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ORIGINAL ARTICLE

Fluctuating environments hinder the ability of female lizards to choose suitable nest sites for their embryos

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Abstract

Nesting behavior is an important part of reproduction that affects maternal fitness. Females of most oviparous species choose microhabitats for nesting that have positive effects on embryo development. However, choosing suitable nest microhabitats could be challenging in environments that fluctuate unpredictably. In many reptiles, females avoid nesting in dry microhabitats because eggs rapidly desiccate. In nature, however, microhabitats with suitable hydric conditions at the time of oviposition may eventually become lethally dry during incubation. We designed an experiment to test whether female lizards (*Anolis sagrei*) avoid nesting in locations with unpredictable fluctuations in substrate moisture and choose sites with stable moist conditions. We provided captive lizards three nest conditions to choose among: 1) substrate that predictably alternated between suitable and lethal moisture conditions, 2) substrate that fluctuated unpredictably between suitable and lethal conditions, and 3) substrate with moisture levels that remained constant. For the constant choice, some females could choose moist substrate (which is suitable for embryos), and others could choose dry substrate (which rapidly desiccates eggs). Females always nested in substrates that were moist at the time of oviposition, regardless of the level of predictability. Additionally, while constantly dry substrate was avoided, females did not distinguish between predictable and unpredictable options, both of which resulted in 100% egg mortality. These results suggest that nest site choice is based on immediate environmental cues, rather than the level of predictability of future conditions of nest sites, which in turn can have negative consequences when environments fluctuate between suitable and unsuitable conditions.

Significance statement

Fluctuating environments pose challenges to females when seeking suitable locations for laying eggs. Female brown anole lizards choose moist nest conditions that facilitate embryo development. However, because moisture levels of nest substrate can unpredictably change, females that avoid nesting in microhabitats with unpredictable moisture fluctuations may have increased fitness. We tested the ability of females to select sites based on moisture predictability by providing them with options where substrate moisture remained stable, fluctuated predictably, and fluctuated unpredictably. Females did not discriminate among stable and fluctuating substrates. Rather, they always nested in moist substrates that were present at the time of oviposition, even if their nest site eventually fluctuated towards lethally dry conditions. Our results show that extreme fluctuations lead to poor choices of nest sites, which is problematic under global change where fluctuations in ambient conditions are expected to increase.

Keywords *Anolis sagrei* · Brown anole · Egg survival · Incubation moisture · Nest-site choice

Introduction

Nest-site choice has an important influence on maternal and offspring fitness in all oviparous organisms (Refsnider and Janzen 2010; Deeming and Reynolds 2015). Because offspring survival depends greatly on the nest environment, natural selection should act on the ability of parents to accurately assess and recognize microhabitats that facilitate

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Extended author information available on the last page of the article embryo development (Mousseau and Fox 1998; Rauter et al. 2002; Brown and Shine 2004; Bakker and Mundwiler 2021). Indeed, studies of many oviparous animals demonstrate that mothers non-randomly select microhabitats for nesting, and actively choose locations or conditions that have positive effects on offspring phenotypes and survival (reviewed in Refsnider and Janzen 2010). Despite this general pattern, however, poor choices of nest habitats are still commonly observed, as nests often succumb to predation, desiccation, or other lethal environmental conditions (Rauter et al. 2002; Warner and Shine 2009; Lyons et al. 2022).

These observations suggest that nest-site choice is challenging for females, and this challenge may be magnified when nest conditions change unpredictably over the developmental period. For example, densities of predator populations fluctuate over time and across space and can influence the location that prey species select for nesting, as well as the success of their nests (Møller 1989; Thomson et al. 2006; Tolvanen et al. 2018; Delaney and Janzen 2020). Temporal changes in temperature and rainfall may also lead to nest mortality if microhabitat conditions chosen by females change before the developing offspring emerge from the nest. Female snapping turtles (*Chelydra serpentina*), for example, nest in open areas (e.g., agricultural fields) in the spring when vegetation is sparse, but crop plants (e.g., sunflowers) shade nests as they grow and change the nest temperature in ways that negatively affect survival and phenotypes of offspring (Freedberg et al. 2011). In this case, important environmental cues to nesting females may not be present at the time of oviposition, and females may have incomplete information about the entire developmental environment; this is critical in species that reproduce during a short seasonal window as they will have limited, if any, opportunity to shift their future nest choice under the changed environment. Alternatively, species that reproduce frequently and experience temporal changes in potential nest environments have the opportunity to gather information and adjust behaviors appropriately to that temporal environmental variation (Pruett et al. 2020). Moreover, the capacity of females to adjust nest-site choice to environmental variation may depend on the level of predictability across potential nest microhabitats (Doligez et al. 2003; Hunter et al. 2016).

