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The effects of thermal and hydric environments on hatching success, embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata*

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Abstract

We examined the effects of thermal and hydric environments on hatching success, the embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata*. The eggs were incubated at four temperatures ranging from 24 to 32°C on substrates with water potentials of 0 and −220 kPa using a 4×2 factorial design. Both thermal and hydric environments affected the water exchange between eggs and their surroundings. Eggs incubated in wetter substrates gained mass throughout the course of incubation, whereas eggs in drier substrates gained mass during the first half of incubation and lost mass thereafter. Hatching success was noticeably higher at 26 and 30°C than at 24 and 32°C, but among treatments, differences in hatching success were not significant. Temperature significantly affected the duration of incubation and most hatchling traits examined. Deformed hatchlings were found in all temperature treatments, with more deformities observed at 32°C. Hatchlings from eggs incubated at different temperatures differed in wet body mass, but the differences stemmed mainly from variation in water contents. Embryos at different temperatures completed development at nearly the same expenditure of energy and catabolized nearly the same amount of lipids, but hatchlings from different temperatures differed in the development condition of carcass at hatching. Hatchlings from eggs incubated at 26°C were larger in SVL than those from other higher or lower incubation temperatures, characteristically having larger carcasses; hatchlings from 32°C eggs were smaller in SVL and had smaller carcasses but larger residual yolks than those from lower incubation temperatures. Hatchlings from eggs incubated at 24°C were shorter in tail length but greater in size (SVL)-specific body wet mass than those from higher incubation temperatures. Within the range from −220 to 0 kPa, the substrate water potential did not affect hatching success, the embryonic use of energy and all hatchling traits examined, and the effects of temperature were independent of the effects of substrate water potential. Therefore, our data add evidence showing that embryonic development in reptiles with pliable-shelled eggs is relatively insensitive to variation in hydric environments during incubation.

Keywords: Reptilia; Squamata; Colubridae; *Elaphe carinata*; Egg; Incubation; Hatching success; Calorimetry; Hatchling trait

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1. Introduction

Thermal and hydric environments affect embryogenesis in reptiles (Packard, 1991; Overall, 1994). Theoretically, embryos can develop successfully at temperatures between the upper and lower limits of incubation temperatures, yielding viable hatchlings (Vinegar, 1974). A high incidence of abnormalities and failure of embryonic development occurs at extreme temperatures, so the proportion of abnormal hatchlings and hatching success can be used to assess the range of viable incubation temperatures for any given species (Ji and Braña, 1999; Lin and Ji, 1998; Ji et al., 1999b). The range of viable incubation temperatures may vary among and within species that differ in distribution and habitat use, and is generally wider in reptiles than in birds (Ferguson, 1985; Deeming and Ferguson, 1991; Lin and Ji, 1998; Ji et al., 1999b). Within this range, temperature may subtly affect the embryonic use of energy and thermally plastic traits of hatchlings (Lin and Ji, 1998; Ji and Braña, 1999; Ji et al., 1999b; Braña and Ji, 2000). Some influence could be ecologically and biologically important, and would, therefore, have a long-term effect on an individual's fitness (Webb and Cooper-Preston, 1989; Burger, 1991, 1998; Van Damme et al., 1992; Allstead and Lang, 1995; Shine, 1995; Shine et al., 1997a,b; Braña and Ji, 2000). In species with TSD (temperature sex determination), temperature also influences the sex ratio of hatchlings (Bull, 1980; Deeming and Ferguson, 1988; Janzen and Paukstis, 1991; Mrosovsky and Pieau, 1991).

Studies examining the effects of hydric environments on eggs and the hatchlings of reptiles are limited, and the conclusions reached seem to be contradictory. Some studies have shown pervasive effects of incubation hydric environments on hatching success, duration of incubation and some important hatchling traits (e.g. mass and linear measurements of hatchlings and size of residual yolk) (Lutz and Dunbar-Cooper, 1984; Packard and Packard, 1984, 1986, 1988; Gutzke and Packard, 1987; Packard, 1991). Others, however, considered that the influence of hydric environments on egg incubation in reptiles, if any, was less important (Tracy, 1980; Hotaling et al., 1985; Plummer and Snell, 1988; Ratterman and Ackerman, 1989; Lin and Ji, 1998; Ji and Braña, 1999; Ji et al., 1999b). This contradiction is not so surprising, however, and suggests that the eggs of different species respond differently to variations in hydric environments encountered during incubation. In addition, investigators using different experimental designs and types of eggs might reach different conclusions on the same topic.

