JOURNAL OF EXPERIMENTAL ZOOLOGY 309A:138–146 (2008)

A Journal of Integrative Biology

Phenotypic Effects of Thermal Mean and Fluctuations on Embryonic Development and Hatchling Traits in a Lacertid Lizard, *Takydromus septentrionalis*

WEI-GUO DU* AND JIA-HUA FENG
Hangzhou Key Laboratory for Animal Science and Technology, School of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, People's Republic of China

ABSTRACT How fluctuating temperatures influence reptilian embryos and hatchlings has attracted increasing scientific attention, but is poorly known. We conducted an incubation experiment with a factorial design of two mean temperatures (24 vs. 28°C) and three diel thermal fluctuations (0, ±3, ±6°C) to determine the effects of diel thermal fluctuations and mean temperature on incubation duration and hatchling phenotypes. Both diel thermal fluctuations and mean temperature significantly affected incubation duration, but not hatching success. Incubation duration increased with increasing temperature fluctuations at a mean temperature of 24°C, but not at a mean temperature of 28°C. The significant interaction between diel thermal fluctuations and mean temperature on hatchling morphology indicated that the effect of thermal fluctuations depended on the mean temperature. Hatchling mass differed significantly between 24±6 and 28±6°C, but not between the two constant temperatures or the temperatures with ±3°C fluctuations. At a mean temperature of 24°C, the effect of thermal fluctuations on hatchling size was marginally significant, with relatively large hatchlings at the constant temperature; at a mean temperature of 28°C, thermal fluctuations had no impact on hatchling size. The locomotor performances were significantly affected by mean temperature rather than diel thermal fluctuations. Therefore, diel thermal fluctuations around a given mean temperature do not affect hatchling phenotypes in a relatively large magnitude, but the influence of diel thermal fluctuations may differ with changing mean temperatures. *J. Exp. Zool. 309A:138–146, 2008.*

How to cite this article: Du W-G, Feng J-H. 2008. Phenotypic effects of thermal mean and fluctuations on embryonic development and hatchling traits in a lacertid lizard, *Takydromus septentrionalis*. J. Exp. Zool. 309A:138–146.

Phenotypic plasticity is critical to our understanding of the ecological processes that involve interaction between environment and organisms. Not only the genotype and reproductive strategies of a mother but also developmental plasticity play an important role to account for phenotypic variation (Shine, 2004a). Environmental factors facing organisms during development are important causes that can induce such phenotypic variations. For example, ambient temperatures may substantially affect developmental time and offspring traits in ectotherms (Hagstrum and Milliken, '91; Liu et al., '95; Deeming, 2004).

As ectotherms, oviparous reptiles provide us excellent model systems to test for the effects of physical condition (in particular, temperature) on offspring phenotypes, because the embryo develops mainly outside the mother's body, and environmental factors such as temperature can thus profoundly affect incubation duration, hatching success, and hatchling traits including morphology, behavior, growth, and gender (Deeming and Ferguson, '91; Janzen and Paukstis, '91; Deeming,

---

Grant sponsor: Zhejiang Province for the Key Discipline of Zoology and Hangzhou Municipal Award for Talents.

*Correspondence to: Wei-Guo Du, Hangzhou Key Laboratory for Animal Science and Technology, School of Life and Environmental Sciences, Hangzhou Normal University, 310036, Hangzhou, Zhejiang, People's Republic of China. E-mail: dwghz@126.com

Received 29 July 2007; Revised 29 November 2007; Accepted 26 December 2007

DOI: 10.1002/jez.442

2004). To date, the evidence that constant temperatures affect egg incubation and hatchling traits is overwhelming (Deeming, 2004). However, owing to the complexity in temporal fluctuations of temperatures experienced by eggs in natural nests, the results from constant-temperature incubation cannot be directly applied to natural systems. In recent years, the topic that how fluctuating temperatures influence reptilian embryos and hatchlings has attracted increasing scientific attention (Shine and Harlow, '96; Shine et al., '97; Ashmore and Janzen, 2003; Georges et al., 2005; Du and Ji, 2006; Les et al., 2007). These studies demonstrated that the effects of fluctuating temperatures on egg incubation are more complicated than those of constant temperatures, and both mean and variance of fluctuating temperatures, significantly affect developmental rate and hatchling traits. For instance, mathematical models predicted that the influence of fluctuating temperatures on developmental rate would depend on thermal mean and thermal fluctuations as well (Georges et al., 2005); in a turtle (*Caretta caretta*) with temperature-dependent sex determination, hatchling sex was determined by the proportion of development that occurred above the threshold temperature for sex determination instead of the proportion of time spent above it (Georges et al., '94).