Nesting behaviors have been studied extensively in reptiles, and most research demonstrates that mothers choose specific microhabitats that are conducive to embryonic development (Refsnider and Janzen 2010; Tiatragul et al. 2020). Many species nest in areas with a specific amount of canopy that differs from what is available at random (e.g., Janzen and Morjan 2001; Doody et al. 2006; Warner and Shine 2008; Sullivan et al. 2022) and that influences nest temperature in ways that have a positive effect on embryo development (Pruett et al. 2019; Tiatragul et al. 2020). Nesting females also choose microhabitats with sufficient substrate moisture (Warner and Andrews 2002; Brown and Shine 2004; Reedy et al. 2013; Doody et al. 2020), which has consequences on embryo metabolism, survival, and a range of offspring phenotypes (Packard et al. 1987; Bodensteiner et al 2014; Warner et al. 2018). Moreover, the parchment shell of many reptile eggs renders embryos highly susceptible to rapid desiccation under dry conditions (Ackerman et al. 1985). Importantly, hydration of nests can fluctuate dramatically in the field (Brown and Shine 2005; Robbins and Warner 2010), especially for species that dig shallow nests in the ground.

We studied the brown anole (*Anolis sagrei*) to determine if nesting lizards assess fluctuations in substrate moisture and subsequently choose sites based on the predictability of substrate moisture levels. The brown anole lays a single egg about every 7–10 days during a long (April to October) and seasonally-changing reproductive period (Pearson and Warner 2018; Hall et al. 2020). This aspect of their reproductive biology enables females to choose nest sites multiple times during the reproductive season and gives them the opportunity to adjust nesting behaviors to seasonal changes in ambient conditions (Pruett et al. 2020). Importantly, as with many reptiles, brown anoles exhibit no parental care after oviposition (but see Delaney et al. 2021). Laboratory and field studies show that female brown anoles consistently choose relatively moist microhabitats for nesting, which has positive effects on egg hatching success (Reedy et al. 2013; Pruett et al. 2020). However, despite this consistent preference for moist nesting environments, the ambient conditions of a given nest microhabitat can fluctuate considerably in ways that could hinder embryo development (Pruett et al. 2020). Thus, selecting a nest site with appropriate moisture content across the entire duration of egg incubation can be challenging if fluctuations are extreme or unpredictable. Moreover, given the small egg size (~0.1 g at oviposition) of *A. sagrei*, brief periods of low moisture rapidly desiccate eggs and kill the embryos (Hall et al. 2022).

Our experiment tested the hypothesis that females choose nest sites with suitable moisture conditions for their developing offspring by avoiding sites that fluctuate unpredictably and reach lethal extremes. By providing females with three options that vary in the pattern of moisture fluctuation (stable, predictably fluctuating, unpredictably fluctuating), we predict that eggs will most frequently be placed in stable moist conditions, and the fewest eggs will be placed in conditions with unpredictable moisture fluctuations. We also quantified the consequences of this nesting behavior by incubating eggs under the different moisture conditions provided to females and measuring egg survival. We expected egg survival to decline under increasingly dry and unpredictable conditions. Overall, results from this study will provide new insights into nest-site choice, and the role of this behavioral maternal effect on offspring survival in fluctuating environments.

Methods

Lizard collection and housing

Lizards (32 males, 64 females) used for this study were collected from a residential area in Palm Coast, Florida on 1–2 February 2020. Lizards were transported to Auburn University and housed in cages under our standard conditions (Hall and Warner 2019) on 3 February. Two females and a male were randomly assigned to each cage. Cages (29 cm tall × 26 cm wide × 39 cm deep) were equipped with reptile cage carpet (ZooMed, Inc) substrate, nesting pots, two bamboo sticks for perching, and artificial vegetation used by lizards for perching and hiding. Cages were sprayed with water daily, and lizards were fed crickets (dusted with vitamins and calcium) three times per week. Room temperature ranged from 21.6—23.3 ºC and the light cycle was set for 10/14 h day/night, and photoperiod was changed to 12.5 h of daylight on 14 February 2020.