Reptiles lay either pliable-shelled eggs or rigid-shelled eggs, and most squamate reptiles lay pliable-shelled eggs. Unlike rigid-shelled eggs, pliable-shelled eggs gain or lose mass rapidly due to the active exchange of water with their surroundings (Vleck and Hoyt, 1991; Vleck, 1991). Consequently, pliable-shelled eggs show greater temporal changes in mass during incubation than do rigid-shelled eggs, providing a greater variation in hydric conditions inside the egg (Vleck, 1991; Ji, 1992; Overall, 1994; Ji et al., 1996, 1997a,b; Lin and Ji, 1998; Ji and Braña, 1999). Due to this difference, we hypothesized that pliable-shelled eggs would be more sensitive to variations in hydric conditions than rigid-shelled eggs and would, therefore, be more suitable to test for the subtle influence of incubation hydric environments.

Elaphe carinata is a large sized colubrid snake, and females normally lay a single clutch with 5–17 pliable-shelled eggs (mean = 9.7) (Ji et al., 2000) per breeding season. The snake is widely distributed in southern China, including Taiwan, northward to the provinces of Henan, Shaanxi and Gansu; it is also found in northern Vietnam and Japan (Ryukyu Island, including the Senkaku Group) (Zhao and Adler, 1993). Previous work on egg incubation of this species focused on the embryonic use of energy and nutrients and the function of residual yolk. Results showed that *E. carinata* embryos use yolk as the source of all organic and most inorganic nutrients, and the eggshell as the additional source of calcium, and the residual yolk could be used to support the subsequent growth of newly emerged hatchlings during their first days of life (Ji et al., 1997a). However, the eggs used in previous work were incubated using just one combination of temperature and moisture, so the extent to which temperature, moisture and their interaction affect eggs and hatchlings remains unclear. Thus, we incubated *E. carinata* eggs at four temperatures ranging from 24 to 32°C on substrates with water potentials of 0 and -220 kPa using a 4×2 factorial design. These temperatures and water potentials were selected because they were mostly within the range of thermal and hydric environ-

ments found in the *E. carinata* nests that were located, although in natural nests, the thermal and hydric conditions normally fluctuate (Ji et al., unpublished data). Our aims were to examine the effects of incubation temperature, substrate moisture and their interaction on the embryonic use of energy and mass, linear measurements, morphology and the composition of newly emerged hatchlings.

2. Materials and methods

Forty-seven gravid female snakes [snout-vent length (SVL): 95.0–144.0 cm; post-oviposition body mass: 276.5–788.0 g] were collected in mid-June 1998 from Lishui and Jiande, Zhejiang, eastern China. The snakes were subsequently transported to our laboratory at Hangzhou Normal College, where they were randomly assigned $50 \times 45 \times 35$ cm^3 (length × width × height) wire cages of which each contained 1–2 individuals. We fed snakes with commercial quail (*Turnix tanki*) eggs and water enriched with vitamins and minerals. Cages were placed in an air-conditioned room, where the temperature varied from 26 to 30°C. Snakes laid eggs between 24 June and 19 July. Cages were checked at least twice every day for eggs and more frequently when females were seen to lay eggs. At oviposition, the eggs were numbered, measured and weighed individually on a GB303 electronic balance (Mettler–Toledo Instruments, China) to the nearest 0.001 g. Detailed data on reproductive female and their eggs will be reported elsewhere. All post-oviposition females were released unharmed at the sites where they were collected.

We incubated the eggs systematically (such that eggs from single clutches were distributed as equally as possible among treatments) at 24, 26, 30 and 32 (± 0.3)°C in plastic containers ($250 \times 180 \times 70$ mm) that were covered with a perforated plastic membrane to retard water loss. The containers contained known amounts of vermiculite and distilled water to produce approximately -220 (1 g water/1 g vermiculite) and 0 kPa (3 g water/1 g vermiculite) water potentials. Substrate water potentials were calibrated with a Wescor HR-33T microvoltmeter (dew point mode) (Lin and Ji, 1998). One-third of the egg was buried in the substrate, with the surface near the embryo being exposed to air inside the container.

We moved containers every day among shelves in the incubators according to a predetermined schedule to minimize any influence of thermal gradients inside the incubator. Incubation temperatures in close proximity to eggs were monitored twice daily using a digital thermometer. Eggs were weighed at 5-day intervals. Containers were weighed daily and, if necessary, distilled water was mixed evenly into substrates to compensate for small evaporative losses and water absorbed by eggs, thereby maintaining the substrate water potential at a constant.

