

## INFLUENCE OF FLUCTUATING INCUBATION TEMPERATURES ON HATCHLING TRAITS IN A CHINESE SKINK, *EUMECES CHINENSIS*

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Effects of fluctuating temperatures on reptilian eggs and hatchlings are poorly known. We carried out a fluctuating-temperature incubation experiment with two treatments ( $27 \pm 3^\circ\text{C}$  vs  $27 \pm 7^\circ\text{C}$ ) to determine the effects of temperature and clutch of origin on incubation duration and the morphology and locomotor performance of hatchlings in a skink, *Eumeces chinensis*. The variance of fluctuating temperatures had no influences on incubation duration, hatching success or hatchling traits. Incubation temperature accounted for over 10% of the total variation in incubation duration and hatchling traits, whereas clutch effects accounted for much more. The discrepancy between these results and those from previous studies may be due to interspecific differences in embryonic response to thermal variance.

*Key words:* egg incubation, maternal effect, temperature

Most embryonic development occurs outside the mother's body in oviparous reptiles, and incubation environments thus can influence egg survival rates and the phenotypic traits of hatchlings (Deeming & Ferguson, 1991). Temperature is one of the most important such environmental factors, and can profoundly affect incubation duration, hatching success, and hatchling traits such as morphology, behaviour, growth and gender (Deeming & Ferguson, 1991; Packard & Packard, 1988; Burger, 1990; Janzen & Paukstis, 1991). Constant-temperature incubation experiments in the laboratory have been conducted on many reptile species. In general, eggs incubated at moderate temperatures produce relative large hatchlings that perform better than those from low or high temperatures (e.g. Packard & Packard, 1988; Van Damme *et al.*, 1992; Phillips & Packard, 1994; Ji & Du, 2001; Ji & Zhang, 2001; Du & Ji, 2003; Du *et al.*, 2003).

In natural nests of reptiles, however, temperatures fluctuate considerably on a daily and seasonal basis (Shine *et al.*, 1996). Therefore, constant-temperature incubation may be biological unrealistic. More recently, some investigators have conducted incubation experiments either in the laboratory or in natural nests to

determine the effect of fluctuating temperatures on incubating eggs and resultant hatchlings (e.g. Shine *et al.*, 1996, 1997; Ashmore & Janzen, 2003; Shine, 2004). These studies suggest that both the variance and the mean of incubation temperatures significantly affect developmental rates and hatchling traits. Nonetheless, few such studies have been conducted and thus, we do not know the generality of any influence of fluctuating temperatures on phenotypic traits of hatchling reptiles. In the current study, we conducted a fluctuating-temperature incubation experiment to determine the effects of thermal regimes on incubation duration, hatchling morphology and locomotor performance in a Chinese skink, *Eumeces chinensis*.

*Eumeces chinensis* is a small (in our study, snout-vent length: 100.5-134.0 mm), diurnal, oviparous skink widely distributed in the central and southern provinces of China (Zhao & Adler, 1993). In May 2003, we captured four gravid females by hand from Quzhou, Zhejiang, eastern China. The animals were housed in a terrarium (110 × 90 × 50 cm) that had been filled with sand and grass to mimic the natural habitat where the lizards were captured. A 60W light bulb was suspended 15 cm above the terrarium floor as a supplementary heating source from 0700-1700 hr. Food (larvae of *Tenebrio molitor*) and water (containing mixed vitamins and minerals) were provided *ad libitum*. Each female constructed a nest individually under the grass where she later deposited a clutch of eggs. The terrarium was checked three times per day for the presence of laid eggs. All eggs were weighed immediately to the nearest mg to minimize any change in egg mass due to water exchange between eggs and their surroundings.

