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Survival of lizard eggs varies with microhabitat in the presence of an invertebrate nest predator

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Abstract

The risk of nest depredation is influenced by numerous factors, including predator density, environmental conditions of the nesting landscape, and nesting behaviors of mothers. Many reptiles choose nest microhabitats that facilitate embryonic development, but little is known about how the risk of nest depredation in different habitats influences egg survival and nesting behavior. To address this knowledge gap, we quantified predator–prey relationships between square-back marsh crabs (*Armases cinereum*) and eggs of the brown anole lizard (*Anolis sagrei*). Our experiments aimed to answer the following questions: (1) are marsh crabs a predator of brown anole eggs, (2) does egg depredation differ among microhabitat types, and (3) how does crab density affect egg survival? We placed viable eggs in three different microhabitats used by nesting females (open area, palm frond, leaf litter), and manipulated the placement of the eggs as either buried or not buried. We also manipulated crab density in a field experiment. Our experiments confirmed square-back marsh crabs as egg predators, and eggs in the leaf litter or eggs buried were the least likely to be depredated. Additionally, eggs in leaf litter and under palm fronds escaped depredation longer than those in the open. Increased crab density also raised the risk of depredation for eggs placed under palm fronds or in open habitats. These results suggest that selection of nest sites by female brown anoles can influence offspring survival in the presence of marsh crabs, and the importance of nest site microhabitat choice may vary with predator density.

Keywords *Anolis sagrei* · *Armases cinereum* · Egg predation · Nesting behavior · Nest-site choice

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Introduction

Predation is a major driver of population regulation and phenotypic selection, and thus, is a critical component of ecology and evolution (Dawkins et al. 1979; Estes et al. 2011). Consequently, the demographic and environmental factors that influence depredation is of broad interest to biologists. Numerous studies demonstrate that depredation dynamics are driven by changes in predator and prey densities (Seip 1991; Vucetich et al. 2002) and can vary among habitats across different landscape scales (Andrén 1995; Bohan et al. 2000). The classic work on predator–prey relationships between lynx and snowshoe hares provides a clear demonstration of these dynamics across time and space (O'Donoghue et al. 1997, 1998a, b). The risk of depredation also varies across life stages; for some organisms, depredation rate is high at early life stages, and shifts close to zero at adult stages (e.g., turtles, crocodilians; Bjorndal et al. 2003; Somaweera et al. 2013). Indeed, predators often target younger age classes because they are relatively slow (Husak 2006; Bro-Jorgensen 2013), small (Allen 2008), and inexperienced (Brown and Chivers 2005; Kuehne and Olden 2012), and thus, less costly to capture than adults (Genovart et al. 2010). Embryos of oviparous species are particularly vulnerable due to their physical limitations and immobility.

Depredation on nests of oviparous species can be intense (Hill 1984; Schmidt and Whelan 1999; Schmidt 2003; Engeman et al. 2005, 2006), and therefore, is an important selective force that has generated a diverse range of parental care behaviors (amphibians: Crump 1996; reptiles: Gans 1996; birds: Gill and Sealy 1996; Cockburn 2006; Colombelli-Négrel and Kleindorfer 2009; Stoddard et al. 2016; insects: Trumbo 2012). In many bird species, for example, parents exhibit elaborate behaviors or morphologies that protect nests from predators (Gill and Sealy 1996; Cockburn 2006; Colombelli-Négrel and Kleindorfer 2009; Stoddard et al. 2016). In addition, certain nest and egg characteristics are favored by selection because they conceal eggs from detection by predators when parents are absent, and when vulnerability is high (Colombelli-Négrel and Kleindorfer 2009; Stoddard et al. 2016). Moreover, most non-avian reptiles lack parental care entirely and eggs within a nest are left to the vagaries of the environment. Thus, maternal choice of nest microhabitat is particularly important for these species because there is no other line of defense from nest predators. Indeed, nests of many turtle species experience extremely high depredation rates (Engeman et al. 2005, 2006), and consequently, natural selection has shaped maternal nesting behaviors so that mothers choose microhabitats that conceal eggs from potential predators (Wilson 1998). However, because the nest environment also has critical impacts on phenotypic development of offspring, there may be a tradeoff for mothers between concealing the nest from predators and maintaining conditions that allow proper embryonic development.

The risk of nest depredation is influenced by demographic parameters of predator populations, environmental conditions of the nesting landscape, maternal nesting behaviors, and the interactions between these factors. For example, mothers can modify their nesting behavior if predator density is high (Emmering and Schmidt 2011), but the degree of this modification is dependent upon the heterogeneity of the landscape (Rosalino et al. 2011), as suitable habitats may not be available or habitat suitability may shift through time (Fischer and Lindenmayer 2007). Interactions among multiple variables make it difficult to understand variation in nest depredation risk and the forces that have shaped maternal nesting behaviors. Experimental studies that decouple these factors and examine their independent and interactive effects will be particularly insightful.

