Journal of Herpetology, Vol. 56, No. 1, 84–91, 2022
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Reducing Populations of an Invasive Ant Influences Survival, Growth, and Diet of Southern Toads (*Anaxyrus terrestris*)

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ABSTRACT.—**The southeastern United States supports some of the greatest levels of amphibian diversity in North America, and several species are in decline. Invasive species in the southeastern United States, such as the red imported fire ant (*Solenopsis invicta*; hereafter RIFA), may be a factor in amphibian declines via depredation, injury of, and/or competition with native amphibians for arthropod prey. Our objective was to assess the influence of RIFAs and RIFA reductions on the diet, growth, and survival of Southern Toads (*Anaxyrus terrestris*). In 2013 and 2014 we randomly assigned juvenile toads into enclosures either treated with an insecticide, hydramethylnon, to reduce RIFAs (hereafter RIFA treatment) or maintained with ambient levels of RIFAs (hereafter control; $n = 4$ enclosures per treatment). The mean proportion of recaptured toads was 9.5 and 21 times greater in the RIFA treatment compared to the control in 2013 and 2014, respectively. Toads in the control enclosures were 23% larger at the end of the study than toads recaptured in the RIFA treatment enclosures, though this was driven largely by differences in toad densities. Toad diets in the control and RIFA treatment enclosures overlapped 94%. When considering the dietary overlap of different ant genera only, the dietary overlap was 44%. Our study provides evidence RIFAs alter amphibian populations and may be contributing to amphibian declines in the southeastern United States. Given the high mortality rates we observed, RIFAs should be considered when developing conservation plans for any amphibian species found in areas where RIFAs are present.**

The southeastern United States supports some of the greatest levels of amphibian diversity in North America (Jenkins et al., 2015), and several of these species are in decline and federally listed as threatened or endangered (e.g., Frosted Flatwoods Salamander [*Ambystoma cingulatum*; U.S. Department of the Interior, Fish and Wildlife Service, 1999; Semlitsch et al., 2017] and Dusky Gopher Frog [*Lithobates sevosus*; U.S. Department of the Interior, Fish and Wildlife Service, 2001]). Though these declines are at least partially attributed to habitat loss and/or overexploitation (Stuart et al., 2004), other factors such as invasive species are likely involved (Kats and Ferrer, 2003). Specifically, in the southeastern United States, the red imported fire ant (*Solenopsis invicta*; hereafter RIFA), may negatively affect amphibian populations via depredation, injury, and/or competition (Wojcik et al., 2001; Allen et al., 2004; Todd et al., 2008).

Red imported fire ants are omnivores and are known to depredate Gopher Tortoise (*Gopherus polyphemus*; Epperson and Heise, 2003; Dziadzio et al., 2016), American Alligator (*Alligator mississippiensis*; Allen et al., 1997), and Bobwhite Quail (*Colinus virginianus*; Mueller et al., 1999) nests, metamorphs of the endangered Houston Toad (*Anaxyrus houstonensis*; Freed and Neitman, 1988), Marbled Salamanders (*Ambystoma opacum*) and Mole Salamanders (*A. talpoideum*; Todd et al., 2008), and other vertebrates (Allen et al., 2004). Aside from direct depredation, RIFAs may have negative effects on species whose diets largely consist of Formicidae (ants), such as amphibians, via ingestion. Specifically, RIFAs may envenomate their predators by stinging them during consumption, ultimately leading to mortality (e.g., Langkilde and Freidenfelds, 2010) and/or reduced fitness (e.g., Myers et al., 2014). Many species of amphibians consume ants regularly, yet little research has assessed the influence of RIFA presence on amphibian diets or population demographics. Nonetheless, recent research has demonstrated positive effects of RIFA reduction using hydramethylnon, a granular insecticide, on herpetofauna diversity and the abundances of some amphibian and reptile species (Allen et al., 2017). To our knowledge, only one study has experimentally assessed the influence of RIFA presence on amphibian population demographics, and it did not demonstrate a relationship between treatment for RIFAs and Houston toad survival (Sirsi et al., 2020). However, the authors suggest this may be because RIFA abundances on their study site were low (Sirsi et al., 2020). Thus, additional research in systems where RIFAs are more abundant and with different species of amphibian are needed (Sirsi et al., 2020).

If the presence of RIFAs negatively influences amphibian populations, treatment to reduce RIFAs may be warranted where threatened or endangered species of amphibians are present. However, RIFA treatment could negatively influence species that consume ants and other invertebrates. Specifically, control of RIFAs at large scales requires the use of insecticides that could also affect nontarget arthropod populations (Williams et al., 2001; Plentovich et al., 2010, 2011). Thus, prior to large-scale implementation of insecticides to control for RIFAs, it is important to quantify not only the potential effects of RIFAs on amphibians, but also the effect that treatment for RIFAs may have on amphibian populations.

