

Effects of Thermal and Hydric Conditions on Egg Incubation and Hatchling Phenotypes in Two *Phrynocephalus* Lizards

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Abstract Flexible-shelled eggs of the lizards *Phrynocephalus przewalskii* and *P. versicolor* were incubated under different thermal and hydric conditions to elicit the effects of incubation environment on hatching success, embryonic development and duration as well as hatchling phenotypes. Embryogenesis of the two species was not sensitive to changes in the hydric environment except *P. przewalskii* incubated in 30°C group. Temperature significantly altered the duration of embryogenesis, with cooler temperatures leading to a longer incubation period. Hatching success was greater at 26 and 30°C than at 34°C. The hatchlings incubated at 26 and 30°C had longer snout-vent length, larger body mass, and better locomotor performance than those incubated at 34°C. Compared to *P. przewalskii*, *P. versicolor* had a shorter incubation period and yielded smaller hatchlings, which then had a higher survival rate in cooler and drier habitats. We conclude that an incubation temperature of 30°C would produce the best balance among developmental rate, hatching success, and post-hatching performance. We speculate that the upper temperature limit for incubation of *P. versicolor* eggs may be slightly higher than 34°C.

Keywords egg incubation, hatchling phenotypes, post-hatching performance, *Phrynocephalus przewalskii*, *Phrynocephalus versicolor*

1. Introduction

The physical conditions (mainly temperature and moisture) important for proper oviparous reptile embryonic development in the natural nest environment directly affect embryogenesis and the resulted hatchlings. Nest temperature has subtle effects on hatching success and the incubation period of eggs, as well as some hatchling phenotypes, such as body size, locomotor performance and behaviour, and may also indirectly affect their growth and survival (Burger, 1989, 1990; Van Damme *et al.*, 1992; Elphick and Shine, 1998; Braña and Ji, 2000; Booth, 2006; Webb *et al.*, 2001; Xu *et al.*, 2005; Hao *et al.*, 2007; Andrews, 2008). In addition, incubation temperature could also determine the sex of the offspring in some species of crocodylians, turtles and lizards (Bull,

1980; Valenzuela and Lance, 2004; Deeming, 2004; Booth, 2006; Schwanz, 2010; Zhang *et al.*, 2010; Warner and Shine, 2011).

Compared to temperature, moisture of the substrate has a small effect on hatchling phenotypes. Previously published work suggests that the nest sites selected by oviparous females for oviposition may influence the water balance of developing eggs, metabolism, growth of the embryos, and size and sub-sequent survival of the hatchlings (Morris *et al.*, 1983; Cagle *et al.*, 1993). Hatchling phenotypes and locomotor performance have also been shown to be affected (Gutzke and Packard, 1987; Packard, 1991, 1999; Miller, 1993; Finkler *et al.*, 2000; Deeming, 2004; Xu *et al.*, 2005). The female's choice of nest site can have profound effects on the phenotypes and survivorship of her offspring (Via *et al.*, 1995; Shine and Harlow, 1996; Brown and Shine, 2004). Thermal and/or hydric variation during incubation have been well studied on a diverse range of squamate reptile species from humid subtropical and tropical habitats. However, few studies work exists for dry desert regions.

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Under the lasting dry climate, it should be more important for females to choose nests with proper moisture for egg incubation.

Here, we selected two toad-headed lizards, *Phrynocephalus przewalskii* and *Phrynocephalus versicolor*, to investigate the effects of incubating conditions on embryonic development and hatchling phenotypes. Both lizards are small and oviparous, living in desert or semi-desert habitat covered by sparse vegetation. *P. przewalskii* is widely distributed in northwestern China, including Inner Mongolia, Ningxia, Gansu, Qinghai and Xinjiang, and *P. versicolor* ranges from Inner Mongolia, Ningxia, Gansu and Xinjiang of China to Mongolia, Kazakstan and Kyrgyzstan (Zhao, 1999). Both habitats where the two lizard species were collected are dry and barren. The annual rainfall is only 370 mm and 280 mm at each habitat respectively. In the present study, lizard eggs were incubated under different thermal and hydric conditions, and we examined whether incubation conditions would affect the following four traits: 1) incubation period and hatching success of females; 2) morphology of hatchlings; 3) locomotor performance of hatchlings; and 4) early growth rate of hatchlings. All these traits are important in egg incubation and closely related to the fitness of species. Furthermore, awareness the effect of incubation conditions on these traits is useful and helpful for protection of the reptiles living dry habitats.

