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Effects of Trematode Parasites on Snails and Northern Leopard Frogs (*Lithobates pipiens*) in Pesticide-Exposed Mesocosm Communities

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Abstract.—**Chemical contamination of aquatic environments is widespread, but we have a limited understanding of how contaminants alter critical host–parasite interactions that can influence disease dynamics. We manipulated Northern Leopard Frog (*Lithobates pipiens*) exposure to pesticides (no pesticides, the insecticide *Bacillus thuringiensis israelensis* [*Bti*], or the herbicide atrazine) and trematode-infected (*Ribeiroia ondatrae* and *Echinostoma* spp.) snails in outdoor mesocosms. *Bti* exposure extended host larval period, and atrazine exposure had a nonsignificant trend toward reducing host survival; however, neither pesticide influenced parasite success nor magnified the effects of parasites on their hosts. Parasites negatively influenced tadpole development and, by metamorphosis, parasitized frogs had severe limb deformities and greater mass than unparasitized frogs. The greater mass in parasitized frogs may have resulted from reduced competition between tadpoles and snails for algal resources because parasites decreased snail abundance in mesocosms. Reduced competition between tadpoles and snails may offset the direct negative effects of trematodes on tadpoles, enabling them to survive with high infection intensities. Trematodes may further facilitate their own success by inducing limb deformities that likely increase anuran consumption by definitive hosts. Our results demonstrate how common pesticides and parasites impact amphibians and suggest that, at environmentally relevant concentrations, these pesticides may not dramatically alter host–parasite dynamics.**

Pesticide exposure can change community dynamics through impacts on multiple community members, leading to important consequences for species interactions (Köhler and Triebskorn, 2013; Allgeier et al., 2019), especially in aquatic environments where pesticide contamination is ubiquitous (Stone et al., 2014). In the midwestern United States, nearly 50% of land use is row-crop agriculture (Bigelow and Borchers, 2017), and freshwater species are often inadvertently exposed to agricultural pesticides that enter wetlands via runoff and drift (Hladik et al., 2014). Pesticides are also purposely introduced into waterbodies to control mosquito populations that may carry diseases (van den Berg et al., 2012). Given the rise in infectious diseases (Daszak et al., 2000), the potential for pesticides to alter host–parasite interactions is of utmost importance.

Many parasites use amphibians as hosts, including the trematode *Ribeiroia ondatrae* that encysts in the developing limb tissue of amphibians, causing limb deformities (Johnson et al., 2002). Another group of trematodes, those in the genus *Echinostoma*, encyst in the kidneys of amphibians and can cause renal failure (Fried et al., 1997). In recent surveys, nearly half of amphibians examined were infected with these trematodes (Stutz et al., 2017), and infections appear to be increasing across many areas in the United States (Johnson and McKenzie, 2009), with some evidence suggesting pesticide exposure plays a role (Rohr et al., 2008a,b; Haas et al., 2017).

Ribeiroia and *Echinostoma* trematodes use amphibians and snails as intermediate hosts, and these hosts can compete for algal food resources (Brönmark et al., 1991). Within snails, trematodes reproduce asexually and produce free-swimming cercariae that leave the snail in search of amphibian hosts. Short-lived cercariae may be vulnerable to environmental factors such as pesticides that can alter their activity, ability to encyst within their host, and survival (Koprivnikar et al., 2007; Rohr et al., 2008a; Mendoza-Estrada et al., 2016). In this way, pesticide exposure could reduce trematode success in aquatic environments.

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Pesticide exposure also can have direct negative effects on amphibians. For example, the commonly used herbicide atrazine, which is frequently detected in surface waters (Stone et al., 2014), may reduce immune responses of amphibians (Brodkin et al., 2007), increasing their susceptibility to trematodes. Likewise, biopesticides such the insecticide *Bacillus thuringiensis israelensis* (*Bti*) are extensively applied to standing waterbodies to control larval mosquito development (van der Berg et al., 2012) and can have sublethal effects on amphibians (Lajmanovich et al., 2015; Allegier et al., 2018, 2019). Sublethal effects may increase the time tadpoles spend at early developmental stages when they are highly susceptible to trematode parasites and , in turn, increase the likelihood of parasite-induced mortality (Johnson et al., 2011a). Furthermore, pesticide-induced alterations in tadpole behavior can change the probability of parasite transmission by altering the likelihood of cercarial encystment (Koprivnikar et al., 2006, 2007).

Depending on pesticide class, indirect effects of pesticides may be equally or more important than direct effects in determining the outcome of host–parasite interactions through changes in the community (Boone and James, 2003; Fleeger et al., 2003; Rohr et al., 2006; Rumschlag et al., 2019). For example, herbicides, such as atrazine, can reduce algal food resources (Fairchild et al., 1998; Boone and James, 2003) that can, in turn, reduce host body condition (Leips and Travis, 1994) and increase host susceptibility to parasites. Conversely, reduced algal resources may decrease the abundance of snails (Johnson et al., 2007) that spread trematodes and compete with tadpoles for food, which could benefit amphibian hosts. Likewise, insecticides such as *Bti* can indirectly influence amphibian–trematode interactions by altering zooplankton populations (Pauley et al., 2015; Allgeier et al., 2019) that act as competitors of tadpoles (Morin et al., 1988) and predators of trematode cercariae (Schotthoefer et al., 2007). Because pesticides can influence hosts and parasites directly while concurrently influencing their community, the outcome of simultaneous exposure may be difficult to predict.