When eggs were found to have pipped, we moved them individually into covered glass containers (250 ml), thereby assuring positive identification of emergent young. The duration of incubation, measured as the number of days to pipping, was recorded for each egg. Upon emergence, mass, SVL and tail length were measured on each hatchling. Deformed hatchlings were excluded from further statistical analyses. Hatchlings ($N = 204$) were then killed by freezing to -15°C for later study. Upon thawing, we separated each hatchling into carcass, residual yolk and fat bodies. The three components were oven dried to constant mass at 65°C, weighed and preserved frozen for the later determination of composition. We assessed the sex of hatchlings by pressing on both sides of the tail base for the presence or absence of hemipenes and considered those with hemipenes as males.

We extracted non-polar lipids from dried samples in a Soxhlet apparatus for a minimum of 5.5 h using absolute ether as a solvent. The amount of lipids in a sample was determined by subtracting the lipid-free dry mass from the total sample dry mass. The total lipid in each hatchling was calculated as the sum of the lipids in its carcass, residual yolk and fat bodies. We determined the energy density of the dried samples using a GR-3500 adiabatic bomb calorimeter (Changsha Instruments, China). Titrations were performed on the residue after calorimetry to correct for nitrogenous wastes. Further corrections were performed for fuse wire burning. We determined ash content (inorganic content) in each sample using a muffle furnace at 700°C for a minimum of 12 h and weighing the remaining ash.

A preliminary statistical analysis showed that there were no between-sex differences in all hatchling traits examined, so we pooled data for both sexes. All data were tested for normality

(Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett test), and Ln transformed when necessary to satisfy the conditions for using parametric tests. We used linear regression analysis, one-way and two-way analyses of variance (ANOVA), one-way and two-way analysis of covariance (ANCOVA), when the assumptions of parametric analyses were met. Non-parametric analyses were used when these assumptions were violated. Values are presented as mean $\pm$ 1 S.E., and the significance level is set at $\alpha = 0.05$.

3. Results

3.1. Changes in egg mass

In all treatments, the final (pre-hatching) mass of eggs was greater than the initial mass of eggs. Eggs incubated in wetter substrates gained mass throughout incubation whereas eggs incubated in drier substrates gained mass during the first half of incubation and lost mass thereafter (Fig. 1a–d). All mass gains were assumed to be a result of the absorption of water. The final mass of eggs was positively correlated with the initial mass of eggs in all treatments (all $P < 0.0001$). Therefore, the initial mass of eggs was used as the covariate and a two-way ANCOVA (with temperature and water potential as the factors) was performed. Incubation temperature ($F_{3,192} = 10.17$, $P < 0.0001$) and substrate water potential ($F_{1,192} = 137.74$, $P < 0.0001$) affected the final egg mass (Fig. 1a–d). Eggs incubated at lower temperatures gained more mass than did eggs at higher temperatures but at the same water potential (one-way ANCOVA, both $P < 0.0001$); eggs incubated in wetter substrates gained more mass than did eggs

