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## Southeastern US Snake Species are Vulnerable to Egg Predation by Red Imported Fire Ants (*Solenopsis invicta*)

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**ABSTRACT:** Invasion and spread of Red Imported Fire Ants (RIFA; *Solenopsis invicta*) is cited as a possible cause for enigmatic reptile declines in the southeastern United States. Reptiles are negatively affected by RIFA through predation of eggs, hatchlings, and adults. We used short-term (12-h) field trials early in incubation to evaluate whether RIFA could successfully depredate intact eggs from six species of native terrestrial oviparous snakes: North American Racer (*Coluber constrictor*), Speckled Kingsnake (*Lampropeltis holbrooki*), Prairie Kingsnake (*L. calligaster*), Rough Greensnake (*Ophedrys aestivus*), Black Ratsnake (*Pantherophis obsoletus*) and Great Plains Ratsnake (*P. emoryi*). Then we used an artificial nest field experiment at the end of incubation to test whether RIFA predation differed between a species that has apparently declined in areas of its range where RIFA has invaded (*L. holbrooki*) and a species that has apparently not declined (*C. constrictor*). We measured pip–hatch and incubation time for each species in the laboratory to determine whether differences in time between pipping and hatching (pip–hatch time) could account for interspecific differences in RIFA predation on eggs. Overall, RIFA predation rates on snake eggs were high for all species (25–67% during early trials, 50–100% at end of incubation), although *P. obsoletus* was only depredated after hatching in the field. *Coluber constrictor* had significantly shorter pip–hatch times than other species, but probability of predation by RIFA did not differ for *C. constrictor* and *L. holbrooki*. Our study provides novel observations of RIFA predation and suggests that time spent in nest, eggshell characteristics, and nest microhabitat may be more important than pip–hatch time in mediating vulnerability of snake eggs to RIFA predation.

**Key words:** Enigmatic declines; Incubation; Invasive species; Reproduction

RED IMPORTED FIRE ANTS (RIFA; *Solenopsis invicta*) are native to South America but have been introduced globally and are ranked among the most damaging invasive species in the world (Lowe et al. 2000). The first potential report of RIFA in the United States was in Mobile, Alabama, USA in 1918; by 1955 RIFA had spread throughout the southeastern United States (Callcott and Collins 1996), and RIFA continues to expand their invasive range northward and westward. As generalist predators, RIFA form large colonies and aggressively defend their colony mound using venomous stings. Their venom makes them a health risk to humans as well as native wildlife (Kemp et al. 2000). Known ecological effects of RIFA include predation of endangered cave invertebrates in Texas, USA (Elliott 1993), predation on live-trapped small mammals (Masser and Grant 1986; Flickinger 1989), reduced Loggerhead Shrike (*Lanius ludovicianus*) and native insect abundance (Allen et al. 2001), and predation on Bobwhite quail (*Colinus virginianus*) chicks (Allen et al. 1995). RIFA are also highly problematic for reptiles and amphibians because they affect their food sources (invertebrates), depredate eggs, and occasionally depredate adults (Wojcik et al. 2001; Todd et al. 2008). Additionally, there is some evidence that the presence of RIFA results in population declines, based on an experimental reduction of RIFA density that led to increased abundance and diversity of a South Carolina, USA reptile and amphibian community (Allen et al. 2017).

Due to their inability to flee, eggs are likely to be the reptile life stage that is most vulnerable to RIFA depredation. Predation by RIFA on reptile eggs has been documented in the laboratory for diverse turtle and snake species (Diffie et al. 2010) and observed in field conditions for Eastern Fence Lizards (*Sceloporus undulatus*) (Newman et al. 2014; Thawley and Langkilde 2016), Six-lined Race-

