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Effects of Thermal and Hydric Environments on Incubating Eggs and Hatchling Traits in the Cobra, *Naja naja atra*

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ABSTRACT.—**We examined in the laboratory the effects of thermal and hydric environments on incubating eggs and hatchling traits of *Naja naja atra* collected from a population near the northern distributional limit of the species in Zhejiang, eastern China. Eggs were incubated at temperatures of 24, 26, 30, and 32 °C on substrates with water potentials of 0 and −220 kPa using a 4 × 2 factorial design. Temperature strongly affected hatching success, incubation length, water uptake by eggs during the course of incubation, and most morphological hatchling traits examined; it even affected embryonic mobilization of inorganic material from the shell. Hatching success was higher at 26 and 30°C but lower at 24 and 32°C. Eggs incubated at 26 and 30°C produced larger and heavier hatchlings that also contained more inorganic material and energy than did eggs incubated at lower and higher temperatures. Hatchlings from eggs incubated at 24 and 32°C were similar in size and mass, but hatchlings from eggs incubated at 32°C had larger residual yolks. Deformed hatchlings were found at 24, 30, and 32°C, the frequency being independent of incubation temperature. At the time of hatching, shells from eggs incubated at 24°C had greater dry mass and contained more ash, suggesting that less inorganic material was withdrawn from the shell by embryos developing at 24°C. Compared with temperature, water potential was not an important source of variation for the hatchling traits we examined, although it did influence water uptake by eggs during incubation and hatching success at 24°C. The effects of temperature were independent of the effects of water potential. Variation in size and mass induced by incubation temperature may be important to posthatching survival and fitness of hatchlings.**

Of all external and internal factors that may potentially influence embryo survival and phenotypic variation in resultant hatchlings, temperature is a particularly important factor. Many oviparous reptiles, compared with birds, have a much wider range of incubation temperatures that yield viable hatchlings (Ferguson, 1985; Deeming and Ferguson, 1991). High incidence of abnormalities and failure of embryonic development occur at extreme temperatures (Vinegar, 1974), and, thus, the ability of tolerance to extreme temperatures by embryos can be one of the reasons that explain the distribution limits of reptiles (Burger, 1991a). The most striking effect of temperature is that warm temperature shortens the incubation length, but more subtle influences were on morphology, behavior, skin-shedding cycle, response to chemicals, and posthatching performance and growth of hatchlings (e.g., Packard and Packard, 1988; Congdon and Gibbons, 1990; Booth and Thompson, 1991; Deeming and Ferguson, 1991; Packard, 1991; Burger, 1991a,b, 1998a,b; Braña and Ji, 2000). In species with environmental sex determination, temperature may also influence sex of hatchlings (e.g., Bull, 1980, 1985; Ferguson and Joanen, 1982; Deeming and Ferguson, 1988; Janzen and Paukstis, 1991; Mrosovsky and Pieau, 1991).

Moisture is another important factor that may influence egg incubation of reptiles. Many investigators indicate that hydric environments influence hatching success, incubation duration, and hatchling traits (e.g., Packard and Packard, 1984, 1988; Packard, 1991; Lutz and Dunbar-Cooper, 1984). Others, however, question whether hydric condition strongly influences hatchling traits (Hotaling et al., 1985; Plummer and Snell, 1988; Ratterman and Ackerman, 1989; Lin and Ji, 1998; Ji and Braña, 1999; Braña and Ji, 2000). The differences may not be so surprising, because investigators using different experimental designs and types of eggs may reach different conclusions on the same topic. In reptiles, pliable-shelled eggs, unlike rigid-shelled eggs, gain or lose mass rapidly because of the more active exchange of water between eggs and their surroundings, providing greater variation in hydric condition inside the egg during incubation (e.g., Vleck, 1991; Vleck and Hoyt, 1991; Lin and Ji, 1998). Thus, pliable-shelled eggs may be more moisture-dependent, and the effects of hydric environments may be more pronounced on pliable-shelled eggs than on rigid-shelled eggs.

The cobra *Naja naja atra* (Elapidae) is one of the most conspicuous elements of the reptile fauna in the southern provinces of China. In

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Zhejiang, the distribution of cobras is very interesting, because it is very common in the central and southern parts (including islands) but absent in the northern part of the province. Largely because of its commercial importance and increasing hunting pressure from local people, the cobra has received considerable attention from investigators (e.g., Hu et al., 1966; Sheng et al., 1988; Huang and Jin, 1990; Ji et al., 1997b). Our objectives were to examine (1) the effects of incubation temperature, substrate moisture, and their interaction on incubating *N. n. atra* eggs and hatchling traits and (2) the range of suitable temperatures for incubating eggs of this over exploited snake.

