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Female Reproductive Cycles of *Phrynocephalus przewalskii* (Lacertilia: Agamidae) in the Tengger Desert, China

Wei ZHAO[1], Nannan YU[1], Yajuan WANG[1],Weihong JI[2] and Naifa LIU[1*]

[1] *School of Life Sciences*, *Lanzhou University*, *Lanzhou* 730000, *Gansu*, *China*

[2] *Institute of Natural Sciences*, *Massey University*, *Private Bag* 102904, *North Shore Mail Centre*, *Auckland* 0745, *New Zealand*

Abstract The timing of reproduction can significantly affect an offspring's fitness, thereby also influencing the fitness of the parents, especially in species inhabiting extreme environments, such as deserts. Female reproductive cycles in *Phrynocephalus przewalskii* were studied from April to September 2008. Significant cycles of gonadal volume were found in all studied populations and the cycles were similar among the various populations. Females began vitellogenesis in April and contained oviductal eggs form May to June. Gonad volume decreased significantly in July and reached minimum volume from August to September. The follicular growth was negatively correlated with increasing precipitation and temperature in all populations. Hatching occurs during summer and early fall, when most of the annual rainfall occurs. Mean clutch size based on all populations was 2.7 ± 0.9 SE (n = 71).

Keyword *Phrynocephalus przewalskii*, lizard, gonad cycle, liver cycle, clutch size, Tengger Desert

1. Introduction

Reproductive traits in lizards usually vary significantly among species and populations, and are often thought to be under proximate or environmental control to some extent (Ballinger, 1983). Reptiles can be divided into two basic groups according to their reproductive cycles: seasonal and non-seasonal. Most lizards from the environments with predictable climate conditions in the tempe-rate zone and seasonal tropics usually have obvious breeding cycles, with courtship, mating and incubation occurring at certain times during the year (Castilla and Bauwens, 1990; Censky, 1995; Huang, 1997; Ramírez-Bautista and Vitt, 1997). However, reproduction of lizard species from non-seasonal tropical regions usually lack reproductive peaks at the population level, with reproductive activities of males and females occurring throughout the year (Benabib, 1994; Ramírez-Sandoval *et al*., 2006). The success of reproduction is significantly influenced by the time when it occurs. Furthermore, the seasonal ti-ming of hatching determines the fitness of juvenile lizards (Olsson and Shine, 1998; Warner and Shine, 2007). Thus, extensive research has focused on the factors which initiate seasonal reproduction. Seasonal reproduction can be initiated by different proximate factors, such as ambient temperature (Gadsden *et al*., 2006), precipitation (Patterson, 1991) and photoperiod (Censky, 1995). Female reptiles inhabiting temperate climates are highly consistent in their seasonality of reproduction (Shine, 1985; Murphy *et al*., 2006) and thermal constraints are responsible for this consistent (Shine, 1985).

Phrynocephalus przewalskii is a small oviparous sand lizard which is distributed widely in northern China and adjacent Mongolia (Urquhart *et al*., 2009). It has been subjected to extensive, systematic and ecological stu-dies (Xu and Yang, 1995; Liu *et al*., 1996; Urquhart *et al*., 2009). Liu *et al*. (1996) found that the male reproductive cycle of *P. przewalskii* from Shapotou in Ningxia, China was affected by photoperiod and exhibited an autumn testes growth without gametogenesis. The result was supported by the study of male reproduction in Minqin of Gansu, China (Xie, 1996). However, we still know little about the female reproductive cycle in *P. przewalskii*.

In this paper, we describe in detail the female reproductive cycles of *P. przewalskii* and address the following questions: What are the annual reproductive cycles of females? Are females' reproductive cycles associated with the same environmental factors that affect males? Are the reproductive cycles varied among different populations?

* Corresponding author: Prof. Naifa LIU, from the School of Life Sciences, Lanzhou University, with his research focusing on evolution, particularly Asian birds, including molecular phylogeny, life history evolution, molecular ecology, ecological genetics and conservation.
E-mail: naifaliu@sohu.com
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2. Material and Methods

A total of 151 *P. przewalskii* were collected from Alax Zuoqi (AZQ) (38.85°N, 105.63°E; 1448 m a.s.l.), Gantang (GT) (37.47°N, 104.50°E; 1614 m a.s.l.) and Minqin (MQ) (38.58°N, 102.99°E; 1369 m a.s.l.) around the Tengger Desert between April and September, 2008. All the specimens were captured during the middle of each month. The snout-vent length (SVL) of each specimen was measured immediately after capture and then the specimens were euthanized. The specimens were fixed (6% formalin) in the laboratory until gonadal analyses were performed.

