

Phenotypic variation in hatchling Chinese ratsnakes (*Zaocys dhumnades*) from eggs incubated at constant temperatures

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ABSTRACT

We incubated eggs of the Chinese ratsnake *Zaocys dhumnades* at four constant temperatures (24, 27, 30 and 30 °C) to examine the effects of incubation temperature on hatching success and hatchling phenotypes. Incubation length increased nonlinearly as temperature decreased, with the mean incubation length being 76.7 d at 24 °C, 57.4 d at 27 °C, 47.3 d at 30 °C, and 44.1 d at 33 °C. Hatching successes were lower at the two extreme temperatures (69% at 24 °C, and 44% at 33 °C) than at the other two moderate temperatures (96% at 27 °C, and 93% at 30 °C). Incubation temperature affected nearly all hatchling traits examined in this study. Incubation of *Z. dhumnades* eggs at 33 °C resulted in production of smaller hatchlings that characteristically had less-developed carcasses but contained more unutilized yolks. Hatchlings from eggs incubated at 27 and 30 °C did not differ in any examined traits. Taking the rate of embryonic development, hatching success and hatchling phenotypes into account, we conclude that the temperature range optimal for incubation of *Z. dhumnades* eggs is narrower than the range of 24–33 °C but should be wider than the range of 27–30 °C.

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1. Introduction

Incubation or gestation temperature affects many aspects of development in reptiles, with inappropriate temperatures having adverse effects on embryos and offspring phenotypes. For example, temperatures over the upper or lower threshold of the range where successful embryonic development can take place not only increase embryonic mortality but also have adverse effects on a wide variety of offspring phenotypes including size, morphology, behavior, physiology, growth, survival and sex in TSD (temperature-dependent sex determination) species (Birchard, 2004; Deeming, 2004; Booth, 2006; Janzen and Phillips, 2006). Unlike viviparous females that have the potential to manipulate their offspring's phenotypes by behavioral thermoregulation, oviparous females do not control thermal conditions for their eggs (except for those brooding eggs; Wang, 1966; Shine et al., 1997a, b). Therefore, in oviparous species not brooding eggs, a female's choice of a thermally appropriate nest site can be a very efficient way through which she selects thermal environments optimal for embryonic development.

The range of temperatures optimal for reptilian embryos is relatively narrow and may vary not only among but also within

species, being broadly correlated with a species' or a population's natural incubation environment (Overall, 1994; Booth, 2004; Deeming, 2004). For example, the optimal incubation temperatures fall within the range from 24 to 26 °C in the checkered keelback *Xenochrophis piscator* (Ji et al., 2001; Lu et al., 2009) and the five-paced pit-viper *Deinagkistrodon acutus* (Lin et al., 2005) from relatively cool habitats but, in the red-banded wolf snake *Dinodon rufozonatum* (Ji et al., 1999; Zhang and Ji, 2002), the king ratsnake *Elaphe carinata* (Ji and Du, 2001a), the stripe-tailed ratsnake *Elaphe taeniura* (Du and Ji, 2008), the gray ratsnake *Ptyas korros* (Du and Ji, 2002), the mucous ratsnake *P. mucosus* (Lin and Ji, 2004), the red-necked keelback *Rhabdophis tigrinus lateralis* (Chen and Ji, 2002) and the Chinese cobra *Naja atra* (Ji and Du, 2001b; Lin et al., 2008) from relatively warm habitats, generally within the range from 26 to 30 °C. In the Chinese skink *Eumeces chinensis*, eggs from a lower latitudinal population have a narrower range of optimal incubation temperatures than do those from a higher latitudinal population, primarily because of more stable thermal environments in the former population (Ji et al., 2002).

The Chinese ratsnake *Zaocys dhumnades* is a large sized (up to 2500 mm total length) oviparous colubrid snake endemic to China (Zhao, 1998). Fecundity, reproductive output and embryonic mobilization of energy and material during embryogenesis at one incubation temperature have been studied using snakes from an island population in Dinghai (30°02'N, 122°10'E), Zhoushan Islands, eastern China (Ji et al., 2000; Du et al., 2007). Eggs of

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Z. dumnades have never been incubated at multiple temperatures. Therefore, the range of incubation temperatures optimal for developing embryos of *Z. dumnades* is currently unknown. To fill this gap, we incubated eggs at four constant temperatures ranging from 24 to 33 °C. Our aims are (1) to examine the influence of incubation temperatures on hatching success and hatchling phenotypes, and (2) to estimate the range of optimal temperatures for embryonic development.