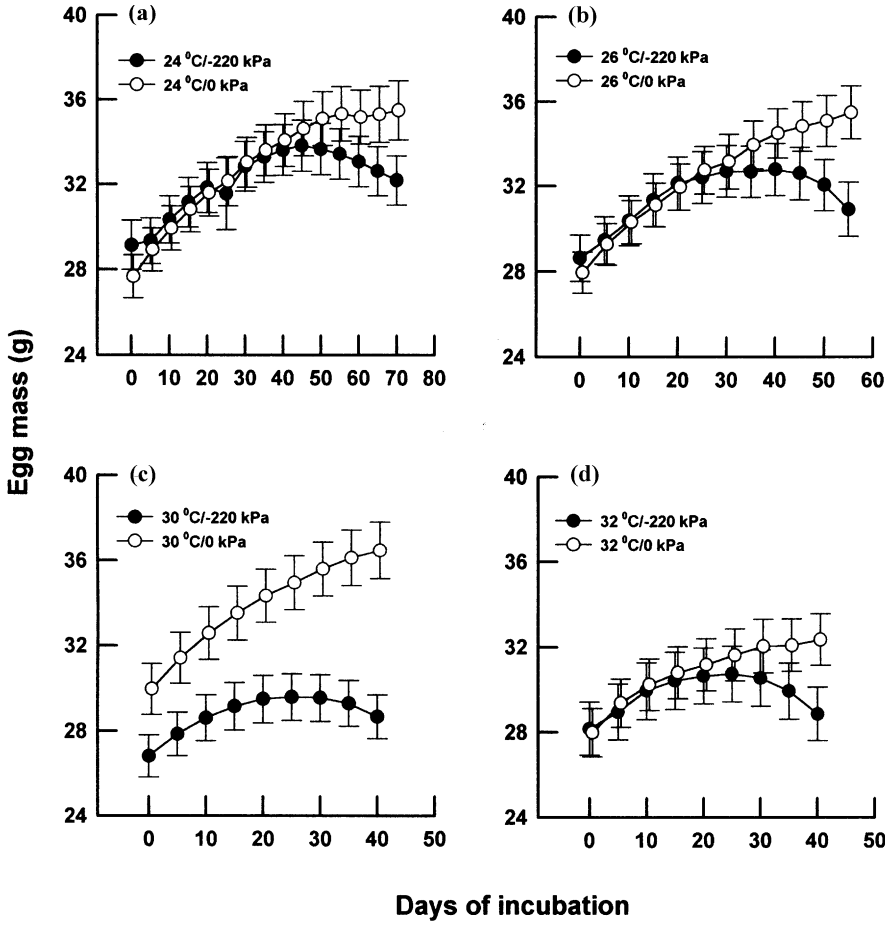

Fig. 1. Temporal changes in mass of viable *Elaphe carinata* eggs incubated in different thermal and hydric environments. Data are expressed as mean and positive and negative bars of standard error.

in drier substrates, but at the same temperature (one-way ANCOVA, all $P < 0.0001$).

3.2. Duration of incubation, hatching success and sex ratio of hatchlings

Initial egg mass did not affect the duration of incubation in all treatments (all $P > 0.05$). Therefore, the covariate was eliminated from consideration and a two-way ANOVA (with temperature and water potential as the factors) was performed on the duration of incubation. Incubation temperature affected duration of incubation ($F_{3,196} = 5034.80$, $P < 0.0001$) and substrate water potential did not ($F_{1,196} = 0.07$, $P = 0.798$). The interaction of temperature and water potential did not affect duration of incubation ($F_{3,196} = 0.38$, $P = 0.769$). Incubation length decreased as temperature increased, but not in a linear way: the average days of incubation decreased 15.9 days from 24 to 26°C, 13.7 days from 26 to 30°C and 1.8 days from 30 to 32°C (Table 1).

Hatching success was noticeably higher at 26 and 30°C than at 24 and 32°C, but among treatment differences in hatching success were not significant ($G = 3.20$, d.f. = 7, $P > 0.75$) (Table 1). A total of 19 hatchlings exhibited trunk and/or tail malformations. Deformed hatchlings were found in seven of the eight treatments; the frequency of deformity was independent of treatments ($G = 7.54$, d.f. = 7, $P > 0.25$) (Table 1). The incubation thermal and hydric environments did not affect the sex ratio of hatchlings ($G = 1.97$, d.f. = 7, $P > 0.95$), but the overall sex ratio was female-biased (females/males = 138/66; $G = 25.97$, d.f. = 1, $P < 0.01$).

3.3. Size and morphology of hatchlings

Incubation temperature affected SVL, tail length, wet body mass, carcass dry mass and residual yolk dry mass of hatchlings, but it did not affect total hatchling dry mass and fat body dry mass (Table 2). Overall, eggs incubated at 26°C produced the largest (SVL) hatchlings, characteristically having the largest carcasses, and those incubated at 32°C produced the smallest hatchlings, characteristically having the smallest carcasses (Table 2). Differences in hatchling wet mass stemmed mainly from variation in water contents, as there were no differences in the total dry mass among hatchlings from different incubation temperatures (Table 2). Hatchlings from eggs incubated at 24°C had shorter tails than did those from higher incubation temperatures (Table 2). More yolk remained unused at hatching in eggs incubated at 32°C, and residual yolks in hatchlings from the three lower incubation temperatures were nearly the same (Table 2).

Compared with incubation temperature, substrate water potential was not an important source of variation for all hatchling traits reported in Table 2. The effects of incubation temperature on the examined hatchling traits were independent of the effects of substrate water potential (Table 2).