To address these issues, we studied nest depredation of brown anole eggs (*Anolis sagrei*) by marsh crabs (*Armases cinereum*). We experimentally examined the effects of crab densities, nest microhabitat, and an index of maternal nesting behavior on egg depredation. This predator–prey system has many features that are amenable for experimentally addressing these effects. First, *A. sagrei* lacks nest attendance and uses a variety of microhabitats for nesting. Females often deposit eggs under cover objects (e.g., logs, leaf litter, rocks: Delaney et al. 2013; Tiatragul et al. 2019; Pruett et al. 2020), which may render eggs more vulnerable to depredation than those buried in a subterranean nest typical of many other reptiles. Laboratory and field studies also demonstrate that females non-randomly select certain microhabitats for nests (Socci et al. 2005; Reedy et al. 2013; Tiatragul et al. 2019), but most studies are interpreted from the perspective of environmental effects on embryo development and rarely consider how microhabitat might impact nest depredation. Second, anole eggs are particularly vulnerable to depredation due to their small size and are important food items for several invertebrate predators (Andrews 1988; Cates et al. 2014). Third, extremely high population densities of adults (Schoener and Schoener 1980; Lee et al. 1989) suggest that the sheer number of anole nests at a given time must be high. Consequently, anole eggs are likely a commonly encountered food item for many predators; this provides an opportunity for predators to develop an efficient search image for this food item (Nams 1997; Schartel and Schauber 2016).

The foraging and burrowing behaviors of marsh crabs have a variety of impacts on their ecosystems (Szura et al. 2017), making the study of this species important wherever they are present. The square-back marsh crab is omnivorous and demonstrates typical crab predatory behaviors in the presence of prey (Buck et al. 2003). Additionally, evidence suggests that they prefer diets that include animal matter, as these diets maximize growth rates (Buck et al. 2003). Given that anole eggs are abundant, immobile, and nutritious (Hall et al. 2018), they are likely highly desirable food items for marsh crabs. Anecdotal observations show that marsh crabs eat anole eggs (Cates et al. 2014). Additionally, depredation rate on invertebrates by marsh crabs decreases in the presence of environmental structures, such as leaf litter (Buck et al. 2003); this suggests that microhabitat of anole nests could have an impact on egg depredation. Another attractive component of this study system is that the density of marsh crabs varies greatly across time and space (Warner, pers. obs.), and is easily manipulated in the field.

We address several questions relevant to the issues described above. First, are marsh crabs a major predator of brown anole eggs? Second, does egg depredation vary among microhabitat types and with egg placement within microhabitats? These first two questions were addressed using indoor and field experiments that simulated maternal placement of eggs in different types of microhabitats that are available in the field. Third, how does crab density affect egg survival, and does density interact with microhabitat and egg placement to influence egg depredation? These last questions were addressed with a field enclosure experiment that manipulated microhabitat type, egg placement and crab density simultaneously. We hypothesized that square-back marsh crabs would prey on anole eggs and that egg survival would vary based on microhabitat, egg placement, and local crab population density. By providing insight into this predator–prey relationship and identifying consequences of oviposition site choice by females, this study will broaden our understanding of the roles these factors play in population and community dynamics.

Materials and methods

Collection of lizard eggs

Our study consisted of indoor and outdoor experiments. For both experiments, eggs were obtained from captive colonies of brown anoles at Auburn University. The colonies consisted of 361 female anoles, which were housed under standard indoor and outdoor captive conditions; the details of female housing are described in Hall & Warner (2018) and Pruett et al. (2020), but relevant details for egg collection are included here. Each cage contained a container of soil as a nesting site for females, and containers were checked two to three times a week for eggs. Once collected, each egg was weighed (to the nearest 0.0001 g) and placed in a Petri dish (top radius: 28 mm, bottom radius: 26 mm, height: 15 mm) half-filled with moist vermiculite (− 150 kPa). The Petri dishes were wrapped in parafilm to prevent water evaporation and to secure the eggs. All eggs were stored in an incubator (Peltier-cooled Incubator IPP55 Plus, Memmert GmbH, Schwabach, Germany) until the onset of the experiment. The incubator was programmed to fluctuate ± 2.4 °C daily around a mean of 20.7 °C, which is similar to the thermal regime of nests early in the season (Pearson and Warner 2018). On two occasions (21 Jun and 17 Jul 2018), egg Petri dishes were placed in a cooler and transported from Auburn, Alabama to Palm Coast, Florida for each experiment (car rides have no effect on hatching success; Hulbert et al. 2017; Pruett et al. 2020). Eggs used in the indoor experiment were 4–37 days post oviposition (mean = 17.5) and eggs used in the outdoor experiment were 3–22 days post oviposition (mean = 13.22). The incubation period at 20.7 °C is ~ 75 days (Pearson and Warner 2018), so the oldest eggs used in our experiment contained embryos that were only about halfway through development.