2. Materials and methods

Ten gravid females of *Z. dumnades* [100–128 mm SVL (snout-vent length); 210–420 g post-oviposition body mass] were collected in June 2005 from a previously studied population in Dinghai, which is approximately 25 km away from the nearest mainland coastline. Females were placed singly in cloth bags and transported to our laboratory in Hangzhou, where they were housed individually in 600 × 600 × 600 mm wire cages. These cages were placed in an indoor animal holding facility where the temperature was controlled within the range from 24 to 30 °C. Food [black-spotted frogs (*Pelophylax nigromaculata*) and rice frogs (*Fejervarya limnocharis*)] was provided *ad libitum*. We checked the cages at least four times daily for freshly laid eggs as soon as the first female laid eggs, such that eggs could be always collected, measured and weighed within 3 h after they were laid. Eggs were individually measured (to the nearest 0.01 mm) for length and width with a Mitutoyo digital caliper and weighed (to the nearest 1 mg) on a Mettler top loading balance. The viability or fertility of freshly laid eggs was judged by the presence of a reddish embryonic disc using a spotlight. Body mass, SVL and tail length were taken for each post-oviposition female.

Eggs were incubated systematically at four constant temperatures, such that eggs from single clutches were distributed as equally as possible among the four temperature treatments. Eggs were individually placed in covered plastic containers (250 ml) with known amounts of vermiculite and distilled water at about –12 kPa water potential (dried vermiculite: water=1:2). One-third of the egg was buried lengthwise in the incubating substrate, with the surface near the embryo exposed to air inside the container. Containers were assigned to four Shellab incubators (Sheldon MFG Inc., USA), with temperatures set at 24, 27, 30 and 33 (± 0.3) °C, respectively. Containers were moved among the shelves in the incubator daily according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. Incubation temperatures in close proximity to eggs were monitored twice daily using a WMZ-3 digital thermometer (Shanghai Medical Instrument, China). Eggs were weighed at 5-d intervals, and the final egg mass was taken 1 d prior to hatching. Containers were weighed every other day, and distilled water was added into the substrate when necessary to compensate for evaporative losses and water absorbed by eggs, thereby maintaining the substrate water potential constant.

The duration of incubation, measured as the number of days to pipping, was recorded for each egg. Hatchlings were collected, measured for SVL and tail length, and weighed a few hours after hatching, and then euthanized by freezing to –15 °C for determination of composition and sex. The killed hatchlings were separated into carcass, residual yolk and fat bodies. The three components of the hatchling were dried in an oven (65 °C) to constant mass, weighed and preserved frozen for later analyses. We determined the sex of hatchlings by pressing on both sides of the ventral tail base with forceps to record the presence or absence of hemipenes; hatchlings with everted hemipenes were recorded as males.

All data were analyzed using the statistics package Statistica (version 5.0 for PC), and data on hatchlings of the same sex were blocked by clutch to avoid pseudo-replication. We used *G* test to examine whether the sex ratio of hatchlings differed among the four treatments. Between-sex differences in incubation length within each treatment and differences in egg mass among the four treatments were examined using one-way analysis of variance (ANOVA). We used Kruskal–Wallis test to examine the differences in incubation length among the four treatments. Changes in egg mass during incubation were analyzed using repeated-measures ANOVA with time step as the within subject factor and temperature treatment as the between subject factor. We used a multivariate analysis of covariance (MANCOVA) with egg mass at oviposition as the covariate or multivariate analysis of variance (MANOVA) to examine the effects of the same factors (sex and incubation treatment) and their interaction on the examined hatchling traits. We used a partial correlation analysis to examine the relationships between the selected pairs of hatchling variables while holding the remaining relevant variables constant. Prior to MANCOVA or MANOVA, the assumption of homogeneity of variances was tested at univariate (Bartlett's test) and multivariate (Box's *M* test) levels. A principal component analysis was used to show positions of hatchlings from different incubation temperatures on a two-dimensional plane. Throughout the paper, values are presented as mean \pm 1 standard error, the significance level is set at $\alpha=0.05$.