Materials and Methods

In early July 1998, we obtained 43 gravid cobras (snout–vent length: 84.0–120.5 cm; postpartum body mass: 126.5–592.0 g) from dealers in Yiwu and Jiande, Zhejiang. Cobras were transported then to our laboratory at Hangzhou Normal College, where they were randomly assigned 1–2 to each 50 × 45 × 35 cm (length × width × height) wire cage placed in an air-conditioned room where the temperature varied from 28 to 30°C. Food (toads and skinks) and water were provided ad libitum. Oviposition occurred between 8 and 23 July. Cages were checked a minimum of six times daily for the presence of eggs so that eggs could be collected, measured, weighed, and numbered promptly, thereby avoiding any uncertainty of initial mass resulting from loss or gain of water. Detailed data on reproductive female and clutches will be reported elsewhere.

We incubated eggs at 24, 26, 30, and 32°C (± 0.3) in plastic containers (250 × 180 × 70 mm) with pierced covers. The containers contained known amounts of vermiculite and distilled water to produce approximately −220 kPa (1 g water/1 g vermiculite) and 0 kPa (3 g water/1 g vermiculite) water potentials (Lin and Ji, 1998). Eggs from single clutches were distributed as equally as possible among treatments. Eggs were buried one-third in the substrate, with the surface near the embryo being exposed to air inside the container. We moved containers among shelves in the incubators daily according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. Incubation temperatures were monitored twice daily using a digital thermometer. Eggs were weighed at five-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 a constant water potential of the substrate. The duration of incubation was defined as the time elapsed from egg laying to hatchling emergence.

Upon emergence, each hatchling was sexed, measured, and weighed. Body measurements included snout–vent length, tail length, and body mass. Deformed hatchlings were excluded from statistical analyses. A total of 191 hatchlings were frozen immediately after measuring and weighing. These hatchlings were later thawed and separated into carcass, yolk sac (residual yolk), and fat bodies. The three components were dried to constant mass at 65°C, weighed, and preserved frozen for later determination of composition.

We extracted nonpolar lipids from dried samples in a Soxhlet apparatus for a minimum of 5.5 h using absolute ether as solvent. The amount of lipids in a sample was determined by subtracting the lipid-free dry mass from the total sample dry mass. We determined energy density of 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. Ash (inorganic material) in samples was determined using a muffle furnace at 700°C for a minimum of 12 h.

A preliminary analysis showed that there were no between-sex differences in hatchling traits except that females had shorter tails; therefore, we pooled data other than tail length for both sexes. All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett's test) prior to further statistical analysis. Ln-transformations were performed when necessary to achieve the conditions for parametric tests. We used two-way analyses of variance (ANOVA), one-way and two-way analysis of covariance (ANCOVA), when the assumptions of parametric analyses were met. In each model of ANCOVAs, we used the initial (the freshly laid) egg mass as the covariate. Nonparametric analyses were used when these assumptions were violated. Throughout this paper, values are presented as mean ± 1 standard error, and significance level is set at $\alpha = 0.05$.

Results

Clutch size and egg mass in our sample averaged 11.1 ± 0.4 g (range 6–17, $N = 43$) and 16.2 ± 0.5 g (range 11.9–29.1, $N = 43$), respectively. Both clutch size ($r^2 = 0.37$, $F_{1,41} = 24.806$, $P < 0.0001$) and clutch mass ($r^2 = 0.53$, $F_{1,41} = 46.591$, $P < 0.0001$) were positively correlated with female SVL.