Northern Leopard Frog (*Lithobates pipiens*) populations are experiencing declines (Johnson et al., 2011b) and are susceptible

to trematode infections (Schotthoefer et al., 2003a,b) and pesticide exposure (e.g., Hayes et al., 2003); therefore, understanding how pesticide exposure alters parasite infections is crucial. The objective of this study was to determine how exposure to either the herbicide atrazine or the insecticide *Bti* influenced the effect of trematode parasites on Northern Leopard Frogs. We selected these pesticides specifically because of their widespread use, probable occurrence in wetland communities, and potential to influence this system based on previous literature. We predicted that exposure to a pesticide or trematodes individually would have negative effects on Northern Leopard Frog behavior, development, and survival and that the severity of the impact of trematodes would vary with pesticide exposure. Although evidence suggests that both pesticides used in this study can negatively influence amphibians and trematodes at the concentrations used here, we predicted each pesticide would more negatively affect amphibians than trematodes and result in increased infection intensity. We did not expect *Bti* or atrazine to cause cercarial morality at the concentrations used in this study as the median lethal concentration of *Bti* for other trematode cercariae is 83.8 mg/L (Mendoza-Estrada et al., 2016), and atrazine does not appear to influence *Echinostoma* spp. cercarial survival at low concentrations (<200 μg/L; Koprivnikar et al., 2006; Rohr et al., 2008a).

Materials and Methods

Animal Collection and Care.—We collected five partial Northern Leopard Frog egg masses on 13 March 2016 from an ephemeral wetland at Talawanda High School (39°29′16′′N, 84°43′42′′W) in Oxford, Butler County, Ohio. The wetland is surrounded by a restored grassland with no obvious point source of pesticide contamination. We held the clutches in the laboratory between 23 and 25°C with a 14 : 10; L : D cycle and changed water daily after hatching. We fed tadpoles TetraMin tropical fish flakes ad libitum daily. When tadpoles were free-swimming (Gosner stage 25 [Gosner, 1960]), we mixed clutches to homogenize genetic variation among treatments and added 30 tadpoles to each mesocosm on 28 March (experimental day 0).

We conducted the experiment in 39 polyethylene mesocosms (1.85 m in diameter; 1,480-L volume) at Miami University's Ecology Research Center (ERC; Oxford, Butler County, Ohio). We added 1000 L of city water on 17 March and 1 kg of leaf litter from a mixed deciduous forest on 18 March to each mesocosm. From 18–26 March, we inoculated each mesocosm with water from local wetlands to establish algal and plankton communities. To provide shade and prevent the colonization of unintended species, we covered each mesocosm with a 0.15-cm fiberglass screen-mesh lid, which allowed the passage of ~50% of ambient light.

We collected planorbid (Planorbidae: *Helisoma trivolvis*) snails from two local ponds between 13 March and 19 April. A pond located in Miami University's ERC (39°31′42.7′′N, 84°43′24.9′′W) contained snails infected with *Echinostoma* spp. based on the presence of collar spines and the shedding timing of cercariae (Szuroczki and Richardson, 2009). A pond in Miami University's Natural Areas (39°31′18.5′′N, 84°42′26.4′′W) had snails that appeared uninfected with trematodes based on lack of cercarial shedding when monitored for ≥72 h. We held all snails individually in a climate-controlled chamber at 25°C with a 14 : 10; L : D cycle and monitored them for cercarial shedding twice daily: once in the morning just after the lights had turned on and once in the afternoon. All cercariae emerged between 0900 and 1400 h during initial screening, supporting our initial identification based on morphology, because *Echinostoma* spp. cercariae generally emerge during the day or with light, whereas *R. ondatrae* cercariae emerge at night (Szuroczki and Richardson, 2009; Orlofske et al., 2015). Although initially we believed that all parasites shed were *Echinostoma* spp., we later discovered snails also had shed *R. ondatrae* during the experiment based on the presence of *R. ondatrae* metacercariae in dissected metamorphs. It is likely that snails had prepatent (i.e., early-stage) *R. ondatrae* infections and transitioned from *Echinostoma*-dominated to *Ribeiroia*-dominated infections, because it is rare for snails to be infected with multiple trematodes simultaneously, but common for large trematode species to outcompete and replace smaller species (Sousa, 1993). Snails received daily water changes after the second screening period and were fed ground algae wafers ad libitum until placement in the mesocosms.