Indoor experiment

The indoor experiment started on 22 Jun 2018 and took place in the garage at our field house in Palm Coast, FL, USA (29′60″ N–81′20″ W). The room maintained temperature similar to the surrounding outdoor temperature, and natural lighting was provided through windows. The experiment involved fifteen plastic cages (45 cm long × 28 cm wide × 30 cm tall), which contained ~ 5 cm of sand/soil substrate, and each cage was equally divided into three microhabitats that are common at our field site: 1) open area, 2) palm fronds, and 3) leaf litter (Fig. 1a). The open microhabitat consisted of sand/soil mixture with no additional debris. The microhabitat with palm fronds consisted of 1 or 2 pieces of palm frond bark on top of the sand/soil. The leaf litter microhabitat consisted of the sand/soil covered in a layer of leaf litter (mostly leaves from live oaks (*Quercus virginiana*); litter depths were kept nearly constant and about 3–5 cm deep, which is typical at our site). The area of each microhabitat was 420 cm^2, and placement of each microhabitat within each cage was randomized. We chose these three microhabitats because they are common at our study site and eggs and/or crabs have been found in each of these habitat types during previous field studies (Delaney et al. 2013; Pruett et al. 2020) and observations.

Ninety brown anole eggs collected from the captive colony were distributed across cages with 6 eggs per cage. Two eggs were placed in each of the 3 microhabitats of each cage. Further, one egg per microhabitat was buried 1 cm under the sand/soil substrate, while the other egg was not buried; these eggs were on the soil surface in

Fig. 1 Experimental enclosures. **a** Representative cage used for the indoor experiment showing different microhabitats for nesting; leaf litter, open area, and palm frond from top to bottom. **b** Representative enclosure for the field experiment showing different microhabitats; palm frond, open area, and leaf litter from left to right. The flags mark egg locations, and the white flags were removed from poles before the experiment began. **c** Block of three field enclosures. The order of microhabitat types in the indoor cages and field enclosures was randomized among replicates

the open habitat, but under a palm frond or leaf litter in the respective microhabitat. Because anole eggs have been found in shallow nests (~ 1 cm) and on substrate surfaces under cover objects (Warner, pers. obs), these positions reflect maternally-chosen placement of eggs. Thirty square-back marsh crabs were collected from a nearby spoil island in the Matanzas River (2.5 km from our field house). Once all the eggs were placed, two crabs were released in each cage, and screen lids were placed on the cages.

The experiment ran for 5 days and ended on 26 Jun 2018. The eggs were checked for depredation once per day between 1500–1600 h. Lids were removed from cages during egg checks and replaced when the egg check was complete. If an egg could not be found, it was considered depredated. Thus, depredation was scored as either "egg absent" or "egg present". Depredation by crabs was the only explanation for the disappearance of eggs in this experiment; indeed, we observed crabs eating the eggs, which validated our use of "egg absent" as an operational definition for depredation.

Field experiment

Our field experiment took place on a spoil island in the Guana Tolomato Matanzas National Estuarine Research Reserve. Before initiation of the field experiment, natural marsh crab population densities were estimated on this spoil island. To do this, three circular drift fence enclosures (2.4 m diameter aluminum flashing) were constructed on the island on 22 Jun 2018. These enclosures were used in a pilot trial to test their ability to hold crabs, and simultaneously used as a drift fence to capture crabs. Each drift fence was 7.5 m in length and contained 5 plastic cups buried along the outside wall of the enclosure as pit fall traps. The cup traps were checked for marsh crabs in the morning, at noon, and in the evening for 5 continuous days. Crabs were counted, marked with a visible dot (using a sharpie marker), and released at each check for each of the three drift fences. The total unmarked crab count for each drift fence was recorded across all 5 days. The lowest count recorded was 21 crabs and the highest count recorded was 52 crabs over the 5-day period. The ratio of the lowest crab count to the highest crab count was roughly 2:5, which was used to determine the number of crabs for the low and high crab density treatments in the field experiment. We also used the Schnabel index (E1) (Schnabel 1938) to estimate the number of crabs around each enclosure:

$$N = \frac{\sum_{i=1}^{m} MiCi}{\sum_{i=1}^{m} Ri} \tag{E1}$$

Ci denotes the number of crabs captured at time *i*, *Mi* denotes the total number of previously-marked crabs at time *i*, and *Ri* denotes the number of marked crabs captured at time *i* (see Table S1). Dead crabs found in the traps were included in the *Ci* count, but unmarked crabs were not included in the *Mi* count. Estimated counts were respectively 125, 191 and 218 around each respective enclosure.