Changes in Egg Mass.—Eggs incubated at 24°C and 0 kPa water potential were excluded from analyses because of the high embryo mortality

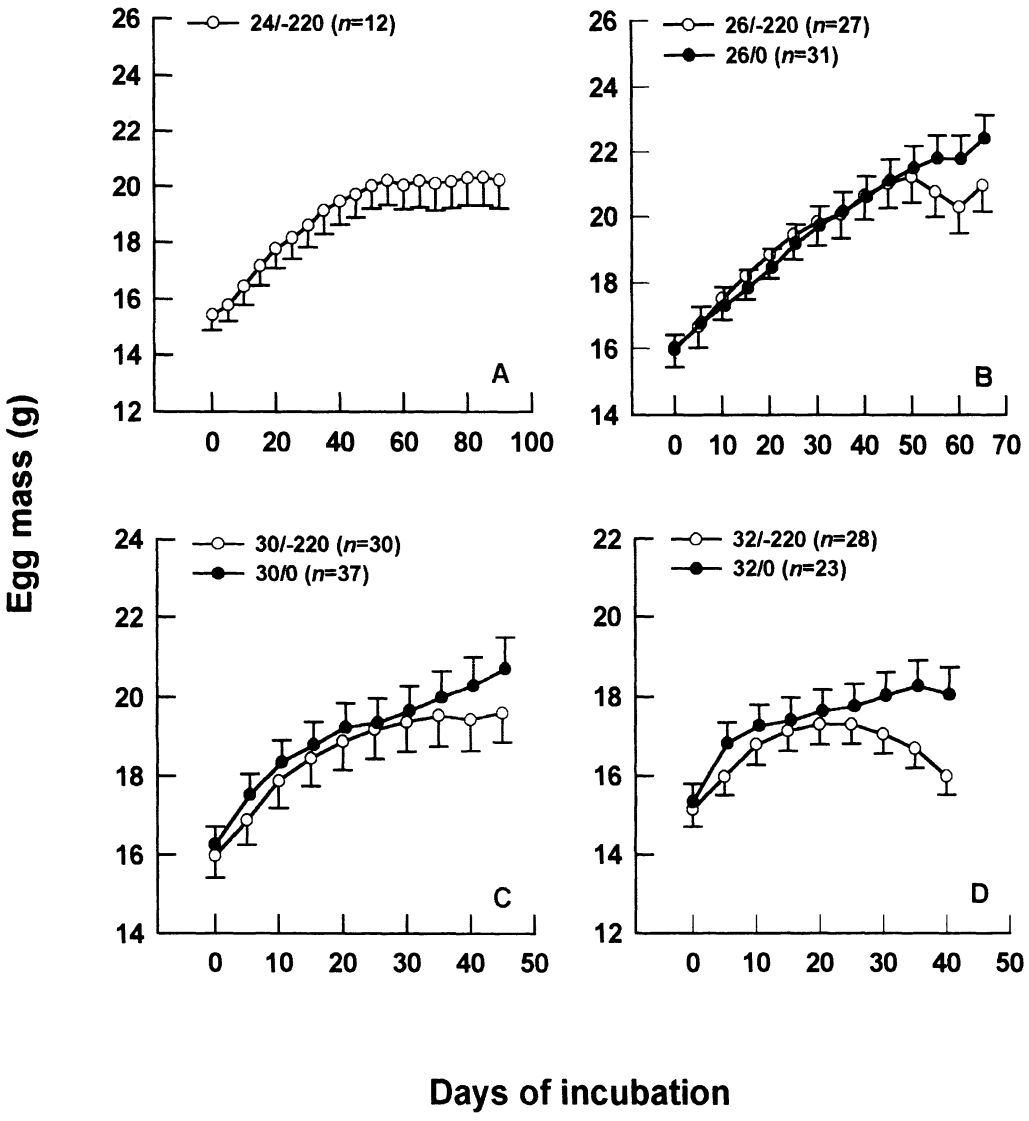

FIG. 1. Temporal changes in mass of *Naja naja atra* eggs incubated in different thermal and hydric environments. Data are expressed as mean and positive or negative standard error, and sample sizes are indicated in parentheses.

(93.2%). Eggs generally gained mass throughout incubation because of a net uptake of water (Fig. 1), with an exception for eggs incubated at 32°C and −220 kPa, which increased in mass during the first half of incubation and declined in mass during the second half (Fig. 1D). The final egg mass was positively correlated with the initial egg mass in all treatments (all $P < 0.001$). Mass gains were dependent on both incubation temperature and substrate moisture (Fig. 1). Eggs incubated at lower temperatures gained more mass than did eggs incubated at higher temperatures but at the same water potential (ANCOVA, in both moisture treatments, $P < 0.0001$). Eggs incubated in wetter substrates gained more mass than did eggs incubated in drier substrates but at the same incubation temperature (ANCOVA, in all temperature treatments, $P < 0.0001$).