Clutch sizes of the four females were 8-13 eggs; and a total of 37 eggs were viable. Each clutch was split into two treatments. The eggs were incubated at  $27 \pm 3^\circ\text{C}$  and  $27 \pm 7^\circ\text{C}$ , respectively. The temperatures in the incubators were programmed to fluctuate over 24 hr. For  $27 \pm 3^\circ\text{C}$  treatment, temperatures increased or decreased  $1^\circ\text{C}$  every two hours; for  $27 \pm 7^\circ\text{C}$ , temperatures increased or decreased about  $1^\circ\text{C}$  per hour. Temperatures inside the containers were recorded every hour by TINYTALK dataloggers (Gemini Pty, Australia). Prior to being transferred into incubators, eggs were placed in containers filled with moist vermiculite substrates (water potential of -12 kPa; Du *et al.*, 2003). We weighed all containers daily and added water to compensate for evaporative losses and water absorbed by eggs throughout the incubation period, such that the water potential of the substrate remained constant.

After the first hatchling pipped its eggshell, containers were checked at least five times each day. Each hatchling was weighed and its locomotor performance was tested immediately after it had hatched. Because locomotor performance is highly sensitive to body temperature in reptiles, we performed trials after placing hatchlings in an incubator at  $30^\circ\text{C}$  for 30 min prior to testing (Du *et al.*, 2003). We assessed locomotor capac-

ity by chasing the lizards along a 1.5 m racetrack with a paintbrush. The locomotor performance of each lizard was recorded with a Panasonic NV-MX3 digital video camera. Videotapes were then examined for sprint speed in the fastest 150 mm interval and maximum distance in a dash.

After the locomotor tests, we euthanized these hatchlings, and took the following measurements: SVL, tail length, head size (length and width), and limb length (including toe length). Subsequently, the hatchlings were dissected into carcass and residual yolk. The two components were oven-dried to constant mass at 65 °C, and weighed.

Hatching success (80.0%, 12/15) of eggs incubated at 27±3 °C was similar to that (86.4%, 19/22) of eggs kept at 27±7 °C (*G*-test, *G*=0.05, *df*=1, *P*>0.05). We analyzed data from these eggs using mixed model multivariate analysis of covariance where temperature was a fixed factor, clutch of origin was a random factor and egg mass was the covariate. These analyses indicated that incubation temperature did not significantly affect incubation duration or hatchling traits (Table 1). Due to low sample size, we conducted a further analysis that regarded each hatchling as an independent sample to test the effects of temperature on hatchling traits, acknowledging that this may not be statistically appropriate. The results also showed that the hatchling traits were not affected by the temperature regimes. To identify the relative magnitude of effects due to temperature *versus* clutch effects, we calculated the percentage variation in hatchling traits due to clutch, temperature and their interaction from the sums of squares in two-way ANCOVAs where egg mass or hatchling SVL was used as the covariate. Temperature effects only accounted for a small amount of the overall variation in incubation duration and hatchling traits,

with no more than 10 % in most traits. In contrast, clutch (maternal) effects accounted for most of the variation in incubation length and hatchling traits (Table 2).

Our study suggests that thermal variation during incubation does not influence hatchling traits in *E. chinensis*. Given that the air temperatures of the locality where the skinks were captured average 25.0 °C and 29.1 °C during June and July when the natural incubations mostly occur (Quzhou Bureau of Meteorology), we believe that the mean temperatures experienced by eggs in natural nests may largely fall in the range of 24~30 °C. Over this range, mean incubation temperatures do not affect hatching traits in this species (Ji & Zhang, 2001). Taken together, these results suggest that the nest temperature exerts relatively little effect on hatchling traits in *E. chinensis*. Instead, most variation in hatchling traits stems from genetic sources and/or maternal effects (Table 2).

Empirical evidence for the influence of fluctuating temperatures on reptile eggs and resultant hatchlings is limited to studies on relatively few species, and such effects are far from universal (Overall, 1994; Shine *et al.*, 1997; Doody, 1999; Andrews *et al.*, 2000; Ashmore & Janzen, 2003). The incubation duration of *E. chinensis* is independent of diel thermal fluctuation, at least over the range of conditions in our study. This result is consistent with that from *Sceloporus undulatus* (Andrews *et al.*, 2000), but not those from other species, in which incubation duration increased (Shine *et al.*, 1997; Ashmore & Janzen, 2003) or decreased (Overall, 1994) with increasing variation in the fluctuating temperatures. Fluctuating temperatures had significant effects on hatchling size and/or locomotor performance in most species of reptiles that have been studied in this respect (Shine, *et al.*, 1997; Doody, 1999; Andrews *et al.*, 2000; Ashmore & Janzen, 2003; Shine, 2004). On the con-