Our objective was to assess the influence of RIFAs and RIFA treatment on amphibian survival, growth, and diet. We used Southern Toads (*Anaxyrus terrestris*) as a model species because they are a common terrestrial amphibian in the southeastern United States and their diet primarily consists of Formicidae species (Moseley et al., 2005). We predicted RIFAs and RIFA treatment would influence survival, growth, and diets of juvenile Southern Toads because they often consume Formicidae and likely interact with RIFAs regularly during the terrestrial portion of their life because RIFAs and Southern

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DOI: 10.1670/20-053

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Toads are both ground dwelling (Jensen, 2005; Tschinkel, 2006). Moreover, one large-scale field experiment demonstrated increases in Southern Toad abundance following use of a granular insecticide to control RIFAs (Allen et al., 2017). To test our hypothesis, we conducted an experiment where we introduced juvenile toads into enclosures where we either reduced RIFAs (RIFA treatment) with a granular insecticide (Hydramethylon) or maintained RIFAs at ambient conditions (control). We assessed the survival and growth of newly metamorphosed, juvenile toads using mark–recapture and the diet of 1-yr-old toads using gut flushing. We expected the RIFA treatment to influence juvenile toad survival and growth positively. Additionally, we predicted the dietary overlap between toads in the control and RIFA treatments would be low for all invertebrate taxonomic groups, and Formicidae separately, because treatment with granular insecticides, like Hydramethylon, can alter invertebrate communities (Williams et al., 2001; Plentovich et al., 2010, 2011). We expected this difference to be greatest when assessing dietary overlap for Formicidae only.

Materials and Methods

Study Site and Enclosures.—We conducted our study on the 12,000-ha research site of the Jones Center at Ichauway (JCI) in Newton, Georgia, USA. At two locations approximately 500 m apart, we constructed eight 10 × 2.5 m (25 m^2) enclosures comprised of 0.9 m aluminum flashing buried 0.3 m into the ground within a mature longleaf pine (*Pinus palustris*) and wiregrass (*Aristida stricta*) savanna (16 total enclosures). We maintained a 0.5-m vegetation-free buffer on the inner and outer perimeter of each enclosure via hand weeding and mowing, respectively. We also placed three 0.5-m^2 wooden boards at the center of each enclosure to provide refugia. Our study area was prescribed burned biannually and a prescribed burn occurred twice outside of the enclosure fencing, once immediately prior to building the enclosures and again in Spring 2014. The RIFA is the most common ant species on the study site, followed by *Solenopsis carolinensis* and *Pheidole* spp. (Stuble et al., 2009).

Study Species.—Similar to RIFAs, Southern Toads are found throughout most of the Atlantic and Gulf Coastal Plain ecoregions in the southeastern United States (Jensen, 2005). Southern Toads inhabit a diversity of terrestrial ecosystems with nearby water sources for breeding and can be found in disturbed areas similar to RIFAs (Jensen, 2005; Tschinkel, 2006). Moreover, both Southern Toads and RIFAs consume arthropods, which may lead to Southern Toads both competing with and acting as a predator of RIFAs (Jensen, 2005; Tschinkel, 2006). As RIFAs consume smaller-sized vertebrates, RIFAs may depredate Southern Toads similar to other documented depredation events on similarly sized amphibians (e.g., Houston Toad; Freed and Neitman, 1988).

Southern Toad egg masses were collected from wetlands located on the JCI and reared to metamorphosis in a 100-L wading pool covered with screening to protect them from depredation. We fed tadpoles crushed rabbit food pellets every other day. We also placed wetland debris and leaf litter on the bottom of the wading pool to provide cover and additional food. After metamorphosis, the toads were moved to another wading pool with leaf debris on the bottom and screening over the top to provide protection from predators. Small flies were able to enter and exit the containers and metamorphs also obtained food from the arthropods within the added litter.

Experimental Design.—Our experiment consisted of a control (n = 8 enclosures) with ambient RIFAs and a treatment (n = 8 enclosures), where we reduced RIFAs by hand broadcasting 1.7 kg/ha of the granular insecticide Amdro® (active ingredient hydramethylnon) between 0800 and 1100 h on 7 June 2013 to reduce RIFA numbers. Amdro was applied inside and within an approximately 10-m buffer surrounding the enclosures. We quantified the relative abundance of RIFAs and other ant species using bait monitoring stations comprised of a piece of hot dog placed into a plastic vial (Agosti et al., 2000). We completed sampling once prestocking in 2013 on 21 June and approximated numbers in the field. During the next four sampling occasions (completed prior to toad recapture sessions or stocking) all ants were preserved in ethanol for later enumeration and identification to genus or species under a stereo microscope. Generally, Amdro should be reapplied when 30% of bait monitoring stations have RIFAs (David Oi, pers. comm.). However, we only captured RIFAs at bait traps during bait sampling on 18 October 2013 and 17 April 2014, and the proportion of bait traps with RIFAs were 25% and 8%, respectively. Thus, to reduce the effects of Amdro on nontarget arthropods, no additional broader scale applications of Amdro were completed. Instead, we immediately spot-treated locations during these two ant sampling sessions where RIFAs were captured by applying Amdro at a rate of 1.7 kg/ha at and within a 0.5-m buffer around each bait station where RIFAs were captured.