Nesting experiment

Each cage contained three nesting pots (plant pots; 8.5 cm tall, 10 cm diameter). The nesting pots were filled with a mixture of potting soil and peat moss, and the substrate in each nesting pot varied in its moisture content and pattern of moisture fluctuation (see details below). The nesting pots were checked for eggs every Monday, Wednesday, and Friday during the experiment, at which point the pots were replaced with a nest pot containing fresh substrate at the assigned moisture content. The first egg was laid 45 days after the onset of the experiment, suggesting that females had ample opportunity to become familiar with the patterns of moisture fluctuations within the three nesting pots before they started to lay eggs. Indeed, females likely become familiar with the nesting microhabitats available to them because they frequently perch on and crawl across the nesting pots and actively move around their cage.

This study comprised two similar choice experiments that were performed simultaneously. The first experiment (termed "moist experiment") provided females with three choices of substrate moisture levels in which to nest. This experiment contained 16 replicate cages, each with three nesting pots (Fig. 1). The substrate in the first of the nesting pots remained constantly moist (85% moisture by mass) during the entire experiment. The substrate moisture level in the second nesting pot fluctuated in a predictable pattern over the duration of the study. To create predictable moisture fluctuations, we randomly chose nest pots to begin with either moist (85% moisture) or dry (0% moisture) substrate, and then we regularly alternated between wet and dry conditions by swapping out old nesting pots with new pots (with assigned substrate moisture) into the cages on Mondays, Wednesday, and Fridays. The substrate moisture levels in the third nesting pot fluctuated in an unpredictable pattern over the duration of the study. To do this, we randomly chose the substrate moisture level (0% versus 85% moisture determined by coin flip) each day the nest substrate was changed. Consequently, some nesting pots had long durations of dry conditions followed by moist conditions (or vice versa), or occasionally fluctuated regularly but for brief periods. The position of each of the three pots was randomized within cages, and the pots stayed in that position over the duration of the experiment.

The second experiment (termed "dry experiment") was almost identical to that described above for the moist experiment except that the substrate of the first pot remained constantly dry (0% moisture), rather than constantly moist substrate. The dry experiment also contained 16 replicate cages and the position of each pot in each cage was randomized.

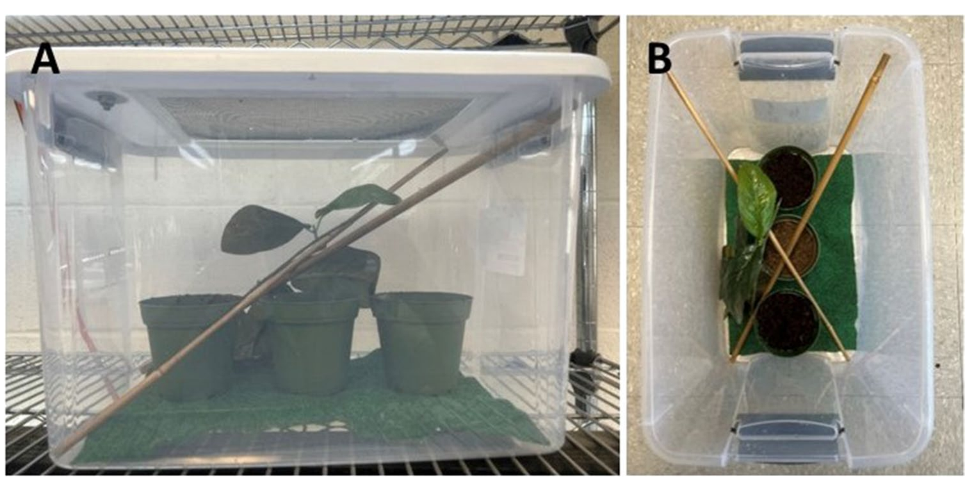

Fig. 1 Arrangement of the interior of a cage from the (**A**) side and (**B**) top views. Each cage contained three pots with different moisture levels for female brown anoles (*Anolis sagrei*) to choose among for nesting

Overall, the moist experiment contained at least one option for females to choose that was constantly suitable for egg incubation (i.e., the constantly moist option). In contrast, females in the dry experiment frequently experienced days when dry (lethal) nest substrate was the only choice available (Table 1). Females in both experiments had an average of 24 days when both moist and dry nest options were simultaneously available (Table 1). Importantly, we chose to conduct two separate experiments (rather than provide all four options simultaneously in one experiment) because we wanted to determine if the level of predictably in substrate moisture fluctuation becomes important if females are forced to avoid a constantly suitable nest microhabitat (i.e., the constantly moist choice). That is, if constantly moist substrate was always an option to females, then they would never need to distinguish between options with predictable vs unpredictable moisture fluctuations because a suitable site would always be available for nesting.