runners (*Cnemidophorus* [= *Aspidoscelis*] *sexlineatus*) (Mount et al. 1981), Cooters (*Pseudemys* spp.) (Allen et al. 2001; Aresco 2004), Sliders (*Trachemys scripta*) (Buhlmann and Coffman 2001), *Ophedrys aestivus* (Conners 1998a), and *Coluber constrictor* (Thawley 2014) in the United States. During laboratory observations, species with more-durable eggshells (e.g., musk turtle, bobwhite quail) were not depredated by RIFA, but these species are still vulnerable to RIFA predation during the pipping stage or right after hatchling emergence (Diffie et al. 2010). This suggests that the amount of time a hatchling reptile spends pipped in the egg or escaping the nest chamber could strongly influence their probability of being depredated by RIFA. Additionally, species may differ in pip–hatch times, and slower pip–hatch times could increase the probability of RIFA predation. Increased ant predation on reptile eggs likely has important population-level consequences, as is the case for tropical anole lizards that have relatively high adult mortality (Andrews 1982). Although evidence shows that RIFA are problematic for native oviparous reptiles in the United States, there are still relatively few studies on vulnerability of reptile species, especially snakes, to RIFA predation.

RIFA are one of the proposed causes for enigmatic reptile declines in the southeastern United States. Enigmatic reptile declines are being reported around the world and are defined as declines for which there is not currently a known cause (Todd et al. 2010). For example, Common Kingsnakes (*Lampropeltis getula* complex) have declined in southeastern portions of their range, including a protected area where habitats have been relatively undisturbed by humans (Krysko and Smith 2005; Winne et al. 2007). Additionally, dramatic local population declines of *L. getula* complex on Paynes Prairie State Preserve in Florida, USA coincided with invasion by polygynous RIFA colonies (Kauffeld 1957; Bartlett 1997). Curiously, other snake species occupying the same range as *L. getula* complex

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are reported as having stable populations, and Copperheads (*Agkistrodon contortrix*) has even increased in abundance, potentially due to release from predation by Kingsnakes (Steen et al. 2014). Even other oviparous snake species such as North American Racers (*Coluber constrictor*) and Ratsnakes (*Pantherophis* spp.) remain common in areas where Kingsnakes have declined and RIFA are present. To date, no studies have tested whether southeastern snake species differ in their vulnerability to egg predation by RIFA under field conditions.

Our study was designed to evaluate interspecific differences in egg predation risk that might contribute to population trends observed for southeastern snake species. Possible factors that might influence predation risk of snake eggs include incubation time, time between pipping and hatching, eggshell durability, and nesting location. We chose to focus on incubation and pip–hatch timing in this study. We examined predation on eggs of six terrestrial snake species (*Coluber constrictor*, *Lampropeltis holbrooki* [= *L. getula holbrooki*], *L. calligaster*, *Opheodrys aestivus*, *Pantherophis obsoletus*, and *P. emoryi*), but particularly focused on comparisons between *L. holbrooki* (the member of the *L. getula* complex occurring at our study sites in Arkansas, USA) and *C. constrictor*, a common sympatric terrestrial and oviparous species that has not experienced widespread population declines. Our study consisted of three components—a short field trial early in incubation, an end-of-incubation field experiment that incorporated hatching behavior, and laboratory observations of hatching behavior to test (1) whether RIFA can depredate eggs from six snake species; (2) whether an apparently declining species (*L. holbrooki*) and stable species (*C. constrictor*) differ in egg predation risk; and (3) whether snake species differ in pip–hatch and incubation times. We hypothesized that *C. constrictor* would experience less predation by RIFA than *L. holbrooki* and have shorter pip–hatch times.

## MATERIALS AND METHODS

### Female Collection and Maintenance

From April to June 2020, we collected gravid female snakes of six terrestrial oviparous species (*Coluber constrictor*, *Lampropeltis holbrooki* [= *L. getula holbrooki*], *L. calligaster*, *Opheodrys aestivus*, *Pantherophis obsoletus*, and *P. emoryi*) in Benton, Washington, Sebastian, and Franklin Counties in Northwest Arkansas. Snakes were housed in 38–76 L aquaria furnished with aspen shavings, a water bowl, and a hide box and kept in a partially shaded, screened outbuilding in Fayetteville, Arkansas that matched ambient temperatures (mean = 13.4–25.8°C) that snakes would experience in the wild. We provided each female with a rectangular plastic container (~26 × 16 × 13 cm) half-filled with moist coconut fiber as a nesting box for egg deposition and offered water ad libitum.