By now, investigators have developed three different methods to detect the effect of fluctuating temperatures on reptilian eggs and hatchlings: egg incubation experiments in natural nests (e.g., Packard et al., '93; Shine et al., '97; Shine et al., 2003), artificial nests (e.g., Castilla and Swallow, '96; Du and Ji, 2003, 2006; Hao et al., 2006), and programmable incubators (e.g., Ashmore and Janzen, 2003; Du et al., 2005b). To disentangle the effects of diel thermal fluctuations and mean temperature on hatchling phenotypes, egg incubation with a factorial design in programmable incubators should be a better choice than field incubation experiments in natural or semi-natural nests, which are not able to tease apart the effect of thermal mean from that of diel thermal fluctuations. Moreover, the field experiments include some confounding factors such as different genetic origins of eggs and/or varying moistures. By contrast, these background noises can be largely controlled by using a split-clutch design and by keeping constant the substrate moisture in incubation experiments at the laboratory. Therefore, such studies would contribute to our understanding of the underlying mechanism whereby fluctuating temperatures induce phenotypic variation in reptilian hatchlings.

Following the above-mentioned idea, we conducted an incubation experiment with a factorial design of two mean temperatures (24 vs. 28°C) and three diel thermal fluctuations (0, ±3, ±6°C) in the laboratory to test for the effects of diel thermal fluctuations and mean temperature on embryonic development and hatchling traits in northern grass lizards (*Takydromus septentrionalis*) from eastern China. This species was used as a subject in our study because phenotypic variation has been addressed in a detailed manner for hatchlings from various constant temperatures as well as an artificial nest (Lin and Ji, '98; Du and Ji, 2006). These results would form a solid background information for the current incubation experiment and facilitate us to further explore the mechanism underneath the effects of fluctuating temperatures on egg incubation and resultant hatchlings. Therefore, our study would provide insights into the effects of diel thermal fluctuations and mean temperature on incubation duration, egg survival, and hatchling phenotypes in lizards.

MATERIALS AND METHODS

Study species

The northern grass lizard, *T. septentrionalis*, is a small (snout–vent length (SVL) of adults ranging from 55 to 80 mm) oviparous lizard, which occupies open grassy areas in the mountainous region of the central and southern provinces of China (Zhao and Adler, '93). From April to August, female *T. septentrionalis* lays clutches of one–five flexible-shelled eggs (Du et al., 2005a). Laboratory and artificial nest incubation experiments have demonstrated that both constant and fluctuating temperatures can significantly affect incubation duration, hatchling size, and sprint speed (Lin and Ji, '98; Du and Ji, 2006).

Thermal profiles of potential nests and experimental design

Given the difficulty in locating nests of *T. septentrionalis* in nature, we measured soil temperatures in their natural habitat to quantify thermal profiles of potential nest sites. In May and July of 2005, we monitored soil temperatures of two typical habitats (dense and scarce vegetations) at 1 hr intervals by placing thermal data loggers (Tinytalk, Gemini data loggers Ltd., Chichester, UK) at a depth of 5 cm (Fig. 1). Given the

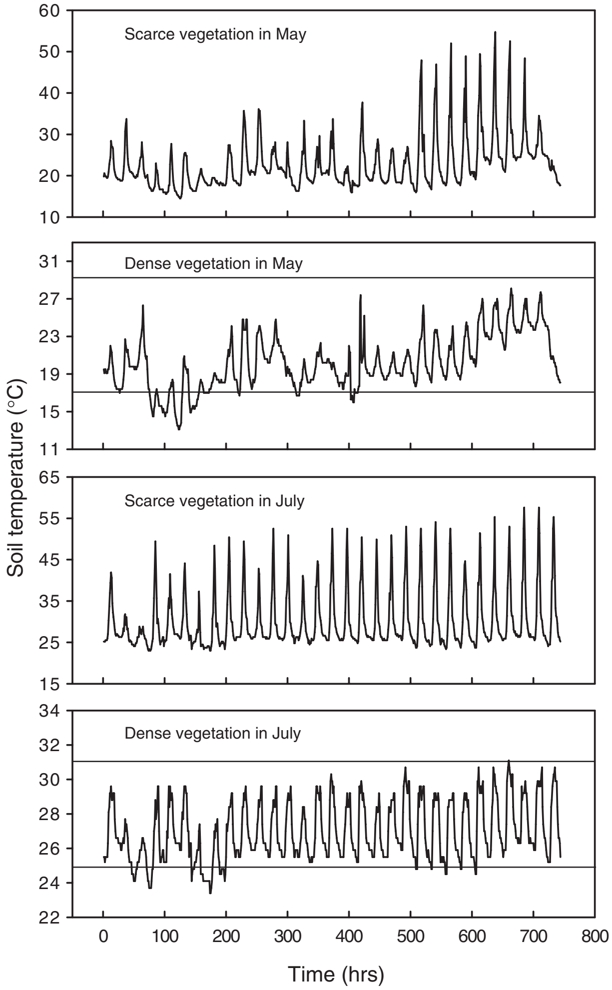

Fig. 1. Thermal features of natural habitats used by the northern grass lizard, *Takydromus septentrionalis*. The graphs show soil temperatures in scarce and dense vegetation during May and July. The reference lines in graphs of dense vegetation in May and July indicate the fluctuating magnitudes of temperatures (24±6 and 28±3°C) mimicked in the laboratory.

extremely high temperatures at open areas that were beyond the maximum thermal tolerance of the embryos in this species (Du and Ji, 2006), these areas were not likely to be used by females as oviposition sites. Instead, females would potentially build their nests in areas with dense vegetation. On the basis of thermal profiles of potential natural nests, we used fluctuating temperatures of 24±6°C to mimic the thermal environment of potential nests in May (Fig 1b) and 28±3°C to mimic that of potential nests in July (Fig. 1d). Accordingly, we designed a 2 mean temperatures (24 vs. 28°C) × 3 diel thermal fluctuations (0, ±3, ±6°C) factorial experiment to detect the effects of diel thermal fluctuations and mean temperature on hatchling traits.