2. Materials and methods

2.1 Animal collection and maintenance The two lizards, *P. przewalskii* and *P. versicolor*, were collected from Jingtai (37°13' N, 104°05' E) and Linze (39°22' N, 100°06' E) in Gansu, China, respectively, in late May of 2011. Most females had mated as we found fertilized eggs in the wild nests when we captured them. Female lizards were brought to the laboratory in Lanzhou University and kept in cages of 800 × 360 × 400 mm (length × width × height), with 5 mm depth of silver sand. Fifteen individuals were raised together in one cage. They were exposed to light for 12 h and kept in dark for another 12 h. A bulb (100 W) was suspended over one side of the cage to create a thermal gradient ranging from 37°C to 25°C for 8 h daily (09:00–17:00 h). Ambient temperatures fell to 20°C overnight. The lizards were observed daily and fed mealworms and water *ad libitum*.

2.2 Egg Collection and Incubation We checked the cages several times every day to see if eggs were laid. Finally, we obtained a total of 263 eggs from 101 female

P. przewalskii and 185 eggs from 66 female *P. versicolor*. The clutch sizes were too small to distribute eggs from each clutch over all treatments. To minimize maternal effects, we assigned eggs from individual clutches randomly to different treatments as Du and Shine (2008) did in scincid lizard *Lampropholis guichenoti* (2008). The collected eggs in each clutch were weighed (± 0.001 g) and then randomly incubated at three constant temperatures (26, 30 and 34 $\pm 0.2^\circ\text{C}$) in 150 mL plastic containers covered with a perforated substrate. As our pilot experiments showed that mold could develop on lizard eggs if the humidity was high (0 to -220 kPa) and undeveloped eggs were often found in extremely low humidity conditions (about -800 kPa, unpublished data), we selected two levels of substrate moisture for the present experiment: -440 kPa (2 g water/5 g vermiculite) and -620 kPa (2 g water/8 g vermiculite). Eggs were partly buried in the substrate over the course of incubation until hatchlings pipped their eggshell. Water was added every two days to compensate for evaporative loss and water taken up by eggs, and containers were moved among shelves in the incubator to minimize any influence of thermal gradient inside the incubator.

2.3 Hatchling morphology and locomotor performance

Hatchlings were collected and phenotypic attributes were measured as soon as they emerged. Body mass was weighed by electronic balance (Sartorius, Göttingen, Germany; with an accuracy of 0.001 g) and morphological traits included SVL, tail length (TL), arm length (AL), leg length (LL), head length (HL) and head width (HW) were taken using a vernier caliper (Shanghai exactitude apparatus factory, Shanghai, China; with an accuracy of 0.02 mm).

Locomotor performance was determined on the second day post-hatching. Prior to each trial, neonates were heated in an incubator to a body temperature of 30°C for 2 h and then locomotor ability was assessed by chasing the lizards along a 1 m racetrack. Each lizard was tested twice with a 1 h rest between trials. The entire locomotor process was recorded by a digital video camera (Canon, Japan). The videos were examined using Ulead VideoStudio 11 (Ulead Systems Inc., Taipei, Taiwan, China) in a computer, and the locomotor performance was analyzed for sprint speed in the fastest 15-cm interval and maximum distance traveled between stops.

2.4 Growth rate and sex identification Hatchlings were reared under uniform conditions (about 30°C during 09:00–17:00 h), fed small mealworms and vitamin-enriched water. Growth rate was assessed through

analysis of the variance of body mass within the initial 30 days. Sex was identified by checking for the presence of hemipenes.

2.5 Statistical analyses The data were tested for normality and homogeneity of variances to meet the assumptions of parametric testing prior to analysis. One- and two- way analysis of variance (ANOVA), multivariate analysis of covariance (MANCOVA), Tukey's post hoc test and linear regression analysis were used to analyse the incubation period, hatchling morphology, locomotor performance, growth rate and sex ratio from different incubation temperatures and moistures. All statistical analyses were tested using SPSS (Release 16.0.0, SPSS, Inc., Chicago, IL). Descriptive statistics were presented as mean \pm SE, and the significance level was set at $\alpha = 0.05$.

