

## RESEARCH ARTICLE

# Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness

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**Funding information**

National Key Research and Development Program of China, Grant/Award Number: 2016YFC0503200; National Natural Science Foundation of China, Grant/Award Number: 31525006

Handling Editor: Robbie Wilson

**Abstract**

1. The fitness consequence of maternal nest-site choice has attracted increasing scientific attention, but field studies identifying the long-term effects of nest-site choice on offspring survival and reproductive success are still rare in vertebrates.
2. To investigate the consequences of nest-site choice in lizards, we quantified the thermal and hydric conditions of nest sites that were chosen by female toad-headed agama (*Phrynocephalus przewalskii*) in the desert steppe of northern China. We also determined the effect of nest-site choice on embryonic development and survival and on offspring growth, survival and maturity by comparing the embryos and offspring from maternally and randomly chosen nest sites.
3. We found that female toad-headed agama chose warm and moist nest sites that improved the developmental rate and survivorship of embryos and promoted the post-hatching growth, sexual maturity, reproduction and fitness of offspring, thereby improving their reproductive success.
4. Such studies on short-lived lizards across multiple stages of embryonic and post-embryonic ontogeny are critical for fully understanding the fitness consequences of nest-site choice.

**KEYWORDS**

embryonic development, hatchling, maternal effect, nest-site choice, oviposition, reptile, temperature

## 1 | INTRODUCTION

The importance of maternal effects in the processes of ecological adaptation and evolutionary change has attracted increasing attention since maternal effects can influence offspring fitness, the evolution of life-history traits and population dynamics (Mousseau & Fox, 1998; Mousseau, Uller, Wapstra, & Badyaev, 2009; Murphy, Goedert, & Morris, 2014; Plaistow & Benton, 2009). In heterogeneous environments, maternal effects can increase offspring fitness by mediating changes in offspring phenotypes (Bernardo, 1996b; Galloway, 2005; Ghalambor, McKay, Carroll, & Reznick, 2007), and the selection of nest sites in oviparous species is a mechanism by which females can regulate the developmental environment of embryos and, therefore, influence

the development and phenotypes of their offspring (Bernardo, 1996a; Mitchell, Maciel, & Janzen, 2015; Refsnider & Janzen, 2010; Spencer, Blaustein, & Cohen, 2002).

Accordingly, nest-site choice is an attractive trait to examine in light of maternal effects. Of the various hypotheses that have been proposed to explain the ecological and evolutionary benefits of this behaviour (Refsnider & Janzen, 2010), the most plausible hypothesis is that nest-site choice maximizes embryo survival and offspring fitness. Female insects, for example, usually lay their eggs at sites with high food availability since their larvae are typically oligophagous and exhibit poor mobility (Wasserberg, White, Bullard, King, & Maxwell, 2013; Wiklund & Friberg, 2008), and fish and frogs typically spawn at sites with high oxygen concentrations and long-term water availability,

both of which are essential for successful embryo development (Rudolf & Rodel, 2005; Smith, Rippon, Douglas, & Jurajda, 2001).

Nonetheless, gaps in our understanding of this field remain. For example, in addition to the importance of nest-site choice in determining embryo survival, animals might maximize reproductive success and offspring fitness by selecting sites based on cues that correspond to major threats (e.g. Spencer, 2002). Accordingly, additional studies on a broad range of taxa are needed to identify general patterns regarding consequences of nest-site choice on fitness. Furthermore, rigorous experimental tests (e.g. field-based nest manipulation), rather than just estimates of the mechanisms by which females select oviposition sites, are needed to elucidate the adaptive significance of nest-site choice (Refsnider & Janzen, 2010). Such field studies can measure ecologically relevant phenotype–fitness relationships that provide meaningful estimates of natural selection in the wild (Warner, 2014). Moreover, the long-term effects of manipulation need to be studied because the overall fitness of offspring encompasses long-term survival and reproductive success (i.e. hatching success, post-hatching survival, and reproductive output at maturity). Therefore, the effects of nest-site choice on fitness need to be evaluated across multiple stages of embryonic and postembryonic ontogeny (Streby, Refsnider, Peterson, & Andersen, 2014; Van de Pol, Bruinzeel, Heg, Van der Jeugd, & Verhulst, 2006).