TABLE 1. ANCOVA results of temperature effects on hatchling traits of *Eumeces chinensis*. ANCOVA with temperature as a fixed factor and clutch of origin as a random factor (Temperature effect 1), and ANCOVA with individual hatchlings as independent samples (Temperature effect 2), were conducted respectively. Egg mass was used as the covariate in analyses of incubation duration, snout-vent length, body mass, carcass and residual yolk. Hatchling SVL was used as the covariate in tail length, head size, limb length and locomotor performance.

	Temperature effect 1	Temperature effect 2
Incubation length	$F_{1,3}=0.98, P=0.37$	$F_{1,28}=0.33, P=0.57$
Snout-vent length	$F_{1,3}=0.12, P=0.76$	$F_{1,28}=0.10, P=0.75$
Wet body mass	$F_{1,3}=0.65, P=0.48$	$F_{1,28}=1.84, P=0.19$
Dry body mass	$F_{1,3}<0.0001, P=0.99$	$F_{1,28}<0.0001, P=0.99$
Carcass	$F_{1,3}=0.05, P=0.84$	$F_{1,28}=0.07, P=0.79$
Residual yolk	$F_{1,3}=0.12, P=0.76$	$F_{1,28}=0.15, P=0.70$
Tail length	$F_{1,3}=0.008, P=0.94$	$F_{1,28}=0.02, P=0.88$
Head length	$F_{1,3}=0.22, P=0.69$	$F_{1,28}=0.17, P=0.68$
Head width	$F_{1,3}=1.60, P=0.33$	$F_{1,28}=0.70, P=0.41$
Forelimb length	$F_{1,3}=2.01, P=0.18$	$F_{1,28}=1.67, P=0.21$
Hindlimb length	$F_{1,3}=0.16, P=0.75$	$F_{1,28}=0.07, P=0.79$
Maximum distance	$F_{1,3}=0.30, P=0.64$	$F_{1,28}=0.23, P=0.63$
Sprint speed	$F_{1,3}=1.64, P=0.22$	$F_{1,28}=0.004, P=0.95$

TABLE 2. Percentage variation in hatchling traits of *Eumeces chinensis* due to clutch, temperature and interaction respectively. Data were calculated from the sums of squares in two-way ANCOVA. Egg mass was used as the covariate in analyses of incubation duration, snout-vent length, body mass, carcass and residual yolk. Hatchling SVL was used as the covariate in tail length, head size, limb length and locomotor performance.

	Clutch (%)	Temp. (%)	Interaction (%)
Incubation length	96.07	3.32	0.61
Snout-vent length	71.75	0.30	27.95
Wet body mass	69.71	0.01	30.28
Dry body mass	83.02	5.28	11.70
Carcass	81.63	1.83	16.54
Residual yolk	73.52	11.07	15.41
Tail length	62.00	2.39	35.61
Head length	48.55	5.47	45.98
Head width	69.57	1.24	29.19
Forelimb length	65.42	21.30	13.28
Hindlimb length	54.43	0.01	45.56
Maximum distance	61.25	5.29	33.46
Sprint speed	83.61	7.30	9.09

trary, our results indicated that hatchling traits were not affected by a wide range of variance of fluctuating temperatures ( $\pm 3\text{ }^{\circ}\text{C} \sim \pm 7\text{ }^{\circ}\text{C}$ ) in *E. chinensis*, though the sample size is low in this study. This discrepancy may represent inter-specific differences in embryonic response to fluctuating temperatures.

It is worth mentioning that most eggs incubated at fluctuating temperatures in our study hatched successfully, even though these eggs briefly experienced extremely low and high temperatures (20~34 °C) each day. Such temperatures induce high embryonic mortality when the eggs were incubated at constant temperatures (Ji & Zhang, 2001). This result supports the conclusion that exposure of eggs to adversely low or high temperatures for short periods may not increase embryonic mortality (Overall, 1994; Andrews *et al.*, 1997; Du & Ji, 2003).

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