Analysis of covariance of linear dimension and mass of hatchlings where hatchling SVL was the covariate showed influence of incubation temperature on morphology of hatchlings. Hatchlings from eggs incubated at 24°C had shorter SVL-specific tail length ($F_{3,202} = 24.50$, $P < 0.0001$) but

Table 1
The effects of thermal and hydric environments on duration of incubation, hatching success, and sex ratio and abnormality of hatchlings in a colubrid snake, *Elaphe carinata*[a]

Temperature (°C)	Moisture	Incubated eggs	Duration of incubation (days)	Hatching success (%)	Sex ratio (♀♀/♂♂)	Abnormality (%)
24	D	35	74.3 ± 0.4 (71.6–77.3)	68.6 (24/35)	12/12	5.7 (2/35)
	W	37	73.8 ± 0.3 (70.3–77.9)	75.7 (28/37)	18/10	8.1 (3/37)
26	D	24	58.0 ± 0.3 (55.4–62.0)	87.5 (21/24)	13/8	4.2 (1/24)
	W	34	58.1 ± 0.3 (54.3–62.8)	82.4 (28/34)	20/8	5.9 (2/34)
30	D	25	44.4 ± 0.2 (42.8–46.6)	92.0 (23/25)	18/5	0 (0/25)
	W	38	44.4 ± 0.2 (42.0–46.8)	89.5 (34/38)	25/9	10.5 (4/38)
32	D	35	42.5 ± 0.2 (40.7–44.4)	65.7 (23/35)	16/7	11.4 (4/35)
	W	34	42.6 ± 0.2 (40.6–44.3)	67.6 (23/34)	16/7	8.8 (3/34)

[a] Data on duration of incubation are expressed as mean ± S.E. (range). D: drier substrate (−220 kPa); W: wetter substrate (0 kPa).

Table 2
Linear dimension, mass and components of *Elaphe carinata* hatchlings from eggs incubated in a different thermal and hydric environments[a]

Hatchling traits	Temperature (°C)					Effects		
	Moisture	24	26	30	32	Moisture $F_{1,195}$	Temperature $F_{3,195}$	Interaction $F_{3,195}$
Initial egg mass (g)	D	28.1 ± 1.2	28.3 ± 1.1	26.8 ± 1.0	28.0 ± 1.3			
	W	28.6 ± 1.0	28.0 ± 0.9	29.9 ± 1.6	28.0 ± 1.1			
Snout-vent length (mm)	D	370.4 ± 5.0	378.5 ± 6.0	360.4 ± 5.0	344.4 ± 5.2	0.09 ns*	13.43***	1.98 ns*
	W	361.9 ± 5.7	374.4 ± 4.6	373.8 ± 5.2	355.2 ± 5.1		24^{b}, 26^{a}, 30^{ab}, 32^{c}	
Tail length (mm)	D	79.8 ± 1.7	87.2 ± 2.3	85.5 ± 1.7	83.1 ± 1.7	0.06 ns*	14.99***	0.56 ns*
	W	77.6 ± 1.8	85.9 ± 1.6	88.5 ± 1.3	84.4 ± 1.5		24^{b}, 26^{a}, 30^{a}, 32^{a}	
Wet body mass (g)	D	20.9 ± 0.9	20.9 ± 0.9	19.4 ± 0.8	19.8 ± 0.8	2.91 ns*	3.87**	1.22 ns*
	W	21.3 ± 0.7	21.1 ± 0.7	21.3 ± 0.8	20.5 ± 0.8		24^{ab}, 26^{a}, 30^{b}, 32^{b}	
Dry body mass (g)	D	5.34 ± 0.25	5.50 ± 0.31	5.15 ± 0.27	5.24 ± 0.27	0.05 ns*	0.57 ns*	2.07 ns*
	W	5.56 ± 0.24	5.37 ± 0.22	5.50 ± 0.26	5.42 ± 0.28			
Carcass (g)	D	3.29 ± 0.14	3.29 ± 0.14	2.90 ± 0.11	2.74 ± 0.23	3.83 ns*	26.99***	1.58 ns*
	W	3.03 ± 0.12	3.25 ± 0.12	3.18 ± 0.12	2.69 ± 0.11		24^{b}, 26^{a}, 30^{b}, 32^{c}	
Residual yolk (g)	D	1.24 ± 0.09	1.34 ± 0.12	1.49 ± 0.15	1.74 ± 0.13	2.95 ns*	12.83***	5.19**
	W	1.73 ± 0.12	1.28 ± 0.09	1.51 ± 0.12	1.95 ± 0.17		24^{b}, 26^{b}, 30^{b}, 32^{a}	
Fat bodies (g)	D	0.81 ± 0.05	0.86 ± 0.07	0.77 ± 0.05	0.76 ± 0.05	0.36 ns*	2.08 ns*	0.37 ns*
	W	0.80 ± 004	0.84 ± 0.05	0.83 ± 0.06	0.77 ± 0.05			

[a] Data are expressed as mean ± S.E. F values correspond to single effects and factor interactions in two-way ANOVAs (with initial egg mass as the covariate). D: drier substrate (−220 kPa); W: wetter substrate (0 kPa). Symbols immediately after F values represent significant levels.