Although many reptiles lay eggs in underground nests and, in most cases, provide no parental care (Angilletta, Sears, & Pringle, 2009; Doody, James, Colyvas, McHenry, & Clulow, 2015; Iverson, Hines, & Valiulis, 2004; Pike, Webb, & Shine, 2010), the embryos are highly sensitive to environmental conditions (e.g. temperature, moisture and oxygen levels) (Deeming, 2004; Du & Shine, 2015). Therefore, oviparous reptiles make excellent models for studying the adaptive significance of nest-site choice, without the confounding effects of post-oviposition parental care. Some investigators have manipulated turtle nests in the field in order to observe the effect of nest-site choice on embryonic development and offspring traits (Mitchell, Maciel, & Janzen, 2013; Mitchell, Warner, & Janzen, 2013; Mitchell et al., 2015; Wilson, 1998). However, detecting the long-term effects of manipulation on offspring fitness is ideally needed to elucidate the adaptive significance of nest-site choice, and this is difficult, if not impossible, in turtles, owing to their extreme longevity. Instead, manipulating the nests of short-lived species, like many lizards, and determining the fitness consequences of nest-site choice across multiple life stages (e.g. embryonic, juvenile and even adult stages) is more ideal (Refsnider & Janzen, 2010). Unfortunately, such studies are rare, mainly owing to the difficulty of locating lizard nests in the field (Angilletta et al., 2009; Doody et al., 2006).

The aim of the present study was to investigate the adaptive significance of nest-site choice in a short-lived lizard, the toad-headed agama (*Phrynocephalus przewalskii*), in the desert steppe of northern China. To achieve this goal, we first quantified the thermal and hydric conditions of maternally chosen nest sites and then assessed the effects of nest-site choice on embryonic development and survival, and on the growth, survival and maturity of offspring by comparing embryos and offspring from real nests at maternally chosen sites and to those from artificial nests at random sites. Using these methods, we

identified the fitness consequences of nest-site choice across multiple life stages to test the hypothesis of maximizing embryonic survivorship and offspring fitness, expecting that (1) the hatching success of eggs from maternally chosen nest sites would be higher than that of eggs from random sites and (2) the fitness (in terms of growth, survival and maturity) of offspring from maternally chosen nest sites would be higher than that of offspring from random sites.

## 2 | MATERIALS AND METHODS

### 2.1 | Study species and site

The desert toad-headed agama, *P. przewalskii*, is a small agamid lizard (to 55 mm snout–vent length) that is widely distributed in northern China (Zhao, Zhao, & Zhou, 1999). From late May to late July, females lay eggs in a small chamber at the end of a burrow in the sand, with a mean clutch size of 3.3 (Qu, Li, Gao, & Ji, 2011; Zeng, Zhao, & Sun, 2013). High incubation temperature (34°C) significantly shortens incubation duration, and decreases hatching success and offspring locomotor performance, but does not affect offspring sex (Tang et al., 2012). Our study site was located at the Shierliancheng Field Station, Institute of Grassland Research of the Chinese Academy of Agricultural Sciences (40°12'N, 111°07'E), which is on the eastern edge of the Hobq Desert. The natural habitat at our study site consists of sandy patches and shrub clusters that are dominated by *Artemisia ordosica*.

### 2.2 | Nest-site choice

From May to July 2015, we captured gravid lizards by hand and measured their body size [snout–vent length (SVL;  $\pm 0.01$  mm) and body mass (BM;  $\pm 0.001$  g)]. Numbers were then painted on their backs using marker pens (Deli permanent marker 6824; Deli, China), and the lizards were released within 1 hr at the location where they were first seen. Afterward, we searched for the marked females every 2 hr from 0900 to 1700 hours on sunny days. Through searching and observation, we located 16 lizard nest sites with known females. We also located another 21 lizard nests by searching fresh and wet soil, which was used by females to cover the entrance of their nest burrows after oviposition. These nests had been constructed by unmarked females on the day that they were discovered, since the wet soil otherwise would be dry in 1 day.