Starting 1 June 2020, we checked cages for eggs at least once per day. Oviposition began on 6 June and finished on 10 July 2020. Following oviposition, we took the postpartum mass of each female snake and then released her at the site of capture. In this way, we obtained 78 *C. constrictor*, 88 *L. holbrooki*, 26 *L. calligaster*, 18 *O. aestivus*, 25 *P. obsoletus*, and 12 *P. emoryi* eggs from 5, 8, 3, 3, 2, and 1 clutches, respectively. We processed eggs from each clutch by labeling

each egg with an identifying number using a fine-tip sharpie, recording mass to the nearest 0.01 g, and length and width to the nearest millimeter. To avoid imparting human scent on eggs and limit potential contact with cutaneous bacteria and fungi, we wore nitrile gloves when handling eggs. We kept clutches together in plastic containers (~26 × 16 × 13 cm or ~18 × 18 × 12 cm) half-filled with a 1:1 mass ratio of vermiculite:water and incubated eggs at 25°C and 80% relative humidity in an environmental chamber (Model no. I41VLC9, Percival Scientific). To maintain a consistent moisture level in egg containers, we recorded mass of all containers and eggs weekly and replenished any evaporated water.

### Field Methods

We conducted field experiments at Kessler Mountain Regional Park (Washington County, Fayetteville, Arkansas; 36.026146°N, 94.204868°W; datum = WGS84). Our field site was on the edge of RIFA invasive range, and RIFA are still rare and very localized in northwest Arkansas, with few colonies persisting through the winter. The primary area we used was an ~20 × 8-m rectangular area on a southwest-facing grassy slope bordering a forest dominated by eastern red cedar (*Juniperus virginiana*) trees. A preliminary survey revealed at least 40 active RIFA mounds on the grassy slope and along the top of the incline by the forest edge. For each RIFA mound surveyed, we recorded the widest diameter of the mound to the nearest centimeter using a measuring tape; percent overstory canopy density using a spherical crown densiometer (Lemmon 1956; Concave Model C, Forestry Suppliers); whether the mound was in the open field or on the forest edge; distance from closest adjacent RIFA mound; and the distance of the mound from the forest edge to the nearest centimeter. We did not determine whether colonies were polygynous.

To test whether RIFA could depredate intact snake eggs early in incubation (0–17 d postoviposition), we set up a small-scale field experiment using eggs from *C. constrictor* ( $n = 20$ ), *L. holbrooki* ( $n = 20$ ), *L. calligaster* ( $n = 3$ ), *O. aestivus* ( $n = 3$ ), *P. obsoletus* ( $n = 3$ ), and *P. emoryi* ( $n = 2$ ). We set individual eggs in 4-mm-deep coconut fiber in small square plastic containers (7.77 × 5.49 × 8.26 cm) with 12, 3-mm holes drilled around the edge. We placed a single container 0.5 m from a RIFA mound. No mounds were reused. Eggs were set out on eight different occasions between 17 June and 15 July 2020 (ambient temperature range = 16.1–31.1°C). We left eggs for 12 h, typically between ~1900 h and 0700 h, and then recorded whether eggs had been depredated. Depredated eggs had RIFA swarming the egg, one or more chew marks or holes in the eggshell, and often leaked fluid.