To assess RIFA effects on toad metamorph survival and growth, we randomly assigned 50 toad metamorphs into four of the eight enclosures in our control and RIFA treatment enclosures on 21 June 2013 and again on 22 May 2014 (400 toads per year; n = 4 enclosures per treatment). A stocking density of 2 toads/m^2 is similar to densities in an enclosure experiment conducted by Todd and Rothermel (2006) and substantially less than metamorph toad densities observed in the wild at other sites (e.g., 16–92 individuals/m^2 at the Savannah River Site in Aiken, SC; Beck and Congdon, 1999). Each toad was given a batch toe clip unique to each enclosure for future identification. If recaptured, each toad received a unique toe clip. At the end of the first year, all toads were removed from the enclosures prior to restocking with new metamorphs. Toad metamorphs were recaptured during two 3-d sessions in 2013 (14–16 September and 19–21 October 2013). Each recapture survey consisted of 20 min of searching the enclosure by hand. At capture, we recorded an individual's unique ID and measured their snout-to-urostyle length (SUL) and weight. Individuals from 2013 were removed from enclosures on 16 March 2014 and 1, 2, and 10 April 2014. Toad metamorphs stocked in 2014 were recaptured in one 3-d session (13–15 July 2014) and one 2-d session (19–20 September 2014).

To assess the influence of RIFA treatment on toad diet, we randomly assigned 48 approximately year-old toads into the four enclosures at each location we were not using for our survival experiments (6 toads per enclosure) in April 2014. Half of these toads were from the RIFA treatment enclosures (n = 24) and half were from the control enclosures (n = 1) or hand captured near buildings on our study site (n = 23). We used toads captured near buildings because so few individuals survived in the control plots. We assessed the diet of 1-yr-old toads after 1 mo via stomach flushing and visually inspecting the contents under a stereo microscope (Solé et al., 2005). Following stomach flushing the toads were released outside of the enclosure near where they were initially found. Arthropods were counted and non-Formicidae arthropods in the stomach

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contents were identified to class or order; Formicidae were identified to genus or species.

Data Analysis.—To assess differences in RIFA abundances between the control and RIFA treatment enclosures, we compared the fit of generalized linear mixed models (GLMMs) with underlying negative binomial or zero-inflated negative binomial distributions that included no fixed effects (the null model) or the fixed effects of RIFA treatment and session (each unique ant sampling session [1–5]) independently, additively, or interactively (10 total models) using the function 'glmmTMB' within the glmmTMB package in program R (Brooks et al., 2017; R Core Team, 2020). We included the bait-trap ID as a random effect to account for nonindependence of samples from the same bait trap across multiple sampling occasions. We considered zero-inflated models because more than 50% of the samples had RIFA abundances of 0.

To assess the influence of RIFA treatment on toad survival, we compared the fit of GLMMs with underlying Poisson distributions that included no fixed effects (the null model) or the fixed effects of RIFA treatment and year independently, additively, or interactively (five total models) using the function 'glmer' within the R package 'lme4' (Bates et al., 2015). We considered the number of recaptured toads as our measure of toad survival because recapture probability likely did not vary across enclosures, as enclosures were located within the same vegetation type. Moreover, as there was no emigration or immigration within enclosures, any loss of toads represented mortality and the number of recaptured toads is representative of toad densities within the enclosures. We included enclosure as a random variable to account for the nonindependence of recapture estimates from the same enclosure between years. We used the 'AICtab' function within the R package 'bbmle' (Bolker and R Development Core Team, 2020) to compare model sets assessing treatment and session or treatment and year effects on RIFA abundances and toad survival, respectively, using Akaike's information criteria corrected (AIC_c) for small sample sizes (Burnham and Anderson, 2002). Models with an ΔAIC_c of <2 were considered competing models. Competing models were model averaged using the 'model.avg' function in the package 'MuMin' (Barton, 2020). All figures were made using the package 'ggplot2' in Program R (Wickham, 2016).

We quantified the influence of RIFA treatment and the number of recaptured toads separately on toad growth (measured as SUL) during the first capture–mark–recapture session in 2013 using GLMMs and included enclosure as a random variable in both models. We completed a Shapiro–Wilks test using the 'stats' package in program R (R Core Team, 2020), which indicated the variable SUL was nonnormal using $P < 0.05$ as a cutoff for significance ($W = 0.94$, $P = 0.02$). When we completed a log-transformation of the SUL, it did not deviate from a normal distribution ($W = 0.96$, $P = 0.07$). Thus, both models were run using the log of SUL as the dependent variable. We only assessed differences in growth in 2013 because so few toads were recaptured in the control in 2014 ($n = 2$). For all models described above, we used the function 'confint' within Program R (R Core Team, 2020) to quantify effect sizes as the 95% confidence interval of the beta parameter associated with the effect of each independent variable on the dependent variables and considered an effect significant if $P < 0.05$ and the 95% confidence interval did not cross 0.