Incubation Length, Hatching Success, and Sex Ratio of Hatchlings.—Duration of incubation decreased as temperature increased (Table 1). A two-way ANOVA (with temperature and water potential as the factors) showed significant influence of temperature on incubation length ($F_{3,182} = 2661.871$, $P < 0.0001$), but the analysis did not show an influence of substrate moisture on incubation length ($F_{1,182} = 0.048$, $P = 0.826$).

TABLE 1. The effects of incubation thermal and hydric environments on duration of incubation, hatching success, and sex ratio and abnormality of hatchlings in the cobra, *Naja naja atra.* Data on incubation duration are expressed as mean ± 1 standard error (range in parentheses).

Temperature (°C)	Moisture (kPa)	Number of incubated eggs	Duration of incubation (d)	Hatching success (%)	Sex ratio (♀♀/♂♂)	Abnormality (%)
24	−220	39	93.1 ± 0.7 (89.3–97.0)	30.8 (12/39)	6/6	2.6 (1/39)
24	0	44	89.8 ± 1.3 (87.8–92.1)	6.8 (3/44)	0/3	6.8 (3/44)
26	−220	35	66.0 ± 0.4 (62.0–70.7)	77.1 (27/35)	12/15	0 (0/35)
26	0	37	67.1 ± 0.5 (63.0–74.3)	83.8 (31/37)	19/12	0 (0/37)
30	−220	35	46.1 ± 0.4 (42.6–50.5)	85.7 (30/35)	15/15	8.6 (3/35)
30	0	47	46.2 ± 0.3 (43.3–50.7)	78.7 (37/47)	22/15	2.1 (1/47)
32	−220	44	42.2 ± 0.2 (40.3–43.8)	63.6 (28/44)	15/13	11.4 (5/44)
32	0	44	42.7 ± 0.3 (40.3–48.0)	52.3 (23/44)	10/13	11.4 (5/44)

In addition, there was no interaction effect of temperature and substrate moisture on incubation length ($F_{3,182}$ = 1.644, P = 0.181).

Survival of embryos to hatching was affected by incubation temperature (G-test, G = 43.939, df = 3, $P < 0.001$) but not by substrate moisture except for eggs incubated at 24°C (G = 6.918, df = 1, $P < 0.01$; Table 1). Hatching success was higher at intermediate temperatures (26 and 30°C) but lower at more extreme temperatures (24 and 32°C; Table 1).

Neither incubation temperature (G = 0.711, df = 3, $P > 0.10$) nor substrate moisture (G = 0.758, df = 1, $P > 0.10$) affected the sex ratio of hatchlings, and the overall sex ratio (females/males = 99/92) did not differ from equality (G = 0.257, df = 1, $P > 0.50$; Table 1). A total of 18 hatchlings exhibited trunk and/or tail malformations. Deformed hatchlings were found at 24, 30, and 32°C, but the frequency was independent of treatments (G = 4.375, df = 7, $P > 0.50$; Table 1).

Size, Mass, and Composition of Hatchlings.—Tails were longer in males than in females (Table

TABLE 2. The effects of incubation thermal and hydric environments on body mass, snout–vent length, and tail length of *Naja naja atra* hatchlings. Data are expressed as mean ± 1 standard error (range). F-ratios correspond to single effects in two-way ANCOVAs (with temperature and moisture as the factors) using the initial egg mass as the covariate. Symbols immediately after F-values represent significant levels: NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Means corresponding to temperatures with different superscripts differ significantly (Tukey's test, α = 0.05; $a > b$)