To test for interspecific differences in RIFA predation near the end of incubation, we set up *C. constrictor* ( $n = 10$ ), *L. holbrooki* ( $n = 11$ ), and *P. obsoletus* ( $n = 3$ ) artificial field nests with three eggs per nest after ~50 d of incubation (~15 d before hatching). We created artificial nests using 4-mm-deep coconut fiber in 7.5-cm-diameter circular plastic containers with 12, 3-mm-diameter holes around the perimeter and a window on the lid made of 3-mm-diameter hardware cloth that allowed RIFA to pass through. We buried artificial nests so that the eggs were at least 10 cm below the soil surface with a plastic bag of soil covering the

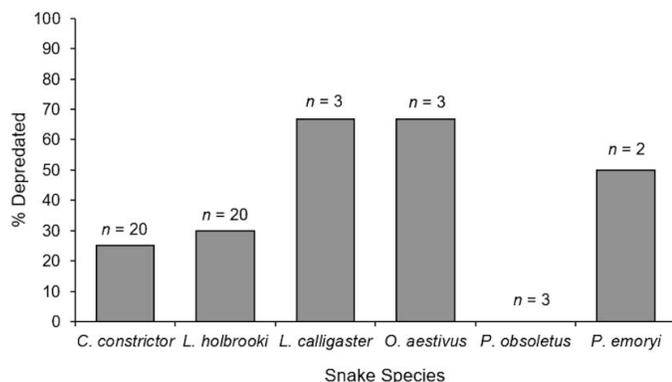


FIG. 1.—Percent of eggs depredated for each species in early incubation field experiment. Sample size (number of eggs) is given for each species.

lid of the artificial nest and a 30.5 × 30.5-cm plywood board on top to limit interference by vertebrate predators and prevent flooding by rain events (artificial nest setup modified from Thawley and Langkilde 2016). We buried artificial nests 1 m from a RIFA mound and monitored them daily to check for predation or successful hatching. We also set up three control nests each for *C. constrictor* and *L. holbrooki*, located at least 300 m away from all RIFA mounds in similar habitat (open field bordered by cedar trees) to establish background predation rates on snake eggs in the absence of RIFA predation. Our artificial nest setup allowed experimenters to visually check eggs for ant activity and predation by removing the bag of soil and lid. We recorded eggs as “depredated before pipping” when RIFA were swarming in and out of multiple holes in the eggshell, “depredated after hatching” when hatchlings were killed after emerging from the egg, and “hatched and survived” when hatchlings were recovered from artificial nests without being swarmed by RIFA. Once eggs in a nest had pipped, the artificial nests were checked twice a day for hatchlings. If an egg was pipped, the hatchling was still present inside the egg but had slit open the eggshell and might occasionally have its head or snout poking out. Once the egg hatched, the hatchling was fully emerged from the eggshell.

#### Laboratory Incubation

After ~50 d of incubation (35 d for *O. aestivus*), we placed the remaining viable snake eggs not used in field experiments (43 *C. constrictor*, 42 *L. holbrooki*, 19 *L. calligaster*, 18 *O. aestivus*, 13 *P. obsoletus*, and 12 *P. emoryi* eggs) in plastic shoeboxes with clear acrylic lids that allowed video monitoring of the eggs. We set a Trophy Cam HD Brown wildlife camera (Model 119874, Bushnell Corporation) to record 30-s videos every 30 min and positioned it above the eggs to record time between when eggs pipped and hatched (hereafter, pip–hatch time) to the nearest 30 min. Once one egg in a clutch had pipped, we visually checked eggs in that clutch twice daily.

#### Statistical Analyses

To test whether the eggs of American Racers (*C. constrictor*) and Kingsnakes (*L. holbrooki*) differed in predation risk early in incubation, we used a binomial logistic regression model with egg as the statistical unit and