Experimental Design.—Using a factorial, randomized design, we manipulated pesticide and parasite exposure: exposure to pesticides (0 μg L^{-1} control, 50 μg L^{-1} atrazine, or 1.16 mg L^{-1} *Bti*) and exposure to parasites (snails infected with *R. ondatrae* and *Echinostoma* spp. or uninfected snails). We randomly selected a subset of mesocosms to terminate after 7 wk to examine treatment effects on tadpoles (3 pesticide treatments × 2 parasite treatments × 3 replicates = 18 mesocosms). We used the remaining mesocosms to determine treatment effects at metamorphosis (3 pesticide treatments × 2 parasite treatments with either 4 replicates [parasites present] or 3 replicates [parasites absent] = 21 mesocosms).

For atrazine treatments, we prepared a stock solution by combining 11.845 g of Atrazine 4L (42.2% purity; WinField United) with 1 L of ultrapure water. We used a commercial formulated product because they are broadly applied in the field and thus reflect real-world exposure. We added 10 mL of the stock solution to mesocosm water in a watering can and spread it out over the surface of each mesocosm on 5 April to achieve desired 50 μg/L concentration. The selected atrazine concentration is within the range recorded in wetlands after runoff events (reservoir and water impoundments, 0–458 μg/L; U.S. Geological Survey National Water Quality Monitoring Council, 2000–2017) and can be toxic to algae (Fairchild et al., 1998). To confirm atrazine concentration, we sent two 1-L composite water samples that we collected by combining three grab samples from the perimeter of pesticide-control and atrazine-exposed mesocosms to Mississippi State Chemical Laboratory the day after pesticide addition and again on 25 May. The measured concentration for atrazine on experimental day 9 and day 58 was 44.5 and 46.6 μg/L, respectively, suggesting no breakdown. The breakdown of atrazine is highly variable in freshwater systems, with the half-life ranging from less than a day to more than a year, depending on environmental factors such as temperature, sunlight, and pH (summarized in Chung and Gu, 2003). Atrazine was not detected in composite control samples.

For *Bti* treatments, we placed one Mosquito Dunk (mass of dunk, 11.262 ± 0.526 g; mass of active ingredients, 1.161 ± 0.054 g [mean ± SD]; Summit Chemical) onto the water's surface of each mesocosm. Mosquito Dunks are a slow-release (≤ 30 days) mosquito control product that contains 10.31% *Bti* active ingredient and are available for purchase at lawn and garden stores. To best imitate use by buyers, we used the product as recommended by the manufacturer: one Mosquito

Dunk for 2.3–9.3 m^2 of surface water (surface water of mesocosm, 2.6 m^2). Although the Mosquito Dunks decreased in size over time, some product remained visible when the first tadpoles began metamorphosing.

To manipulate parasite exposure, we randomly assigned five snails (individual mass, 0.527 ± 0.127 g [mean ± SD]) from each treatment group to each mesocosm. All mesocosms received one snail on 29 March and two snails on both 12 April and 20 April. We added snails to all mesocosms to attempt to control for the effects of competition between snails and tadpoles. On 1 June, we counted the total number of snails visible (i.e., adults added initially plus juvenile snails resulting from reproduction) within each mesocosm to determine the influence of treatments on snail abundance.

Response Variables.—To examine how exposure to parasites and pesticides influenced swimming behavior in tadpoles, five tadpoles were haphazardly collected from all mesocosms for use in behavioral assays 6 wk after addition to mesocosms. On the day of collection, individual tadpoles were randomly placed in a 1-L plastic container with water from their original mesocosm. We placed containers in a 2 × 3 block and recorded activity for 20 min. The recording for each tadpole was analyzed using video tracking software (Ethovision XT7, Noldus Information Technology, Wageningen, Netherlands) from which we determined time spent moving for each tadpole. We excluded the first 5 min of each video from analysis to allow for tadpole acclimation. We returned tadpoles to their original mesocosm to allow for continued development.

Seven weeks after addition to mesocosms, we drained and searched three mesocosms of each treatment to assess tadpole development. We staged (Gosner, 1960) and weighed each tadpole. Tadpoles were then euthanized using a 1% solution of buffered MS-222 (tricaine methanesulfonate), placed in Bouin's Solution (Sigma-Aldrich) for 24 h to fix tissues, and then transferred to 75% ethanol until the time of dissection. To determine *Echinostoma* spp. infection prevalence in tadpoles, the kidney complex from each tadpole was examined for presence/absence of metacercarial cysts. The preservation process with Bouin's Solution damaged the cysts and amphibian tissue, so we could only determine the prevalence of *Echinostoma* spp. and not the intensity of infection. We were also unable to determine prevalence or intensity of *R. ondatrae* infection in tadpoles because of damaged caused by the Bouin's Solution. At this point in the experiment, hindlimb abnormalities were not apparent in any tadpoles.

We checked the remaining mesocosms daily for metamorphs (presence of at least one front limb; Gosner 42 [Gosner, 1960]), and they were held in the laboratory until tail resorption. We weighed each metamorph, recorded time and survival to metamorphosis, and determined the prevalence of limb deformities. Metamorphs were euthanized as described above, preserved in 10% neutral-buffered formalin for 48 h to prevent damage of tissue and metacercarial cysts, and then placed in 75% ethanol. We terminated the experiment on 25 and 26 July (experimental day 119 and 120, respectively) and thoroughly searched each mesocosm for tadpoles (12 remaining tadpoles with ≤3 tadpoles per mesocosm).