3. Results

3.1 Effects of thermal and hydric conditions on egg incubation and hatchling phenotypes in lizards *P. przewalskii*

Incubation length, hatching success and sex ratio of hatchlings: Hydric environments had no significant influence on incubation length and hatching success at the same incubation temperature, while temperature had notable effect on incubation length ($F_{1,98} = 1217.942$, $P < 0.001$). Higher temperature shortened the duration of incubation in a nonlinear way, and the average days of incubation decreased 6–7 days from 26 to 30°C and 14 days from 30 to 34°C. Temperature also affected the hatching success, and eggs incubated at 26 and 30°C yielded more hatchlings than those at 34°C (Table 1).

No hatchling exhibited trunk or tail malformations. The sex ratio did not differ significantly among the treatments ($R^2 = 0.009$, $P = 0.5$), and the overall sex ratio was 61 females: 53 males in *P. przewalskii* (Table 1).

Hatchling morphometric traits: Hatchling morphometric traits did not differ between sexes. Hydric environments had no effect on offspring morphology at 26 and 34°C, but significantly affected the hatchlings' body mass and most morphometric traits at 30°C. Compared to the -620 kPa group at 30°C treatment, hatchlings incubated from -440 kPa had larger body mass, SVL, TL, AL, HL and HW (Table 2).

Incubation temperatures significantly influenced all morphometric traits of hatchlings (Table 2). Neonates hatched at 26 and 30°C were larger than those at 34°C. Growth rate of hatchlings was not affected by incubation temperature ($F_{2,47} = 1.296$, $P = 0.29$) and substrate moisture ($F_{1,47} = 1.908$, $P = 0.174$; Figure 1). After 30

Table 1 Incubation length, hatching success and hatchling sex ratio of *P. przewalskii* and *P. versicolor* hatchlings under different thermal and hydric environments. Data on duration of incubation are expressed as mean \pm SE (range), and sex ratio includes those that died at the late stage of incubation.

Species	Temperature/ moisture (°C/ kPa)	n	Duration of incubation (d)	Hatching success (%)	Sex ratio (♀/♂)
<i>P. przewalskii</i>	26/-440	16	45.94 \pm 0.43	40.00 (16/40)	8/8
	26/-620	26	46.15 \pm 0.34	50.00 (25/50)	12/14
	30/-440	19	32.61 \pm 0.24	52.78 (19/36)	11/8
	30/-620	20	32.30 \pm 0.22	43.48 (20/46)	8/12
	34/-440	17	26.12 \pm 0.64	36.17 (17/47)	13/7
	34/-620	16	25.00 \pm 0.38	31.82 (14/44)	7/9
<i>P. versicolor</i>	26/-440	27	44.25 \pm 0.53	64.29 (27/42)	17/10
	26/-620	11	43.83 \pm 0.75	52.38 (11/21)	5/7
	30/-440	22	30.59 \pm 0.20	75.86 (22/29)	14/8
	30/-620	14	30.86 \pm 0.25	66.67 (14/21)	8/6
	34/-440	4	24.50 \pm 0.65	11.11 (4/36)	2/4
	34/-620	8	23.33 \pm 0.33	22.22 (8/36)	7/3

days of growth with sufficient food and water, hatchlings produced from different incubating treatments showed no significant difference in body mass.

Locomotor performance of hatchlings: Change in the hydric environment had no significant influence on the locomotor performance of neonate *P. przewalskii*, but hatchlings from the lower incubation temperature group exhibited better locomotor performance. Neonates of *P. przewalskii* from 26°C had the maximum sprint speed ($F_{2,58} = 7.453$, $P < 0.001$) and longest distance traveled between stops ($F_{2,58} = 9.314$, $P = 0.001$; Table 3).

3.2 Effects of thermal and hydric conditions on egg incubation and hatchling phenotypes in lizards *P. versicolor*

Incubation length, hatching success and sex ratio of hatchlings: Hydric conditions had no significant effect on incubation length and hatching success of *P. versicolor*. Temperature significantly influenced the duration of incubation ($F_{1,98} = 484.761$, $P < 0.001$), with the cooler temperature resulting in longer incubation period. Eggs incubated at 26 and 30°C had notably higher hatching success, but much lower at 34 °C (Table 1).