The field experiment started about one month later (on 20 Jul 2018). For this experiment, 12 enclosures were constructed on the same island. Each enclosure consisted of a circular drift fence (2.4 m diameter, 15.5 cm tall). Drift fences were dug ~ 6 cm into the ground and supported with PVC pipe stakes around the outside. The two ends of the drift fence were attached to each other by duct tape, and a rectangular piece of aluminum flashing was secured perpendicular on top of the duct taped portions to prevent crab escape. The enclosures were then divided into the same three microhabitats used in the indoor experiment: open area, palm fronds, and leaf litter; these three areas were rectangular in shape (1.7 m × 0.57 m) and located in the central part of the circle (Fig. 1b.) The open area consisted of open space (mostly sand) with no additional debris. The microhabitat with palm fronds consisted of 1 or 2 full palm frond branches on the ground. The leaf litter microhabitat consisted of a layer of leaf litter covering the ground (mostly leaves from live oak trees). The location of each microhabitat within each enclosure was randomized.

The experiment consisted of 4 blocks of 3 enclosures (Fig. 1c). In each block, one enclosure was a control (no crabs), one enclosure had low crab population density (10 crabs released), and one enclosure had high crab population density (25 crabs released), which represent the same ratios from the crab population study. Each block was positioned in areas of similar canopy cover.

The 144 brown anole eggs collected from the captive colony at Auburn University were distributed across all 12 enclosures, with 12 eggs per enclosure. Four of these eggs were placed in each of the three microhabitats; 2 eggs placed above ground and 2 buried under 1 cm of ground substrate. Importantly, eggs placed above ground in the leaf litter and palm frond treatments were still underneath these substrates, and not placed on top of them. Each egg location was marked by a survey flag on a wire pole, so that each egg could be found again during egg checks (Fig. 1b). The flag was removed from each survey flagpole to avoid the possibility of crabs using it as a visual cue for food availability. Square-back marsh crabs were collected from the same island. Once all the eggs were in place, crabs were released into the enclosures the next morning based on their respective crab density treatments. All enclosures were thoroughly sprayed with freshwater at 0800 h and 1500 h each day to provide water for the crabs.

The experiment ran for 7 days and ended on 27 Jul 2018. The eggs were checked for depredation every 2 days. If an egg could not be found, it was considered depredated. Similar to the indoor study described above, we observed some crabs eating eggs in the enclosures, which again validated our use of "egg absent" as an operational definition for depredation. Because we observed some crab mortality and escape from the enclosures, we added crabs to the enclosures after each egg check; specifically, 5 crabs were added to the low density enclosures and 12 crabs were added to the high density enclosures to maintain crab densities near our target ratio (~2:5). These numbers were equal to half the number of the crabs that were originally placed in each treatment. At the end of the study, all drift fence enclosures were thoroughly checked for crabs. At this time, an average of 8.25 crabs (range 6–10) remained in the low density enclosures and 23.5 crabs (range 22–26) remained in the high density enclosures.

Statistical analyses

For the indoor experiment, the effects of microhabitat (open, palm frond, and leaf litter), placement position (buried vs not buried), and their interaction on egg depredation were analyzed using an ordinal logistic regression model with a Hessian optimization (package MASS: Venables and Ripley 2002; package car: Fox and Weisberg 2019; R Core Team 2020). The dependent variables consisted of five ordinal levels corresponding to the cumulative presence/absence of eggs at each egg check. The five levels were "depredated before check 1", "depredated between check 1 and check 2", "depredated between check 2 and check 3", "depredated between check 3 and check 4" or "still present at the end of the experiment". The age of the egg when the experiment started was also added as a covariate (see Table S2). We did not include "Cage" as a random effect because we only had one egg per treatment in each cage.

For the field experiment, we used the same analysis described for the indoor experiment, but there were only four ordinal levels ("depredated before check 1", "depredated between check 1 and check 2", "depredated between check 2 and check 3", "still present at the end of the experiment"). Independent variables consisted of crab density, microhabitat and placement location, as well as their two-way interactions (see Table S3). Age of the egg was also used as a covariate. Since there were only two eggs per treatment in each enclosure, we did not add "enclosure" as a random effect.

Results

For the indoor experiment, the probability of egg depredation varied among microhabitats ($\chi^2_2 = 46.32$, $P < 0.00001$); eggs placed under leaf litter were 44 times less likely to be depredated by crabs than eggs placed in the palm frond microhabitat (Fig. 2, Table 1, Table S2) and 132 times less likely than those in open habitat. Eggs placed in the open