*ns $P > 0.05$; **$P < 0.01$; ***$P < 0.001$. Means with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, a > b > c).

greater SVL-specific body mass (dry mass, $F_{3,202} = 4.65$, $P < 0.004$; wet mass, $F_{3,202} = 4.99$, $P < 0.003$) than did those from higher incubation temperatures.

3.4. Embryonic use of energy and material

Hatchlings from different incubation temperatures did not differ in energy and lipid contents (Table 3). This indicated that, within the temperature range from 24 to 32°C, embryos of *E. carinata* completed development at nearly the same expenditure of energy and catabolized nearly the same amount of lipids. Incubation temperature significantly affected embryonic use of ash (inorganic materials), as indicated by the fact that hatchlings from different incubation temperatures differed in ash contents (Table 3). Hatchlings from eggs incubated at 26°C apparently contained more ash than did those from eggs incubated at 32°C (Table 3), indicating that more inorganic materials were transferred from the egg into the hatchling when eggs were incubated at 26°C.

Again, substrate water potential was not an important source of variation for energy, lipid and ash contents of hatchlings (Table 3). The effects of incubation temperature on these hatchling traits were also independent of the effects of substrate water potential (Table 3).

4. Discussion

4.1. Water flux and influence of substrate water potential

In this study, a net mass gain (and, hence, a net water gain) occurred in eggs in all treatments (Fig. 1). However, eggs incubated at different temperatures had different patterns of water exchange even when substrate water potentials were identical, and eggs incubated in different water potentials had different patterns of water exchange even when temperatures were identical. Eggs incubated at lower temperatures absorbed more water than eggs at higher temperatures, largely due to the longer developmental time and smaller transpirational losses of water from the egg at lower temperatures. This result is consis-

Table 3
The effects of incubation thermal and hydric environments on energy contents, non-polar lipid mass and ash mass of *Elaphe carinata* hatchlings[a]

Temperature (°C)	Moisture	*N*	Energy (kJ)	Non-polar lipids (g)	Ash mass (g)
24	D	24	130.7 ± 7.0 (81.2–197.0)	1.34 ± 0.08 (0.68–2.07)	0.56 ± 0.02 (0.38–0.72)
	W	28	133.1 ± 6.0 (76.7–197.9)	1.46 ± 0.07 (0.83–2.18)	0.58 ± 0.02 (0.37–0.87)
26	D	21	139.1 ± 9.9 (77.8–258.2)	1.36 ± 0.10 (0.67–2.22)	0.59 ± 0.03 (0.41–0.85)
	W	28	127.6 ± 5.7 (70.9–184.6)	1.34 ± 0.07 (0.55–2.02)	0.59 ± 0.02 (0.41–0.83)
30	D	23	122.8 ± 7.4 (79.4–223.6)	1.28 ± 0.08 (0.58–2.02)	0.55 ± 0.02 (0.41–0.78)
	W	34	132.1 ± 7.5 (75.9–262.2)	1.36 ± 0.09 (0.70–2.71)	0.59 ± 0.02 (0.39–0.91)
32	D	23	123.5 ± 7.1 (73.7–194.4)	1.32 ± 0.09 (0.74–2.25)	0.56 ± 0.02 (0.41–0.82)
	W	23	128.6 ± 7.0 (82.6–195.5)	1.40 ± 0.09 (0.64–2.06)	0.56 ± 0.02 (0.39–0.83)
Effects	Temperature	$F_{3,195}$	1.16 ns*	0.66 ns*	3.36** (24^{ab}, 26^{a}, 30^{ab}, 32^{b})
	Moisture	$F_{1,195}$	0.49 ns*	0.56 ns*	0.35 ns*
	Interaction	$F_{3,195}$	1.50 ns*	2.08 ns*	1.21 ns*

[a] Data are expressed as mean ± S.E. (range). *F* ratios correspond to single effects in two-way ANOVAs (with temperature and moisture as the factors) using the initial egg mass as the covariate. D: drier substrate (−220 kPa); W: wetter substrate (0 kPa). Symbols immediately after *F* values represent significant levels.