No hatchlings exhibited trunk or tail malformations. The sex ratio did not differ significantly among treatments ($R^2 = 0.493$, $P = 0.69$), with the overall sex ratio of 50 females : 36 males (Table 1).

Hatchling morphometric traits: Hatchling morphometric traits did not differ between sexes. Hydric environments did not influence offspring morphology, whereas temperature significantly affected all

Table 2 Morphological phenotypes of *P. przewalskii* and *P. versicolor* hatchlings. *F* values correspond to single effects and factor interactions in two-factor ANOVA (for SVL) or ANCOVAs (with SVL as the covariate, for other hatchling traits). Data are expressed as mean ± SE. Symbols immediately after *F* values represent significant levels: *ns* (not significant) $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Means with different superscripts differ significantly (Tukey's test, $P = 0.05$; a > b > c).

Species	Temperature/ moisture (°C/ kPa)	n	Body mass (g)	SVL (mm)	Tail length (mm)	Arm length (mm)	Leg length (mm)	Head length (mm)	Head width (mm)
<i>P. przewalskii</i>	26/-440	16	0.96 ± 0.03	26.67 ± 0.33	36.17 ± 1.32	10.89 ± 0.14	18.84 ± 0.28	8.34 ± 0.06	6.99 ± 0.10
	26/-620	26	0.91 ± 0.03	25.98 ± 0.32	36.52 ± 0.60	11.05 ± 0.11	18.47 ± 0.16	8.33 ± 0.08	7.00 ± 0.05
	30/-440	19	0.98 ± 0.03	27.18 ± 0.28	40.00 ± 0.76	11.77 ± 0.20	19.99 ± 0.21	8.23 ± 0.06	7.12 ± 0.07
	30/-620	20	0.84 ± 0.01	25.68 ± 0.29	36.56 ± 0.68	11.20 ± 0.16	18.85 ± 0.20	8.04 ± 0.08	6.81 ± 0.07
	34/-440	17	0.84 ± 0.03	25.26 ± 0.34	38.03 ± 0.71	11.20 ± 0.30	18.63 ± 0.22	7.84 ± 0.09	6.68 ± 0.12
	34/-620	16	0.88 ± 0.04	25.51 ± 0.42	39.88 ± 0.83	11.51 ± 0.14	19.00 ± 0.26	7.94 ± 0.11	6.69 ± 0.07
	Moisture	$F_{1,98}$	3.97*	5.38*	0.32 <i>ns</i>	0.04 <i>ns</i>	2.56 <i>ns</i>	0.27 <i>ns</i>	2.07 <i>ns</i>
	Temperature	$F_{2,98}$	-440 ^a , -620 ^b	3.83*	5.36**	4.14*	8.17**	12.73***	7.69**
	Interaction	$F_{2,98}$	26 ^a , 30 ^{ab} , 34 ^b	3.25*	5.71**	26 ^b , 30 ^a , 34 ^a	5.77**	26 ^c , 30 ^b , 34 ^c	1.41 <i>ns</i>
			4.43*	31.14 ± 1.29	10.00 ± 0.39	16.91 ± 0.68	7.53 ± 0.29	6.40 ± 0.25	
<i>P. versicolor</i>	26/-440	27	0.81 ± 0.03	24.65 ± 0.32	31.14 ± 1.29	10.00 ± 0.39	16.91 ± 0.68	7.53 ± 0.29	6.40 ± 0.25
	26/-620	11	0.78 ± 0.05	25.17 ± 0.48	31.83 ± 0.69	10.00 ± 0.21	17.88 ± 0.31	7.98 ± 0.25	6.63 ± 0.14
	30/-440	22	0.85 ± 0.01	25.91 ± 0.20	36.58 ± 0.46	11.23 ± 0.12	19.07 ± 0.19	7.97 ± 0.08	6.86 ± 0.08
	30/-620	14	0.87 ± 0.03	25.93 ± 0.25	36.73 ± 0.33	11.34 ± 0.20	18.84 ± 0.22	7.90 ± 0.08	6.84 ± 0.08
	34/-440	4	0.74 ± 0.04	25.88 ± 0.43	37.38 ± 0.55	10.56 ± 0.30	18.75 ± 0.63	7.78 ± 0.02	6.48 ± 0.06
	34/-620	8	0.74 ± 0.02	24.61 ± 0.45	35.67 ± 0.60	10.78 ± 0.16	18.03 ± 0.17	7.88 ± 0.09	6.66 ± 0.07
	Moisture	$F_{1,77}$	0.00 <i>ns</i>	0.46 <i>ns</i>	0.06 <i>ns</i>	0.09 <i>ns</i>	0.00 <i>ns</i>	0.39 <i>ns</i>	0.32 <i>ns</i>
	Temperature	$F_{2,77}$	5.89**	3.98*	9.79***	6.00**	3.06 <i>ns</i>	0.24 <i>ns</i>	1.20 <i>ns</i>
	Interaction	$F_{2,77}$	26 ^a , 30 ^b , 34 ^c	1.66 <i>ns</i>	26 ^b , 30 ^a , 34 ^{ab}	26 ^b , 30 ^a , 34 ^{ab}	0.03 <i>ns</i>	0.65 <i>ns</i>	0.18 <i>ns</i>
			0.21 <i>ns</i>	0.29 <i>ns</i>	0.03 <i>ns</i>	0.65 <i>ns</i>	0.49 <i>ns</i>	0.18 <i>ns</i>	