Collection and husbandry of lizards

In April 2006, we captured a total of 45 adult *T. septentrionalis* (30 females and 15 males) by noose from Quzhou, Zhejiang, eastern China. The lizards were taken to our laboratory in Hangzhou, and were housed in 60 × 40 × 30 cm terraria. Each terrarium contained ten females plus five males. We provided 5 cm-thick sand and grass on the bottom of terrarium to mimic the natural habitat where the lizards were collected, and a 60 W light bulb at the central of terrarium as a supplementary heating source from 0700 to 1700 hr. Sufficient food (larvae of *Tenebrio molitor*) and water (containing mixed vitamins and minerals) were provided ad libitum. We palpated the females every 5 days, and transferred those females with shelled oviductal eggs into 20 × 15 × 20 cm glass terraria individually. The glass terraria were filled with 2 cm-depth moist sand in which the females could produce their eggs.

Incubating eggs and measuring hatchling traits

We checked the small terraria five times daily to collect freshly laid eggs. The eggs were weighed promptly to minimize the egg mass change owing to loss or gain of water. A total of 128 eggs were incubated in six programmable incubators (Ningbo Life Science and Technology Ltd, Ningbo, China) in which the temperatures were set at constant temperatures of 24 and 28°C or fluctuating temperatures of 24±3, 24±6, 28±3, and 28±6°C, respectively. The three and six degrees of fluctuations were set on 24-hr cycles. Eggs from a single clutch were randomly assigned to different temperature treatments to minimize the family effect. We incubated eggs in jars containing vermiculite with water potential of −12 kPa (Lin and Ji, ’98), and the jars were placed on the same shelf and moved in a predetermined schedule to avoid potential influence of thermal gradient inside the incubators. We weighed all jars every two days and added water to compensate for evaporative losses and water absorbed by eggs. Thermocron iButton temperature loggers (DS1921, ±1°C, MAXIM Integrated Products/Dallas Semiconductor Ltd, Sunnyvale, CA) were put into the jars to record the exact thermal environment experienced by eggs (Fig. 2).

Toward the end of incubation(Lin and Ji, ’98; Du and Ji, 2006), we checked the incubators and

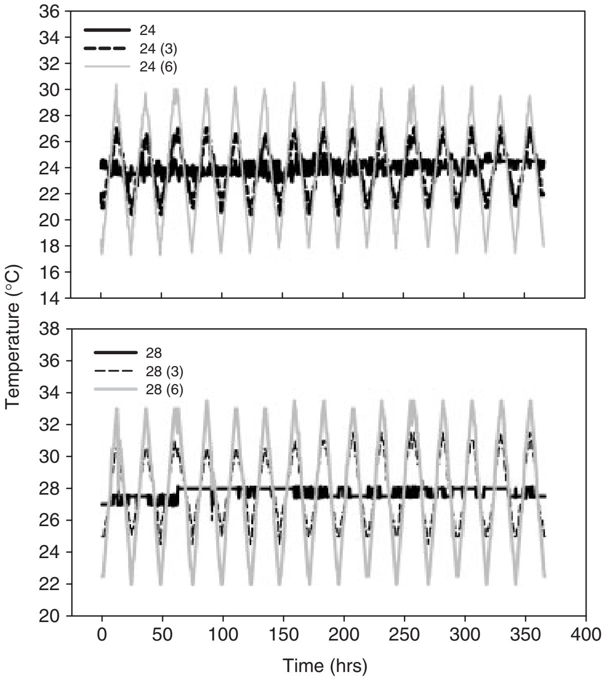

Fig. 2. Temperature profiles of incubation environments that *Takydromus septentrionalis* eggs experienced in the programmable incubators within 15 days.

collected hatchlings at least three times a day. Each hatchling was weighed, SVL and tail length were measured locomotor performance was determined. We assessed locomotor capacity by chasing the lizards along a 1 m racetrack with a paintbrush after the hatchlings had been placed into an incubator at 30°C for 30 min (Lin and Ji, '98; Du and Ji, 2006). The locomotor performance of each lizard was recorded with a Panasonic NV-MX3 digital video camera (Beijing, China). We then transferred the video to a computer to measure the sprint speed in the fastest 250 mm interval and the average speed in the 1 m racetrack. After the test, the hatchlings and their parents were released to the sites where they were originally captured.