When pots were checked for eggs (on Mondays, Wednesdays, Fridays), we carefully removed each pot, poured the soil into a bin, and sifted for eggs. We recorded the cage number and pot where each egg was found and weighed each egg. All eggs were either discarded or used in an incubation experiment described below; this removed a potential biological cue that might influence female nest site choice (Dees et al. 2020). We were not able to record data blindly with respect to the three possible choices because we needed to record this information immediately after finding eggs in nest pots. Additionally, whether the substrate was moist or dry was obvious when eggs were collected. A subset of the eggs was used in an egg incubation experiment described below. The nesting experiment was terminated on 20 April 2020.

Egg incubation experiment

To determine the consequences of the different nest choices, forty eggs were randomly assigned to four incubation treatments that mimicked the substrate conditions that females had available to them in the nesting experiment. These eggs were collected towards the end of the nesting experiment (6–13 April 2020) until we obtained our target sample size (n = 40) and haphazardly assigned to the incubation treatments. These eggs were produced by females from 21 different cages. All eggs were incubated in glass jars (59 ml) filled with the soil substrate used for the nesting experiments described above. The glass jars were covered with plastic wrap and sealed with a rubberband, and then placed in an incubator set at a constant 28 ºC. The moisture content of the incubation substrate varied among the following four incubation treatments ($n = 10$ eggs per treatment): 1) constantly moist (85% water content), 2) constantly dry (0% water content, 3) predictably fluctuating, and 4) unpredictably fluctuating. In the predictably fluctuating treatment, we alternated substrate moisture each time we moved eggs to a new jar with either moist or dry substrate. Eggs in the unpredictably fluctuating treatment were also moved to new jars with moist or dry substrate, but the moisture content was determined by coin flip. The eggs in the constant moist and dry treatments were also moved to new jars with fresh substrate (to control for potential movement effects), but the substrate moisture levels remained constant. One hundred percent egg survival in our constantly moist treatment (see Results) suggests that egg movement was not detrimental. All eggs were moved to new jars with their assigned substrate every Monday, Wednesday, and Friday during incubation.

Eggs were checked daily for signs of mortality (e.g., dimpled or shriveled appearance due to desiccation), and if an egg appeared desiccated the date was recorded. We continued to monitor desiccated eggs for several days due to the potential for eggs to rehydrate after being moved to a moist substrate. However, desiccated eggs never recovered, and therefore the date of egg death was determined as the day the egg showed initial signs of desiccation.

While the percentage of moisture in the substrate in our nesting and egg experiments were comparable to that in the field measured across multiple nest sites and times during the nesting season (Pruett et al. 2020; Hall et al. 2022), we did not use substrate from our field site. Thus, we cannot make specific comparisons of water availability to eggs between our lab study and previous field studies since water potential is influenced by substrate type. Although we do not know exactly how our manipulations relate to nest conditions in the field, natural nests undoubtedly experience moisture fluctuations that range between suitable (moist substrate option) and unsuitable (dry substrate option) levels for eggs, which we simulated in our laboratory study.

Table 1 Average number of days (range of days) when female brown anoles (*Anolis sagrei*) had different options to choose among wet and dry conditions during the experiment. These averages are based on the number of days from when the first egg was laid (18 March) to the termination of the experiment (20 April); i.e., a 33-day period

Potential choices for nesting	Wet experiment	Dry experiment
Only dry substrate available	0	9.00 (3–16)
Both wet and dry substrates available	24.06 (18–28)	24.00 (17–30)
Only wet substrate available	8.94 (5–15)	0