We dissected a random subset of 10 metamorphs from each parasite-absent mesocosm and all metamorphs from parasite-present mesocosms to quantify *Echinostoma* spp. infection intensity. We removed the kidney complex from each metamorph and sectioned each kidney into posterior and anterior segments. We placed each segment between microscope slides, applied pressure to the slides to expose present cysts, and manually quantified *Echinostoma* spp. cysts. To quantify *R. ondatrae* infection intensity, we necropsied a random subset of 10 metamorphs from each mesocosm. We removed the host's skin, mandible, tail reabsorption site, muscle, and all internal organs; placed the tissues between two petri plates; and applied pressure to expose present cysts. Totals of both *Echinostoma* spp. and *R. ondatrae* were combined to determine total trematode infection intensity. For the subset of animals used to quantify *R. ondatrae* intensity, we also determined the severity of deformities, that is, the number of independent deformities, per animal by using protocols detailed in Johnson et al. (2001).

Statistical Analysis.—We used analysis of variance (ANOVA) to determine the effects of parasites, pesticide exposure, and their interaction on the amount of time tadpoles spent moving (log transformed), tadpole mass, and Gosner developmental stage. We used a generalized linear model with a logit link function and a binomial distribution to test for treatment effects on tadpole survival, survival to metamorphosis, and the prevalence of limb deformities in individual metamorphs. The effects of parasites, pesticide exposure, and their interaction on time to metamorphosis and mass at metamorphosis were analyzed using ANOVAs. Using only parasite-present mesocosms, we tested for the effects of pesticide exposure on deformity severity measured as the total number of independent deformities and intensity of infection (*Echinostoma* spp., *R. ondatrae*, and both) using ANOVAs with endpoints averaged by mesocosm. We analyzed the effects of parasites, pesticide exposure, and their interaction on snail abundance (square root transformed) using an ANOVA. All variables were tested for assumptions of ANOVA using the Shapiro–Wilk test for normality and the Levene's test for homogeneity of variance and were transformed as noted above (i.e., tadpole behavior and snail abundance) if they did not meet the assumptions. We followed all analyses with Dunnett's multiple comparison tests to test for differences among pesticide treatments. All analyses were performed using R 3.6.1 (R Core Team, 2019) with mesocosm as the experimental unit.

Results

Tadpole Responses.—All tadpoles exposed to parasites were infected with *Echinostoma* spp., whereas *Echinostoma* spp. metacercariae were absent from all tadpoles from the parasite-absent mesocosms. Although tadpole survival was not affected by parasites (Table 1), parasites reduced tadpole mass by 21% and delayed development by 3.8 ± 0.6 (mean ± SE) Gosner developmental stages compared with individuals in the parasite-absent treatment (Fig. 1; Table 2). There was no effect of pesticide exposure or the interaction of parasites and pesticide exposure on tadpole mass or development (Table 2). Pesticide exposure marginally influenced tadpole survival, with Dunnett's test showing this effect was attributable to atrazine exposure (Table 1). Average survival was 55 ± 5% (mean ± SE) in atrazine-exposed mesocosms compared with 66 ± 5% in the no-pesticide control. Parasites, pesticide exposure, and their interaction did not affect tadpole movement ($F_{1–2,33} \leq 0.383$; $P \geq 0.685$); tadpoles spent an average 13% (±3% SE) of the trial time moving.

Metamorph Responses.—All metamorphs that were dissected from parasite-present mesocosms were infected with both *R. ondatrae* and *Echinostoma* spp., and no parasites were observed in metamorphs from the parasite control. Across all mesocosms, total infection intensity ranged from 398 to 4,386 cysts per animal (1,935.2 ± 85.2 [mean ± SE], $n = 122$). *R. ondatrae* infection

TABLE 1. Summary of generalized linear model and Dunnett's multiple comparison test (MCT) for Northern Leopard Frog tadpole survival, survival to metamorphosis, and number of animals with deformities. Significant effects ($\alpha \leq 0.05$) are in bold.

Response variable	Source of variation	df	χ^2	*P*	Dunnett's MCT *P*
Tadpole survival	Parasite	1	0.941	0.332	
	Pesticide	2	4.918	0.086	Atrazine $P = 0.057$ *Bti* $P = 0.220$
	Parasite × pesticide	2	0.407	0.816	
Survival to metamorphosis	Parasite	1	1.715	0.190	
	Pesticide	2	4.115	0.128	Atrazine $P = 0.088$ *Bti* $P = 0.772$
	Parasite × pesticide	2	0.241	0.886	
No. of metamorphs with deformities	Parasite	1	363.25	**<0.001**	
	Pesticide	2	11.090	**0.004**	Atrazine $\mathbf{P = 0.022}$ *Bti* $P = 0.847$
	Parasite × pesticide	2	0.000	1.000	