3. Results

3.1. Incubation length, hatching success and sex ratio of hatchlings

The 10 females laid clutches ranging in size from 9 to 14 between 28 June and 14 July. Of the 106 eggs incubated, 80 hatched (Table 1). Hatching successes were apparently lower at the two extreme temperatures (24 and 33 °C) than at the other two moderate temperatures (Table 1). Incubation temperature did not affect the sex phenotype of hatchlings ($G=4.12$, $df=3$, $P>0.10$). Incubation length was independent of egg mass (simple linear regression; all $P>0.121$), and did not differ between the sexes within each treatment (one-way ANOVA; all $P>0.201$). The mean values for incubation length differed among the four treatments ($H_{3,N=67}=61.53$, $P<0.0001$). Incubation length decreased as incubation temperature increased, but not in a linear pattern. The mean value shortened by 19.3 d from 24 to 27 °C, by 10.1 d from 27 to 30 °C, and by 3.2 d from 30 to 33 °C (Table 1).

3.2. Temporal changes in egg mass

The mean values for initial egg mass did not differ among the four treatments ($F_{3,59}=0.23$, $P=0.875$) and between the sexes ($F_{1,59}=0.39$, $P=0.534$), and the temperature \times sex interaction was not a significant source of variation in egg mass ($F_{3,59}=1.75$, $P=0.166$). Temporal variation in egg mass was evident within each treatment (repeated-measures ANOVA, all $P<0.0001$), with eggs gaining net mass during incubation because of absorption of water in all treatments. The mean values for final egg mass differed among the four treatments (ANCOVA with initial egg mass as the covariate, $F_{3,62}=21.73$, $P<0.0001$), with eggs at the two extreme temperatures gaining more mass than did those at the two moderate temperatures (Tukey's *post-hoc* test, all $P<0.0002$) (Fig. 1).

Table 1
Effects of incubation temperature on incubation length, hatching success and the sex ratio of hatchlings.

Temperature (°C)	Incubated eggs	Incubation length (d) ^a	Hatching success (%)	Sex ratio (♀/♂)
24	26	76.7 ± 0.3 (74.7–79.7)	69.2 (18/26)	10/8
27	26	57.4 ± 0.2 (56.1–60.8)	96.2 (25/26)	18/7
30	27	47.3 ± 0.1 (45.6–48.8)	92.6 (25/27)	11/14
33	27	44.1 ± 0.2 (43.0–44.9)	44.4 (12/27)	7/5

Data on duration of incubation are expressed as mean ± SE (range).

^a Data from the same clutch are blocked.

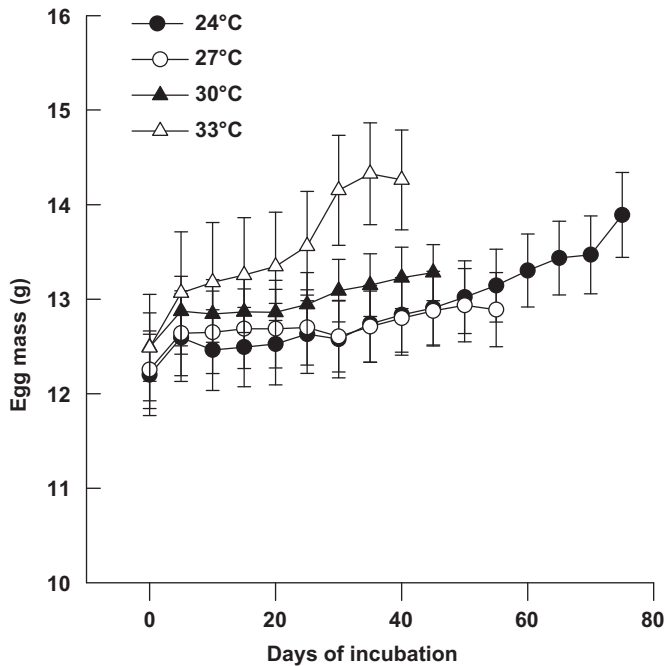


Fig. 1. Temporal changes in mass of *Zaocys dhumnades* eggs incubated at four constant temperatures. Data are expressed as mean ± SE.

