.* 2014, 2018). While these previous studies concern the impacts of individual pesticides, our results highlight that these effects can be generalised to pesticide class. Furthermore, epidemiologic models support that top–down and bottom–up mechanisms initiated by chlorpyrifos and atrazine can increase transmission rates of trematodes (Halstead *et al.* 2018). Parasite exposure might also have been influenced by direct effects of pesticides on parasites. While we did not observe any direct relationships between pesticides and trematode infections (e.g. Fig. S1), we did not quantify any measurements of cercarial survival or

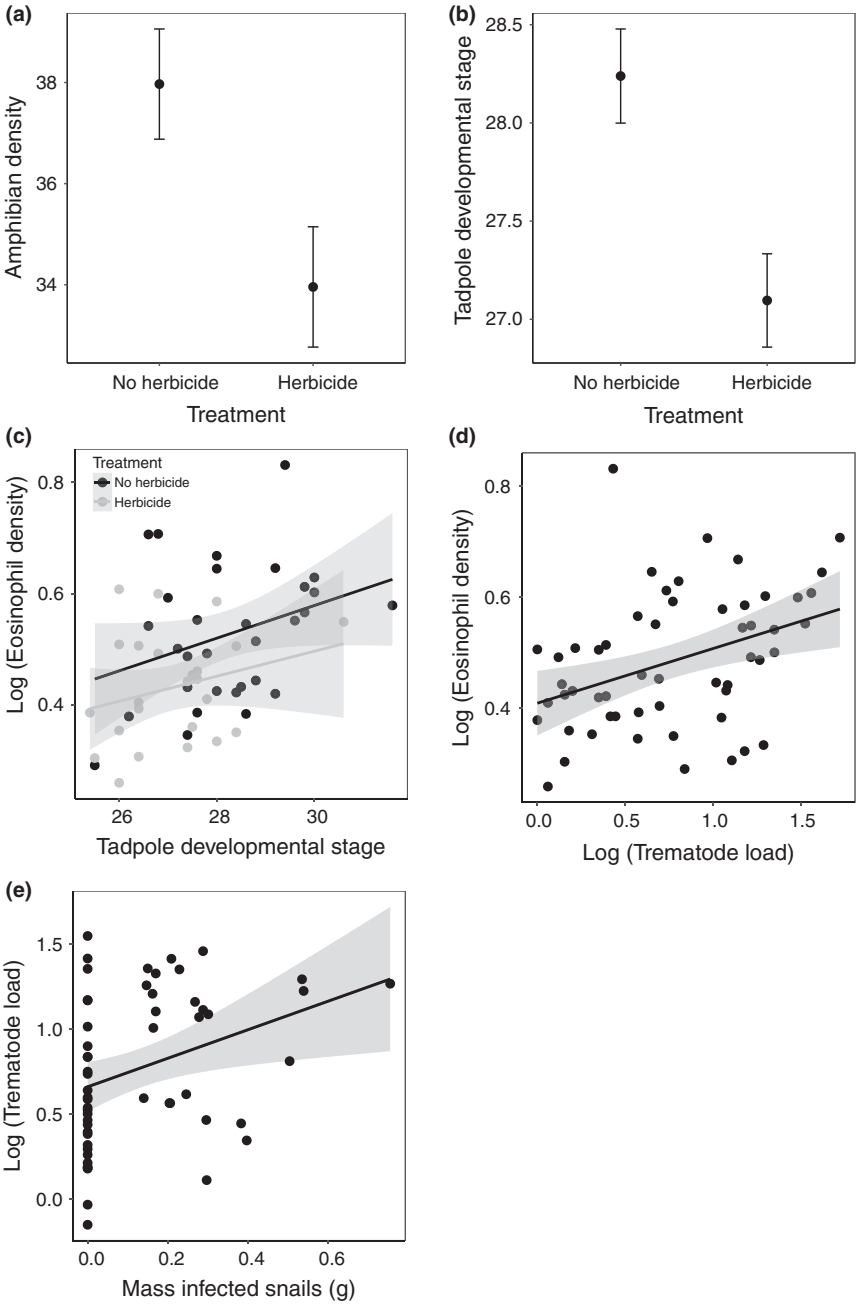



**Figure 4** Significant regression and covariance relationships included in the path model (see Fig. 3). (a) Amphibian density is lower in mesocosms with herbicide exposure than without herbicide exposure. In the path model, amphibian density was divided by 10 to get the variance estimate on a similar scale as the other variables in the model. (b) Tadpole development, as indicated by Gosner stage, is slower with than without herbicide exposure. (c) Tadpole developmental stage is positively associated with eosinophil densities with and without herbicide exposure, (d) trematode infection load and eosinophil densities covary and (e) trematode infection load is associated with mass of trematode-infected snails when controlling eosinophils. In (a) and (b), points represent treatment means across mesocosms. Bars are standard errors with the exception of Poisson standard errors for amphibian density (number of amphibians per mesocosm).

infectivity. Neither did we examine other parasite stages or hosts in the complex life cycle of trematodes, which could also be influenced by pesticides. While direct effects of pesticides on trematodes or other hosts, including snails, could be important, previous studies on trematodes suggest these pathways are likely unaffected. In amphibian–*Echinostoma trivolvis* and human–*Schistosoma* systems, studies show no direct effects of multiple pesticides at environmentally relevant concentrations on trematode egg viability; survival of miracidia (first larval stage); survival and infectivity of cercaria; and snail survival, growth and fecundity (Rohr *et al.* 2008a; Raffel *et al.* 2009; Halstead *et al.* 2018).

Third, our results show that herbicides, but not insecticides, increase susceptibility to trematodes by slowing host development