Data analysis

We used *G* test to check the influence of temperature regimes on hatching success (Zar, '99). The effects of diel thermal fluctuations and mean temperature on incubation duration and hatchling morphology were detected using the analysis of covariance (ANCOVA) with initial egg mass as the covariate, and a further individual ANCOVA on temperature regimes with same mean temperature or fluctuations were conducted, respectively, in these cases the interaction between diel thermal fluctuations and mean temperature was significant. We also performed a two-way ANCOVA with hatchling SVL as the covariate to determine whether diel thermal fluctuations and mean temperature affect the body shape and locomotor performance of hatchlings.

TABLE 1. Incubation duration and hatching success of hatchlings at different temperature regimes in the northern grass lizard, Takydromus septentrionalis

Temperature regime (°C)	Mean temperature (°C)	Hatching success (%)	Incubation duration (d)
24	24.0	18/22 (81.8)	42.5±0.2
24±3	24.0	21/24 (87.5)	43.8±0.3
24±6	23.7	15/19 (79.0)	45.8±0.3
28	27.8	18/21 (85.7)	30.0±0.2
28±3	28.0	15/20 (75.0)	30.2±0.2
28±6	27.7	18/22 (81.8)	30.5±0.2

Data on incubation duration are expressed as mean±SE (range). Mean temperatures were calculated from the iButton temperature data in each incubator.

RESULTS

Mean temperature, diel thermal fluctuations, and their interaction significantly affected incubation duration (mean temperature, $F_{1,98} = 4620.1$, $P < 0.00001$; diel thermal fluctuations, $F_{2,98} = 29.5$, $P < 0.00001$; interaction, $F_{2,98} = 16.9$, $P < 0.00001$), but initial egg mass did not influence incubation duration ($F_{1,98} = 0.16$, $P = 0.69$). Given the significant interaction between diel thermal fluctuations and mean temperature, we further conducted separate ANCOVAs for each mean temperature. At a mean temperature of 24°C, diel thermal fluctuations significantly affected incubation duration, with longer incubation duration at larger thermal fluctuations ($F_{2,50} = 33.3$, $P < 0.00001$); at a mean temperature of 28°C, however, diel thermal fluctuations had no significant impact on incubation duration ($F_{2,47} = 1.3$, $P = 0.27$; Table 1). Hatching success was equally high for eggs incubated at different temperature regimes (*G* test, $G = 1.47$, df = 5, $P > 0.05$; Table 1).

A preliminary analysis indicated that SVL, tail length, and body mass of hatchlings from different incubation treatments were positively correlated with initial egg mass (all $P < 0.05$). After the influence of initial egg mass had been removed statistically, both diel thermal fluctuations and mean temperature did not significantly

TABLE 2. Effects of diel thermal fluctuations and mean temperature on hatchling traits in the northern grass lizard, Takydromus septentrionalis

	Diel thermal fluctuations		Mean temperature		Interaction	
	$F_{2,98}$	P	$F_{1,98}$	P	$F_{2,98}$	P
Snout–vent length	0.94	0.40	0.62	0.44	2.64	0.08
Body mass	0.17	0.85	0.97	0.33	3.83	0.03
Tail length	1.71	0.18	3.16	0.08	1.90	0.15
Average speed	1.23	0.30	8.15	0.005	0.00	0.99
Sprint speed	1.77	0.18	9.80	0.002	0.34	0.71

A two-way ANCOVA with initial egg mass or hatchling snout–vent length as the covariate was employed on morphological traits or locomotor performances, respectively. ANCOVA, analysis of covariance.

TABLE 3. Individual ANCOVA results on snout–vent length, body mass, and tail length of hatchling Takydromus septentrionalis

	Snout–vent length	Body mass	Tail length
Thermal fluctuation effect at 24°C	$F_{2,50} = 2.85$, $P = 0.07$	$F_{2,50} = 2.04$, $P = 0.14$	$F_{2,50} = 2.02$, $P = 0.14$
Thermal fluctuation effect at 28°C	$F_{2,47} = 0.60$, $P = 0.55$	$F_{2,47} = 1.95$, $P = 0.15$	$F_{2,47} = 0.04$, $P = 0.96$
Thermal mean effect at constant temperature	$F_{1,33} = 1.32$, $P = 0.25$	$F_{1,33} = 1.77$, $P = 0.19$	$F_{1,33} = 1.08$, $P = 0.31$
Thermal mean effect at fluctuating temperature (±3°C)	$F_{1,33} = 0.06$, $P = 0.80$	$F_{1,33} = 2.94$, $P = 0.10$	$F_{1,33} = 1.11$, $P = 0.30$
Thermal mean effect at fluctuating temperature (±6°C)	$F_{1,30} = 3.62$, $P = 0.07$	$F_{1,30} = 4.88$, $P = 0.03$	$F_{1,30} = 3.44$, $P = 0.07$

The analyses were conducted on the treatments of thermal mean and fluctuations separately, and initial egg mass was used as the covariate. ANCOVA, analysis of covariance.