intensity ranged from 139 to 1,049 cysts per animal (508.5 ± 20.4, $n = 122$), with high densities in the tail reabsorption site, mandible, and basal tissues of the hindlimbs. *Echinostoma* spp. infection intensity ranged from 40 to 4,205 cysts per animal (1,411.4 ± 78.8, $n = 170$). Parasites significantly increased the presence of deformities in metamorphs (Table 1). Approximately 86% (±5% SE, $n = 203$) of metamorphs from each parasite-present mesocosm had some form of limb deformity, including missing and extra limbs, skin webbing, and boney triangles (Fig. 2). One metamorph with absent hindlimbs appeared to be exhibiting edema, but dissection revealed that this condition had resulted from either lack of cloacal development or a cloacal obstruction that prevented defecation (Fig. 2). Edema was not apparent in any other metamorph. On average, parasitized animals had 2.6 (±0.2 SE, $n = 122$) unique deformities, whereas no deformities were found in the absence of parasites.

Although pesticide exposure did not interact with parasites to influence the number of metamorphs with deformities, there was a main effect of pesticides on the prevalence of deformities, with Dunnett's test revealing that this effect was driven by atrazine (Table 1). Atrazine, but not *Bti*, significantly reduced the presence of deformities in parasitized metamorphs relative to no-pesticide exposure (Fig. 3). However, there was high variation in the prevalence of deformities between atrazine mesocosms; the percentage of metamorphs that had deformities from the four atrazine and parasite-present ponds were 38, 73, 87, and 100%. There was no effect of pesticide exposure on the severity of deformities ($F_{2,9} = 0.439$; $P = 0.6579$) or the intensity of infection for either parasite species observed among parasitized frogs ($F_{2,9} \leq 1.631$; $P \geq 0.2486$). Unlike with the prevalence of deformities, Dunnett's tests did not reveal individual effects of atrazine or *Bti* on the severity of deformities or parasite infection intensity ($P \geq 0.1820$).

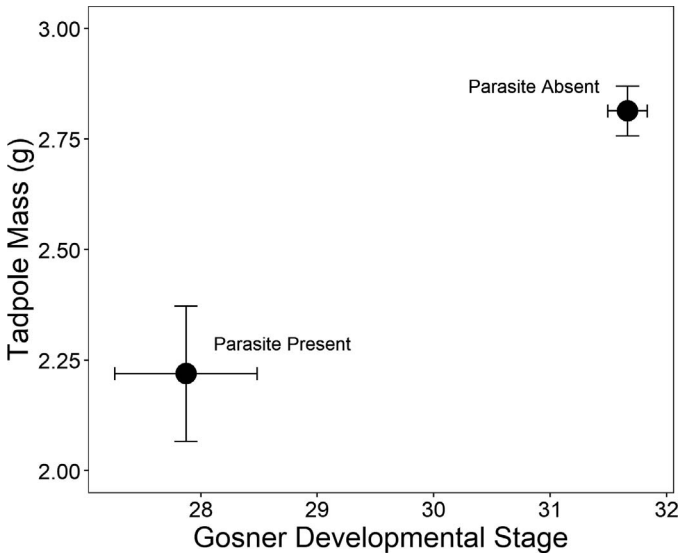

FIG. 1. Effects of parasites on tadpole responses. Mass and Gosner developmental stage for Northern Leopard Frog tadpoles exposed to different parasite treatments (absent, present) for 7 wk. Plotted values are means ± 1 SE.

Survival to metamorphosis was not significantly impacted by parasites, pesticide exposure, or their interaction (Table 1). However, as with tadpole survival, atrazine resulted in a trend of lower survival to metamorphosis on average (Fig. 4A; Table 1). Parasites increased time to metamorphosis by 6% (Fig. 4B) and mass at metamorphosis by 29% (Fig. 4C; Table 2). Although mass at metamorphosis was not influenced by any other treatment combination, there was a significant interaction between parasites and pesticide exposure on time to metamorphosis, with *Bti* exposure causing longer larval periods regardless of parasite treatment, whereas other treatments showed longer larval periods only with exposure to parasites (Fig. 4B; Table 2).

Snail Abundance.—On experimental day 65, 1 wk before the emergence of the first metamorph, snails were visible in all but one mesocosm. In parasite-present mesocosms, only large snails, assumed to be the snails originally added at the beginning of the experiment, were visible (average snail abundance, 2.3 ± 0.4 [mean ± SE]; range, 0–5 snails per mesocosm). In parasite-absent mesocosms, juvenile snails were abundant, and their high density and large size made it impossible to distinguish them from the snails originally added to the mesocosms (average snail abundance, 159.2 ± 14.8; range 105–238 snails per mesocosm). Parasite exposure reduced the total number of snails in each mesocosm ($F_{1,15} = 429.86$; $P < 0.001$; Fig. 5), whereas effects of pesticide exposure did not ($P \geq 0.2249$).

DISCUSSION

Pesticides have the potential to alter host–parasite interactions through impacts on parasite survival and infectivity; host growth, survival, and susceptibility; and through changes in the food web. In this study, we found that parasites can have substantial effects on amphibians even in the presence of pesticides, suggesting that, at environmentally relevant concentrations, *Bti* and atrazine do not negatively influence parasite success or magnify the effects of parasites on their hosts.