Once each nest was located, we quantified the basal vegetation cover (%) in a  $1 \times 1$  m<sup>2</sup> quadrat (Figure S1a), which provided information on the relative patchiness of sun and shade available to a lizard (Zeng et al., 2014). We also determined the thermal features of the ground surface ( $1 \times 1$  m<sup>2</sup>) of 19 nests using infrared images taken by a thermal-imaging camera (thermaCAM T640; FLIR Systems, Inc., USA; Figure S1b). We analysed the images using special-purpose software (FLIR Tools 4.0) to record the mean temperatures over the entire area covered by the images. Then, we carefully excavated each nest and measured its depth ( $\pm 0.1$  cm). We placed an iButton temperature logger (DS1921; Maxim Integrated Products, Ltd., USA) at the bottom of

each nest to record temperatures hourly, and measured nest moisture every 2 weeks throughout incubation by taking four underground soil samples (at the depth of nests) just beside each nest (within 10 cm). We weighed the wet mass of the soil samples immediately and then determined their dry weight after oven-drying the soil at 105°C for 24 hr. We calculated the water content of the soil as: water content = (wet mass – dry mass)/wet mass × 100%. For comparison, we also chose a random site within 10 m of each nest to quantify the vegetation cover (Figure S1c), thermal features of the ground surface (Figure S1d) and the temperature and moisture of underground soil, using the same methodology described above. We identified the direction of each random site from the corresponding nest by the direction of a marker pen after tossing it straight up into the air, and the distance (in m) of the random site to the nest by haphazardly picking one of 10 slips of paper that were labelled 1–10 (Warner & Shine, 2008).

## 2.3 | Effects of nest-site choice on embryonic survivorship and offspring traits

### 2.3.1 | Construction of nests at maternally chosen and random sites

We excavated the located nests to collect (93 eggs from 37 nests) and weigh ( $\pm 0.001$  g) the eggs. We returned roughly half the eggs from each clutch to the original chamber, and restored the burrow using a plastic corrugated tube (inner diameter 2.5 cm, mean length 25 cm). Meanwhile, we incubated the other half of the eggs from each clutch in an artificial nest that mimicked the natural nest in depth and possessed a chamber that contained the eggs; we constructed the inclined burrow and chamber in the same way that we restored the natural nests. For both types of nests, we plugged the entrance of the burrow with a piece of cotton mixed with sand.

### 2.3.2 | Egg incubation and offspring traits

We collected the eggs and transferred them to the laboratory after they incubated in the field for an average of 38 days (34–43 days), with the incubation period decreasing as nest temperatures increased from May to July. In the laboratory, we individually incubated the eggs in plastic cups (200 ml) that contained a 5-cm-thick layer of sand collected from the field with a water content of 4%. We added water to the sand every 3 days in order to maintain a relatively constant water content. We also sealed the cups with plastic wrap to minimize evaporation of water from the soil and kept them at 26°C in an incubator (PT2499; Exo-Terra, Canada).

We checked the cups for newly emerging hatchlings every day and, after hatching, measured [SVL ( $\pm 0.01$  mm) and BM ( $\pm 0.001$  g)] and individually toe-clipped the offspring for identification. A total of 65 hatchlings successfully hatched from 37 nests, which we released into two circular enclosures (16 m diameter, 0.5 m high, protected by bird nets) at the study site, with 34 and 31 lizards in the two enclosures. The maximum density of the lizards in the enclosures (0.17 and 0.15 lizards/m<sup>2</sup>) was similar to that in the natural habitat at our study site (0.12 lizards/m<sup>2</sup>)

(Zeng et al., 2016), although the actual density in each enclosure was lower because we released the lizards gradually, and some of them died during the experiments. In addition to naturally available food sources, we provided mealworms (about 40% of total lizard mass) on the ground of enclosures weekly until late September. We recaptured the hatchlings before and after winter (late September in 2015 and spring [mid-May] and summer [late June] in 2016), re-measured their body size (SVL and BM) and then returned them to the enclosures. We recorded survival of the hatchlings, and calculated their growth rate before (from hatch date to late September in 2015) and after winter (from mid-May to late June in 2016) as the daily change in body size (SVL and BM). We palpated the abdomens of female hatchlings to assess their reproductive status. We transferred gravid yearlings to individual small glass terraria (200 × 150 × 200 mm) that contained a 20-mm-thick layer of moist sand. We checked each terrarium at least three times a day for freshly laid eggs, weighing each egg to 0.001 g and recording clutch sizes.