affect SVL, tail length, or body mass (Table 2). Interestingly, the interaction between diel thermal fluctuations and mean temperature was significant on body mass, and marginally significant on SVL (Table 2). Separate ANCOVAs on different treatments of thermal mean and fluctuations demonstrated that the influence of diel thermal fluctuations on SVL was marginally significant at 24°C, with relatively larger hatchlings from the constant temperature than from fluctuating temperatures (Table 3; Fig. 3); hatchling size and mass did not differ significantly between 24 and 28°C when eggs were incubated at constant and fluctuating (±3°C) temperatures, but showed substantial differences between the two temperatures with wider fluctuations (±6°C) (Table 3; Fig. 3). In addition, the diel thermal fluctuations and mean temperature did not affect the body shape of the lizards. When SVL was controlled constant, neither body mass (mean temperature, $F_{1,98} = 0.21$, $P = 0.65$; diel thermal fluctuations, $F_{2,98} = 0.78$, $P = 0.46$; interaction, $F_{2,98} = 1.82$, $P = 0.17$) nor tail length (mean temperature, $F_{1,98} = 2.48$, $P = 0.12$; diel thermal fluctuations, $F_{2,98} = 1.15$, $P = 0.32$; interaction, $F_{2,98} = 0.60$, $P = 0.55$) varied among temperature regimes.

Locomotor performances (both sprint speed and average speed) of the lizards were significantly correlated with their SVL (all $P < 0.05$). The locomotor performances were profoundly affected by mean temperature (Wilks-$F_{2,97} = 5.27$, $P < 0.01$), but not by diel thermal fluctuations (Wilks-$F_{4,194} = 1.52$, $P = 0.20$). Also, no significant interaction on locomotor performances was found between diel thermal fluctuations and mean temperature (Wilks-$F_{4,194} = 0.34$, $P = 0.85$). Hatchlings from 24°C had higher sprint speed and average speed than their counterparts from 28°C (Fig. 4).

To identify the relative importance of the effects caused by diel thermal fluctuations, mean temperature, or their interaction, we calculated the percentage variation in hatchling traits owing to diel thermal fluctuations, mean temperature, and their interaction from the sums of squares in two-way ANCOVAs where egg mass or hatchling SVL was used as the covariate. The interaction between diel thermal fluctuations and mean temperature accounted for the majority of the variations in hatchling size (SVL and body mass), whereas mean temperature effects accounted for most of the variations in locomotor performances (Table 4).

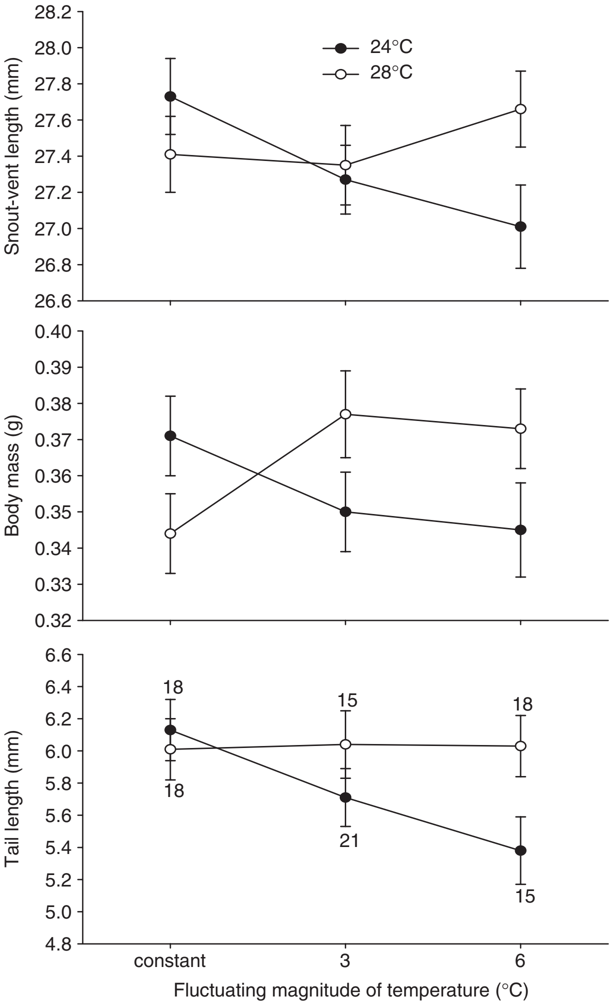

Fig. 3. Snout–vent length, body mass, and tail length of hatchling *Takydromus septentrionalis* incubated at different thermal regimes. Data are expressed as adjusted mean ± SE. Initial egg mass was used as the covariate in an ANCOVA, and was set at 0.304 g to calculate the adjusted means. Numbers up or below the error bar in the lower graph are sample sizes and apply to all graphs within this figure.