TABLE 2. Summary of ANOVA and Dunnett's multiple comparison test (MCT) for Northern Leopard Frog tadpole mass, tadpole developmental stage, and mass at and time to metamorphosis. Significant effects ($\alpha \leq 0.05$) are in bold.

Response variable	Source of variation	df	*F*	*P*	Dunnett's MCT *P*
Tadpole mass	Parasite	1,12	14.830	**0.002**	
	Pesticide	2,12	1.986	0.180	Atrazine $P = 0.954$ *Bti* $P = 0.228$
	Parasite × pesticide	2,12	0.977	0.404	
Developmental stage	Parasite	1,12	32.690	**<0.001**	
	Pesticide	2,12	0.896	0.434	Atrazine $P = 0.713$ *Bti* $P = 0.304$
	Parasite × pesticide	2,12	0.410	0.673	
Mass at metamorphosis	Parasite	1,15	12.935	**0.002**	
	Pesticide	2,15	0.136	0.874	Atrazine $P = 0.828$ *Bti* $P = 0.940$
	Parasite × pesticide	2,15	1.152	0.342	
Time to metamorphosis	Parasite	1,15	31.572	**<0.001**	
	Pesticide	2,15	1.225	0.322	Atrazine $P = 0.585$ *Bti* $P = 0.960$
	Parasite × pesticide	2,15	7.221	**0.006**	

Parasite exposure severely impacted limb development in amphibians, with more than 85% of exposed metamorphs having some form of limb deformity. The malformation types and rates found in this study mirror those observed in the field (Meteyer et al., 2000), suggesting that this experimental design adequately mimicked wetland conditions. Deformities can limit mobility (Goodman and Johnson, 2011), increasing the vulnerability of metamorphs and juveniles to predation in the terrestrial habitat, which likely contributes to the lack of adult amphibians observed with severe deformities in the field (Johnson et al., 2001). Because metamorph survival has a disproportionate impact on population growth (Biek et al., 2002), reductions in survival early in the terrestrial life stage could reduce population viability.

Parasite exposure also reduced tadpole mass and Gosner developmental stage, consistent with the results of other studies (Koprivnikar, 2010; Johnson et al., 2011a, 2012; Marino et al., 2014). Decreased development in tadpoles translated to an increased larval period for metamorphs, yet parasite-exposed metamorphs were larger at metamorphosis. Although *Echinostoma* spp. are known to induce edema in their tadpole hosts (Fried et al., 1997), which could contribute to increased mass, in this study, we did not observe edema in metamorphs. Instead, this increase in mass at metamorphosis may be attributable to longer larval periods and reduced competition between snails and tadpoles for food resources in parasite-present mesocosms. By the end of the experiment, snail abundance in parasite-present mesocosms was 75% lower than that in parasite-absent mesocosms and likely reduced competition for periphyton algal resources between tadpoles and snails (Holomuzki and Hemphill, 1996; Rohr and Crumrine, 2005). The reduced abundance of snails in parasite-present mesocosms is likely driven by trematode-induced castration of snails (Huffman et al., 2009) as is evident from the

FIG. 2. Example Northern Leopard Frog metamorphs observed with limb deformities. (Top left) Extra limbs. (Top right) Extra limb and boney triangle. (Bottom left) Boney triangle. (Bottom right) Absent limbs.

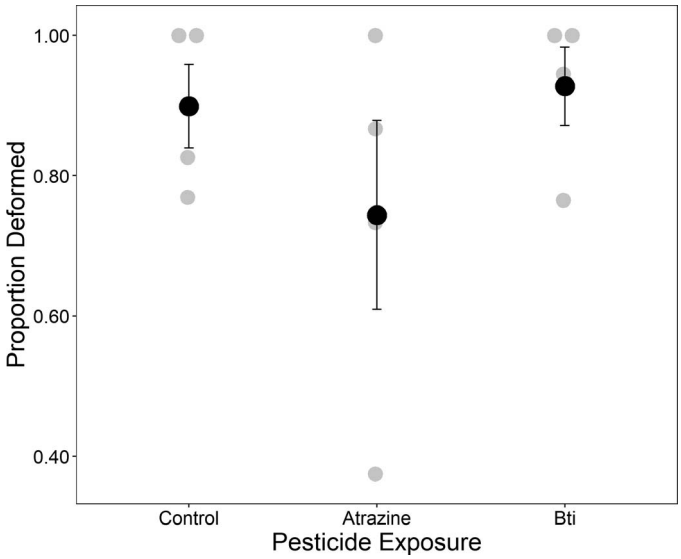

FIG. 3. Influence of pesticide treatment on the proportion of metamorphs with deformities. Proportion of parasitized Northern Leopard Frog metamorphs with deformities exposed to pesticide treatments (control, atrazine, *Bti*). Plotted values are means ± 1 SE. The gray points indicate proportion of metamorphs deformed within each mesocosm.