## 2.4 | Statistical analyses

We calculated mean daily mean, mean daily minimum, mean daily maximum, mean daily range and variance of the nest temperature data recorded by the iButtons. We arcsine square root-transformed vegetation cover and nest moisture data prior to statistical analyses. We used paired *t*-tests to compare the microhabitat variables of the nest sites and random sites.

We analysed the effect of nest site on egg survival using a generalized linear mixed model with a binomial distribution and a logit link function and with clutch as a random factor. We also used a generalized linear mixed model to detect the effect of nest site on hatchling survival, with clutch and enclosure as random factors. We used a binomial logistic regression to investigate the relationship between hatchling survival and hatchling phenotypes (initial body size and hatch date), and we used the survival of individual hatchlings, rather than the clutch mean value, because binomial logistic regression indicated that clutch did not influence hatchling survival (all stages,  $p > .200$ ). Meanwhile, we evaluated the effects of nest site on the duration of incubation and the initial body size of hatchlings using mixed-model ANOVAs or ANCOVAs (egg mass as a covariate for hatchling morphology), with clutch as a random factor. We also evaluated the effects of nest site on the morphology and growth rate of the recaptured hatchlings using mixed-model ANOVAs, with clutch and enclosure as random factors. We used linear regression to assess the relationships between hatchling growth rates and hatchling phenotypes.

We performed all analyses using IBM SPSS 20, and we used Kolmogorov–Smirnov and Levene' tests to check for normality and homogeneity of variance. We express the data as  $M \pm SE$ .

## 3 | RESULTS

### 3.1 | Nest-site choice

The natural lizard-constructed nests were  $14.9 \pm 0.3$  cm deep, on average, and located in sites with vegetation cover that was 42% sparser

**TABLE 1** Microhabitat variables of maternally chosen *Phrynocephalus przewalskii* nest sites and random sites

Variable	Nest site	Random site	Statistic
Vegetation cover (%)	8.6 ± 1.4	50.3 ± 2.9	$t_{36} = -13.840, p < .001$
Surface temperature (°C)	41.7 ± 0.6	35.6 ± 0.9	$t_{18} = 9.115, p < .001$
Nest moisture (%)	3.4 ± 0.1	3.0 ± 0.1	$t_{36} = 3.935, p < .001$
Nest temperature (°C)			
Mean	25.9 ± 0.1	24.7 ± 0.2	$t_{36} = 8.901, p < .001$
Minimum	22.3 ± 0.1	22.2 ± 0.1	$t_{36} = 0.500, p = .620$
Maximum	30.0 ± 0.2	27.3 ± 0.3	$t_{36} = 13.413, p < .001$
Range	7.8 ± 0.2	5.1 ± 0.2	$t_{36} = 14.034, p < .001$
Variance	8.1 ± 0.3	3.8 ± 0.3	$t_{36} = 13.536, p < .001$

The majority of the microhabitat variables were obtained from 37 nests, but ground surface temperature was only measured for 19 nests. Data are expressed as  $M \pm SE$ .  $p$  values in bold are statistically significant.