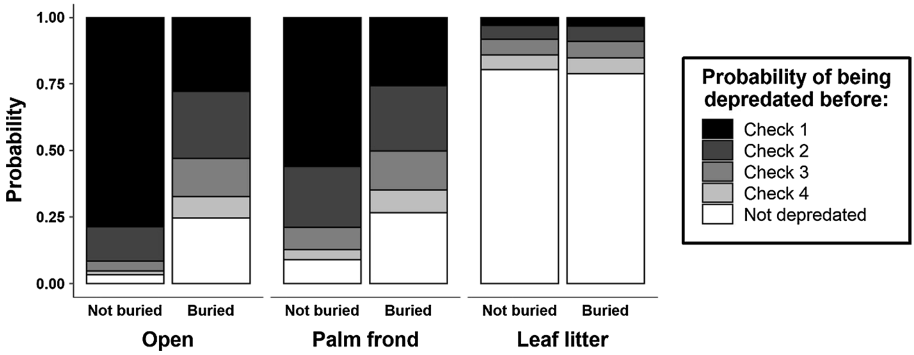

Fig. 2 Probability of lizard egg depredation during the indoor experiment

Table 1 Predicted probabilities of egg depredation by crabs in the indoor experiment.

	Probability of being depredated before				
	Check 1	Check 2	Check 3	Check 4	Not depredated
Open area					
Not buried	0.79 (12)	0.13 (2)	0.04 (0)	0.01 (1)	0.03 (0)
Buried	0.28 (5)	0.25 (4)	0.14 (0)	0.08 (0)	0.25 (6)
Palm frond					
Not buried	0.56 (9)	0.23 (2)	0.08 (2)	0.04 (1)	0.09 (1)
Buried	0.26 (2)	0.24 (5)	0.15 (4)	0.08 (1)	0.27 (3)
Leaf litter					
Not buried	0.03 (1)	0.05 (0)	0.06 (1)	0.06 (1)	0.80 (12)
Buried	0.03 (0)	0.06 (1)	0.06 (1)	0.06 (1)	0.79 (12)

For the four columns for each egg check, the values are the probabilities of depredation with the number of eggs depredated in parentheses. For the column labelled "Not depredated", values are the probability of not being depredated during the experiment with the number of eggs that were not depredated in parentheses

were 3 times more likely (based on odds ratio of 0.33, Table S2) to be depredated by crabs than those in the palm frond microhabitat. The probability of egg depredation also varied with egg placement ($\chi^2_1 = 10.34$, $P = 0.0013$); buried eggs were 4 times less likely to be depredated than those that were not buried. This trend did not differ across microhabitats ($\chi^2_2 = 3.73$, $P = 0.16$), even though egg placement (buried vs not buried) tended to impact the probability of depredation in the open habitat (odds ratio of 2.61) more than in the palm frond habitat; egg placement (buried vs not buried) did not affect the probability of depredation in the leaf litter (odds ratio of 0.25, Table S2). Eggs that were not buried tended to be depredated within the first day of the experiment (i.e., before check 1) whereas buried eggs tended to be depredated within the first 2 days (Table 1, Fig. 2). Egg age was not related to the probability of being depredated ($\chi^2_1 = 0.183$, $P = 0.67$, Table S2).

For the field experiment, the probability of egg depredation varied significantly among crab density treatments ($\chi^2_2 = 32.5$, $P < 0.00001$; Fig. 3). Eggs placed in the control treatment (no crabs) were 4.63 and 125 times less likely to be depredated than those in the low

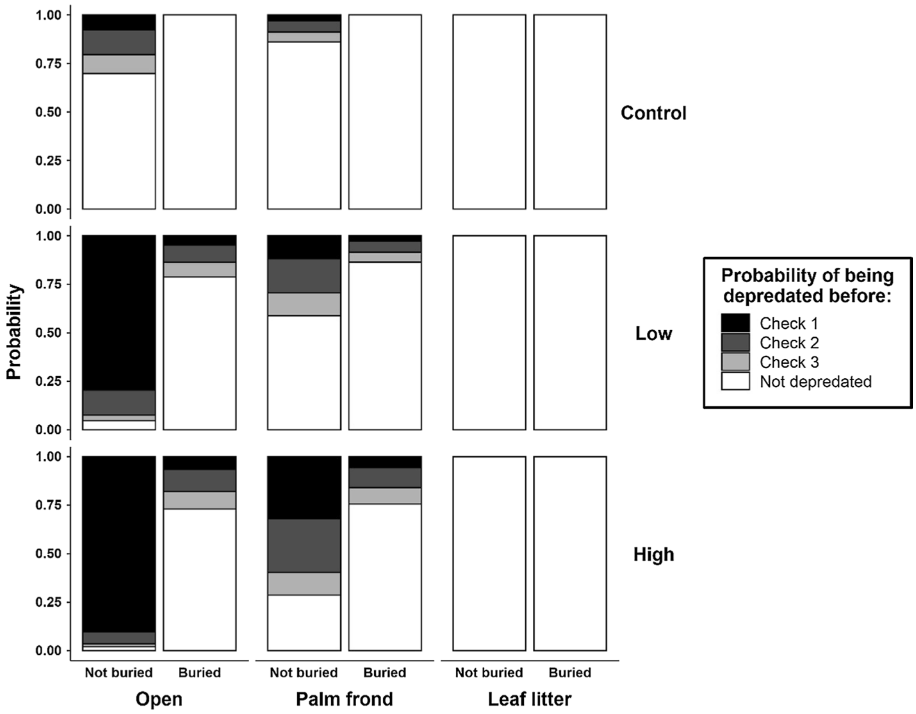

Fig. 3 Probability of lizard egg depredation during the field experiment. Each panel represent a crab density treatment ("Control", "Low" and "High")