To assess effects of RIFA treatment on toad diets within the enclosures, we calculated the frequency of occurrence (FO) and relative occurrence (RO) of each prey order and for the ant genera relative to all taxonomic groups and ant genera only within the stomach contents of the toads we collected (Balakrishna et al. 2016). We also calculated Pianka's index of dietary overlap for all taxonomic groups and Formicidae separately (Pianka, 1973). We completed all calculations associated with toad diets in Excel.

Results

During our first sampling occasion prestocking in 2013, we captured no RIFAs in the RIFA treatment enclosures, whereas RIFAs were present in >80 % of vials collected from the control enclosures. For the remaining five ant sampling occasions, our most supported model had a zero-inflated negative binomial distribution and included the variables treatment, session, and their interaction (Table 1). We found no interactive effect of treatment and session or an effect of session on RIFA abundances (Tables 2 and 3). Averaged across all sampling occasions, RIFA abundances were 20 times greater in the control (242 individuals/vial, SE = 34) compared to the RIFA treatment (12 individuals/vial, SE = 11; Tables 2 and 3).

When assessing effects on the number of toads recaptured, our most supported model had an underlying Poisson distribution and included RIFA treatment and year additively as effects (Table 1). We found one competing model ($\Delta AIC_c < 2$) that also had a Poisson distribution and included the RIFA treatment and year interaction (Table 1). Thus, we report the findings from the averaged model. Using the mean proportion of recaptured toads as an index of survival, we found the mean proportion of recaptured toads was 2.8 times greater in 2013 (mean = 7.9, SE = 2.8) compared to 2014 and 9.5 and 21 times greater in the RIFA treatment compared to the control in 2013 and 2014, respectively (Table 3; Fig. 1). We found no interactive effect of treatment and year on the number of recaptured toads (Table 3).

The SULs of toads in the control at initial release ranged from 8 to 14 mm (= 9.3 mm) in 2013 and 10 to 14 mm (= 12.9 mm) in 2014. In the RIFA treatment the SULs of toads ranged from 7 to 14 mm (= 9.9 mm) in 2013 and 11 to 15 mm (= 13.0 mm) in 2014. Using SULs as a measure of growth, toad growth was approximately 23% greater in the control (= 34.0 mm, SE = 0.55) compared to the RIFA treatment (= 27.7 mm, SE = 0.36; Table 3; Fig. 2). Toad growth negatively correlated with the number of recaptured toads (Table 3; Fig. 3).

Of the 48 toads stocked into the enclosures for the dietary study, we were able to recapture and gut flush 36, half in the control and half in the RIFA treatment enclosures. Two toads had one item or less in their stomach contents and were not included in further analyses. We identified 17 taxa (identified to phylum, order, or class) within the toad stomach contents and 9 genera of ants (Table 4). Toad diets in the control and RIFA treatment enclosures overlapped 94%. When considering only the portion of their diets comprised of ants, dietary overlap was 44%.

Though the FO of Formicidae was equal, the RO was 22.9% greater in the control compared to the RIFA treatment enclosures. Toads in both the control and RIFA treatment enclosures consumed RIFAs, but the RO of RIFAs was twice as high in the control enclosures compared to the RIFA treatment enclosures. Among Formicidae, toads in the control enclosures consumed 5.4 times more *Pheidole* sp. and 5.7 times less *Nylanderia* sp. compared to toads in the RIFA treatment enclosures.

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TABLE 1. The dependent and independent variables, underlying distributions, relative differences between each model's Akaike's Information Criteria corrected for small sample size values and the top model (ΔAIC_c), degrees of freedom (df), and model weights (w_i) associated with models run to assess the influence of red imported fire ant (RIFA) treatment and capture session (1–5) or RIFA treatment and year (2013 and 2014) on the number of RIFAs captured at bait stations (RIFA abundances) and the number of Southern Toad (*Anaxyrus terrestris*) recaptures (number of recaptures) in an experiment where toad metamorphs ($n = 50$ toads per enclosure per year) were stocked into enclosures with ambient or reduced RIFAs ($n = 4$ per treatment) located in Newton, Georgia, USA.