Females with an SVL larger than 43 mm had the potential to reproduce and were considered sexually matured (Liu *et al.*, 1996). The maximum length and maximum width of follicles were measured to 0.1 mm, and the volume was calculated with the formula for the volume of an ellipsoid: $V=4/3\Pi ab^2$, where a is half of the shortest diameter and b is half of the longest diameter. Then, we defined the total volume of all the follicles as gonad volume. The liver mass and all the follicles mass of females were measured to 0.001 g. We then defined the total mass of all the follicles as gonad mass. Body condition of individuals was defined as the residuals from general linear regression of ln (body mass - gonads mass) vs. ln (snout-vent length). Then, the variation of fat body was estimated by body condition. Clutch size was estimated from the counts of vitellogenic follicles and oviductal eggs. Climatic data for a 30-year period, monthly mean temperature, mean photoperiod and mean precipitation were collected from the Chinese National Climatic Data Center (CDC; Figure 1).

Because organ mass or volume usually varies with body size, we first performed regressions of ln-transformed organ mass or volume data against ln-SVL. For significant regressions, the residuals from the relationship of variables on SVL were calculated to produce SVL-adjusted variables. These residuals were used to describe the organ mass or volume and reproductive cycles (Ramírez- Bautista and Vitt, 1997). One-way ANOVA on the organ mass or volume residuals (with month as the factor) was performed to determine whether significant monthly variation existed (Ramírez-Bautista *et al.*, 2006). General li-near regression was used to determine the role of climatic variables in reproductive cycles. Follicles and oviductal eggs of females were examined, and their conditions were classified according to the following four stages: nonvitellogenic follicles, vitellogenic follicles, oviductal eggs, and vitellogenic follicles plus oviductal eggs. The clutch size was estimated by counting the vitellogenic follicles or oviductal eggs.

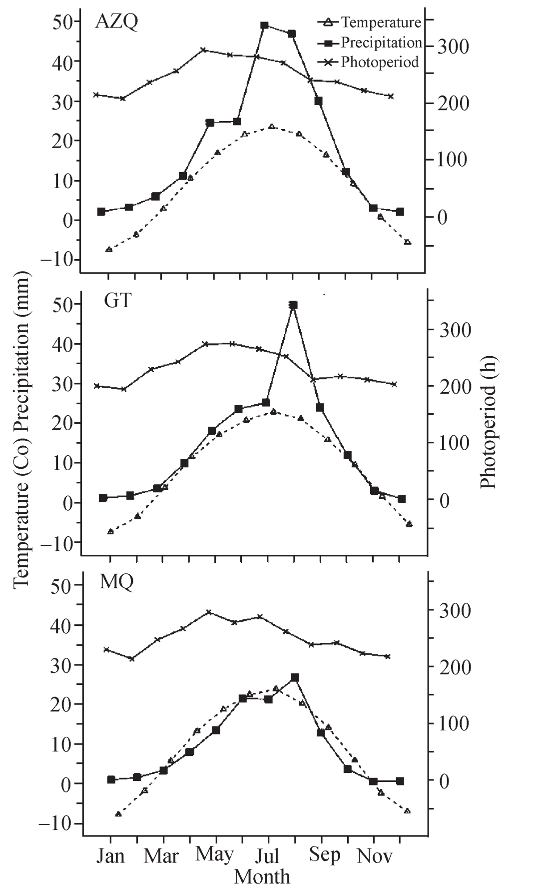

Figure 1 Monthly historical mean precipitation, temperature, and photoperiod recorded in Alxa Zuoqi (AZQ), Gantang (GT) and Minqin (MQ). The data are obtained from the Chinese National Climatic Data Center (CDC, from 1979 to 2008).

When the vitellogenic follicles or oviductal eggs were coexisted we only counted the oviductal eggs. Person Correlation or Partial Correlation was used to analyze the relationship between female SVL and clutch size. All statistics are presented as mean ± SE and all the analyses were performed with SPSS 16.0 for windows.