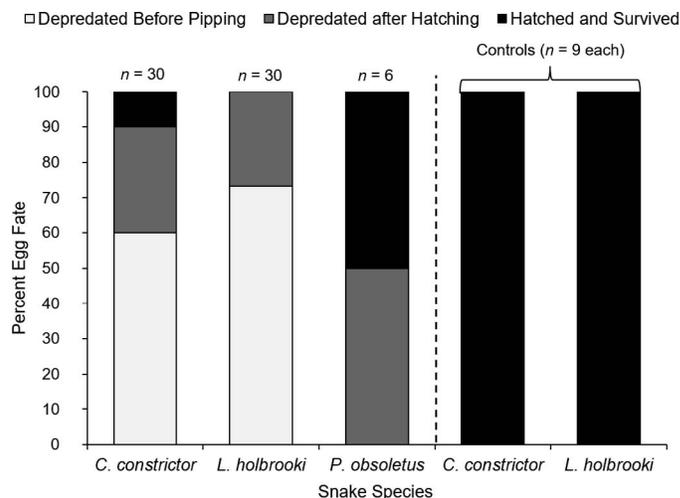


FIG. 2.—Percent of eggs depredated before pipping, depredated after hatching, or hatched and survived for each species in the artificial nest field experiment at the end of incubation. All eggs in control nests hatched and survived.

clutch as a random effect. We fit the mixed model using Gauss–Hermite quadrature, which is more accurate than Laplace approximation, and used a likelihood ratio test to determine the *P*-value for the fixed effect, species (Bolker et al. 2009). To validate the model, we used the dispersion\_glm function in the blmeco package to calculate a dispersion factor and check for overdispersion (Korner-Nievergelt et al. 2015) and plotted residuals. Small sample sizes precluded statistical analysis of survival and predation risk for other snake species (*L. calligaster*, *O. aestivus*, *P. obsoletus*, and *P. emoryi*). Thus, we evaluated predation risk qualitatively as proportion depredated for these species (Fig. 1).

For artificial nest field experiments at the end of incubation, we constructed Kaplan–Meier survival curves for eggs. We then used a log-rank test to determine whether *C. constrictor* and *L. holbrooki* eggs placed near RIFA mounds differed in survival. Two *C. constrictor* eggs in this experiment were excluded from analyses because the hatchlings had pipped within nests that were being depredated by RIFA and were removed from artificial nests early due to an experimenter error. Sample sizes for *P. obsoletus* were too low for statistical comparisons so we qualitatively reported proportion depredated (Fig. 2). We also did not include two artificial nests (one *P. obsoletus* and one *L. holbrooki*) that were depredated by mammals (identified by nests dug up and eggshells destroyed and empty) the day after they were placed in the field. We ran a Cox proportional hazards model to test whether egg survival was correlated with RIFA mound diameter, average distance to forest edge, or canopy cover.

In 2019 we did a preliminary study and recorded pip–hatch and incubation times for eggs from one clutch of *C. constrictor* (*n* = 3), three clutches of *L. holbrooki* (*n* = 23), one clutch of *L. calligaster* (*n* = 3), and one clutch of *O. aestivus* (*n* = 3). To increase our sample size, we pooled data from 2019 and 2020 and used year as a random effect in the models. To determine whether snakes differed in pip–hatch or incubation time, we used linear mixed effect models with

species as the fixed effect and clutch as a random effect. We tested for significance of clutch as a random effect using a likelihood ratio test (LRT) with restricted maximum likelihood (REML) to compare models with and without the random effect term. Based on these analyses, we determined that clutch did not need to be included as a random effect for pip–hatch time ( $df = 1$ ,  $\chi^2 = 0$ ,  $P > 0.99$ ), but it was important for incubation time ( $df = 1$ ,  $\chi^2 = 245$ ,  $P < 0.001$ ). When the fixed effect was significant, we used a post hoc Tukey contrast multiple comparison of means with `glht()` in R package `multcomp` to examine species differences in pip–hatch and incubation times. We checked assumptions of models with visualizations of residual plots. All analyses were conducted in R version v3.6.1 (R Core Team 2019) using `car`, `MASS`, `multcomp`, and `lme4` packages (Hothorn et al. 2008; Bates et al. 2015; Fox and Weisberg 2019; Venables and Ripley 2002).