3.3. Size, mass and composition of hatchlings

Snout-vent length, tail length, body mass and carcass dry mass were affected by incubation temperature (Wilks' $\lambda=0.172$, $df=15$, 149 , $P<0.0001$), but not by sex (Wilks' $\lambda=0.888$, $df=5$, 54 , $P=0.253$) and the temperature \times sex interaction (Wilks' $\lambda=0.877$, $df=15$, 140 , $P=0.945$). Snout-vent length, tail length and carcass dry mass were smaller in the 33 °C treatment than in other three treatments (Tukey's *post-hoc* test, all $P<0.0002$); body wet mass was greatest in the 24 °C treatment and smallest in the 33 °C treatment, with the other two treatments in between (Tukey's *post-hoc* test, all $P<0.05$); body dry mass did not differ among the four treatments (Tukey's *post-hoc* test, all $P>0.269$) (Table 2). Residual yolk dry mass and fat body dry mass were affected by incubation temperature (Wilks' $\lambda=0.212$, $df=6$, 116 , $P<0.0001$), but not by sex (Wilks' $\lambda=0.999$, $df=2$, 58 , $P=0.969$) and the temperature \times sex interaction (Wilks' $\lambda=0.957$, $df=6$, 116 , $P=0.861$). Residual yolk dry mass was greatest in the 33 °C treatment and smallest in the 30 °C treatments, with the 24 and 27 °C treatments in between; fat body dry mass was smaller in the 24 °C treatment than in the other three treatments (Tukey's *post-hoc* test, all $P<0.001$). A series of partial correlation analysis showed that: hatchling SVL was positively correlated with carcass dry mass ($r=0.79$, $t=10.37$, $df=63$, $P<0.0001$) and fat body dry mass ($r=0.39$, $t=3.41$, $df=63$, $P<0.001$), but negatively correlated with residual yolk dry mass ($r=-0.32$, $t=2.72$, $df=63$, $P<0.001$);

carcass dry mass was not correlated with residual yolk dry mass, nor with fat body dry mass (both $P>0.05$); residual yolk dry mass was not correlated with fat body dry mass ($P>0.05$).

A principal component analysis resolved two components (with eigenvalues ≥ 1) from seven hatchling variables, accounting for 76.1% of the variation in the original data (Table 3). The first component (52.9% variance explained) had high negative loadings for the egg size-free values of SVL, tail length, hatchling wet mass, hatchling dry mass and carcass dry mass, and the second component (23.2% variance explained) had a high positive loading for the egg size-free value of residual yolk dry mass (Table 3). Hatchlings incubated at the four temperatures differed in their scores on both the first (ANOVA, $F_{3,63}=16.77$, $P<0.0001$; 24^b, 27^b, 30^b, 33^a, Tukey's *post-hoc* test, $a>b$) and second axes (ANOVA, $F_{3,63}=14.16$, $P<0.0001$; 24^a, 27^b, 30^b, 33^a, Tukey's *post-hoc* test, $a>b$), and could be divided into three groups: hatchlings incubated at 27 and 30 °C, hatchlings incubated at 24 °C, and hatchlings incubated at 33 °C (Fig. 2).

4. Discussion

Consistent with studies of other oviparous reptiles (Birchard, 2004; Deeming, 2004), the results of this study show that incubation temperature affects hatching success, incubation length, and body dimensions (SVL and tail length) and body composition of hatchlings in *Z. dhumnades*. Incubation temperature did not affect the sexual phenotype of hatchlings, thus signifying that *Z. dhumnades* is among reptiles where the sexual phenotype of offspring is not determined by embryonic temperature (Janzen and Phillips, 2006). Our study also adds evidence that incubation of eggs at relatively low or moderate temperatures produces larger hatchlings (Lu et al., 2009).