3. Results

3.1 Clutch size Data from April to June were used to estimated the clutch size. The mean clutch size based on vitellogenic follicles or oviductal eggs did not differ among populations (ANOVA, P >0.05 in all cases), so the data were pooled. The mean clutch sizes of the three populations were 3.1 ± 0.7 (ranging from 2 to 4, n = 19, AZQ), 2.6 ± 0.2 (from 1 to 6, n = 24, GT) and 2.4 ± 0.1 (from 1 to 4, n = 28, MQ), respectively. Mean clutch sizes were correlated with female SVL (r = 0.461, $F_{1,69}$ = 18.65, P < 0.001) and ANCOVA analysis indicated that no significant difference of clutch size existed among populations ($F_{2,67}$ = 2.87, P = 0.064). The mean clutch size of all the populations was 2.7 ± 0.9 SE (n = 71).

Among these females with vitellogenic follicles or oviductal eggs, the females with vitellogenic follicles and oviductal eggs were simultaneously found in all the studied populations (AZQ, 1 vs. 19; GT, 3 vs. 24; MQ, 2 vs. 28), which indicated that they might have at least 2 clutches in the same year. According to our field research, neonate was found from July to September in all populations.

3.2 Reproductive cycle Forty-two females were used to study the reproductive cycle in Alax Zuoqi. There was a significant relationship between ln SVL and ln (gonad volume), ln (liver mass) (ANOVA, $P < 0.001$ in all cases). The residuals from these regressions were used to describe the gonadal volume and liver mass cycle (Figure 2). There were significant monthly variations in gonads volume ($F_{5,36} = 12.44$, $P < 0.001$), and livers mass ($F_{5,36} = 10.77$, $P < 0.001$), while no significant monthly variation was found in body condition ($F_{5,36} = 0.78$, $P = 0.574$). The females with enlarged vitellogenic follicles or oviductal eggs were observed from April to August. The volume of gonads was associated with precipitation ($r = -0.665$, $F_{1,40} = 31.73$, $P < 0.001$) and temperature ($r = -0.455$, $F_{1,40} = 10.45$, $P = 0.002$), but not with photoperiod ($r = 0.303$, $F_{1,40} = 4.04$, $P = 0.051$). Gonad volume of individual females was correlated with liver mass ($r = 0.348$, $F_{1,40} = 5.51$, $P = 0.024$), but not with body condition ($r = -0.057$, $F_{1,40} = 0.174$, $P = 0.678$).

Forty-seven females were used to study the reproductive cycle in Gantang. SVL had a significant effect on ln (liver mass) ($r = 0.729$, $F_{1,45} = 50.899$, $P < 0.001$), but no effect on ln (gonad volume) ($r = 0.220$, $F_{1,45} = 2.28$, $P = 0.138$). The residual from regression of ln (liver mass) vs. ln SVL was used to describe the liver cycle, while the ln-transformed data of gonad volume was used to describe gonad cycle (Figure 3). Significant monthly variations were found in gonad volume ($F_{4,42} = 26.48$, $P < 0.001$) and body condition ($F_{4,42} = 3.34$, $P = 0.018$), but not in liver mass ($F_{4,42} = 2.33$, $P = 0.071$). The gonad volume was associated with precipitation ($r = -0.631$, $F_{1,45} = 29.76$, $P < 0.001$) and temperature ($r = -0.567$, $F_{1,45} = 21.36$, $P < 0.001$), but not with photoperiod ($r = 0.091$, $F_{1,45} = 0.37$, $P = 0.544$). The gonad volume of individual females was not correlated with liver mass ($r = 0.143$, $F_{1,45} = 0.94$, $P = 0.336$) or body condition ($r = -0.106$, $F_{1,45} = 0.51$, $P = 0.480$).