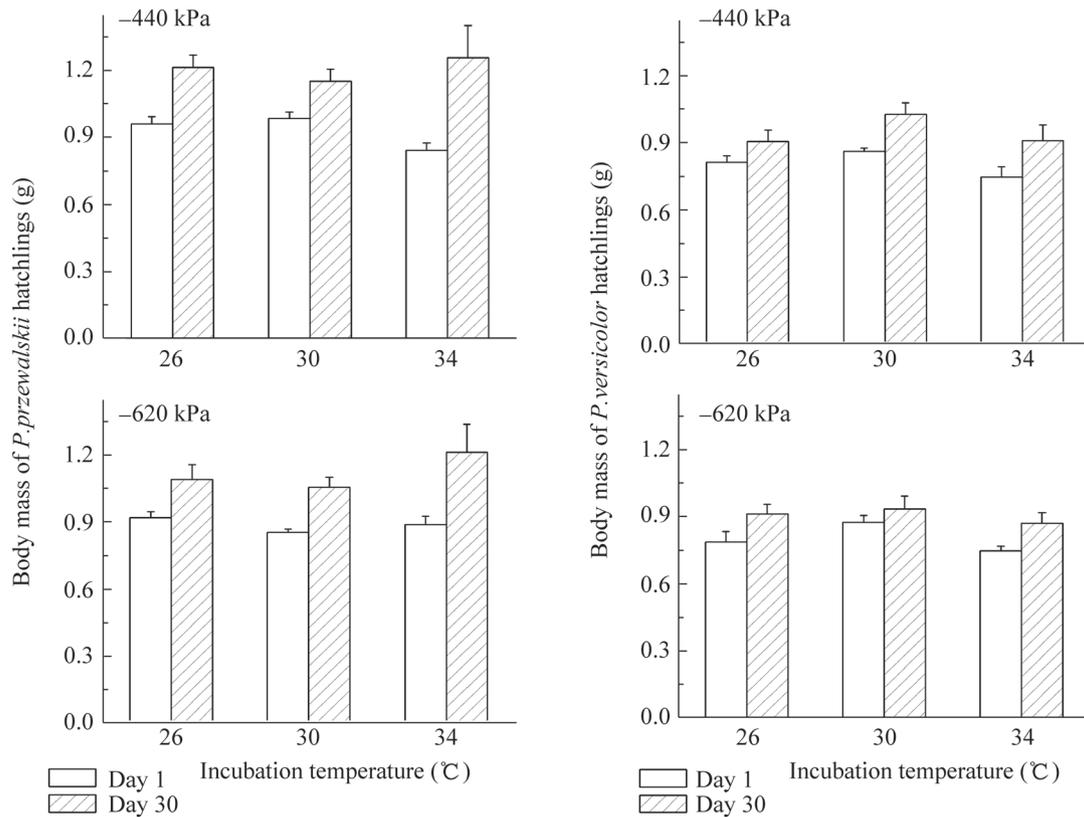


Figure 1 Early growth rate of hatchling body mass in oviparous lizards *P. przewalskii* and *P. versicolor* incubated at different thermal and hydric environments.