Variables	Distribution[a]	ΔAIC_c	df	w_i
RIFA abundances				
Treatment * Session	ZNB	0	10	0.953
Treatment	ZNB	7.1	6	0.028
Treatment + Session	ZNB	7.8	8	0.02
Treatment	NB	51.4	4	<0.001
Treatment + Session	NB	53.3	5	<0.001
Treatment * Session	NB	53.6	6	<0.001
Null	ZNB	73	4	<0.001
Session	ZNB	74.2	6	<0.001
Null	NB	86.6	3	<0.001
Session	NB	88.6	4	<0.001
Number of toads recaptured				
Treatment + Year	Poisson	0	4	0.6671
Treatment * Year	Poisson	1.4	5	0.3312
Year	Poisson	12	3	0.0017
Treatment	Poisson	18.6	3	<0.001
Null	Poisson	30.6	2	<0.001

[a] ZNB, zero-inflated negative binomial; NB, negative binomial.

DISCUSSION

Our study supports anecdotal and observational evidence suggesting RIFAs as a factor in amphibian population declines in the southeastern United States. Direct depredation by RIFAs is common in vertebrates (Allen et al., 2004) and was likely a main factor underlying the lower survival of Southern Toads in our control enclosures. Nonetheless, consumption of RIFAs by toads, competition with RIFAs for arthropod prey, and/or behaviors associated with avoiding RIFAs may also have contributed to reduced survival. Similar to the Southern Toads in our study, Eastern Fence Lizards (*Sceloporus undulatus*), purple martin, and Northern Bobwhite Quail (*Colinus virginianus*) consume RIFAs (Langkilde and Freidenfelds, 2010; Long, 2013; Myers et al., 2014; Helms et al., 2016). Effects of RIFA consumption on these vertebrates are mixed, leading to reduced survival in Eastern Fence Lizards and Northern Bobwhite Quail (Langkilde and Freidenfelds, 2010; Myers et al., 2014). Conversely, consumption of RIFA queens during their dispersal decreased the amount of time purple martins spent foraging, though effects on population parameters are unknown (Helms et al., 2016). Eastern Fence Lizards will also consume RIFAs as a defense strategy when attacked (Langkilde and Freidenfelds, 2010) and toads may display this same behavioral response. Moreover, Southern Toads display antipredator behaviors in response to the presence of RIFAs by moving more frequently to avoid RIFAs (Long et al., 2015). If individuals moved more while attempting to avoid RIFAs in the control treatment in our study, increased energy expenditures, increased depredation by other predators, and/or increased stress could be another explanation for reduced survival rates.

Interestingly, a recent experimental study did not find any effects of RIFAs on Houston Toad survival (Sirsi et al., 2020). However, the authors indicated the abundances of RIFAs on their study site in Texas were relatively low (Sirsi et al., 2020). Though the abundances of RIFAs in our study are not directly comparable to Sirsi et al. (2020) because they averaged pitfall and bait-trap data, differences in the abundances of RIFAs may be an explanation for our disparate results regarding toad survival. The contrast in our findings from Sirsi et al. (2020) demonstrate the importance of understanding the effects of invasive species within the context of different ecoregions, as these effects might be influenced by local variation in resources and environmental conditions. For instance, although they did not quantify survival, Allen and colleagues found Southern Toad abundances increased when RIFAs were reduced with hydramethylnon in a similar landscape system to our study (Allen et al., 2017). Thus, RIFAs can lead to reductions in the abundances of even a common species, like the Southern Toad, potentially via reductions in toad metamorph survival, as we found in our study, and via other mechanisms (e.g., adult survival and recruitment) that we did not quantify.

Though toad growth was greater in the control treatment in our study, growth also was negatively related to the number of recaptured toads within each enclosure, indicating toad growth

TABLE 2. Mean and standard error (SE) of the number of red imported fire ants (*Solenopsis invicta*; RIFAs) captured at bait stations ($n = 3$ per enclosure per session) over five capture sessions completed in enclosures with ambient (control) or reduced (RIFA treatment) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

	Control		RIFA treatment	
Session date	Mean	SE	Mean	SE
9 September 2013	258	77	0	0
18 October 2013	249	85	53	53
17 April 2014	267	71	5	4
17 May 2014	104	45	0	0
7 July 2014	335	89	0	0

TABLE 3. The independent and dependent variables, beta (b) parameters and their 95% confidence intervals (CI), z- or t-values, and P values (P) associated with top models explaining the influence of red imported fire ant (RIFA) treatment and session (1–5), RIFA treatment and year (2013 and 2014), and RIFA treatment or the number of recaptures on the number of RIFAs captured at bait stations (RIFA abundances; $n = 3$ stations per enclosure per session), the number of Southern Toad (*Anaxyrus terrestris*) recaptures, and the log of the snout–urostyle lengths (SUL) of recaptured toads (log SUL [$n = 5$ toads in the control and 46 toads in the treatment, respectively]) in an experiment where toad metamorphs ($n = 50$ toads per enclosure per year) were stocked into enclosures with ambient (control) or reduced (treatment) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