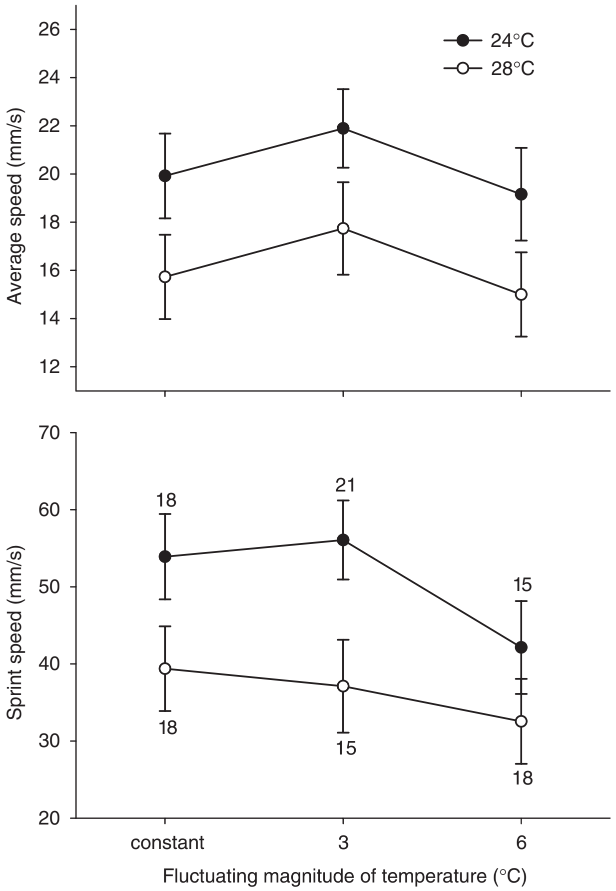

Fig. 4. Locomotor performance (average and sprint speeds) of hatchlings in *Takydromus septentrionalis* incubated at different thermal regimes. Data are expressed as adjusted mean ± SE. Hatchling snout–vent length was used as the covariate in an ANCOVA and was set at 27.42 mm to calculate the adjusted means. Numbers up or below the error bar in the lower graph are sample sizes and apply to all graphs within this figure.

DISCUSSION

The mean and variance of temperatures are two important features of thermal environments. Previous studies have suggested that both of them have significant effects on incubating eggs and hatchlings in reptiles (Deeming, 2004). Our incubation experiment with a factorial design in the lizard *T. septentrionalis* demonstrates that (1) the interaction between diel thermal fluctuations and mean temperature is significant on embryonic development and hatchling morphology, the effects of diel temperature fluctuations are significant when the mean temperature is kept at 24°C rather than 28°C; the difference in hatchling morphology is evident between the two mean temperatures with large fluctuations (±6°C), but not between the constant temperatures or those with low fluctuations (±3°C); (2) in accord with the previous studies on this species (Lin and Ji, '98; Du and Ji, 2006), constant temperatures significantly affect incubation duration but not hatchling morphology within the range of 24–28°C.

Diel thermal fluctuations significantly affected incubation duration when eggs were incubated at thermal regimes with mean temperature of 24°C but not at those with mean temperature of 28°C, with longer incubation duration at more fluctuating temperatures. This result is consistent with former studies on this species (Du and Ji, 2006) and other reptilian species as well (Shine et al., '97; Ashmore and Janzen, 2003; Hao et al., 2006).

TABLE 4. The percentages of variations in hatchling traits of Takydromus septentrionalis owing to thermal mean and fluctuations, and their interaction, respectively. Data were calculated from the sums of squares in two-way ANCOVAs

	Diel thermal fluctuations (%)	Mean temperature (%)	Interaction (%)
Snout–vent length	24.09	7.90	68.01
Body mass	3.72	10.87	85.41
Tail length	33.00	30.42	36.58
Average speed	23.17	76.83	0.00
Sprint speed	25.29	69.83	4.88

Egg mass was used as the covariate in analyses of snout–vent length, body mass, and tail length. Hatchling SVL was used as the covariate in locomotor performance. ANCOVA, analysis of covariance; SVL, snout–vent length.

Some other studies, however, show that increasing temperature fluctuations reduce (Overall, '94; Shine and Harlow, '96) or does not affect (Andrews et al., 2000; Webb et al., 2001; Du et al., 2005b) incubation duration. Similarly, the effects of fluctuating temperature on insect development are also conflicting among species. Developmental times at constant temperatures are generally shorter above the temperature range of 25–30°C, but longer below this range than at fluctuating temperatures with the same means, whereas there are no significant differences in developmental times in some species (Hagstrum and Milliken, '91; Liu et al., '95).

The relationship between development rate and temperature is linear over a range of moderate temperatures, within which the impact of diel thermal fluctuations on developmental time does not differ considerably from that of constant temperatures (Sharpe and Demichele, '77). Such consistency, however, does not exist any more at the extreme temperatures and the differential effects of diel thermal fluctuations would become evident (Georges et al., 2005). The effect of diel thermal fluctuations on development time thus depends on the mean of fluctuating temperatures. In our study, 24±6°C treatment rather than 24±3°C treatment could take the embryo to temperatures that may inhibit or retard its development for part of each day and hence led to a longer incubation duration than a constant temperature of 24°C. By contrast, both 28±3 and 28±6°C treatments might not expose the embryos to extremely high temperatures that could considerably inhibit their development; accordingly incubation durations at these two treatments were not significantly different from that at a constant temperature of 28°C. Therefore, for a certain species, the effect of fluctuating temperatures on development time is not only affected by thermal regimes but also associated with thermal sensitivity of embryonic development. For future studies, it is worthy to determine the developmental zero and the thermal reaction norm for developmental rate, which could differ among species and thus cause inter-specific differences in the response of embryonic development to fluctuating temperatures.