Temperature (°C)	Moisture (kPa)	N	Egg mass (g)	Body mass (g)	Snout–vent length (cm)	Tail length (cm)	
						Female	Male
24	−220	12	15.4 ± 0.6 (12.5–19.2)	11.4 ± 0.5 (8.0–15.8)	25.8 ± 0.4 (22.7–28.0)	4.4 ± 0.1 (4.2–4.7)	4.8 ± 0.1 (4.4–5.4)
24	0	3	15.7 ± 1.4 (12.9–17.4)	11.1 ± 1.0 (9.3–12.7)	25.5 ± 1.0 (23.5–26.9)	—	4.7 ± 0.1 (4.5–5.0)
26	−220	27	16.0 ± 0.6 (11.3–22.5)	12.2 ± 0.5 (8.9–17.7)	26.9 ± 0.2 (25.1–29.3)	4.5 ± 0.1 (4.1–4.8)	5.2 ± 0.1 (4.7–5.9)
26	0	31	16.0 ± 0.5 (11.7–21.1)	12.5 ± 0.4 (8.2–17.4)	27.0 ± 0.2 (23.4–29.9)	4.5 ± 0.1 (3.6–5.5)	5.0 ± 0.1 (4.6–5.8)
30	−220	30	16.0 ± 0.5 (11.7–21.4)	12.6 ± 0.5 (8.6–18.4)	27.1 ± 0.2 (24.1–28.9)	4.7 ± 0.1 (4.3–5.3)	5.0 ± 0.1 (4.0–5.6)
30	0	37	16.3 ± 0.5 (12.0–22.8)	12.7 ± 0.4 (9.4–17.4)	27.2 ± 0.2 (25.0–31.0)	4.6 ± 0.1 (4.0–5.5)	5.0 ± 0.1 (4.2–5.6)
32	−220	28	15.1 ± 0.4 (11.4–20.2)	11.6 ± 0.4 (8.7–15.6)	25.8 ± 0.2 (22.9–27.9)	4.4 ± 0.1 (4.0–4.9)	4.8 ± 0.1 (4.3–5.3)
32	0	23	15.3 ± 0.5 (12.7–19.3)	11.4 ± 0.4 (9.1–14.9)	25.7 ± 0.3 (23.1–28.4)	4.3 ± 0.1 (3.9–5.0)	4.7 ± 0.1 (4.2–5.0)
Effects F	Temperature			4.735** 24^b, 26^a, 30^a, 32^b	16.273** 24^b, 26^a, 30^a, 32^b	5.278** 26^{ab}, 30^a, 32^b	3.313* 24^b, 26^a, 30^a, 32^b
	Moisture			0.635^{NS}	0.260^{NS}	0.511^{NS}	2.074^{NS}
	Interaction			1.814^{NS}	0.424^{NS}	0.044^{NS}	0.095^{NS}

TABLE 3. The effects of incubation thermal and hydric environments on total dry mass and dry masses of carcass, residual yolk, and fat bodies of *Naja naja atra* hatchlings. Data are expressed as mean ± 1 standard error (range). *F*-ratios correspond to single effects in two-way ANCOVAs (with temperature and moisture as the factors) using the initial egg mass as the covariate. Symbols immediately after *F*-values represent significant levels: NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Means corresponding to temperatures with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$; $a > b$)

Temperature (°C)	Moisture (kPa)	*N*	Dry body mass (g)	Carcass dry mass (g)	Residual yolk dry mass (g)	Fat body dry mass (g)
24	−220	12	2.63 ± 0.12 (1.84–3.64)	1.56 ± 0.06 (1.07–1.75)	0.67 ± 0.08 (0.34–1.40)	0.41 ± 0.03 (0.26–0.53)
24	0	3	2.59 ± 0.27 (2.07–2.97)	1.58 ± 0.12 (1.32–1.71)	0.61 ± 0.07 (0.50–0.75)	0.41 ± 0.09 (0.23–0.53)
26	−220	27	2.93 ± 0.15 (1.98–4.63)	1.73 ± 0.06 (1.38–2.48)	0.71 ± 0.10 (0.23–2.38)	0.48 ± 0.03 (0.26–1.06)
26	0	31	2.97 ± 0.13 (2.14–4.70)	1.75 ± 0.05 (1.26–2.48)	0.74 ± 0.08 (0.24–2.53)	0.47 ± 0.02 (0.14–0.77)
30	−220	30	3.07 ± 0.17 (1.79–5.15)	1.78 ± 0.06 (1.15–2.41)	0.77 ± 0.08 (0.27–1.99)	0.51 ± 0.03 (0.21–0.89)
30	0	37	3.11 ± 0.14 (1.98–5.18)	1.72 ± 0.04 (1.32–2.29)	0.90 ± 0.10 (0.32–2.59)	0.48 ± 0.02 (0.20–0.75)
32	−220	28	2.81 ± 0.11 (1.94–4.04)	1.62 ± 0.04 (1.31–2.06)	0.75 ± 0.07 (0.39–1.65)	0.43 ± 0.02 (0.16–0.60)
32	0	23	2.75 ± 0.12 (2.05–4.31)	1.55 ± 0.04 (1.13–1.97)	0.80 ± 0.10 (0.40–2.52)	0.40 ± 0.02 (0.21–0.58)
Effects *F*	Temperature		2.862* $24^b, 26^a, 30^a, 32^{ab}$	5.959*** $24^b, 26^a, 30^a, 32^b$	3.989** $24^b, 26^b, 30^b, 32^a$	2.183^{NS}
	Moisture		0.446^{NS}	1.289^{NS}	0.198^{NS}	0.901^{NS}
	Interaction		0.436^{NS}	1.767^{NS}	0.336^{NS}	0.206^{NS}