Sixty-two females were used to study the reproductive cycle in Minqin. Like in Gantang, the residual from regression of ln (liver mass) vs. ln SVL (linear regression, $r = 0.42$, $F_{1,60} = 13.137$, $P = 0.001$) and the ln-trans-

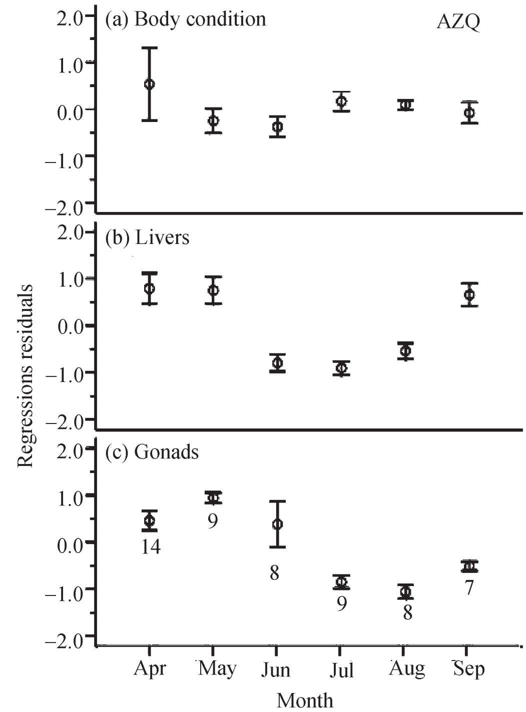

Figure 2 Female gonads, liver mass and body condition cycles of *P. przewalskii* obtained from Alax Zuoqi. The data are residuals from a regression of ln (body mass - gonads mass) (g), ln liver mass (g) and ln gonads volume (mm^3) against ln SVL. The values in all the three panels are shown as mean ± SE. The same below.

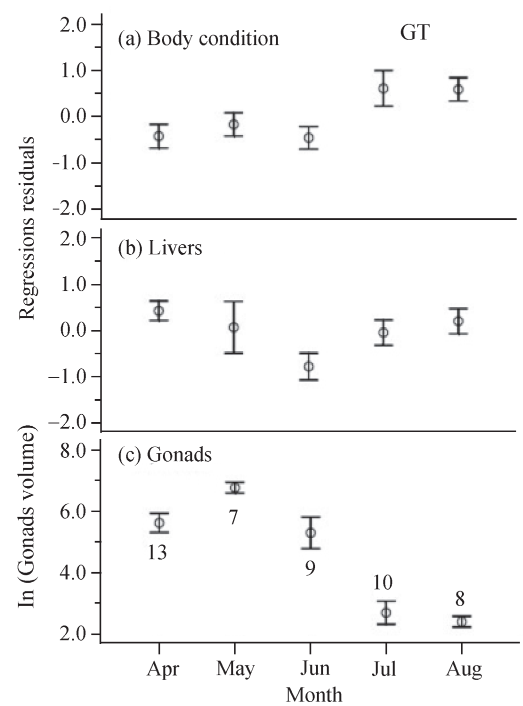

Figure 3 Female gonads, liver mass and body condition cycles of *P. przewalskii* obtained from Gantang.

formed data of gonad volume (linear regression, $r = 0.069$, $F_{1,60} = 0.29$, $P = 0.593$) were used to describe the reproductive cycle (Figure 4). Significant monthly variations were found in gonad volume, liver mass and body condition (ANOVA, $P < 0.001$ in all cases). Gonadal volume was associated with precipitation ($r = -0.651$, $F_{1,60} = 44.22$, $P < 0.001$), temperature ($r = -0.373$, $F_{1,60} = 9.70$, $P = 0.003$) and photoperiod ($r = 0.322$, $F_{1,60} = 6.92$, $P = 0.011$). Female individual gonad volume was correlated with liver mass ($r = 0.472$, $F_{1,60} = 17.18$, $P < 0.001$), but not with body condition ($r = 0.165$, $F_{1,60} = 1.68$, $P = 0.199$).

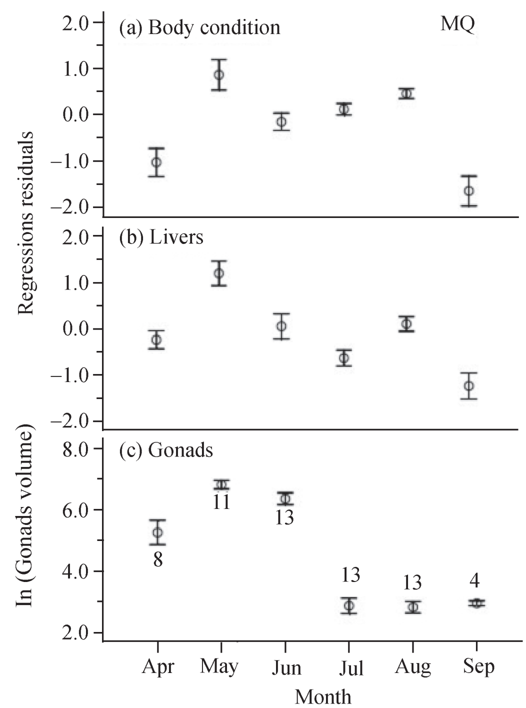

Figure 4 Female gonads, liver mass and body condition cycles of *P. przewalskii* obtained from Minqin.