and high density treatments, respectively. Eggs placed in the high density treatment were 4 times more likely to be depredated (based on odds ratio of 0.26) than those in the low density treatment; this pattern resulted in increased probability of egg depredation as crab density increased (Fig. 3, Table 2, Table S3).

The probability of egg depredation also varied significantly among microhabitats ($\chi^2_2 = 32.45$, $P < 0.00001$). No eggs in the leaf litter microhabitat were depredated; consequently, those in leaf litter were $6*10^8$ times less likely to be depredated by crabs than those placed under palm fronds and $1.2*10^8$ times less likely than eggs in open habitat. Eggs placed in open habitat were 24 times more likely (based on odds ratio of 0.042) to be depredated by crabs than those under palm fronds. The probability of egg depredation varied significantly with egg placement ($\chi^2_1 = 24.86$, $P < 0.00001$); eggs that were buried were 2.51 times less likely to be depredated than those that were not buried (Table S3).

The probability of egg depredation was also influenced by interactions among independent variables (Figs. 3, 4, Table 3, Table S3) but only the interaction between placement and microhabitat was statistically supported (density treatment x microhabitat: $\chi^2_4 = 1.80$, $P = 0.77$; density treatment x placement: $\chi^2_2 = 0.84$, $P = 0.66$; microhabitat x placement: $\chi^2_2 = 6.20$, $P = 0.045$). For instance, the probability of egg depredation did not differ among crab density treatments or position (buried vs not buried) for eggs in leaf litter habitat (Fig. 3, Table 3, Fig. 4), but these factors influenced egg depredation in the other microhabitats. Egg placement in open areas had a greater influence on the likelihood of being depredated than egg placement in the two other microhabitats (Fig. 4a). Additionally,

Table 2 Predicted probabilities of egg depredation by crabs in the field experiment

	Control (no crabs)				Low density				High density			
	Probability of being depredated before:				Probability of being depredated before:				Probability of being depredated before:			
	Check 1	Check 2	Check 3	Not depredated	Check 1	Check 2	Check 3	Not depredated	Check 1	Check 2	Check 3	Not depredated
Open microhabitat												
Not buried	0.07 (0)	0.13 (2)	0.10 (0)	0.70 (1)	0.79 (6)	0.13 (0)	0.03 (1)	0.05 (1)	0.90 (7)	0.07 (0)	0.01 (1)	0.02 (0)
Buried	0 (0)	0 (0)	0 (0)	1 (8)	0.05 (0)	0.09 (2)	0.07 (0)	0.79 (6)	0.07 (1)	0.11 (1)	0.09 (1)	0.73 (5)
Palm frond microhabitat												
Not buried	0.03 (0)	0.06 (1)	0.05 (0)	0.86 (7)	0.12 (1)	0.17 (1)	0.12 (1)	0.59 (5)	0.32 (3)	0.27 (2)	0.12 (0)	0.29 (3)
Buried	0 (0)	0 (0)	0 (0)	1 (8)	0.03 (0)	0.05 (0)	0.05 (2)	0.87 (6)	0.06 (0)	0.10 (2)	0.08 (1)	0.76 (5)
Leaf litter microhabitat												
No buried	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)
Buried	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (8)

For the columns for each egg check, the values are the probabilities of depredation with the number of eggs depredated in parentheses. For the columns labelled “Not depredated”, values are the probability of not being depredated during the experiment with the number of eggs that were not depredated in parentheses

