

Mating Ethogram of a Video-aided Study of Mating and Parturition in Captive Chinese Crocodile Lizards (*Shinisaurus crocodilurus*)

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Abstract Mating behavior and parturition are important components in the life history of any species. The endangered Chinese crocodile lizard (*Shinisaurus crocodilurus*) is suffering dramatic population decreases in the wild. Successful captive breeding can provide individuals to be reintroduced into their natural habitat and help to restore the wild populations. Studying the mating behavior and parturition of these lizards in captivity will enable us understand how is this ancient species reproduction and optimize artificial reproduction protocol. We studied the mating behavior and parturition of Chinese crocodile lizards in captivity using video recordings at the Daguishan National Nature Reserve, China, from 2015 to 2017. We analyzed pre-copulation and copulation, which occurred in mid-March to late May from all studied years. Pre-copulation and copulation lasted on average 7.51 ($n = 11$) and 27.45 ($n = 11$) minutes, respectively. We found that large-bodied male lizards have longer copulation periods, a pattern seen in other lizard species. In the mating event, males always initiated sex, while the females were passive. The duration of parturition lasted 153.8 ± 26.8 ($n = 46$) minutes, and larger females had heavier clutches. In addition, even though Chinese crocodile lizards represent an ancient lizard clade with primitive characteristics, they still abide by the reproduction patterns seen in most other lizard species.

Keywords lizard, mating and reproduction, behavior, quantitative study

1. Introduction

Natural and sexual selection produced several reproductive strategies in order to maximize species breeding success. Some examples are polygamy and polyandry, which allow for more opportunities for males and females to

reproduce (Anderson and Iwasa, 1996; Lewis *et al.*, 2000). In many lizard species, females will copulate with multiple males and bear the offspring of multiple males in a single clutch. These examples are sand lizards (*Lacerta agilis*) (Olsson and Madsen, 2001), adder snakes (*Vipera berus*), or painted turtles (*Chrysemys picta*) (Pearse *et al.*, 2002). This multiple paternity strategy is often related to the coexistence of conflicting male mating strategies (Gullberg *et al.*, 1997; Laloï *et al.*, 2004).

Sexual dimorphism interpreted by body size and

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morphology difference often play important role in reproduction process (Ryan, 1997; Sun, 2006; He *et al.*, 2011). Many studies have demonstrated that males with larger body sizes have more advantages during mating competition than smaller individuals. This occurs because larger males are stronger and can move across larger ranges of distance (Lewis *et al.*, 2000; Pearse *et al.*, 2002; Kamath and Losos, 2018), while intra-sexual competition favors large male size and other physical attributes, such as bite force, while inter-sexual selection, usually in the form of female choice, favors traits in males that make them attractive to females (Ryan, 1997; Du *et al.*, 2005).

After mating, animals have select their reproductive modes. Among the 8008 known extant squamate species, 6658 species are oviparous, 1137 are viviparous, some reproduce asexually (Pyron *et al.*, 2013), and some species such as Chinese crocodile lizards are ovoviviparous (Zhang, 2002), and which means that the mother protects the embryos and hatches the egg inside the body. Then she delivers the baby lizard just after it is well developed. In Chinese crocodile lizard, this reproductive mode is likely to be an adaptation to its unstable environment (Tang and Zhang, 1986; Zhang, 2002; Sun, 2006).

Shinisauridae is a monotypic family that contains the single species, *Shinisaurus crocodilurus*. The adult male and female are 143.6 ± 1.5 mm ($n = 69$), and 147.1

± 2.3 cm ($n = 41$) on SVL and weight 72.7 ± 2.2 g and 79.9 ± 3.5 g respectively (He *et al.*, 2011). All lizards will hibernate at end of October and wake up at March next year, adult males isolated live one another, while adult females live with their newly borne infant lizards (Zhang, 2002; He, 2010). Based on fossil evidence, this lineage of squamates likely originated during the end of the Pleistocene (Zhang, 2002; Pyron *et al.*, 2013; Huang *et al.*, 2015) and once distributed in Europe and North America (Smith, 2017; Conrad, 2006). Previous literature on Chinese crocodile lizard distribution, populations, endangered status, and conservation indicated that this species inhabits the provinces of Guangdong and Guangxi in southern China and northern Vietnam (Gong *et al.*, 2006; Huang *et al.*, 2008; van Schingen *et al.*, 2014) (Figure 1), and that crocodile lizards prefer small streams in broadleaf forests (Wu *et al.*, 2007; Wu *et al.*, 2012). They tend to be more active during the day, and sleep by perching above small pools along streams during the night (Zhang, 2002; Zeng, 2003; Wu *et al.*, 2007).