Incubation of *Z. dhumnades* eggs at constant temperatures revealed that incubation length decreased nonlinearly as incubation temperature increased (Table 1). Such a pattern is widespread among reptiles, although the mean incubation length at a given temperature may differ among species that differ in phylogeny, egg size and/or embryonic stage at oviposition (Table 4). For example, embryonic stages at oviposition, grouping around Zehr's (1962) stage 27, do not differ between the multi-banded krait *Bungarus multicinctus* and the Chinese cobra *Naja atra*, but the mean incubation lengths at identical temperatures are shorter in *B. multicinctus* than in *N. atra* (85.0 versus 92.4 d at 24 °C, and 41.0 versus 46.1 d at 30 °C; Table 4) largely because eggs are much smaller in the former species (~ 5 –11 g; Ji et al., 2007) than in the latter species (~ 12 –29 g; Ji and Wang, 2005). The five-paced pit-viper *D. acutus* provides another example where noticeably short incubation lengths (36.4 d at 24 °C, and 15.7 d at 30 °C) result primarily from that females retain eggs for prolonged periods of time before oviposition (ZH Lin et al., 2005).

Incubation of reptilian eggs at high temperatures accelerates embryonic development (Deeming, 2004). Nonetheless, as in

Table 2
Descriptive statistics for size, mass and composition of hatchlings incubated at different temperatures.

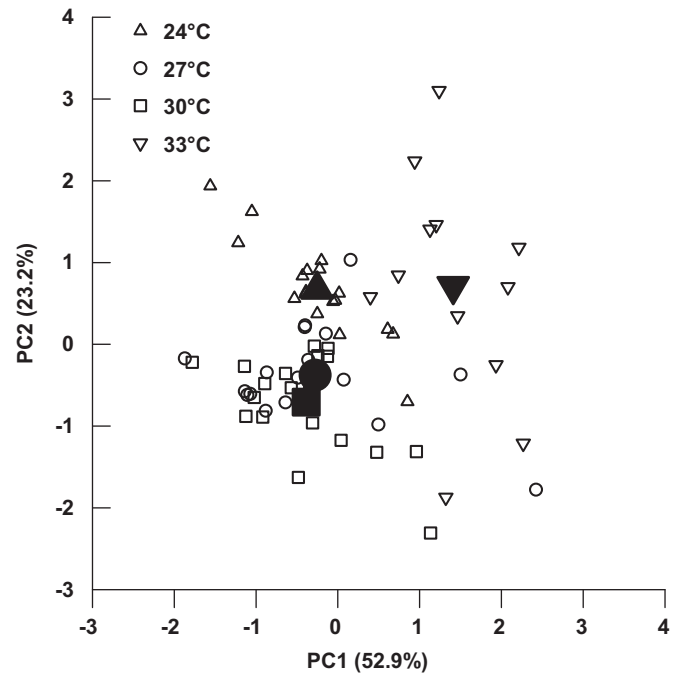
Variable	Incubation temperatures (°C)							
	24	27	30	33	24	27	30	33
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
N	10	8	10	7	10	10	7	5
Initial egg mass (g)	12.7 ± 0.6 9.8–15.5	11.5 ± 0.5 10.4–14.4	11.9 ± 0.5 10.0–14.5	12.7 ± 0.7 10.0–15.1	12.5 ± 0.5 9.7–15.2	12.5 ± 0.5 9.7–15.2	11.8 ± 0.6 10.11–13.8	13.4 ± 1.1 10.0–16.0
Snout-vent length (mm)	281.7 ± 4.9 252.0–303.0	279.9 ± 4.3 262.0–291.0	283.9 ± 5.6 245.0–300.7	294.6 ± 5.4 265.0–306.0	288.2 ± 5.5 251.0–305.0	288.2 ± 5.5 251.0–305.0	247.0 ± 6.6 215.0–270.0	264.6 ± 9.1 230.0–281.2
Tail length (mm)	101.3 ± 1.8 91.0–108.0	97.8 ± 2.1 90.0–107.0	97.0 ± 2.5 83.5–110.0	100.9 ± 2.6 90.0–109.0	99.9 ± 2.5 89.0–112.5	99.9 ± 2.5 89.0–112.5	83.9 ± 4.0 70.0–103.0	88.0 ± 3.6 75.0–96.2
Hatchling wet mass (g)	8.6 ± 0.5 6.1–10.6	8.0 ± 0.4 6.8–10.0	7.8 ± 0.3 5.9–8.9	8.6 ± 0.5 6.8–10.4	8.1 ± 0.4 5.7–9.8	8.1 ± 0.4 5.7–9.8	7.66 ± 0.44 6.37–9.33	8.38 ± 0.64 6.52–10.32
Hatchling dry mass (g)	2.33 ± 0.12 1.56–2.81	2.13 ± 0.10 1.78–2.52	2.14 ± 0.09 1.60–2.53	2.36 ± 0.13 1.87–2.71	2.24 ± 0.11 1.51–2.72	2.24 ± 0.11 1.51–2.72	2.08 ± 0.13 1.69–2.58	2.30 ± 0.19 1.74–2.88
Carcass dry mass (g)	1.72 ± 0.09 1.17–2.15	1.58 ± 0.08 1.30–2.04	1.50 ± 0.07 1.05–1.76	1.65 ± 0.10 1.36–2.09	1.60 ± 0.07 1.15–1.93	1.60 ± 0.07 1.15–1.93	1.18 ± 0.07 0.99–1.53	1.39 ± 0.11 1.09–1.68
Residual yolk dry mass (g)	0.42 ± 0.03 0.22–0.53	0.39 ± 0.02 0.33–0.47	0.26 ± 0.03 0.15–0.38	0.32 ± 0.08 0.16–0.70	0.23 ± 0.02 0.14–0.40	0.23 ± 0.02 0.14–0.40	0.59 ± 0.08 0.39–1.00	0.55 ± 0.11 0.26–0.84
Fat body dry mass (g)	0.18 ± 0.02 0.11–0.28	0.16 ± 0.03 Nil–0.36	0.38 ± 0.03 0.20–0.48	0.40 ± 0.03 0.25–0.53	0.40 ± 0.03 0.23–0.51	0.40 ± 0.03 0.23–0.51	0.32 ± 0.03 0.23–0.44	0.36 ± 0.02 0.29–0.44