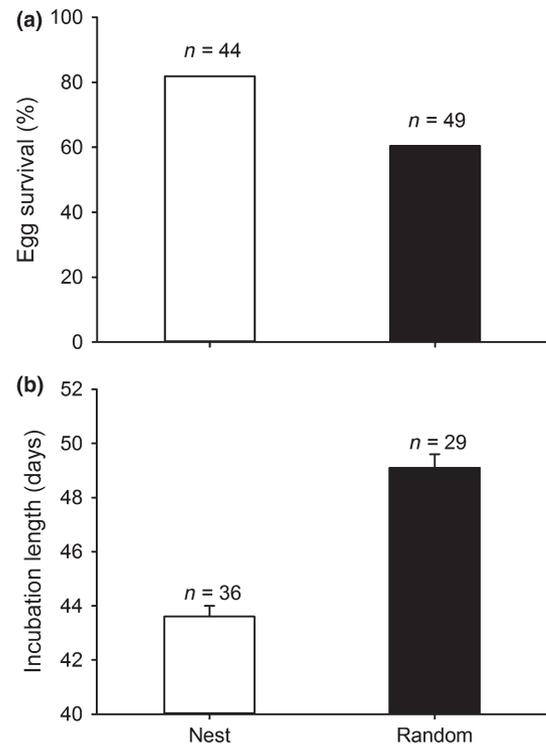
than the vegetation cover of the random sites (Table 1, Figure S1). As a result, nest sites were warmer and wetter than random sites, with higher ground surface temperatures, mean and maximum nest temperatures, temperature range and variance, and moisture content (Table 1).

### 3.2 | Effects of nest-site choice on embryonic survivorship and offspring traits

Eggs spent an average of 83.0% (66.1%–97.6%) of their total incubation in the field, and 65 (from 33 clutches) of the 93 eggs (from 37 clutches) hatched successfully. Eggs from the maternally chosen sites had an 18% higher probability of hatching than those from the random sites ( $F_{1,91} = 5.469, p = .022$ ; Figure 1a), and the eggs from the maternally chosen sites hatched 5 days earlier ( $F_{1,31} = 74.969, p < .001$ ; Figure 1b).

On average, hatchlings from the two nest types had similar snout-vent lengths and body masses. However, hatchlings from the maternally chosen sites grew faster before winter, which allowed them to reach larger body sizes before winter; although there were no differences in the growth rates of the two groups after winter, the difference in body size was maintained through the following summer (Table 2; Figures 2 and 3). Moreover, the rate of body mass increase before winter was inversely related to hatch date ( $r^2 = 0.547, p < .001$ , Figure S2).

The survival of offspring from the maternally chosen ( $n = 36$ ) and random ( $n = 29$ ) sites did not differ by the end of the first active season (prior to winter) [61.1 and 58.6%, respectively,  $F_{1,63} = 0.021, p = .884$ ], the following spring [38.9% and 31.0%, respectively,  $F_{1,63} = 0.367, p = .547$ ], or the following summer [33.3% and 31.0%, respectively,  $F_{1,63} = 0.017, p = .898$ ]. Offspring survival also was independent of phenotype ( $p > .20$ ). However, by the end of the experiment, a higher proportion of female offspring from the maternally chosen sites had reached maturity and were gravid during the first year of life, when compared to those from the random sites [62.5% (5 of 8) vs. 0% (0 of 6); Fisher's exact test,  $p = .031$ ]. Each of the female yearlings from the maternally chosen sites laid a single clutch of eggs, with a mean clutch size of two eggs and a mean egg mass of 0.505 g.



**FIGURE 1** Survival rate (a) and incubation duration (b) of *Phrynocephalus przewalskii* eggs incubated in maternally chosen nests (white bar) and random sites (black bar). Numbers above the bars indicate sample sizes