## RESULTS

In the early incubation field experiment, RIFA successfully pierced the eggshells and depredated five *Coluber constrictor* and six *Lampropeltis holbrooki* eggs (out of 20 each), two *L. calligaster* and two *Opheodrys aestivus* eggs (out of three each), and one out of two *Pantherophis emoryi* eggs. None of the three *P. obsoletus* eggs set out were depredated after 12 h (Fig. 1). Egg sample sizes for *L. calligaster*, *O. aestivus*, *P. obsoletus*, and *P. emoryi* were too small for statistical comparisons, but *C. constrictor* and *L. holbrooki* did not differ in predation probability during 12-h field trials early in incubation (Binomial Logistic Regression,  $df = 1$ ,  $\chi^2 = 0.29$ ,  $P = 0.59$ ). The dispersion factor of the model was 0.9, which is less than 1.4, indicating our model was not overdispersed.

In the artificial nest field experiment near the end of incubation, all but three *C. constrictor* eggs were depredated (18 depredated before pipping and 9 depredated after hatching), all *L. holbrooki* eggs were depredated (22 before pipping and 8 after hatching), and 50% of the six *P. obsoletus* eggs were depredated after hatching (the other three eggs hatched and survived). No eggs in control nests (nine eggs each for *C. constrictor* and *L. holbrooki*) located away from RIFA mounds were depredated; all control eggs successfully hatched and survived (Fig. 2). Egg sample sizes for *P. obsoletus* were too small for statistical comparisons, but *C. constrictor* and *L. holbrooki* did not differ in survival during field nest experiments (Log-rank test,  $df = 1$ ,  $\chi^2 = 0.4$ ,  $P = 0.5$ ). Number of days to predation was similar for *C. constrictor* (8.60 d  $\pm$  0.81 SE) and *L. holbrooki* (9.55 d  $\pm$  1.82 SE) eggs.

Relative risk of death for eggs did not differ with RIFA mound size (Cox Proportional Hazards Model,  $df = 3$ ,  $z = -1.02$ ,  $P = 0.31$ ) or distance from forest edge ( $df = 3$ ,  $z = 0.03$ ,  $P = 0.98$ ) but significantly differed with increased overstory canopy density or canopy cover ( $df = 3$ ,  $z = -4.61$ ,  $P < 0.01$ ). Greater canopy cover resulted in reduced hazard of death (HR = 0.95, 0.94–0.97 CI).

Species differed significantly in their pip–hatch times (linear mixed effect model [LMEM],  $\chi^2 = 34.4$ ,  $P < 0.004$ ; Supplemental Table S1), with *C. constrictor* having significantly shorter pip–hatch times than all species tested (all  $P < 0.01$ ) except *L. calligaster* ( $P = 0.11$ ; Fig. 3A). *Coluber*

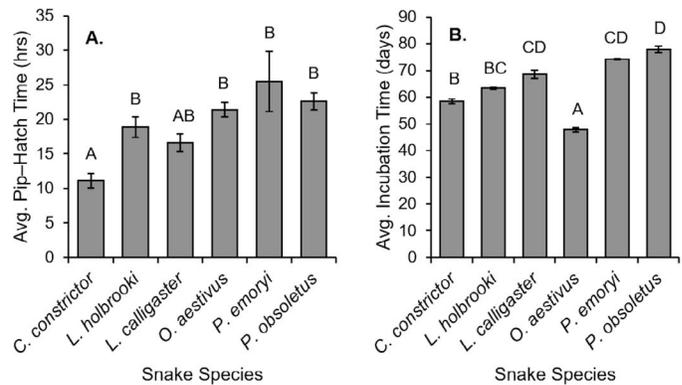


FIG. 3.—Average time between pipping and hatching (A) and average incubation time (B) for eggs of six snake species. Letters above bars (i.e., A, B, C, D) indicate significant differences between species. Error bars are  $\pm$  1 SE.