Table 3

Loading of the first two axes of a principal component analysis on seven hatchling variables.

	Factor loading	
	PC 1	PC 2
Snout-vent length	– 0.886	–0.282
Tail length	– 0.884	–0.140
Hatchling wet mass	– 0.745	0.592
Hatchling dry mass	– 0.736	0.569
Carcass dry mass	– 0.889	–0.027
Residual yolk dry mass	0.440	0.844
Fat body dry mass	–0.243	–0.370
Variance explained (%)	52.9	23.2

Size effects are removed in all cases by using residuals from the regressions on initial egg mass. Variables with the main contribution to each factor are in bold.

**Fig. 2.** Positions of hatchlings from eggs incubated at four constant temperatures in the space defined by the first two axes of a principal component analysis based on seven hatchling variables. Effects of egg size were removed using residuals from the regressions of corresponding variables on initial egg mass. Larger black symbols show the mean values of scores on the two axes.

other reptilian taxa, incubation temperatures that maximize the rates of embryonic development do not maximize hatching success and offspring phenotypes in snakes (Lu et al., 2009 and included references). Theoretically, maternal fitness is maximized in females producing the greatest number of surviving young (Bernardo, 1996; Agrawal et al., 2001; Roff, 2002). Therefore, the highest embryonic mortality at 33 °C provides an inference that maternal fitness would be substantially reduced in *Z. dhumnades* if females deposit their eggs in nests at temperatures close to 33 °C. Incubation of *Z. dhumnades* eggs at 33 °C also resulted in production of smaller hatchlings shorter in SVL and tail length (Table 2). In snakes, body dimensions at hatching are tightly associated with the developmental condition of carcass, with hatchlings from eggs incubated at high temperatures often having less-developed (and thus, lighter) carcasses but more unutilized (residual) yolks (Lu et al., 2009 and included references). The finding that more residual yolks remained unutilized at 33 °C

Table 4
The mean incubation lengths and hatching successes (in parentheses) of 10 species of oviparous snakes at different incubation temperatures.