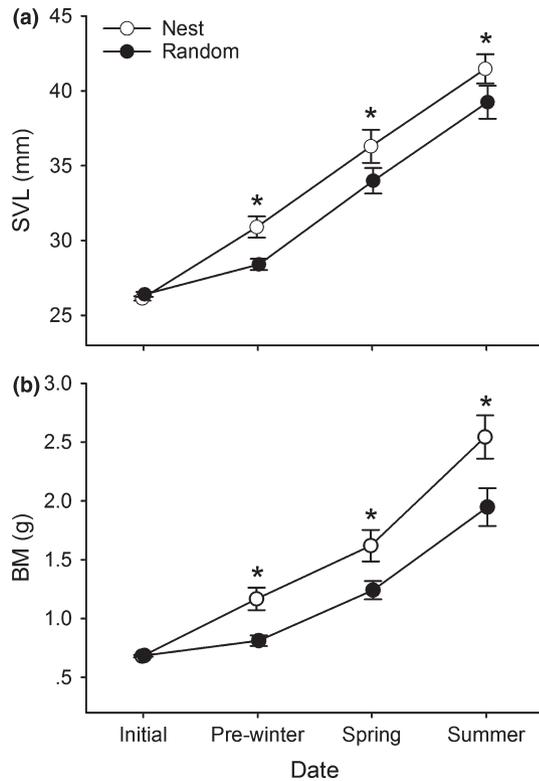
## 4 | DISCUSSION

Our study indicated that nest-site choice has positive effects on both embryonic development and offspring traits in lizards. Toad-headed agama chose warm and moist sites for their nests that promoted embryonic development and survivorship, as well as post-hatching growth, sexual maturity and fitness of their offspring. Below, we discuss potential mechanisms of nest site induced developmental plasticity and its importance for offspring fitness.

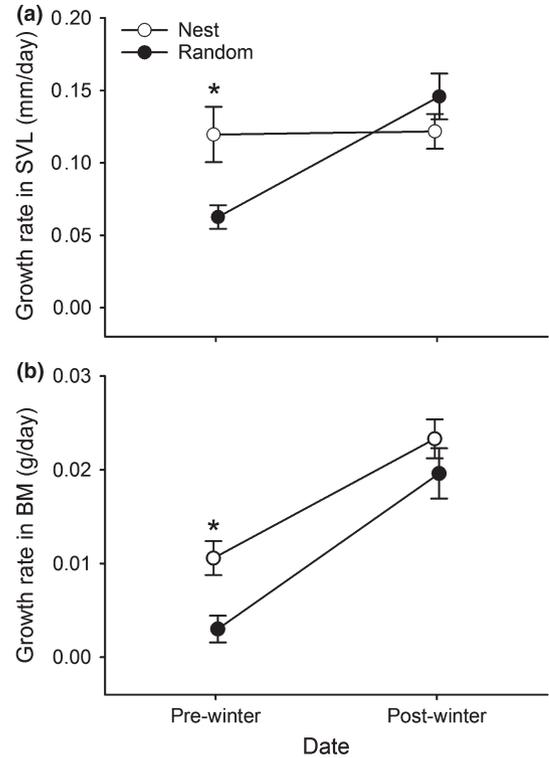
**TABLE 2** Statistical analysis of phenotypes of *Phrynocephalus przewalskii* hatchlings from maternally chosen nests and random sites

Variable	Covariate	Statistic
Snout-vent length (mm)		
Initial	Egg mass	$F_{1,30} = 0.717, p = .404$
Pre-winter		$F_{1,9} = 9.346, p = .014$
Spring		$F_{1,3} = 13.959, p = .033$
Summer		$F_{1,3} = 21.077, p = .019$
Body mass (g)		
Initial	Egg mass	$F_{1,30} = 0.370, p = .547$
Pre-winter		$F_{1,9} = 6.946, p = .027$
Spring		$F_{1,3} = 10.347, p = .049$
Summer		$F_{1,3} = 11.559, p = .042$
Growth rate in SVL (mm/day)		
Pre-winter		$F_{1,9} = 7.555, p = .023$
Post-winter		$F_{1,3} = 0.009, p = 0.931$
Growth rate in BM (g/day)		
Pre-winter		$F_{1,9} = 6.550, p = .031$
Post-winter		$F_{1,3} = 3.653, p = .152$

*p* values in bold are statistically significant.