Variables	b	95% CI	z or t	P
RIFA abundances				
Treatment	−3.25	−6.25 to −0.26	−2.13	0.03
Session	−0.33	−1.17 to 0.52	−0.76	0.45
Treatment * Session	0.07	−0.83 to 0.97	0.15	0.88
Number of toad recaptures				
Treatment	2.39	1.36 to 3.42	4.55	<0.001
Year	−1.15	−2.26 to −0.04	2.03	0.04
Treatment * Year	0.79	−1.64 to 3.22	0.64	0.52
log SUL				
Treatment	0.19	0.05 to 0.32	2.64	0.047
Number of recaptured toads	−0.01	−0.02 to −0.008	−4.31	<0.001

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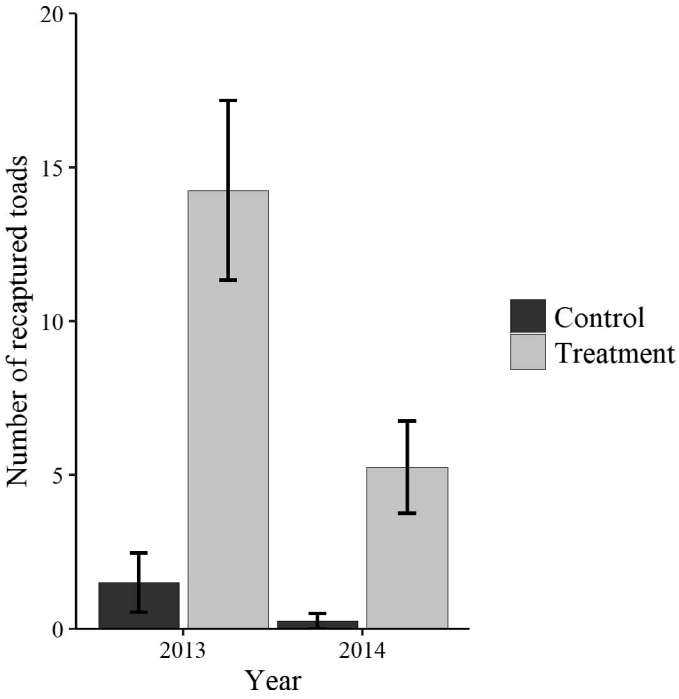

FIG. 1. Mean and standard error of the number of Southern Toads (*Anaxyrus terrestris*) recaptured in an experiment where toad metamorphs ($n = 50$ toads per enclosure per year) were stocked into enclosures with ambient (control) or reduced (treatment) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

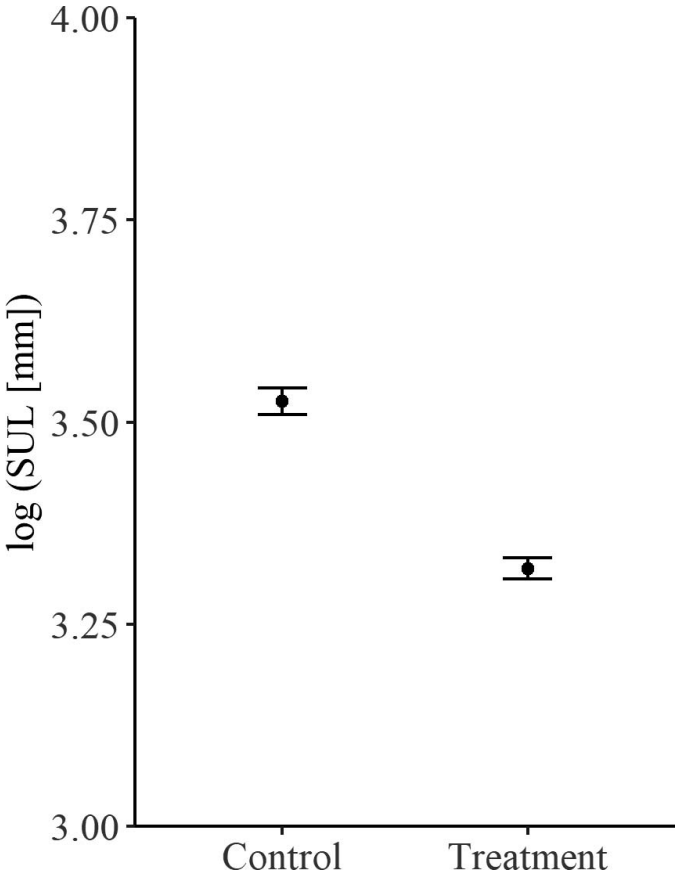

FIG. 2. Mean and standard error of the log of the snout–urostyle length (SUL; mm) of Southern Toads (*Anaxyrus terrestris*) in an experiment where toad metamorphs ($n = 50$ toads per enclosure) were stocked into enclosures with ambient (control; $n = 5$ recaptured toads) or reduced (treatment; $n = 46$ recaptured toads) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

is indirectly related to reductions in RIFA numbers via effects on toad densities. These results were similar to a recent study that demonstrated no effects of RIFA treatment on Houston Toad metamorph growth but a negative correlation between Houston Toad growth and density (Sirsi et al., 2020). The correlation between toad growth and density in our study suggests reduced toad growth in the RIFA treatment compared to the control treatment was caused by intraspecific competition, rather than differences in prey availability related to RIFA treatment. Though this effect could influence toad populations because growth rate in anurans has been linked with lifetime reproductive success (Calder, 1984; Cabrera-Guzmán et al., 2013), we suggest the effect of competition may not be as strong in the natural environment where movements are not restricted.