Statistical analyses

All statistical analyses were performed in R (R Core Team 2022). First, we used a chi-square test to compare the proportion of eggs that were in moist versus dry substrate at the time of oviposition, regardless of whether substrate moisture content was constant or fluctuating. For our second set of analyses, nest choice was recorded as presence or absence of eggs (i.e., binomial dependent variable) in a given nesting pot, and our analyses excluded days from any given cage when no eggs were laid. We used generalized linear mixed models with a binomial distribution (glmer function in the lme4 package; Bates et al. 2015) to quantify choice of nest moisture conditions among the different options available to females. The data in the moist and dry experiments were analyzed separately from each other, however, we excluded the constantly dry option in the dry experiment because this choice was never used by females (see Results). For each analysis, the dependent variable was egg presence/absence, and the independent variable was the nest site option (i.e., the three choices available to the females). Because multiple eggs were obtained from a given cage, the cage number was a random effect in our models. In a final analysis of nest site choice, we included the proportion of time that moist soil was available throughout the duration of the experiment as an additional independent variable in the generalized linear mixed model. This is important because females might choose the stable moisture option most frequently simply because the constantly moist option was available 100% of the time in the moist experiment, whereas moist conditions were available on average 50.7% and 53.2% of the time for the predictable and unpredictable options, respectively.

The effect of incubation treatment on egg survival to hatching was analyzed with a chi-square test. These results were visualized as the number of days it took for all eggs to desiccate in each of our treatments.

Results

Eighty-five eggs were laid between 18 March and 20 April 2020, produced by females in 25 of the 32 cages. Females always nested in pots that contained moist substrate at the time of oviposition, regardless of the pattern of moisture fluctuation (i.e., 100% of the eggs were laid in moist substrate; $\chi^2 = 68.0$, $p < 0.001$). In the moist experiment, females nested most frequently in the constantly moist substrate and least frequent in the unpredictably fluctuating substrate ($Z = -2.71$, $p = 0.007$; Fig. 2A). Females also chose the predictably fluctuating substrate less frequently than the constantly moist substrate, but this difference was marginally non-significant ($Z = -1.79$, $p = 0.073$). However, this trend disappeared when egg presence in each nesting option was adjusted for the proportion of time that moist substrate was available; the presence of eggs in the constantly moist substrate was not different from that in the predictably ($Z = 0.79$, $p = 0.431$) and unpredictably ($Z = 0.53$, $p = 0.599$) fluctuating substrates. For the dry experiment, females never nested in the constantly dry option (Fig. 2B). While all eggs laid in the dry experiment were in the fluctuating options, females showed no preference for the predictable or unpredictable option ($Z = -0.04$, $p = 0.971$), and eggs were only laid on days when moist conditions were available.

Egg hatching success varied among incubation treatments ($\chi^2 = 40.0$, $p < 0.001$; Fig. 3). All the eggs incubated under constantly dry condition were desiccated by day 6, whereas all the eggs in the predictably and unpredictably fluctuating treatments were desiccated by day 8 and 13, respectively. All eggs successfully hatched in the constantly moist treatment.

Discussion

Reproductive females face multiple challenges when locating suitable microhabitat for nesting. Mothers must navigate complex landscapes that vary in densities of nest predators, competition for resources and nesting habitat, and a range of abiotic factors (Rauter et al. 2002; Refsnider et al. 2015; Drake and Martin 2020; O'Brien et al. 2020). Temporally fluctuating environments pose another challenge because the

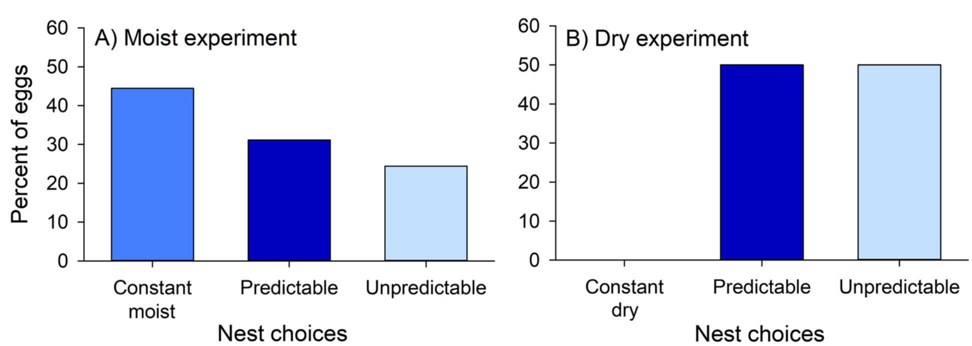

Fig. 2 Percentage of eggs found in nesting pots with constant levels of substrate moisture, predictably fluctuating patterns, and unpredictably fluctuating patterns of substrate moisture. The moist experiment (**A**) contained a choice of a constantly moist substrate; $n = 45$ eggs were laid in this experiment. The dry experiment (**B**) contained a choice of a constantly dry substrate; $n = 40$ eggs were laid in this experiment