**FIGURE 2** Snout-vent length (a) and body mass (b) of *Phrynocephalus przewalskii* offspring from maternally chosen nests (white circles) and random sites (black circles) at different stages [initial (at hatching), pre-winter (late September 2015), spring (mid-May 2016) and summer (late June 2016)]. Asterisks indicate significant differences



**FIGURE 3** Growth rates of the snout-vent length (a) and body mass (b) of *Phrynocephalus przewalskii* offspring from maternally chosen nests (white circles) and random sites (black circles) at different stages [initial (at hatching), pre-winter (late September 2015), spring (mid-May 2016) and summer (late June 2016)]. Asterisks indicate significant differences

The maximization of embryo survival is one of the most important reasons for the nonrandom selection of nest sites by oviparous animals (Refsnider & Janzen, 2010). This principle is supported by our finding that hatching success was greater in maternally chosen sites than in random sites. Incubation temperatures profoundly affect the survival of reptile embryos, with higher survivorship at optimally warm temperatures and lower survivorship at thermal extremes (Du & Shine, 2015). For example, striped mud turtles (*Kinosternon baurii*) construct nests at more densely vegetated sites to protect embryos from extremely high temperatures, as their small adult body size constrains their nest depth (Wilson, 1998). Warmer conditions (to a point) also accelerate the development of reptile embryos (Du & Ji, 2006; Shine, Madsen, Elphick, & Harlow, 1997), which may enhance hatching success by reducing nest predation risk (Hepp, Kennamer, & Johnson, 2006). Thermal conditions are similarly important in maximizing embryo survival in other ectotherms, including fish and amphibians. For example, river-spawning salmonids prefer warmer-than-average sites in temperate regions, presumably to avoid freezing (Geist et al., 2002), and amphibians sometimes oviposit communally to improve embryo survival in cool weather (Caldwell, 1986). The hydric conditions of nest sites also are essential for embryo survival in reptile species with parchment-shelled eggs (e.g. most lizards and snakes) because the eggs need to absorb water from the environment in order to complete development successfully (Du, 2004; Ji

& Du, 2001; Packard & Packard, 1988; Socci, Schlaepfer, & Gavin, 2005). Thus, locating nest sites with suitable moisture is especially important for species living in arid environments (e.g. deserts). To that end, we found that nests of toad-headed agama were not only warmer but also wetter, which is inconsistent with the traditional finding that warmer nests have lower soil moisture (Warner & Shine, 2008; Wilson, 1998). Our result seems counterintuitive, as high soil temperatures of open areas can increase soil evaporative potential and thus decrease soil moisture. However, the relationship between soil moisture and vegetation is more complex than that. For example, shrubs also can increase soil evaporative potential by extracting water (Obrist, Yakir, & Arnone, 2004). Moreover, shrubs can decrease soil water recharge by intercepting rainfall before it reaches the soil, which may decrease soil moisture. Such situations are more common in arid areas because rainfall is often too scarce to moisten the soil beneath shrubs (Barchuk, Valiente-Banuet, & Diaz, 2005). Like most reptiles, amphibian species select oviposition sites with sufficient amounts of water and avoid habitats that are prone to desiccation because their eggs lack a protective shell and, thus, are less efficient at retaining moisture (Figiel & Semlitsch, 1995; Rudolf & Rodel, 2005). In addition to temperature and moisture, other nest-site factors that can affect hatching success are oxygen levels, competition, and predation. For example, many species select oviposition sites that reduce the risk of egg predation (Martin, 1993; Vredenburg, 2004) or conspecific disturbance and competition (Vasconcelosneto & Monteiro, 1993). Therefore, the role of biotic factors in nest-site choice of reptiles is also of great interest.

Optimizing offspring phenotypes is another important reason for choosing a particular nest site. Our results are consistent with the hypothesis that maternally chosen oviposition sites enhance offspring fitness in this way (Mitchell, Warner, et al., 2013; Reedy, Zaragoza, & Warner, 2013; Refsnider & Janzen, 2010). Toad-headed agama chose nests with thermal and hydric environments that accelerated offspring development, growth and maturity. The faster growth rate of lizards from maternally chosen sites may be due to optimum nest conditions [nests are warmer and wetter than random sites, but cooler than detrimentally high temperature (Tang et al., 2012)]. Indeed, the reaction norm of reptile performance as a function of incubation conditions is roughly normally distributed (Doody, 1999): embryos that develop in intermediate conditions usually become offspring with better performance (e.g. faster running speed and growth rate) than those from too cold and dry or too hot and wet conditions (Booth, 2006; Brown & Shine, 2006; Deeming, 2004; Packard, 1999; Shine & Brown, 2002; Tang et al., 2012). Alternatively, early hatching might expose offspring to more favourable environmental conditions and provide them with a longer growth period prior to winter (Uller & Olsson, 2010; Warner & Shine, 2007). Regardless, faster growth during early life stages accelerates maturity in this species, as it does in other lizards (Angilletta, Niewiarowski, Dunham, Leache, Porter, 2004; Angilletta, Steury & Sears, 2004). Although the sample sizes are modest, our results are consistent with the fact that wild hatchlings from eggs incubated at nest sites can reach maturity in 1 year (Li et al., unpubl. data). For many short-lived animals, earlier maturity is favoured by natural selection