Through studies of sexual dimorphism in Chinese crocodile lizard, it is clear that there are differences in coloration and head size. He *et al.* (2011) measured 110 individuals in the Luokeng Nature Reserve (hereafter LKNR), one of the main Chinese crocodile lizard populations in Guangdong Province in China, and found

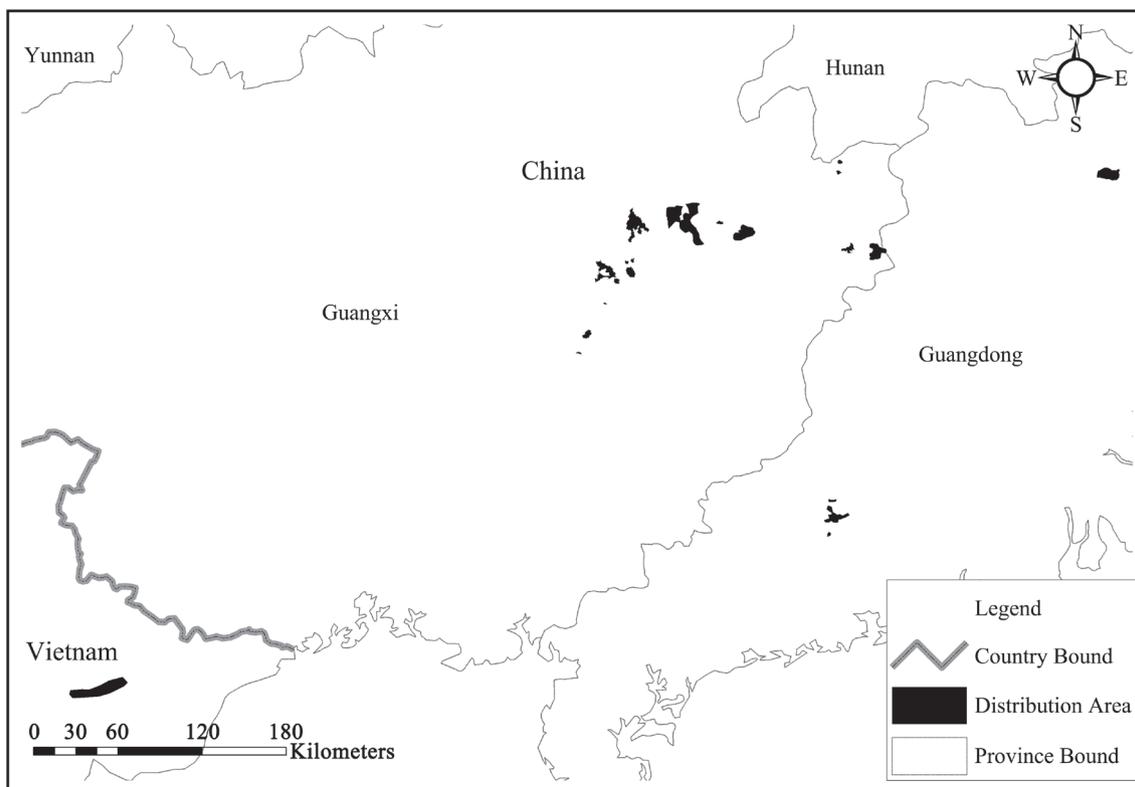


Figure 1 Distribution map of Chinese crocodile lizard.

that the males had a scarlet or light blue patch on their chests and bellies that are maintained throughout the whole year. However such coloration has its expression increased during the breeding season, while females lack this coloration (He *et al.*, 2011; Zhang 2002; Yu *et al.*, 2009). Courtship and copulation in captivity documented there were seven steps in courtship and copulation in LKNR (Yu *et al.*, 2009), however no data on relationship of weight of male and copulation duration and weight of female and clutch has documented.

Unfortunately like many other species in the region, the illegal pet trade and habitat loss are resulting in severe population declines (Huang *et al.*, 2008; van Schingen *et al.*, 2014; Lucie, 2018). In fact, there are fewer than 1000 individuals in China and approximately 200 individuals in the Yen Tu Nature Reserve, Quang Ninh Province, northeastern Vietnam (Li and Xiao, 2002; Gong *et al.*, 2006; Huang *et al.*, 2008; Quyet and Ziegler., 2003; van Schingen *et al.*, 2016). Given its small population size and continued fragmentation of their habitat, establishing a self-sustaining captive breeding population of Chinese crocodile lizard, which would then be reintroduced into the wild, may be an effective approach their conservation (Robert, 2009).