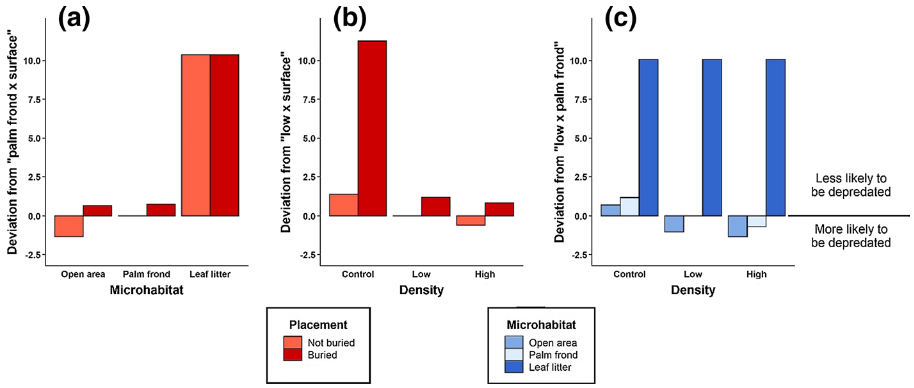

Fig. 4 Representation of the interactions between variables used in the field experiment. Values on the y-axis refer to the degree of deviation from a reference parameter, such that values > zero refer to a reduced probability of egg predation, and those < zero have an increased probability of egg predation. **a** Effects of egg placement within each microhabitat. The reference parameter is unburied eggs in palm frond microhabitat. **b** Effects of egg placement within each density treatment. The reference parameter is unburied eggs in the low density crab treatment. **c** Effects of microhabitat within each density treatment. The reference parameter is eggs in palm frond microhabitat in the low density crab treatment

unburied eggs were more likely to be depredated in the high density treatment than in the low or control density treatments (Fig. 4b). Also, unburied eggs in open habitats did not differ in probability of depredation between the low and high density crab treatments, but within the palm frond microhabitat, eggs in the low crab density treatment were less likely to be depredated than those in the high density treatment (Fig. 4c). Unburied eggs were quickly depredated by crabs in the open habitat, as indicated by high depredation probability at check 1 for the low and high density treatments. Unburied eggs that were placed under palm fronds were found by crabs primarily by the time we performed check 2 (Table 2, Fig. 3); for eggs that were buried, the probability of depredation did not vary appreciably among the three time periods. Egg age was not related to the probability of depredation ($\chi^2_1 = 0.61$, $P = 0.43$).

Discussion

Nest depredation has important consequences on population dynamics and is a strong selective force on reproductive behaviors, such as nest habitat choice by females. Our experiments were designed to provide insight into the factors that influence crab depredation on lizard eggs and their implications for egg survival and maternal nesting behavior. We first confirmed in indoor and field settings that marsh crabs readily depredate eggs when given the opportunity. This is an important finding given that depredation of vertebrate eggs by invertebrate predators is rarely documented in terrestrial ecosystems (Andrews 1988; Chalcraft and Andrews 1999; Warkentin 2000; Thawley and Langkilde 2016) and may be an underappreciated source of variation in egg survival in many oviparous vertebrates. Second, we detected a statistically significant difference in egg depredation across microhabitats within our indoor and field experiments. Additionally, eggs that were buried experienced less depredation than those that were not buried in both the indoor and field settings. Third, we demonstrated that increased crab density raised the probability of egg depredation in the field.

Nesting and egg ecology are poorly studied in *Anolis* lizards. Nevertheless, sources of egg mortality have been attributed to extreme thermal or hydric conditions (Pruett et al. 2020; Tiatragul et al. 2020), as well as fire ants (*Solenopsis*) (Andrews 1988; Chalcraft and Andrews 1999). We now report that marsh crabs are another predator of anole eggs, but the importance of this egg predator is likely limited to anole populations that inhabit salt marsh habitat. Given the abundance of marsh crabs in the southeastern United States (Teal 1958; Seiple 1979; Buck et al. 2003) and across our study islands, depredation rates of anole eggs by these predators may also be relatively high. Indeed, the marsh crab is considered a critical component of terrestrial food webs in coastal habitat (Ho and Pennings 2008), and although these scavengers forage primarily on partially-decomposing plant matter, they also readily consume animal prey when available (Buck et al. 2003; Kiskaddon et al. 2019). Consequently, marsh crabs may be a major source of lizard egg mortality where they coexist and also impose significant pressure on nest site choices of female anoles. Indeed, many of the eggs that were depredated in our experiments were detected by crabs very quickly (i.e., before our first check), particularly those exposed in open habitat.

The likelihood of egg depredation varied among microhabitats. We showed that eggs under leaf litter were less likely to be depredated than eggs in the other microhabitats (Figs. 2, 3); this trend was statistically supported in both experiments, and no eggs placed under leaf litter were depredated by crabs in the field experiment (Table 2). In the indoor experiment, egg depredation in the open area and palm frond microhabitats were relatively similar, but in the field experiment, egg depredation was lower in the palm fronds. We never observed marsh crabs under or between the leaf litter at any time during the experiments, while they spent most of their time exploring the open area and hiding under the palm fronds. This observation is consistent with both previously reported trends of marsh crab refuge use (Seiple and Mueller 1992) and our personal observations of free-ranging marsh crabs frequently walking through open areas and sheltering under palm fronds. This suggests that the crabs may not be actively searching for eggs, but instead depredating eggs opportunistically as they happen upon them, which is common foraging behavior of many arthropod generalists (Snyder and Evans 2006). Additionally, depredation probability before check 1 was very high in the open microhabitat (Figs. 2, 3) when eggs were not buried in the substrate. Being placed under palm frond decreased this probability yet most of depredation was likely to occur before check 2. This further supports the opportunistic foraging behavior of marsh crabs. Had the field experiment extended beyond 7 days, crabs may have eventually detected eggs in leaf litter. Indeed, given the ~ 40-day incubation period of *A. sagrei* eggs under field temperatures (Pearson and Warner 2016), there is ample time for foraging crabs to discover eggs in this microhabitat. Nevertheless, leaf litter appears to be the best microhabitat for anole eggs, as it reduces predator detection time and also retains moisture relatively well compared to some other microhabitats (Tiatragul et al. 2019), which is critical for successful egg incubation (Packard and Packard 1980).