since it can increase lifetime fecundity and compensate for negative effects of juvenile mortality (Oli & Dobson, 1999; Stearns & Koella, 1986). The fitness consequences of nest-site choice may also appear at multiple life stages (Miller, 2008; Reedy et al., 2013; Streby et al., 2014). For example, our results reveal that nest-site choice favoured both embryonic survivorship and more rapid growth and earlier maturity in hatchlings.

The ecological and evolutionary drivers of nest-site choice can be more complex than maximizing embryonic survival and offspring fitness. Alternative reasons include maximizing maternal survival, proximity to suitable habitat for offspring, maintaining natal philopatry and indirect nest-site choice *via* mate choice (Refsnider & Janzen, 2010). For example, females may choose nests to minimize predation on themselves, rather than on their offspring. Indeed, female pythons (*Liasis fuscus*) select warm sites that do not require costly maternal brooding, thereby reducing predation on the females but increasing the risk of egg predation (Madsen & Shine, 1999). However, such phenomena may be absent in our study system because toad-headed agama construct nests in areas with sparse vegetation, where predation risk is high (Zeng et al., 2016).

Previous laboratory experiments improved our understanding of how lizards choose nest sites on the basis of environmental factors (Reedy et al., 2013; Warner & Andrews, 2002). By manipulating nest conditions in the field, we further clarified the fitness consequences of nest-site choice. Such field studies are critical for elucidating the ecological significance of nest-site choice (Mitchell, Warner, et al., 2013; Warner, 2014). However, long-term studies on the effects of maternally chosen nest sites on offspring survival and reproduction are also needed (Clark & Shutler, 1999; Huang & Pike, 2011; Refsnider, Bodensteiner, Reneker, & Janzen, 2013) since the fitness consequences of nesting behaviour may occur at the embryo, juvenile and adult stages. Repeating and extending well-designed nest manipulation experiments of short-lived lizard taxa, as in the present study and several previous studies (e.g. Mitchell, Warner, et al., 2013), holds great promise for elucidating the adaptive significance of this fascinating maternal behaviour. In addition, comparing the fitness consequences of nesting behaviour between turtles and lizards, which generally have different life spans, would be of interest in future studies because nest-site choice *a priori* might be expected to be less critical in the former than in the latter.

## ACKNOWLEDGMENTS

We thank Z-G. Zeng, X-Z. Han, X-K. Han and Q-Y. Li for their assistance in the field and to anonymous reviewers for their helpful comments. We are grateful to the staffs at the field station for logistic support. Ethics approval and protocol (IOZ14001) for the collection, handling, and husbandry of the study animals was given by the Animal Ethics Committee at the Institute of Zoology, Chinese Academy of Sciences. This work was supported by grants from the National Natural Science Foundation of China (31525006) and the National Key Research and Development Program of China (2016YFC0503200). All authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

W.G.D. and S.R.L. conceived the ideas and designed methodology; S.R.L., X.H., Y.W., J.H.B. and Y.P.Z. collected the data; S.R.L. and B.J.S. analysed the data; W.G.D., S.R.L. and F.J.J. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.28524> (Li et al., 2017).

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**How to cite this article:** Li S-R, Hao X, Wang Y, et al. Female lizards choose warm, moist nests that improve embryonic survivorship and offspring fitness. *Funct Ecol*. 2018;32:416–423. <https://doi.org/10.1111/1365-2435.12995>