In this study, we aim to thoroughly describe and quantify the mating behavior and parturition of a captive breeding population using video playback analyses, which will help the understanding of the reproduce strategy of this ancient species and optimizes the protocol for artificial reproduction.

2. Materials and Methods

2.1. Study site Guangxi Daguishan National Nature Reserve (hereafter DGSNNR) is located in Hezhou municipality, province of Guangxi, China, between 24°02'–24°07' N and 111°47'–111°53' E, with a total area of 3 780 hm² (8.77 km from East to West and 8.9 km from South to North). DGSNNR is higher in the northeast and lower in the southwest, ranging from 210 m to 1 024 m. The climate in the reserve is humid subtropical with monsoons whole year. The yearly average temperature is 19.3 °C, monthly temperature 9.3 °C in January and 28.8 °C in July. Annual precipitation is 2 056 mm with 82.2% average humidity. The reserve has 88.3% forest coverage, with vegetation belonging to monsoon evergreen broadleaved forests, including *Olea europaea*, *Cryptocarya chinensis*, *Castanopsis tonkinensis*, *Castanopsis hystrix*, *Castanopsis eyreivar*.

The reserve has extremely rich biodiversity. It

documented 269 species of terrestrial animals, belonging to 197 genres, 85 families, 29 orders and 4 classes. The Chinese crocodile lizard is one of three national first class protected animals in China (Tan, 2014).

2.2. Captive samples The captive breeding pool was a water pool of approximate of 2 m wide and 3 m long with soil on the bottom. One meter high wall made of cement and brick surrounded the pool, which prevents the crocodile lizards from escaping. We used plastic pipe to lead the running water from a nearby natural stream to provide water into the water pool, which kept the water in the pool constant. We used water pools for breeding research in mid-March and later May, and other time of the year for feeding.

2.3. Data collection and analysis During the research, we released one pair of adult male and adult female in estrus into one water pool at one time and used Haikang DS-2CD300D video camera to focus on the whole captive breeding pools (Wang *et al.*, 2010). We stopped video recording after the lizards finishing copulation and separation. We took the video back to office and replay the video, measured copulation time as when the male lizard first approached the female in which the male ejaculated and both separated each other.

To study parturition, we kept 46 pregnant females in different water pools with every camera focusing on different individual for the entire process. Camera recorded from the first delivery to the last. We took the videos back to the office to replay and study. The duration of parturition was measured as the time of initial delivery of the first offspring until the time of the last offspring. The lizard keeper took all the baby lizards out for special care after the last baby lizard was born.

We used a total of 16 videos for copulation and 40 for parturition with total of 280 hours of video recording from 2015 to 2017 for this study. We replayed the video footage, carefully analyzed each target individual's behavior. We calculated the duration time of each behavior based on the time on video screen that showed as hour-minute-second, and we converted the time units into decimals for our analyses (e.g. 25 minute 30 second was converted into 25.5 minutes).

We measured the weight of each individual (adults and offspring) using an electronic scale with accuracy of 0.01 g. Using SPSS 16.0, we calculated the mean and standard deviation of mating behavior duration. We used male body weight and copulation duration as two variables to examine the relationship between them, and variables of female body weight and clutch

weight, female body weight and clutch size to check the relationship between each pair. We used ordinary least square regression method to run the regression test. We also used calibrate and electronic scale to measure the new born baby's length of the snout vent length (VSL) and weight respectively.

We used a noninvasive protocol to collect data, which was approved by the Forestry Administration of province of Guangxi, and DGSNNR.

3. Results

3.1. Mating behavior Mating usually occurred from mid-March to late May after female lizard delivery. Of the 16 videos of copulation we recorded, eleven events of copulation were successful and five failed because the adult female finally refused to accept her partner and the lizard keeper took the pairs out of the water pool.