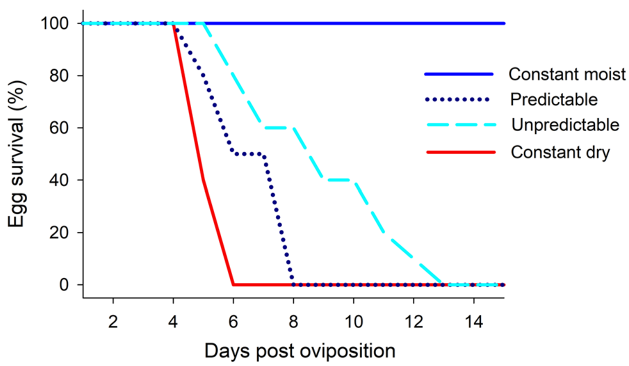

Fig. 3 Duration of egg survival after oviposition under four incubation moisture treatments. Incubation temperature was 28 °C, which results in about a 30-day incubation period; only data up to day 15 are shown here, as egg survival was either 100% (moist constant treatment) or 0% (other treatments) after day 13 of incubation

conditions available at the time of oviposition may not be a reliable indicator of the environment at a later point of embryo development (Freedberg et al. 2011; but see Janzen 1994). Females that are able to assess potential nest environments, and subsequently use past cues upon which to base their nest-site choice, would have increased fitness compared to those that choose sites randomly. In this study, we tested whether female lizards avoid nest sites that fluctuate between suitable (moist) and lethal (dry) conditions for their embryos, and we assess whether the degree of predictability in moisture fluctuations influences their choice of nest site. We showed that females always choose moist conditions at the time of oviposition, and they do not distinguish between stable and fluctuating (predictably and unpredictably) microhabitats. Thus, we found no evidence that information about the substrate moisture history is used by females when making decisions about their nest site. Instead, females rely on cues present at the time of oviposition.

Similar to many oviparous vertebrates, brown anoles choose nest locations with conditions that are conducive to embryo development (Reedy et al. 2013; Pruett et al. 2020). This pattern was also apparent in this study, but only at the time of oviposition; females chose nest sites when the substrate was moist, and they avoided nesting on days when only dry conditions were available. This behavior was pronounced in the dry experiment, where moist conditions were not always available. Indeed, dry conditions were the only option for nesting females in the dry experiment for an average of 9 days per cage (and as much as 14–16 days in some cages; Table 1) across the duration of the study; this represents an average 27.3% (and up to 48.5%) of the total duration of the experiment, and females never nested on those occasions. This lack of nesting on days when moist conditions were not available suggests that females retain eggs until moist conditions eventually become available.

Similar patterns have been observed in other reptile species, where nesting behaviors are often observed during or after rainfall (Brown and Sexton 1973; Stamps 1976; Burke et al. 1994; but see Geller et al. 2022). Given the large surface area to volume ratio of brown anole eggs, waiting for suitable hydric conditions may be advantageous as it would ensure that the small eggs do not rapidly desiccate immediately after oviposition.

Nest-site choice based on immediate and past cues is well documented in animals (e.g., Janzen and Morjan 2001; Elphick et al. 2013; Kivelä et al. 2014). For example, collared flycatchers use cues that provide information about past nest success (Kivelä et al. 2014). Similarly, brown anoles and other lizards choose nest sites that contain evidence of past hatching success (e.g., hatched eggshells in the substrate) (Radder and Shine 2007; Pike et al. 2011; Dees et al. 2020). While these studies provide evidence that females use biotic cues (i.e., those from previous conspecifics) to signal suitable nest microhabitats, these are still cues that females assess at the time of nesting (rather than prior to nesting, which we evaluated in our experiment). Other *Anolis* species have the capacity for rapid associative learning and to solve simple tasks when rewarded with a prey item (Leal and Powell 2012), but whether females have the capacity to learn and respond to past information from abiotic cues (e.g., past patterns of nest moisture) that have no immediate benefit to them is less understood. Our results show that immediate cues are used by females, and we provide no evidence that females use past information about substrate moisture when choosing a nest site. Importantly, substrate moisture can be relatively unpredictable in nature as it varies with a wide range of other factors (e.g., precipitation, evaporation rate, solar radiation). Other environmental cues that fluctuate, such as temperature, may also be unpredictable over time but may correlate with a temporally predictable variable (i.e., shade cover by vegetation) that may be the actual cue for nesting. Thus, while past information about substrate moisture may not be used by females when making choices about nest microhabitat, females may use past information about other environmental factors and adjust nesting behavior accordingly. Studies like ours, but that manipulate other variables, would provide insight into this possibility.