* ns, $P > 0.05$; ** $P < 0.05$. Means corresponding to temperatures with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, a > b).

tent with that reported for some other reptiles, e.g. *Pituophis melanoleucus* (bull snake) (Gutzke and Packard, 1987), *Podarcis muralis* (Mediterranean wall lizard) (Ji and Braña, 1999) and *Takydromus septentrionalis* (northern grass lizard) (Lin and Ji, 1998). The decline in mass late in incubation when eggs were incubated in drier substrates is most likely due to a greater rate of transpirational losses of water from the egg. Clearly, the external thermal and hydric environments encountered by developing eggs significantly affect the hydric conditions inside the egg. This fact leads to the following question: do the hydric conditions a hatchling experienced during embryogenesis subtly affect its development condition at hatching? If so, then any resulting differences in hydric conditions among eggs of *E. carinata* in different thermal and hydric environments would, to some extent, affect hatchling phenotypes. Our data, however, indicate that such effects are unlikely to occur in *E. carinata*, because hatching success, incubation length, the embryonic use of energy, and sex, size, mass and composition of hatchlings are all unaffected within the range of water potential considered in this study. Therefore, we conclude that eggs of *E. carinata* are insensitive to variation in incubation hydric conditions within the range of water potential we used (−220 to 0 kPa). The hydric insensitivity of egg incubation is considered to be unusual amongst reptiles, but has been recorded in some reptiles with pliable-shelled eggs (Muth, 1980; Tracy, 1980; Packard and Packard, 1987; Plummer and Snell, 1988; Thompson, 1990). Because the hydric dependence of egg incubation does exist in many reptiles (Packard et al., 1980, 1982, 1981, 1983; Morris et al., 1983; Gutzke and Packard, 1985, 1987; Packard and Packard, 1986; Packard, 1991), we tend to support the conclusion that the eggs of different reptilian species may respond differentially to variance of hydric environments (Lin and Ji, 1998; Ji and Braña, 1999).

4.2. Influence of incubation temperature

Thermal variance affects many aspects of egg

incubation in *E. carinata*, including the aforementioned water exchanges between eggs and their surroundings. Our results show that the incubation temperature affects hatching success, incubation length, the embryonic use of energy and linear dimension, morphology and composition of hatchlings.

Significant differences in linear dimension and wet body mass were found among hatchlings from eggs incubated at different temperatures, with hatchlings from eggs incubated at 26°C being larger (SVL) and heavier than their siblings incubated at lower or higher temperatures (Table 2). However, the differences in wet body mass of hatchlings result mainly from variation in the water content, because there are no significant differences in dry mass among hatchlings from different temperatures. Our finding that eggs incubated at medium temperatures such as 26°C produce larger hatchlings is consistent with reports for other reptiles (Gutzke et al., 1987; Packard et al., 1989; Burger, 1990; Van Damme et al., 1992). The larger hatchling size has an association with the greater carcass dry mass (= total hatchling dry mass − fat body dry mass − residual yolk dry mass) (Ji et al., 1997a, 1999a,b,c; Ji and Braña, 1999; Ji and Sun, 2000). Therefore, the larger size of hatchlings from eggs incubated at 26°C is primarily attributed to their larger carcasses, and the smaller size of hatchlings from 32°C is primarily attributed to their smaller carcasses (Table 2). However, size at hatching is not a good indicator for judging the exact size to which newly emerged young will grow prior to feeding, because they may use resources in the residual yolk to increase size during their first post-hatching days (Packard, 1991; Ji et al., 1997a, 1999a,b,c; Ji and Sun, 2000). For example, smaller hatchlings of *E. carinata* may increase size (SVL) following the depletion of residual yolk, thereby compensating for their smaller size at hatching (Ji et al., 1997a). The reverse relationship between carcass mass and residual mass has also been reported for some reptiles (Packard, 1991; Ji et al., 1997a, 1999a,b,c; Ji and Braña, 1999; Ji and Sun, 2000).