Table 3 Effect of incubation temperature and water potential on hatchling locomotor performance of *P. przewalskii* and *P. versicolor* tested at 30°C.

Species	Temperature/ moisture (°C/ kPa)	N	Sprint speed (m/s)	Maximal distance between stops (m)
<i>P. przewalskii</i>	26/-440	10	1.26 ± 0.08	0.76 ± 0.05
	26/-620	12	1.24 ± 0.07	0.68 ± 0.05
	30/-440	13	1.23 ± 0.05	0.67 ± 0.04
	30/-620	13	1.14 ± 0.07	0.64 ± 0.04
	34/-440	10	1.02 ± 0.07	0.56 ± 0.05
	34/-620	6	0.93 ± 0.04	0.45 ± 0.03
<i>P. versicolor</i>	26/-440	27	0.82 ± 0.07	0.65 ± 0.06
	26/-620	11	0.84 ± 0.06	0.63 ± 0.04
	30/-440	22	1.12 ± 0.05	0.73 ± 0.04
	30/-620	14	1.09 ± 0.06	0.64 ± 0.05
	34/-440	4	0.94 ± 0.10	0.65 ± 0.07
	34/-620	8	1.01 ± 0.07	0.61 ± 0.05

morphometric traits of hatchlings. Offspring produced at 26 and 30°C were larger than those at 34°C (Table 2).

Growth rate of hatchlings was not affected by incubation temperature ($F_{2,32} = 0.888$, $P = 0.422$) and

substration moisture ($F_{1,32} = 0.642$, $P = 0.429$; Figure 1). After 30 days of growth, hatchlings from different incubation temperatures had a similar body mass.

Locomotor performance of hatchlings: Hydric environments had no significant influence on neonate locomotor performance of *P. versicolor*, but the hatchlings from lower incubation temperature group exhibited better locomotor performance. Neonate offspring from 30°C had the maximum sprint speed ($F_{2,50} = 9.770$, $P < 0.001$; Table 3).

4. Discussion

The relative importance of external conditions is likely to be especially high in oviparous species, where embryogenesis is not buffered by the maternal system as in viviparous species (Deeming, 2004). The physical conditions inside natural nests can vary considerably depending on the soil type, depth, and degree of vegetation cover (Burger and Zappalorti, 1986; Burger, 1993; Pike, 2011); sensitivity of hatchling phenotypes to incubation conditions will be maximized if nests are

not maternally attended (Du and Shine, 2008). In the present study, the relatively high hatching success rates indicated that the hydric regimes (from -620 to -440 kPa) we set provided proper conditions for the development of embryos of *P. przewalskii* and *P. versicolor*. High hatching success rate was also achieved when *P. versicolor* (Wulatehouqi population) eggs were incubated at approximately -12 kPa water potential (Qu *et al.*, 2011), which indicated that the embryos of *P. versicolor* can develop well in a wider range of moisture conditions. As described in the results, water potentials had no significant impact on incubation length, hatching success, or hatchling's morphometric traits in most treatments, except *P. przewalskii* hatched in the 30°C group. In that group, eggs kept in moist substrates produced larger hatchlings than those kept in drier substrates. Generally, embryos developed in wetter environments often grew faster than in drier settings, for embryos incubated in moist conditions could consume energy reserves in the yolk more rapidly (Morris *et al.*, 1983). We speculated that at 30°C the *P. przewalskii* embryos might be more sensitive to the hydric condition, but this need more experiments and provide further information.