*constrictor* emerged from their eggs the quickest (mean pip–hatch time = 11.14 h  $\pm$  1.06 SE), and *L. holbrooki* averaged 7.8 h (70%) longer, *L. calligaster* 5.5 h (49%) longer, *O. aestivus* 10 h (92%) longer, *P. emoryi* 14 h (129%) longer, and *P. obsoletus* 11 h (103%) longer pip–hatch times than *C. constrictor* (Supplemental Table S1). Species also differed significantly in their incubation period lengths (LMEM,  $\chi^2 = 65.6$ ,  $P < 0.001$ ). *Opheodrys aestivus* had shorter incubation times than any other snake (all  $P < 0.02$ ), *C. constrictor* had shorter incubation times than *L. calligaster* and the *Pantherophis* species (all  $P < 0.05$ ), and *P. obsoletus* had longer incubation times than *L. holbrooki* ( $P < 0.01$ ; Fig. 3B; Supplemental Table S1).

## DISCUSSION

Eggs of all snake species tested in this study except *Pantherophis obsoletus* were depredated during 12-h early incubation trials, suggesting that leathery snake eggshells (and even eggs with a granular surface such as those of *Coluber constrictor*) can be readily punctured by RIFA. Lack of predation on control nests during the end of incubation experiment indicates that proximity to RIFA colonies substantially increases predation risk for snake eggs, providing additional support to the idea that in areas where they have invaded, RIFA are potentially important egg predators. Although *C. constrictor* had significantly shorter pip–hatch times, *L. holbrooki* and *C. constrictor* eggs did not differ in probability of predation by RIFA in either early incubation trials or the end of incubation experiment. Additionally, we found that placement of the artificial nest in areas with greater canopy cover reduced probability of predation, suggesting that the role of nest microhabitat warrants further investigation. Thus, our results suggest predation on eggs by RIFA is a threat to southeastern snake species but do not provide direct support for increased egg vulnerability to RIFA predation as a possible mechanism for enigmatic declines of *L. getula* complex.

Contrary to our predictions, pip–hatch time was not an important determinant of species' vulnerability to predation by RIFA. Overall, snake species significantly differed in pip–hatch and incubation times, with *C. constrictor* emerging from their eggs faster than most other snake species, and *O. aestivus* incubating faster than other snake species. Our

observation of *O. aestivus* having the fastest incubation time matches previously published incubation times (Ernst and Ernst 2003). *Coleuber constrictor* had a significantly shorter pip-hatch time than did *L. holbrooki* but did not differ in probability of predation. The time that hatchlings spend in the nest after hatching may be a more important driver of predation risk than pip-hatch time. We observed that the fluid in pipped eggs seemed to deter ants, and RIFA typically did not enter the eggs in field nests during pipping but attacked hatchlings after they emerged. This observation of hatchling mortality primarily after emergence matches previous studies (Conners 1998b; Buhlmann and Coffman 2001), although one study suggested that turtles were most vulnerable while still in the egg after pipping (Allen et al. 2001). Our experimental design did not test how fast hatchlings were able to escape the nest and disperse. A more natural setup would be needed to evaluate the role time spent in the nest after hatching might play on hatchling vulnerability.

Excluding *P. obsoletus*, predation rates in our study were high, with 25–67% of eggs depredated during the 12-h field trials and 90–100% during the end of incubation field experiment. It is possible that these rates were inflated relative to natural predation rates, given that eggs were set very close to RIFA mounds in artificial nests and were disturbed during daily monitoring. However, our predation rates are within values observed by previous studies. Studies using artificial field nests found rates of RIFA predation on eggs to be 24% for *Sceloporus undulatus* over only 15–20 d of incubation (Thawley and Langkilde 2016) and 60%, 50%, and 27% for *Trachemys scripta*, depending on distance from RIFA colony (Buhlmann and Coffman 2001). Weeklong laboratory observations of RIFA predation on various snake, turtle, and bird eggs found predation rates of 100% for *Malaclemys terrapin*, *Trachemys scripta scripta*, *Chrysemys picta picta*, and *Elaphe* (= *Pantherophis*) *obsoleta quadrivittata*, 92% for sea turtles (*Caretta caretta*), 75% for Burmese pythons (*Python molurus bivittatus*), and 0% for bobwhite quail (*Colinus virginianus*), *Sternotherus odoratus*, and *Apalone ferox* (Diffie et al. 2010). These data, combined with our study, suggest that although some eggs might resist RIFA predation in short-term trials, over the entire course of incubation nests in proximity to RIFA mounds are likely to experience relatively high rates of predation (60–100%).