Contrary to our predictions, the overall dietary overlap of toads in the control and RIFA treatment was high. Though we found high overall dietary overlap, the overlap when considering Formicidae only was relatively low. Amdro® is also made and marketed to target *Pheidole* sp., which likely explains why we observed a decrease in the proportion of *Pheidole* sp. in the diet of toads in the RIFA treatment. Moreover, on our study site, RIFAs are the dominant ant on the landscape (Stuble et al., 2009) and a previous study at Ichauway found that RIFAs and native ant species had similar recovery rates relative to their abundances following treatment with hydramethylnon, the active ingredient in Amdro (Stuble et al., 2011). However, treatment led to an increased species richness of native ants (Stuble et al., 2011). Thus, the lower dietary overlap of Formicidae we observed between toads in the control and RIFA treatment is likely because treatment with hydramethylnon alters native ant communities. These changes in ant communities would influence the availability of different ant genera, such as *Pheidole* sp., as a food resource for toads. Though we did find differences in toad diets between treatments, it is important to recognize these differences in diet between toads in the control and RIFA treatment may be related to prey availability, which we did not measure. Future studies should measure arthropod availability on the landscape to determine if differences in toad diets are driven by RIFAs or prey availability.

Given the effects of RIFAs we observed on toad survival, treatment for RIFAs using hydramethylnon, other similar insecticides designed to target RIFAs, or noninsecticide methods (e.g., hot water treatment; King and Tschinkel, 2008) should be considered as a tool for amphibian conservation in certain circumstances. The longleaf pine ecosystem where we completed our study relies on frequent fires and the presence of an open canopy, which creates an open, disturbed landscape preferred by RIFAs (Jose et al., 2007; Tschinkel, 2006). Thus, native amphibians in this and other similar ecosystems, may benefit from treatment for RIFAs. At a small scale (e.g., around breeding wetlands), treatment of RIFA mounds using hot water may be a feasible approach to minimize potentially negative effects treatment with insecticides may have on nonnative arthropods (e.g., Williams et al., 2001; Plentovich et al., 2010, 2011). However, when applied correctly, hydromethylnon and similar granular insecticides have minimal effects on nontarget arthropods because RIFAs are dominant and recruit to bait faster than other arthropods (Gibbons and Simberloff, 2005). Moreover, observed negative responses of arthropods to hydro-

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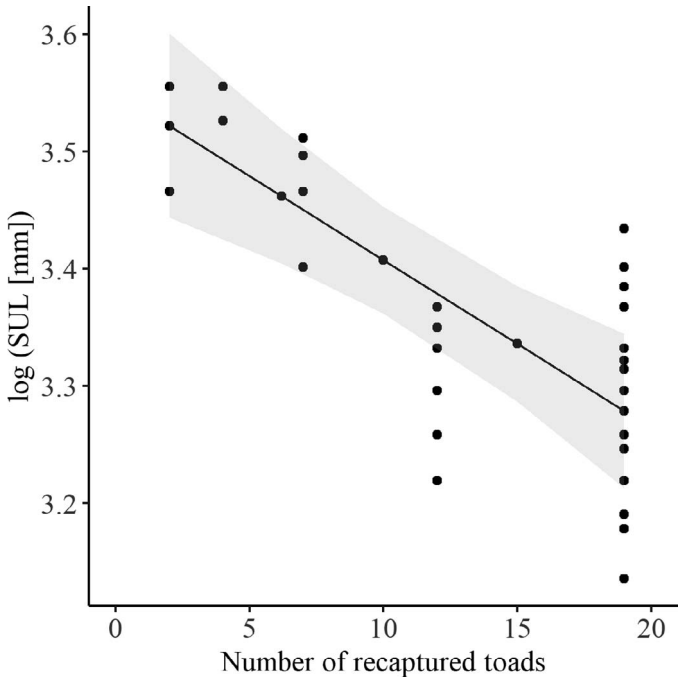

FIG. 3. Regression plot displaying all observations and the fit line with associated 95% confidence intervals showing the relationship between the log of the snout–urostyle length (SUL; mm) of Southern Toads (*Anaxyrus terrestris*) and the number of toads captured in an experiment where toad metamorphs ($n = 50$ toads per enclosure) were stocked into enclosures with ambient (control; $n = 5$ recaptured toads) or reduced (treatment; $n = 46$ recaptured toads) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

methylnon and other insecticide treatments have been relatively short lived, and studies have demonstrated positive responses of arthropod communities to RIFA reductions with insecticides (e.g., Epperson and Allen, 2010; Stuble et al. 2011; Epperson et al., 2021). Granular insecticides can also be broadcast over large areas, whereas hot water treatment would be challenging to apply at larger scales.