The available evidence on the influence of fluctuating temperature that comes from three kinds of experiments, egg incubation in natural nests, artificial nests, and programmable incubators, is limited by now and varies among different species. Firstly, fluctuating temperatures in natural or artificial nests changed seasonally, and the effects of fluctuating temperature on hatchling traits (either morphology or performance) are basically significant (Overall, '94; Shine et al., '97; Shine et al., 2003; Shine, 2004b; Du and Ji, 2006). Secondly, programmable incubators provide embryos thermal regime with fixed diel fluctuations, and the experimental results are sometimes conflicting: the effects of fluctuating temperature on hatchling traits are significant in some reptilian species (Shine and Harlow, '96; Doody, '99; Ashmore and Janzen, 2003), but not in others (Andrews et al., 2000; Du et al., 2005b). The contrast results among different species or experiments imply that (1) the reaction norm of embryos on fluctuating temperature may differ among species, and thus more work on various species would be appreciated for completely understanding the effects of fluctuating temperature; (2) the thermal environments that eggs experience during incubation are actually different between natural nests and programmable incubators. Given that the effects of fluctuating temperature on hatchling traits depend on the proportion of development at a particular temperature and the extremely high and low temperatures are able to inhibit the development of embryos (Georges et al., '94, 2005), the differences in thermal regimes between the two methods may in turn induce variation in hatchling phenotypes.

This study provides explanations to the underlying mechanism of the phenomenon indicated by our previous artificial nest incubation that thermal fluctuations are positively correlated with locomotor performance, but not with hatchling SVL and mass (Du and Ji, 2006). The mean temperatures and diel thermal fluctuations in the artificial nest ranged from 23.4 to 27.2°C and from 6.8 to 11.7°C, respectively (Du and Ji, 2006). Most, if not all, hatchlings from such temperature regimes would not differ considerably in morphology according to the results from this study (Table 3). Therefore, the non-significant relationship between thermal fluctuations and hatchling SVL and mass discovered in the artificial nest incubation is reasonable. Nonetheless, this by no means de-emphasizes the importance of interaction between thermal mean and fluctuations, which suggests that the effect of thermal fluctuations depends on thermal means (Table 3). For example, hatchling mass differed significantly between 24±6 and 28±6°C, but not between the two constant temperatures or the temperatures with ±3°C fluctuations. This is because, at 24±6 and 28±6°C, *T. septentrionalis* embryos may experience extremely high and/or low temperatures that probably have different impacts on hatchling traits compared with moderate temperatures and in turn cause the difference in hatchling size between them. Such interactions on hatchling traits have also been demonstrated in other reptilian species (Mullins and Janzen, 2006). Given the non-significant effects of diel thermal fluctuations on locomotor performances, the positive relationship between thermal fluctuations and locomotor performance found in the artificial nest incubation does not get support from this study. Potential explanations to this discrepancy are that this positive relationship may stem from (1) the effects of mean temperatures and/or seasonal change in temperature fluctuations; or (2) genetic difference among individuals used in the artificialnest incubation rather than thermal effects, i.e., in the nest, early eggs experiencing wider temperature fluctuations may also intrinsically have better performances (Olsson and Shine, 1997).

Given the significant influence of fluctuating temperatures on embryonic development and hatchling traits in *T. septentrionalis* (Du and Ji, 2006; this study), thermal environments of nests selected by the mother would play an important role in determining hatchling fitness. Apparently, a mother may determine the nest environments and in turn affect hatchling traits and fitness by selecting the time of reproduction and the location of nests. For example, eggs from early clutches are probably exposed to low temperatures and thus have long incubation period; nests covered by dense vegetation may have lower and less variable temperatures than those under scarce vegetation. This implies that nest selection by a female can be an important ecological process that would impose profound impacts on offspring survival and thus the sustainability of population.

In conclusion, diel thermal fluctuations may retard embryonic development and thus induce longer incubation duration, but such effects largely depend on the mean temperature that the thermal fluctuations surround. The influences of diel thermal fluctuations on hatchling phenotypes are not significant within a relatively large magnitude of diel fluctuations, but may differ with the changing mean temperatures. Therefore, this study emphasizes that the mean and variance of fluctuating temperatures interact intimately to impose significant influence on embryonic development and offspring fitness in reptiles.