Our data show that hatchlings of *E. carinata* from different incubation temperatures do not differ in energy and lipid contents (Table 3). This indicates that, over the temperature range from 24 to 32°C, embryos of *E. carinata* complete development at nearly the same expenditure of energy and catabolize nearly the same amount of lipids. However, newly emerged young from different temperatures differ in the development condition of carcass, with hatchling from 32°C having smaller carcasses but larger residual yolks than those from lower temperatures (Table 2). The result that more yolks remain unused at hatching when eggs are incubated at high temperatures has been reported for all studied reptiles and is, therefore, not surprising (Beuchat, 1988; Phillips et al., 1990; Van Damme et al., 1992; Phillips and Packard, 1994; Ji and Braña, 1999). In *E. carinata*, the unused yolk at hatching (i.e. residual yolks) is far from trivial and can be used for maintenance and carcass growth during the first 3 post-hatching weeks when the hatchlings are fasted at temperatures ranging from 26 to 38°C (Ji et al., 1997a). Thus, hatchlings from 32°C can be expected to show a subsequent growth of carcass following the depletion of residual yolk. The post-hatching carcass growth may substantially increase the SVL of hatchlings (Ji et al., 1997a, 1999a,b,c; Ji and Sun, 2000), thereby making it possible for hatchlings from 32°C to grow to the size of newly emerged young from low incubation temperatures. Because a part of the energy in the residual yolk is used for maintenance (Ji et al., 1997a) and hatchlings from 32°C do not differ from those from lower temperatures in energy contents, embryos of *E. carinata* developing at 32°C actually have a lower efficiency of converting energy reserves to tissue. On the contrary, embryos of *E. carinata* developing at 26°C complete development at nearly the same expenditure of energy as do those at other lower or higher temperatures, but produce larger hatchlings with larger carcasses (Table 2), showing a higher efficiency of converting energy reserves to tissue.

Our data show the influence of incubation temperature on the morphology of hatchlings, with hatchlings from eggs incubated at 24°C having shorter tails and greater size (SVL)-specific wet body mass than do their siblings from higher temperatures. However, the explanation for the cause and ecological consequence of the thermal dependence of these morphological traits remains unknown at this time.

4.3. The suitable temperatures for incubating eggs of E. carinata

Embryos of *E. carinata* at 24°C take almost 2.5

months to complete development (Table 1), so the majority of newborns from this temperature are expected to appear between late September and early October. Because hatchlings of *E. carinata* need at least 3 weeks to absorb the yolk sac and do not feed during this period (Ji et al., 1997a), their growth period prior to the onset of the first winter (late November) through feeding is approximately 1 month or less. In squamates, incubation length increasingly increases as temperature decreases (e.g. Van Damme et al., 1992; Overall, 1994; Lin and Ji, 1998; Ji et al., 1999c; Ji and Braña, 1999; Braña and Ji, 2000). This pattern is also found in *E. carinata*: incubation length decreases by 15 days when the temperature increases from 24 to 26°C, and by 14 days when the temperature increases from 26 to 30°C (Table 1). Taking the pattern into account, we expect that hatchlings of *E. carinata* from temperatures lower than 24°C would have to take the risk of losing the growth period prior to first winter. In addition, the prolonged duration of incubation at lower temperatures increases the exposure of eggs to the effects of adverse biotic (increased microbial contamination) and abiotic factors (extreme thermal and hydric conditions) in the incubation environment of the eggs, which may substantially reduce hatching success.

Because an increase in incubation temperature from 30 to 32°C only shortens the incubation length by approximately 1.9 days in *E. carinata*, the ecological advantage of the shortened incubation length can be negligible. Actually, eggs of *E. carinata* incubated at 32°C exhibit a noticeable higher level of embryo mortality and produce smaller hatchlings than do eggs incubated at lower temperatures (Table 1). In addition, our unpublished data show that none of *E. carinata* eggs can be hatched when they are incubated at 33°C, although eggs are tolerant of the temperatures higher than 33°C for a brief period when they are incubated in a fluctuating thermal regime. Thus, we conclude that the temperature of 32°C is near the upper threshold for successfully incubating eggs of *E. carinata*.

Taken together, our results indicate that temperatures within the range from 24 to 32°C are, overall, suitable for incubating eggs of *E. carinata*. Temperatures outside this range can adversely affect egg incubation of the species either by depriving a hatchling of its growth period prior to the onset of the first winter or by dramatically increasing embryonic mortality. Because the effects of incubation temperature on post-hatching performance and behavior of hatchlings were not examined in this study, and because size alone is not a good indicator of a hatchling's 'quality', we do not know what constant temperatures are more suitable for incubating the eggs of *E. carinata*. However, taking the energy expenditure and the rate of embryogenesis into account, we consider that the relatively better constant incubation temperatures for *E. carinata* are within the temperature range between 26 and 30°C.

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