Pre-copulation began with adult male displaying courtship behavior, followed by approaching and chasing his partner, and ended with successful neck biting on her (Table 1). Throughout this process, the females were relatively passive while the males initiated each mating behavior. Courtship interpreted when the male displayed head nodding up and down to the female repeatedly on the land or in water inside the pool. Male displayed courtship behavior immediately after the lizard keeper released them into the pool simultaneously, while female sometime responded to male's head-nodding behavior with her head-nodding too, sometime not (Table 1). We interpreted approaching as the male fast moved close to the female, which sometimes occurred at the same time as courtship behavior (Table 1). The male would then chase the female, and the females often escape. Following the neck-biting attempt behavior, which was when a male bit a female on the neck. Neck-biting attempts often failed by female's escaping, and the males had to try 5–6 times before he successfully held female by neck-biting. After neck-biting was successful, the crocodile lizards would proceed on to copulation (Table 1). The behaviors of courtship, approach, chase and neck-biting were continuous and difficult to separate and count, we used re-copulation to combine the total duration time of courtship, chase and unsuccessful biting. Pre-copulation lasted on average 7.5 ± 4.4 min ($n = 11$) (Table 2).

Mating behavior and duration of Chinese crocodile lizard copulation included three steps: 1) holding the female via neck biting, 2) contacting the cloaca, and 3) ejaculation (Figure 2). Males would bite the female on the neck from either side (Figure 2). If the male bit the



Figure 2 Typical mode of copulation of Chinese crocodile lizard (male with orange color on both body sides).

female from the right side, the male would use its left front limb to pin down the female on her back and the left rear limb to hold the female's pelvic region. If the male bit the female from her left side, the male would use its right limbs to hold down the female in the same manner. While holding the female, the male used its tail to raise the female's tail and forced his cloaca into the female's in this posture. The male's abdomen would then contract and expand rhythmically until ejaculation (Figure 2). Separation demonstrated the end of copulation when the male released the female from neck biting and physically separated from the female. Copulation on average lasted 27.5 ± 4.3 min ($n = 11$) (Table 2).

When we regressed copulation duration against male body weight (Figure 3), there was a statistically significant positive relationship (Pearson correlation, $r = 0.859$, $n = 11$, $P = 0.001 < 0.005$). Linear regression coefficient is significant, which demonstrate the male body weight has linear relationship with copulation duration ($P = 0.047 < 0.05$) (Figure 3).

3.2. Parturition Female parturition lasted from mid March until early May in one year, between waking up from hibernation and before copulation. Forty five of the 46 parturition events recorded were during this time period, but one female gave birth in late October before hibernation. After delivery of the final offspring, females always rested. The animal keeper took all the baby lizards out of the captive breeding pools for human-mediated manual feeding.

Parturition duration consisted of pre-parturition and parturition. In the pre-parturition period, pregnant females showed restlessness by moving back and forth and sometimes jumping into the water to swim, and quickly climbing out. The pregnant females would repeatedly move her rear limbs up and down while her abdomen

Table 1 Mating ethogram of Chinese crocodile lizard.

Behavior and posture*		Male	Female
Pre-copulation	Courtship	Active display	Responding sometime
	Chase	Fast running to female	Escaping
	Neck-biting	Biting female on neck	Escaping
Copulation	Body position	Most part above female	Most part below male
	Tail position	Below female	Above male
	Cloaca position	Facing up to female's	Facing down to male's
	Ejaculation	Abdomen expanding and contract	No much difference
	Separation	Releasing neck-biting	Escaping

Table 2 Mating behavior and duration of Chinese crocodile lizard.

Case No	Per-Cop time (mins)	Cop-duration (mins)
1	3.16	21.08
2	5.33	27.04
3	21.4	23.2
4	7.33	23.45
5	2.81	61.27
6	6.58	27.57
7	5.71	27.5
8	12.33	26.48
9	11.83	23.32
10	14.33	35.34
11	15.03	28.65
Mean \pm SD	7.5 \pm 4.4	27.5 \pm 4.3

contracted and expanded until the first baby lizard within the amnion flowed out of her cloaca on land, the baby lizard acted soon. On average, the duration of the delivery from first to last offspring was 153.8 ± 26.8 min ($n = 46$) based on the video data. There was a range of two to 11 baby lizards per clutch, with a mean of 6.2 ± 1.8 offspring per clutch ($n = 46$). Of the 247 delivered offspring, 147 survival (59.6%), 65 died lizard (26.3%) and 35 undeveloped eggs (14.1%).

In the 46 parturition events studied, 14 females were weighed before pregnancy, and their clutch weight and clutch sizes were recorded as well. Based on these data, the female Chinese crocodile lizards on average weighed 115.9 ± 22 g before pregnancy. These 14 females delivered on average 6 ± 2 baby lizards per clutch, with an average weight of 25.34 ± 3.6 g per clutch.