Our experiment contained two extremes of moisture that fluctuated between conditions that yield complete egg survival (moist) to complete mortality (dry) with no options that result in partial egg survival. While the rapid shifts in nest hydration in our experiment may occur in nature, fluctuations are likely more gradual (Robbins and Warner 2010) with intermediate moisture levels that result in egg survival at some intermediate level. If females had an intermediate option (e.g., 30–50% moisture in the nesting substrate), overall egg survival would likely have been greater than what we show here for our fluctuating treatments (Reedy et al.

2013). Nevertheless, 3–4 days under dry conditions would kill most eggs (Fig. 3), and such conditions would have likely appeared at some point during the ~ 30-day incubation period. Thus, our impression is that consequences of nest site choice on egg survival would not have changed even if intermediate moisture levels were occasionally available. Moreover, since females showed no evidence of using cues from substrate moisture fluctuations that regularly reached 100% lethal conditions, then it is unlikely they would have the capacity to avoid nest sites that eventually become lethally dry even if intermediate substrate moisture was occasionally available. Our results, however, may have changed if substrate options fluctuated within a moisture range that is sub-lethal or that results in partial egg mortality (e.g., conditions that never drops to 0% moisture). These possible outcomes remain to be assessed in future experiments.

Our study has implications for understanding variation in nesting behavior among species with different temporal patterns of egg production (e.g., species with single vs multiple egg clutches) or those that inhabit environments that differ in availability of nesting sites. The frequent production of single-egg clutches across a long reproductive season may provide anoles an opportunity to spread their eggs throughout their habitat as a bet-hedging strategy (Rand 1967). Such a strategy may be beneficial for species that reproduce frequently, but may be detrimental for species that reproduce once or twice per season. Thus, consequences of nest-site choice on maternal fitness may be reduced in *Anolis* lizards compared to species that produce one or two clutches in a season, and anoles may therefore be relatively less “choosy” of nest microhabitats based on the degree of environmental predictability. For species or populations that have a limited range of nest sites, early information about potential nest sites could be particularly important and females may use such cues for nesting. Comparisons of nesting behavior among taxa with different patterns of reproduction or nest site availability will provide insight into the factors that drive the evolution of maternal nesting behaviors.

Environments are often unstable, which imposes many challenges that force individuals to adjust their behaviors. When faced with uncertainty, organisms must be able to assess their surroundings and make decisions based on the information they gather. Indeed, the capacity for nesting females to assess past and present information about potential nest sites can directly impact their reproductive success (Brown and Shine 2004; Löwenborg et al. 2010; Zhao et al. 2016; Li et al. 2018). While nesting behavior of reptiles has received much research attention, many questions about the choices females make and the cues that they use remain unanswered, particularly in *Anolis* lizards (Pruett et al. 2022). Here, we showed that female brown anoles chose nest sites with moisture conditions that are suitable for embryo development at the time of oviposition, but they do not use past information about moisture fluctuations. Consequently, these lizards choose unsuitable nest sites (with moisture fluctuations) just as frequently as suitable sites (with stable moist conditions), which suggests that extreme fluctuations could lead to poor choices of nest sites by females. Overall, these findings highlight the importance of understanding the consequences of fitness-related behaviors in temporally fluctuating environments, particularly in the context of global change where extreme fluctuations in temperature and moisture are predicted to increase.

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Author contribution Conceptualization (DAW, JEP, AF, RLK); data collection (JEP, AF, RLK); data analysis (DAW); writing and editing (DAW, CK, JEP, AF, RLK).

Data availability Data is publically available at the Auburn University data repository (AUrora): http://dx.doi.org/10.35099/aurora-518

Declarations

Ethics approval This project was approved by the Auburn University Institutional Animal Care and Use Committee (protocol #2019–3639). All applicable international, national, and/or institutional guidelines for the use of animals were followed.

Competing interests The authors declare no competing interests.

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