The position of eggs (buried vs not buried) had an effect on egg depredation in both experiments. Eggs buried under substrate appeared to be more protected from crabs (except for eggs placed in the leaf litter where position had no effect) than those on top of the substrate – this was particularly evident in the open microhabitat, which is not surprising given that these eggs were not concealed and visible to predators, unlike the buried eggs and those in other microhabitats. This was also confirmed by our finding in the indoor experiment that unburied eggs had a higher probability of depredation near the beginning of the experiment (before check 1) than buried eggs (Table 1, Fig. 2). Importantly, based on our observations, anoles never lay eggs on the open surface, and thus, this treatment (open/not buried) is not necessarily a biologically-relevant nest site in open habitat. Nevertheless, because nearly all eggs in this treatment disappeared in enclosures with crabs, yet most eggs in the open remained present in the treatment with no crabs, there is strong evidence that egg disappearance was due to depredation by the crabs, rather than other predators that could have entered our field enclosures; e.g., fire ants (Thawley and Langkilde 2016) or small nocturnal rodents.

Egg depredation varied among crab density treatments but was driven mostly by differences between the control treatment (with no crabs) and the two experimental density treatments. Yet, estimates suggest that, as predicted, egg depredation increased as crab densities increased, indicating that brown anole eggs are at increased risk of depredation during times when marsh crabs are abundant. However, it is unclear how the enclosures may have altered marsh crab foraging behavior. Marsh crabs eat leaf litter yet prefer animal matter when it is present (Buck et al. 2003), suggesting that anole eggs could be preferred. Additionally, capture-mark-recapture studies have shown that square-back marsh crabs are mostly found within 3 m of their established refuge (Seiple and Mueller 1992). Therefore, our enclosures (1.2 m radii) are only slightly limiting in terms of their size and we provided ample food (leaf litter and anole eggs), water, and a refuge (palm fronds). Considering this, crab foraging conditions in our experiment appear suitable and relatively similar to natural conditions. Further research is needed for understanding spatial and temporal dynamics of egg predator populations and their influence on anole populations, and our study provides the groundwork for exploring these broader topics. Indeed, given the long reproductive season of *A. sagrei* (Hall et al. 2020), it is likely that crab densities reach their peak during times when eggs are present, but this aspect of crab phenology has not been explicitly studied. The establishment of this new study model is particularly important considering that mortality at the egg stage explains variation in population density better than mortality at the other life stages for lizards (Andrews 1988).

The patterns of egg depredation that we document suggest that aspects of predator populations (density), environmental conditions of the landscape (microhabitat), and maternal behavior (choice of egg location) are all factors that contribute to variation in nest success. Egg depredation increased with crab density, but the leaf litter microhabitat appeared to protect eggs from predators even under relatively high density. The impacts of egg placement on survival appeared to be dependent on microhabitat. Although more work is needed to understand the interactions between crab density and nest site choice of anoles, we provide evidence that lizards that choose leaf litter for nesting protect their eggs against depredation in the presence of marsh crabs. This result is consistent with lab studies showing that some anoles prefer to nest under leaves rather than bury their eggs in open soil (Socci et al. 2005). Changes in habitat use by lizards in the presence of predators is previously documented (Losos et al. 2004; Calsbeek and Cox 2010), but more research is needed to examine how maternal preference for nest microhabitat shifts in the presence of predators. Previous explanations for nest site choices include improved embryo survival and increased offspring fitness (Li et al. 2018), but our work also suggests that depredation pressure could be a major driver of nest site choice. Potential explanations for oviposition site choice vary across species (Refsnider and Janzen 2010), and study systems that are amendable to experimental manipulation will advance our understanding of this variation. Thus, the predator–prey relationship described in this study provides a viable system for answering fundamental questions about egg-stage depredation in vertebrate animals and its consequences on maternal nesting behaviors.

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Author contributions DW originally formulated the idea for this study, and all authors designed and performed the experiment. ADS and AF analyzed the data. The first draft of the manuscript was written by ADS and all authors contributed to subsequent versions of the manuscript. All authors read and approved the final manuscript.

Data availability The dataset generated during this study is available on the supplementary information files.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This research followed the protocols approved by the Auburn University Animal Care and Use Committee (Protocol #: 2017–3027), and the Guana Tolomato Matanzas National Estuarine Research Reserve.

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