4. Discussion

4.1 Reproductive cycle Our results indicate that the females of *P. przewalskii* have reproductive cycle similar to that of the other temperate lizards: ovulation occurs in mid- to late spring; eggs are laid in late spring or early summer (Shine, 1985; Murphy *et al*., 2006). Females from all studied populations of *P. przewalskii* respond to the same environmental cues, such as precipitation and temperature. Gonadal volume was found associated with increasing photoperiod only in Minqin, which indicates that photoperiod might be a minor factor.

Thermal constraints are important for female reproduction, especially in the areas of temperate climate, because summer is the only time of year when soil temperature and insolation are high and constant enough to permit rapid embryonic development (Shine, 1985). Our study also found a significant correlation between gonad activity and temperature. This relationship may reflect a thermal constraint.

For desert lizards, higher reproductive success often correlates with increased rainfall (Robinson, 1990). In arid ecosystems, highly variable and unpredictable precipitation often becomes the driver of biological processes (Noy-Meir, 1973). This might be due to the increased food availability from annual plant growth and phytophagous insects (Whitford and Creusere, 1977). The reproductive activity of *P. przewalskii* was negatively correlated with precipitation, so that offspring can hatch in summer when food is abundant. Our field investigation suggests that neonates were to be found during July and August when precipitation was high. Although all the three factors possibly influence the reproductive activity of this species, the timing of rainfall may be the ultimate cue for reproduction through its effects on egg and offspring survival (Andrews and Sexton, 1981).

Research indicates that in other lizard species the liver is involved in the synthesis or storage of lipids for reproductive activity and egg development (Ramírez-Bautista and Vitt, 1997; Feria-Ortíz *et al*., 2001; Ramírez-Sandoval *et al*., 2006). The significant positive correlation between gonad volume and livers mass in Alax Zuoqi and Minqin indicates that livers might be involved in reproductive activity. The energy used in egg development comes from food and/or lipids. Although no correlation between gonad volume and body condition was found, significant monthly variation in body condition was found in Gantang and Minqin. The decrease in the body condition in June indicates that egg development depended on the catabolism of lipids to some extent. But neither seasonal variation in body condition, nor linear relationship between gonads volume and body condition was found in Alax Zuoqi, which indicated that the energy stored in lipids was not used in egg development. Our results suggested that the source of the energy for egg development might be different among populations.

4.2 Clutch characters In this study, specimens simultaneously have both enlarged vitellogenic follicles and oviductal eggs in May and June among all the populations, while having no vitellogenic follicles in August. This evidence is always used as an indictor of multiple clutches (Ramírez-Sandoval *et al*., 2006). But several other studies on Chinese *Phrynocephalus* indicated that the females of both alpine species (Wang and Macey, 1993; Huang and Liu, 2002) and desert species (Chen *et al*., 1993; Liu *et al*., 1996; Guo and Zhao, 2001) produced one clutch per year. Because of atresia of previtellogenic and vitellogenic follicles (Aldridge and Semlitsch, 1992), we cannot determine the clutch frequency through our small simples.

5. Conclusion

Our results indicate that the females of *P. przewalskii* from different populations have a similar reproductive cycle and respond to the same environmental cues, such as precipitation and temperature. The effects of thermal constraint of embryonic development and food avai-lability on offspring survival may be the best explanation. According to previous research, the reproductive cycle of males was affected by photoperiod, but not by temperature or precipitation, so more detailed work is needed to explain this difference. The energy used in reproduction is also ambiguous, and some experimental manipulations will be needed and may be helpful to determine the source of energy utilized by females. We also find that the females of *P. przewalskii* have the potential to produce more than one clutch per year, but we can not determine the clutch frequency without a culture experiment.

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