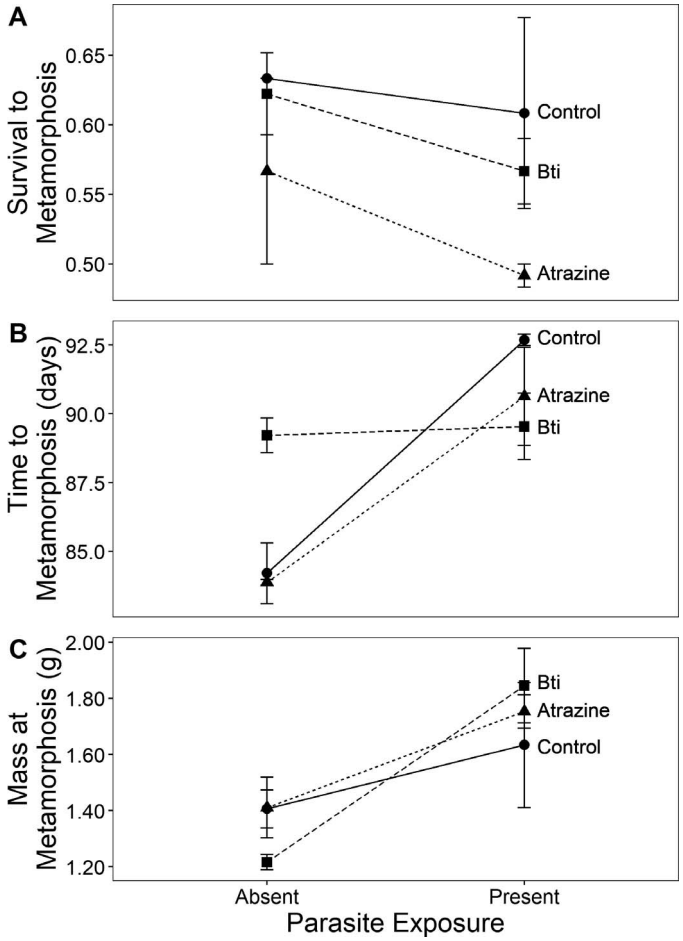

FIG. 4. Influence of treatments on metamorph responses. (A) Proportion of Northern Leopard Frog tadpoles exposed to pesticide treatments (control, atrazine, *Bti*) and parasite treatments (absent, present) that survived to metamorphosis. (B) Larval period of Northern Leopard Frogs exposed to pesticide treatments (control, atrazine, *Bti*) and parasite treatments (absent, present). (C) Mass at metamorphosis of Northern Leopard Frogs exposed to pesticide treatments (control, atrazine, *Bti*) and parasite treatments (absent, present). Plotted values are means ± 1 SE.

absence of juvenile snails in parasite-present mesocosms. However, *Echinostoma* spp. can also use snails as a second intermediate hosts and can cause morality in juvenile snails (Ponder and Fried, 2004), which could further contribute to the lack of juvenile snails in parasite-present mesocosms. Reduced snail numbers in parasite mesocosms did not increase tadpole size early in development, but changes in food resources may have become more important later in the larval period (Leips and Travis, 1994), and competition between tadpoles and snails may have been limited early in the experiment when snail numbers were low. This study suggests that the direct negative effects of parasites on tadpole mass may reduce with development or even reverse with decreased competition between tadpoles and snails for algal food resources (not measured here, but demonstrated by others [Holomuzki and Hemphill, 1996; Rohr and Crumrine, 2005]).

Many studies have observed increases in mortality in amphibians exposed to *R. ondatrae* (Schotthoefer et al., 2003b; Johnson et al., 2011a) and to a lesser degree with *Echinostoma* spp. (Fried et al., 1997; Schotthoefer et al., 2003a), but that was not the case in the present study. Similar survival whether in the

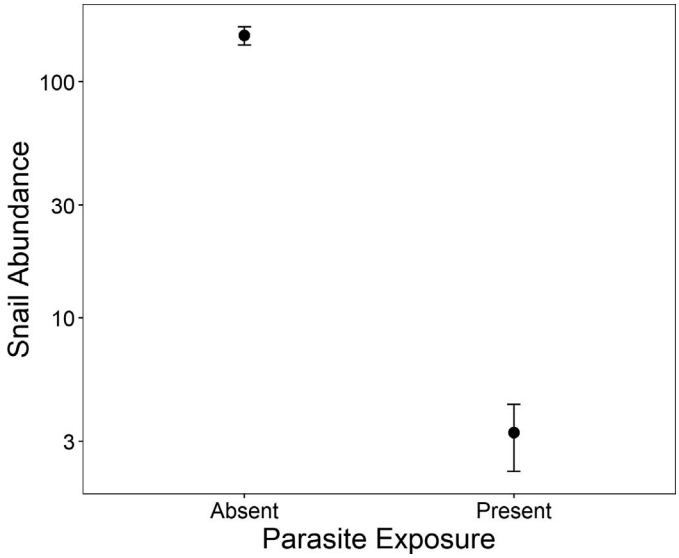

FIG. 5. Influence of parasite infection on total snail abundance. Number of original and juvenile snails observed in each parasite treatment (absent, present) on experimental day 65. Plotted values are means ± 1 SE. The y-axis is log transformed.