The phenomenon that nest temperatures affect embryonic development and hatchling traits is now overwhelming in reptiles. The incubation thermal conditions used in the present study profoundly affected incubation length, hatching success, and most hatchling traits, and yielded an equal offspring sex ratio in *P. przewalskii* and *P. versicolor*. Incubation length decreases as incubation temperature increases but not in a linear pattern, and the average days of incubation decreased 6–7 days from 26 to 30°C and 14 days from 30 to 34°C. Either lower or higher incubation temperatures may have detrimental effect on embryonic development and perhaps lead to developmental arrest. In some species of snake, turtles, skinks and lizards, the upper limit of temperature for successfully incubating eggs is around 32–34°C, at which none or less eggs were successfully incubated (Chen and Ji, 2002; Chen *et al.*, 2003; Xu *et al.*, 2005). Our experiments in the lizards *P. przewalskii* and *P. versicolor* have also shown that high incubation temperature of 34°C induced a low hatching success (only 36% in *P. przewalskii* and 11%–22% in *P. versicolor*). Compared with the previous work that embryos of *P. versicolor* (Wulatehouqi population) were well developed at 32°C with the hatching success rate being 75.9% (Qu *et al.*, 2011), we speculate that the eggs' upper temperature limit of temperature for *P. versicolor* egg incubation may be slightly higher than 34°C.

The dependence of neonatal morphology and behavior on thermal conditions during embryogenesis is common, and it has been reported on a diverse array of snakes (Burger, 1989, 1990; Du and Ji, 2008; Mickelson and Downie, 2010), lizards (VanDamme *et al.*, 1992; Braña and Ji, 2000; Booth, 2006) and turtles (Booth, 1998; Eiby and Booth, 2011). Reptile eggs incubated at relatively low and/or moderate temperatures produce well-developed (larger) hatchlings after long incubation time (Gutzke and Packard, 1987; Van Damme *et al.*, 1992; Elphick and Shine, 1998; Ji and Zhang, 2001; Du and Ji, 2002; Qu *et al.*, 2011). The results of the present study provide a support for the previous conclusions. Hatchling size has been thought to be an important determinant of fitness in many lizards. Larger hatchlings may have advantages in hunting prey and avoiding predators (Van Damme *et al.*, 1992; Braña and Ji, 2000; Ji and Zhang, 2001), and may have prior access to limited resources and better chances to survive the first active season and winter (Ferguson and Fox, 1984; Garland *et al.*, 1990). Incubation temperatures might modify locomotor ability of hatchling *P. przewalskii* and *P. versicolor* as well. Hatchlings incubated at 26 and 30°C were well-developed and had better performance in the racetrack than those incubated at 34°C. High incubation temperatures often produce negative effects on the development of hatchling morphology and fitness (Braña and Ji, 2000; Rodríguez-Díaz *et al.*, 2010). Our finding may indicate that hatchling locomotor ability could be optimized at low incubation temperatures in *P. przewalskii* and *P. versicolor*. However, after 30 days of growth no significant difference in body mass was found between the hatchlings from different incubation conditions. It could be deduced that the disparity of initial body mass can be compensated by the differential growth rates in these two species and more field-based investigations on early stage growth would provide verification.

Compared to *P. przewalskii*, *P. versicolor* exhibited a shorter incubation period and yielded smaller hatchlings under the same condition, which suggested that ecological factors could potentially affect the embryogenesis. The habitat of *P. versicolor* is cooler (annual mean temperature: -3.1°C vs. -2.7°C) and drier (average mean rainfall: 280 mm vs. 370 mm) than that of *P. przewalskii*. The shorter incubation period could arise from faster development or advanced development before oviposition (Du *et al.*, 2010).

Optimum incubation temperature is normally defined as the temperature required to achieve maximum hatchability. In considering the length of incubation

and the quality of the hatchlings, we conjecture that the temperature close to 30°C is optimum for incubation of both species. On one hand, eggs incubated at high temperature induced a low hatching success. On the other hand, eggs incubated at low temperatures would have longer incubation time but shorter growth period before the onset of winter dormancy (Elphick and Shine, 1998; Du and Ji, 2002; Rodríguez-Díaz *et al.*, 2010), which may also delay reproductive maturity and affect lifetime fitness (Warner and Shine, 2007). Moreover, low incubation temperatures in the nests could affect hatchling survival because the longer the hatching is delayed, the longer the eggs are exposed to adverse biotic or abiotic factors (Rodríguez-Díaz *et al.*, 2010). Eggs incubated at 30°C have higher hatching success and shorter incubation time, and produced well-developed hatchlings with excellent locomotor performance. Thus, we could consider that the optimum incubation temperature is 30°C for the eggs of *P. przewalskii* and *P. versicolor*.

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