ACKNOWLEDGMENTS

We thank L.J. Zhu, H.Y. Hu for their assistance in the field and laboratory and two anonymous reviewers for their suggestive comments on an earlier version of this paper. The first author also thanks Rick Shine and the University of Sydney for providing the facilities and literatures during the preparation of the manuscript. This research was funded by grants from local government of Zhejiang Province for the Key Discipline of Zoology and Hangzhou Municipal Award for Talents. Animal collection and the experiments carried out in this study comply with the current laws of China.

LITERATURE CITED

Andrews RM, Mathies T, Warner DA. 2000. Effect of incubation temperature on morphology, growth, and survival juvenile *Sceloporus undulates*. Herpetol Monogr 14:420–431.

Ashmore GM, Janzen FJ. 2003. Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. Oecologia 134:182–188.

Castilla AM, Swallow JG. 1996. Thermal dependence of incubation duration under a cycling temperature regime in the lizard, *Podarcis hispanica* atrata. J Herpetol 30:247–253.

Deeming DC. 2004. Reptilian incubation: environment and behaviour. Nottingham: Nottingham University Press.

Deeming DC, Ferguson MWJ. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: Deeming DC, Ferguson MWJ, editors. Egg incubation, its effect on embryonic development in birds and reptiles. Cambridge: Cambridge University Press. p 147–171.

Doody JS. 1999. A test of the comparative influences of constant and fluctuating incubation temperatures on phenotypes of hatchling turtles. Chelon Conserv Biol 3:529–531.

Du WG, Ji X. 2003. The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*. J Therm Biol 28:279–286.

Du WG, Ji X. 2006. Effects of constant and fluctuating temperatures on egg survival and hatchling traits in the northern grass lizard (*Takydromus septentrionalis,* Lacertidae). J Exp Zool 305A:47–54.

Du WG, Ji X, Zhang YP, Xu XF, Shine R. 2005a. Identifying sources of variation in reproductive and life-history traits among five populations of a Chinese lizard (*Takydromus septentrionalis*, Lacertiade). Biol J Linnean Soc 85:443–453.

Du WG, Shou L, Shen JY, Lu YW. 2005b. Influence of fluctuating incubation temperatures on hatchling traits in a Chinese skink, *Eumeces chinensis*. Herpetol J 15: 139–142.

Georges A, Limpus C, Stoutjesdijk R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily length of exposure. J Exp Zool 270:432–444.

Georges A, Beggs K, Young JE, Doody JS. 2005. Modelling development of reptile embryos under fluctuating temperature regimes. Physiol Biochem Zool 78:18–30.

Hagstrum DW, Milliken GA. 1991. Modeling differences in insect developmental times between constant and fluctuating temperatures. Ann Entomol Soc Am 84:369–379.

Hao QL, Liu HX, Ji X. 2006. Phenotypic variation in hatchling Mongrolian racerunners *Eremias argus* from eggs incubated at constant versus fluctuating temperatures. Acta Zool Sin 52:1049–1057.

Janzen FJ, Paukstis GL. 1991. Environmental sex determination in reptiles: ecology, evolution, and experimental design. Q Rev Biol 66:149–179.

Les HL, Paitz RT, Bowden RM. 2007. Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. J Exp Zool 307A:274–280.

Lin ZH, Ji X. 1998. The effects of thermal and hydric environments on incubating eggs and hatchlings of the grass lizard, *Takydromus septentrionalis*. Zool Res 19: 439–445.

Liu SS, Zhang GM, Zhu J. 1995. Influence of temperature variations on rate of development in insects: analysis of case studies from entomological literature. Ann Entomol Soc Am 88:107–119.

Mullins MA, Janzen FJ. 2006. Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. Herpetologica 62:27–36.

Olsson M, Shine R . 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. J Evol Biol 10:369–381.

Overall KL. 1994. Lizard egg environments. In: Vitt LJ, Pianka ER, editors. Lizard ecology: historical and experimental perspectives. Princeton: Princeton University Press. p 51–72.

Packard GC, Miller K, Packard MJ. 1993. Environmentally induced variation in body size of turtles hatching in natural nests. Oecologia 93:445–448.

Sharpe PJH, Demichele DW. 1977. Reaction kinetics of poikilotherm development. J Theor Biol 64:649–670.

Shine R. 2004a. Adaptive consequences of developmental plasticity. In: Deeming DC, editor. Reptilian incubation: environment, evolution and behaviour. Nottingham: Nottingham University Press. p 187–210.

Shine R. 2004b. Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. Funct Ecol 18:43–49.

Shine R, Harlow PS. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. Ecology 77:1808–1817.

Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. Ecology 78:2559–2568.

Shine R, Elphick MJ, Barrott EG. 2003. Sunny side up: lethally high, not low, nest temperatures may prevent oviparous reptiles from reproducing at high elevations. Biol J Linnean Soc 78:325–334.

Webb JK, Brown GP, Shine R. 2001. Body size, locomotor speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii*, Colubridae): the influence of incubation environments and genetic factors. Funct Ecol 15: 561–568.

Zar JH. 1999. Biostatistical analysis. New York: Prentice-Hill Inc.

Zhao E, Adler K. 1993. Herpetology of China. Oxford, OH: SSAR.