Species	Incubation temperatures (°C)										References
	22	24	26	27	28	30	32	33			
<i>Dinodon rufozonatum</i> (red-banded wolf snake)	N	76.6 (81)	N	N	47.6 (61)	46.1 (77)	N	N	ji et al. (1999); Zhang and Ji (2002)		
<i>Eliaphis carinata</i> (king ratsnake)	N	74.0 (72)	58.1 (85)	N	N	44.4 (91)	42.6 (67)	N	Ji and Du (2001a)		
<i>Eliaphis taeniura</i> (stripe-tailed ratsnake)	N	86.0 (78)	N	66.3 (79)	N	53.9 (79)	50.5 (41)	N	Du and Ji (2008)		
<i>Pituophis melanoleucus</i> (pine snake)	N	101.0 (27)	73.4 (87)	N	N	60.0 (97)	58.0 (77)	54.1 (–)	Burger et al. (1987); Burger (1991)		
<i>Phyas korros</i> (gray ratsnake)	N	99.0 (75)	72.2 (87)	N	N	54.7 (91)	48.7 (53)	N	Du and Ji (2002)		
<i>Phyas mucosus</i> (mucous ratsnake)	N	105.4 (90)	N	78.0 (100)	N	57.8 (100)	N	51.3 (79)	ZH Lin and Ji (2004)		
<i>Rhabdophis tigrinus lateralis</i> (red-necked keelback snake)	N	45.0 (100)	N	32.7 (100)	N	27.3 (96)	N	26.0 (17)	Chen and Ji (2002)		
<i>Xenochrophis piscator</i> (checkered keelback snake)	N	66.8 (77)	50.0 (87)	48.8 (89)	N	36.7 (75)	32.2 (30)	F	Ji et al. (2001); Lu et al. (2009)		
<i>Bungarus multicinctus</i> (multi-banded krait)	N	85.0 (87)	N	61.6 (80)	N	41.0 (87)	N	N	Ji et al. (2007)		
<i>Naja atra</i> (Chinese cobra)	F	92.4 (18)	66.6 (81)	N	59.8 (88)	46.2 (82)	42.4 (56)	F	Ji and Du (2001b); LH Lin et al. (2008)		
<i>Demagkistrodon acutus</i> (five-paced pit-viper)	N	36.4 (91)	28.7 (90)	N	21.8 (85)	15.7 (48)	F	F	ZH Lin et al. (2005)		

N: temperatures at which eggs have never been incubated; F: temperatures at which eggs cannot be incubated.

suggests that *Z. dhumnades* shares this feature. Residual yolk can be used by hatchling snakes for maintenance and somatic tissue growth in their first post-hatching days, with the depletion of residual yolk always followed by a subsequent carcass growth (Ji et al., 1997, 1999; Ji and Sun, 2000). It is possible for small hatchling snakes to increase their body dimensions by using residual yolk, but this entails additional energetic costs associated with carcass growth (Ji et al., 1997, 1999; Ji and Sun, 2000; Du et al., 2007). Accordingly, we believe that *Z. dhumnades* embryos at 33 °C would at least consume more energy to grow to the size of embryos developing at the two moderate temperatures. The mean incubation length at 33 °C is approximately 3.2 d shorter than that at 30 °C in *Z. dhumnades*, suggesting that the advantage of the decreased incubation length and thus, increased growth period prior to the onset of the first winter due to an increase in incubation temperature from 30 to 33 °C is ecologically less important.

Though producing well-developed hatchlings (Table 2), incubation of eggs at 24 °C reduced hatching success (Table 1). Moreover, the majority of hatchlings incubated at 24 °C appeared in the second half of September, so the growth prior to the onset (November) of the first winter for these hatchlings would be less than 2 months if they were released to the field. Therefore, the disadvantage of incubating *Z. dhumnades* eggs at 24 °C could be ecologically significant. For reptilian eggs in natural nests, the prolonged incubation length at low temperatures increases exposure of eggs to the effects of adverse biotic (increased microbial contamination and predation risk) and abiotic factors (extreme thermal and hydric conditions) in the general environment of the eggs, which potentially reduces hatching success.

Eggs incubated at 27 and 30 °C also produced well-developed hatchlings that did not differ in any examined traits. The extremely high hatching successes provide strong evidence that these two temperatures fall within the range optimal for incubation of *Z. dhumnades* eggs. Taking the rate of embryonic development, hatching success and hatchling phenotypes into account, we conclude that the temperature range optimal for incubation of *Z. dhumnades* eggs is narrower than the range of 24–33 °C but should be wider than the range of 27–30 °C.

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