Minimizing disturbance may also reduce effects of RIFAs on amphibians. For instance, clear-cutting within a >30-yr-old managed loblolly pine (*P. taeda*) forest led to high mortality rates (30%) associated with RIFAs in Mole and Marbled Salamanders 17 mo post clear-cutting (Todd et al., 2008). A study completed within this same forest system immediately post clear-cutting documented a 2% mortality rate in Mole Salamanders associated with RIFAs (Rothermel and Luhring, 2005). Differences in mortality rates between these studies are likely the result of RIFA colonization correlating positively with time since disturbance (Porter et al., 1988) and suggests RIFAs would not have been a significant source of mortality in these salamanders predisturbance (Todd et al., 2008). Ultimately, reduced disturbance should reduce suitability for RIFAs (Tschinkel, 2006), although this may conflict with other management goals, such as timber harvest or maintenance of native pine forests with prescribed fire.

Our study provides evidence that RIFAs alter amphibian populations and may be contributing to amphibian declines in the southeastern United States. Given the high mortality rates we observed, RIFAs should be considered when developing conservation plans for any amphibian species found in areas where RIFAs are present. Species associated with disturbance-reliant ecosystems or those found as remnants within disturbed

TABLE 4. Total abundance, frequency of occurrence, and relative occurrence of Arthropods in the stomach contents of Southern Toads (*Anaxyrus terrestris; n* = 18 per treatment) stocked into enclosures with ambient (control) or reduced (treatment) RIFAs ($n = 4$ enclosures per treatment) located in Newton, Georgia, USA.

	Total abundance		Frequency of occurrence		Relative occurrence	
Taxonomy	Control	Treatment	Control	Treatment	Control	Treatment
Acari	1	0	6.3	0.0	0.5	0.0
Araneae	20	27	68.8	76.5	9.4	17.6
Blattodea	1	5	6.3	29.4	0.5	3.3
Coleoptera	18	24	47.1	47.1	8.5	15.7
Collembola	0	3	0.0	18.8	0.0	2.0
Chilopoda	6	1	25.0	6.3	2.8	0.7
Diplopoda	5	5	31.3	25.0	2.3	3.3
Diptera	2	1	12.5	6.3	0.9	0.7
Gastropoda	0	1	0.0	6.3	0.0	0.7
Hemiptera	5	10	25.0	37.5	2.3	6.5
Hymenoptera (Formicidae)	142	67	81.3	81.3	66.7	43.8
Formicidae (unidentified)	4	2	12.5	12.5	1.9	1.3
Solenopsis invicta	29	9	50	37.5	13.6	5.9
Brachymyrmex sp.	13	8	56.25	18.75	6.1	5.2
Camponotus sp.	11	0	18.75	0	5.2	0.0
Pheidole sp.	68	9	31.25	37.5	31.9	5.9
Cyphomyrmex sp.	8	2	25	6.25	3.8	1.3
Nylanderia sp.	8	33	18.75	31.25	3.8	21.6
Hypoponera sp.	0	2	0	6.25	0.0	1.3
Solenopsis sp.	1	0	6.25	0	0.5	0.0
Trachymyrmex sp.	0	2	0	6.25	0.0	1.3
Lepidoptera	3	3	12.5	12.5	1.4	2.0
Opiliones	1	0	6.25	0	0.5	0.0
Orthoptera	2	0	12.5	0	0.9	0.0
Trichoptera	2	1	6.25	6.25	0.9	0.7
Nematoda	5	5	12.5	18.75	2.3	3.3
Total	213	153				

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landscapes in need of restoration would be most affected. Additionally, though Southern Toads are relatively common (Jensen, 2005) we cannot ignore the negative effects reductions in the survival of toad metamorphs associated with RIFAs could have on Southern Toad populations, along with more imperiled amphibian species. If mortality associated with RIFAs is additive, this could have large, negative implications for populations of amphibians over time, including species considered common.

Acknowledgments.—We are thankful to the field technicians in the herpetology and wildlife labs at the JCI that collected this data and the JCI for funding our study. All toads used in our study were collected under the Georgia Department of Natural Resources Scientific Collecting Permit 29-WJH-13-164. All data collection for this research was completed by technicians at the JCI. The JCI is privately owned and at the time of this research did not have an Institutional Animal Care and Use Committee (IACUC). However, the research was carried out following guidelines presented in the "Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research," which is compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (HACC, 2004).

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Accepted: 10 June 2021.
Published online: 11 March 2022.

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