Significant test for the correlation indicated there is significant relationship between the female weight and clutch weights (Pearson correlation, $r = 0.575$, $n = 14$, $P = 0.031 < 0.05$), no significant relationship between female's weight and clutch size in clutch (Pearson correlation, $r = 0.324$, $n = 14$, $P = 0.258 > 0.05$). Linear regression coefficient between female weight and baby weights is significant, which demonstrates female body weight has linear relationship with total baby weight in clutch ($P = 0.01 < 0.05$) (Figure 4) and coefficient is not significant between female weight and baby number in clutch ($P =$

$0.14 > 0.05$) (Figure 5).

3.3. New born baby lizard and parental care The lizard offspring's snout-vent-length (SVL) was 100–129 mm and weighed 3–4.5 g on average respectively. There is a triangular-shaped yellow spot on the head (Figure 6), which faded at around 2–3 months. The newborn can move independently soon after delivery. The mother does not provide any parental care, even when the offspring were physically close to her.

4. Discussion

Our study provides a thorough description and quantification of mating behaviors and parturition in Chinese crocodile lizards. Video playback showed that sexually mature females often escaped during pre-copulation, which resulted in five cases of copulation failure. This behavior may be a form of female choice, where the female selects the stronger male by seeing which male is able to chase, catch, and hold her through the act of neck biting. Similar strategies were observed in other species, such as *Liopholis whitii*, *Anolis sagrei*, *Ameiva exsul* (Halliwell *et al.*, 2017; Kamath and Losos, 2018; Lewis *et al.*, 2000). The data of age, head size and bite force for male and clutch size, weight and age for female are very important for prediction the female partner selection in further study.

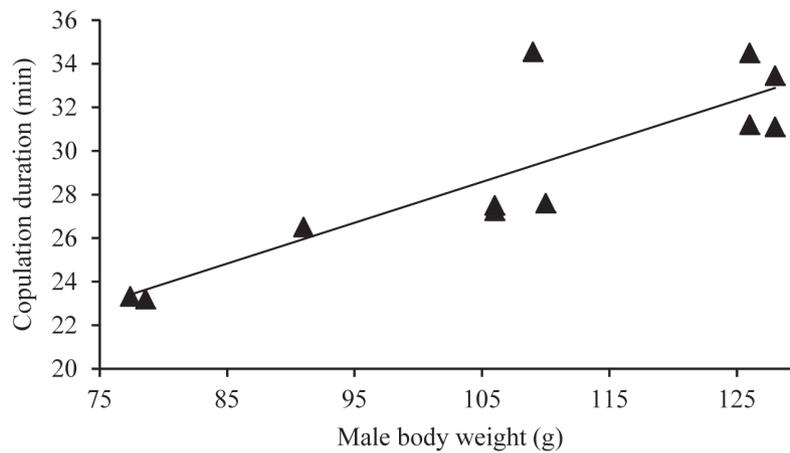


Figure 3 Linear regression between male's weight and copulation duration of Chinese crocodile lizard (demonstrates the heavier of the male, the longer of his copulation).

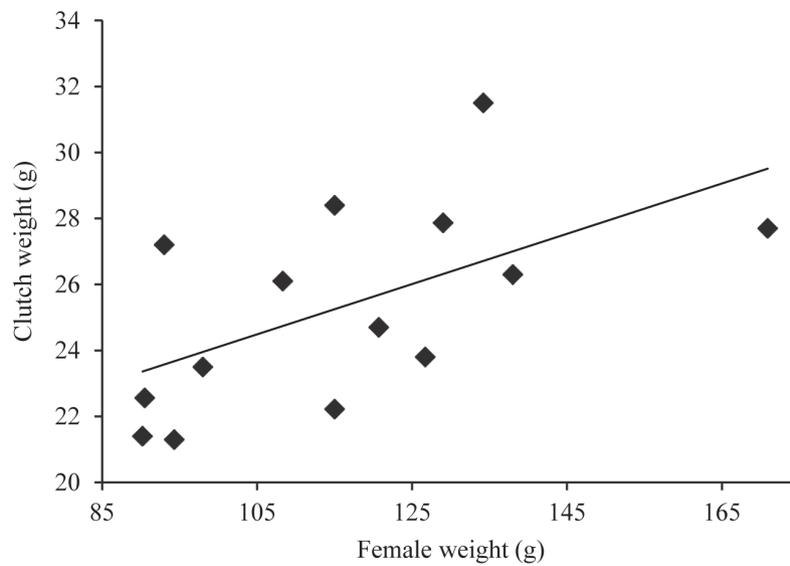


Figure 4 Linear regression between female's weight and clutch weight of Chinese crocodile lizard (demonstrates the heavier of the female, the heavier of her clutch).