and suppressing host immunity. Generally amphibian susceptibility to trematode infections is high at early life stages (Johnson *et al*. 2011). When amphibian hosts are exposed to herbicides, the cost of detoxification likely slows developmental rate (Rohr & McCoy 2010), which increases time in early susceptible life stages. Similarly, high conspecific densities have been found to slow developmental rates of amphibians, resulting in more time in early life stages and higher trematode loads (Raffel *et al*. 2010). In addition, other studies have found that herbicide exposure can disrupt amphibian immune responses (Mann *et al*. 2009; Rohr & McCoy 2010; Knutie *et al*. 2018). In contrast to the effects of herbicides, we did not observe an effect of insecticides on host susceptibility. While insecticides reduced densities of tadpole predators, which might have increased tadpole foraging (Peacor & Werner 2000), this effect did not result in any effect on amphibian developmental rate or susceptibility, possibly because the cost of detoxification of insecticides was greater than or equal to the increase in energy intake associated with any increase in tadpole foraging. Alternatively, the benefit from increased tadpole foraging could have been negated by decreased periphyton and high competition.

Given that the production of pesticides is predicted to increase two to five times by the year 2050 (Tilman *et al*. 2001), understanding mechanisms that underlie human-altered changes in transmission cycles is critical to predict the spread of parasites. Our results show that pesticides can alter parasite exposure via cascading trophic pathways and host susceptibility via indirect physiological pathways. These results highlight the importance of community context (LoGiudice *et al*. 2003; Pedersen & Fenton 2007; Johnson *et al*. 2015) and simultaneous contributions of parasite exposure and host susceptibility. Most notably, we show that the effects of pesticides on susceptibility and exposure can be generalised to pesticide class and type within a community context. Our findings advance community ecology by showing that contaminants that share chemical structures or environmental targets can have similar direct and indirect effects on disease dynamics. Future studies might also consider the generalisable impacts of pesticides on 'tolerance' to parasite infection, the per-parasite fitness costs on hosts. Considering generalised contaminant effects will reduce complexities in predicting how thousands of contaminants influence ecological communities.

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## AUTHORSHIP

J.T.H., T.R.R. and J.R.R. designed the experiment, H.J.C., N.H., J.T.H., T.R.R. and J.R.R. conducted the experiment, S.L.R. and J.R.R. conducted the analyses, S.L.R wrote the manuscript and all authors contributed to the editing.

## DATA ACCESSIBILITY STATEMENT

Data are available on Figshare Repository: https://doi.org/10.6084/m9.figshare.7719200.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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