Although sample sizes were small, *P. obsoletus* eggs were not depredated by RIFA in the 12-h early incubation trials and were not depredated until after hatching in the end of the incubation field experiments. Eggs of all other snake species were depredated during the early incubation trials, suggesting that some aspect of eggshell structure protected *P. obsoletus* eggs from being penetrated by RIFA. However, it is worth noting that Yellow Ratsnakes (*Elaphe* [= *Pantherophis*] *obsoletus quadrivittata*) were depredated by RIFA in lab experiments after seven days (Diffie et al. 2010), indicating that it is possible for RIFA to depredate *Pantherophis* spp. eggs before hatching. In our study, *P. obsoletus* eggs were about twice the volume and mass of other species, on average. Large egg size results in greater surface area, possibly making it more difficult for RIFA to aggregate in one location and penetrate the eggshell. In the presence of predatory rats, larger seabird eggs had more-

durable shells and higher survival probability than smaller eggs and took longer for rats to depredate (Latorre et al. 2013). In birds, eggshell thickness and durability typically scales with mass (Ar et al. 1979; Rahn and Paganelli 1989), so *P. obsoletus* eggs likely also have thicker eggshells, but there are relatively few studies on reptile eggshell thickness. Although we did not measure eggshell thickness or durability in this study, it has been suggested that reptile eggshell durability can influence vulnerability to RIFA predation (Diffie et al. 2010), perhaps affording species such as *P. obsoletus* some degree of protection from RIFA.

Nest site selection by female snakes could also mediate vulnerability of snake eggs to RIFA predation. Our study found that distance of artificial nest from forest edge was positively correlated with probability of predation, likely because all nests in the end of the incubation field experiment, except for two *C. constrictor* nests on the forest edge, were depredated. This trend can be explained by RIFA preference for sunny, open areas and increased foraging activity correlated with lower canopy cover (Brown et al. 2012). Female snakes may protect their eggs from RIFA by nesting in alternative locations and could actively avoid nesting near RIFA mounds; furthermore, if they attempted to nest in proximity to a RIFA mound, they would likely be attacked and deterred from laying eggs there. Nest depth and substrate could also be important factors influencing snake egg vulnerability to RIFA predation. *Pantherophis obsoletus* and *O. aestivus* often nest in tree hollows above the ground, a microhabitat less commonly used by RIFA (Ernst and Ernst 2003; Trauth et al. 2004; Gibbons 2017). A lack of basic natural history information on snake nesting behavior limits our ability to understand the role that nest microhabitat could play in vulnerability of snake eggs to predation. Given the secretive nature of snakes, we encourage naturalists to continue to record and publish observations of snake nesting behavior to contribute to our growing knowledge of the role that reproductive behavior may play in snake population declines.

Our study is among the first to provide direct data indicating that RIFA predation may be a critical source of mortality for oviparous snakes, yet many questions remain. We still do not know how RIFA locate reptile eggs or exactly which factors allow RIFA to pierce some reptile eggshells but not others. Future studies should examine additional species traits that might contribute to egg vulnerability to RIFA predation, such as eggshell thickness and durability, nest microhabitat, and nesting behavior. Additionally, while this study does provide a potential mechanism through which RIFA might cause widespread oviparous snake declines, the link between RIFA introductions and enigmatic reptile declines is still tenuous. Additional mechanistic field studies of vulnerable oviparous snakes and long-term data on snake populations are needed to determine whether invasion by RIFA is responsible for population declines. The results of this study contribute to the growing body of research into the role RIFA invasions might play in global reptile declines.

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#### SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at <https://doi.org/10.1655/Herpetologica-D-21-00004.1.S1>.

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