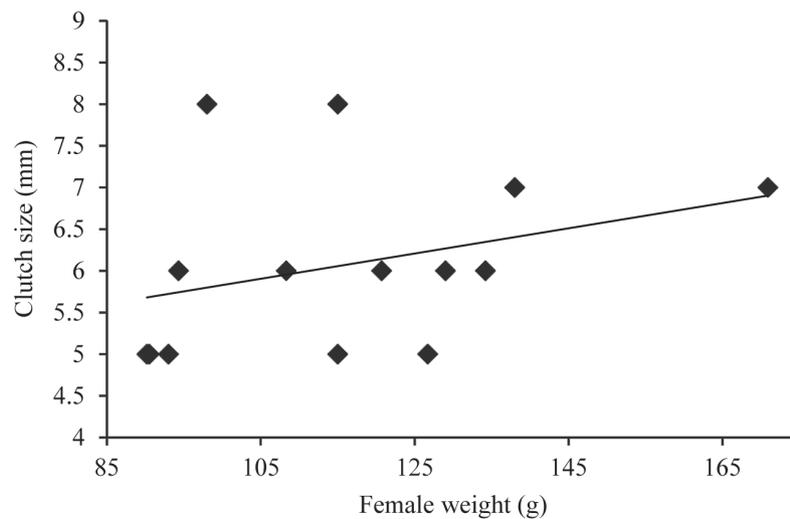


Figure 5 Linear regression between female's weight and clutch size of Chinese crocodile lizard (demonstrates the heavier of the female, the larger of her clutch).



Figure 6 New born baby lizards of Chinese crocodile lizard (indicates light yellow spot on the head).

We have shown that neck biting, which allows the male to hold down the female, is a crucial behavior during copulation. Therefore, we can suggest that males with a stronger bite force would be more successful in mating with females. He (2010) showed that there was a significant difference in male and female bite force (14.74 N for adult males and 13.55 N for adult females), which is correlated with sexual dimorphism in head length and width. Other possible functions for strong bite forces in males could be related to foraging behavior or territory defense, but more studies are necessary to fully understand the selection for a stronger bite force in males, however it has been studied that bite force difference between male and female was associated with different preys between the sex (Herrl *et al.*, 2018) and varied within same clad (Meyers *et al.*, 2018).

Previous studies have shown that copulation duration depends on male mating order, with the first male mating longer because of the lack of a rival male (Olsson *et al.*, 2004). We did not acquire data on mating order of Chinese crocodile lizard before we carry out the experiment, but we analyzed the correlation between the male body weight and copulation duration. Results indicated there was significant positive relationship between them even with our small sample size, demonstrating that larger male Chinese crocodile lizards are able to copulate for longer periods of time. Additionally, heavier male body weight indicates strength and health of the male individual, which may influence the duration of copulation (Lewis *et al.*, 2000).

Female body weight range from 93 g to 170.9 g and is associated to clutch weight in Chinese crocodile lizards, which represent female reproductive output. Egg weight in other reptile species is usually associated with the

mother's body weight (Sinervo & Licht, 1991; Du *et al.*, 2005) because the amount of space available within the female abdomen influences her total reproductive allocation, enabling her to modify her output. Therefore heavier females usually produce heavier clutches. This is also the case for Chinese crocodile lizards. Fitch (1970) suggested that in most species, female reproductive output might increase with female age and larger females produced larger clutch sizes and more eggs. It would be useful for future studies to analyze the correlations amongst clutch size, clutch weight and female age.

We found that the heaviest female Chinese crocodile lizard in our study was almost double the weight of the lightest one. However, the heaviest female did not produce double the number of offspring of the lightest female. In many species, clutch size varies with the female body size as she grows larger, but space in the abdomen available for reproduction is limited, resulting in a nonlinear relationship between clutch size and female body weight (Du *et al.*, 2005). Previous studies indicated that the maximum clutch size for Chinese crocodile lizard is eight individuals (Zhang, 2002; Tang and Zhang, 1986), indicating that clutch size is dependent on abdomen size, not body size.

In general, Chinese crocodile lizards represent an ancient but prominent lizard clade, and we have shown that they have similar reproductive strategies of many other lizards.

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