presence or absence of parasites is surprising given that infection intensity in this study was higher than levels shown to cause high mortality in other studies (Schotthoefer et al., 2003a,b). Timing of exposure of the tadpoles to parasites may have moderated their impact on survival, as demonstrated by Schotthoefer et al. (2003a,b), who found that the probability of survival is not altered in Northern Leopard Frogs when exposure to *R. ondatrae* and *Echinostoma* spp. occurs after limb-bud formation. Furthermore, Northern Leopard Frogs may be less susceptible to parasite-induced mortality than other species, as anurans with larger body sizes may have reduced risk for severe pathology (Johnson et al., 2012). We have no reason to suspect that simultaneous exposure to both parasites would reduce the probability of encystment for either species, although previous *Echinostoma* infections may reduce *R. ondatrae* infections (Hoverman et al., 2013). However, in this study, average *R. ondatrae* infection intensity was more than two times higher than the greatest infection measured in the field (highest *R. ondatrae* load, 236; Johnson et al., 2002), suggesting that *Echinostoma* infections did not reduce the probability of *R. ondatrae* encystment. Likewise, we would not expect coinfection by trematodes to bolster survival of metamorphs (Johnson and Hoverman, 2012).

Surprisingly, neither pesticide substantially increased susceptibility of Northern Leopard Frogs to parasites or to the consequences of parasite exposure, but time to metamorphosis was significantly affected by an interaction between pesticides and parasites. Although parasite exposure increased time to metamorphosis in control and atrazine-exposed ponds, Northern Leopard Frogs exposed to *Bti* had long larval periods in both the presence and absence of parasites. Although longer larval periods can allow amphibians to maximize mass at metamorphosis (Wilbur and Collins, 1973), anurans exposed to *Bti* in this study in the absence of parasites were not significantly larger, despite having extended larval periods, suggesting a cost of exposure. On average, individuals exposed to *Bti* from parasite-absent mesocosms were the smallest, suggesting a direct negative effect of *Bti* on developing amphibians (similar to Allgeier et al., 2019). However, in the

presence of parasites, metamorphs exposed to *Bti* were the largest, suggesting that parasite exposure was beneficial to growth via decreased competition resulting from a reduction in snail abundance. Given that *Bti* can cause intestinal damage in amphibians (Lajmanovich et al., 2015), it is possible that animals exposed to *Bti* were unable to obtain adequate nutrients in parasite-absent mesocosms where competition for resources was high. By reducing snail density, and thus competition between tadpoles and snails for food resources, parasite presence may have offset the negative effects of *Bti* on tadpoles. It is also worth noting that in this study, *Bti* had effects on anuran metamorphosis even though by the end of the experiment *Bti* exposure was likely minimal because only a single Mosquito Dunk was added to each mesocosm. Given that the product's manufacturer recommends adding Mosquito Dunks to ponds every 30 d, we might expect amphibians developing in wetlands with Mosquito Dunks reapplication to experience more negative effects as a result of extended exposure to *Bti*. This possibility warrants further investigation of the effect of *Bti* on developing amphibians, especially those with relatively long larval periods like Northern Leopard Frogs.

Atrazine can negatively affect immune function in Northern Leopard Frogs at concentrations below those used in this study (21 μg/L; Brodkin et al., 2007); yet, we did not detect any difference in trematode load or the severity of deformities in parasitized metamorphs exposed to atrazine relative to controls. Despite having no effect on parasite load, we observed a decrease in the prevalence of deformities in metamorphs with atrazine exposure, which was driven by a reduction in the number of deformed metamorphs in one mesocosm. Although we do not have an explanation as to why many metamorphs in this mesocosm did not have deformities, as the abundance and location of *R. ondatrae* metacercarial cysts are consistent with those within other atrazine mesocosms, removing this mesocosm would remove the effect of atrazine on the number of metamorphs with deformities.

Apart from a marginal reduction in survival of tadpoles and metamorphs, atrazine did not significantly influence endpoints measured in this study. Atrazine exposure may have had negative impacts on survival early in the experiment when tadpoles were at the greatest risk for mortality (Calef, 1973), possibly by reducing algal food resources (Fairchild et al., 1998; Boone and James, 2003). Reductions in tadpole density early in the experiment may have compensated for reductions in food resources, leading to no decrease in development in surviving individuals and no additional decreases in survival in the weeks leading to metamorphosis.

Conclusions.—Parasite exposure had strong effects on Northern Leopard Frog metamorphosis by slowing development and causing severe limb deformities. Reduced competition between tadpoles and snails mediated by trematode-induced castration appeared to mitigate the direct negative effects of parasites and *Bti* on Northern Leopard Frog tadpoles. By limiting reproduction and/or causing mortality in their snail hosts, trematode parasites generated large-bodied amphibians that survived to metamorphosis, despite having high infection intensities. These parasites may further facilitate their transmission to definitive hosts by inducing limb deformities in their amphibian hosts that make them vulnerable to predation. Our results highlight the significant role these parasites can have on aquatic communities and the need to examine how these relationships may be affected by changing environments.

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