2), but this difference did not result from the effects of incubation environments (Ji et al., 1999a). Hatchlings from eggs incubated at 26 and 30°C were larger and heavier than those from eggs incubated at 24 and 32°C, and length and mass of hatchlings from eggs incubated at 24 and 32°C were nearly the same (Table 2).

Temperature significantly affected hatchling dry mass, carcass dry mass, and residual yolk dry mass, but it did not affect fat body dry mass (Table 3). Hatchlings from eggs incubated at 26 and 30°C had greater total dry mass and carcass dry mass than did those from eggs incubated at 24°C, but these hatchlings had nearly the same residual yolks. Hatchlings from eggs incubated at 32°C had nearly the same carcass dry mass as did those from eggs incubated at 24°C, but the former hatchlings had larger residual yolks than did the latter ones.

Hatchlings from eggs incubated at 26 and 30°C contained more energy and ash than did those from eggs incubated at 24 and 32°C (Table 4). There were no significant differences in levels of nonpolar lipids among hatchlings from eggs incubated at different temperatures (Table 4). Shells from eggs incubated at 24°C had greater dry mass and contained more ash at the time of hatching (Table 4).

Substrate moisture was not an important source of variation for any hatchling trait we examined, and it was also not an important source of variation for shell dry mass and shell ash mass at the time of hatching (Tables 2–4). There were no significant interaction effects of temperature and substrate moisture on any of the hatchling traits examined (Tables 2–4).

DISCUSSION

The Effects of Substrate Moisture.—The final mass of incubating *N. n. atra* eggs varied among treatments and was dependent on the initial egg's mass, incubation temperature, and substrate moisture. Clearly, substrate moisture affected water uptake by *N. n. atra* eggs during the course of incubation, thereby affecting the hydric environments inside the egg. However, substrate moisture only affected hatching success at 24°C. Embryonic use of energy and sex, size, mass, and composition of hatchlings were all unaffected within the moisture range we considered. Moreover, unlike some embryonic turtles that tend to remain in the egg longer before hatching in wetter substrates (Morris et al., 1983; Packard and Packard, 1986; Packard, 1991), the incubation duration of *N. n. atra* eggs was unaffected by the substrate moisture. Thus, we conclude that embryonic development of *N. n. atra* is insensitive to substrate moisture, and

TABLE 4. The effects of incubation thermal and hydric environments on energy content, lipid mass, and ash mass of *Naja naja atra* hatchlings and dry mass and ash mass of shells at hatching. Data are expressed as mean ± 1 standard error (range). *F*-ratios correspond to single effects in two-way ANCOVAs (with temperature and moisture as the factors) using the initial egg mass as the covariate. Symbols immediately after *F*-values represent significant levels: NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$. Means corresponding to temperatures with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$; $a > b$)

Temperature (°C)	Moisture (kPa)	*N*	Hatchling			Eggshell	
			Energy (KJ)	Nonpolar lipid (g)	Ash mass (g)	Dry mass (g)	Ash mass (g)
24	−220	12	62.8 ± 2.9 (45.6–85.9)	0.72 ± 0.04 (0.53–0.99)	0.27 ± 0.01 (0.18–0.37)	0.30 ± 0.01 (0.26–0.37)	0.065 ± 0.002 (0.055–0.076)
24	0	3	61.3 ± 6.9 (47.6–69.7)	0.73 ± 0.10 (0.52–0.83)	0.27 ± 0.03 (0.23–0.31)	0.30 ± 0.03 (0.25–0.33)	0.070 ± 0.005 (0.061–0.077)
26	−220	27	71.0 ± 3.5 (46.9–110.1)	0.81 ± 0.04 (0.48–1.33)	0.30 ± 0.01 (0.20–0.45)	0.29 ± 0.01 (0.22–0.39)	0.066 ± 0.002 (0.050–0.099)
26	0	31	71.2 ± 3.2 (45.5–121.7)	0.83 ± 0.05 (0.36–1.76)	0.31 ± 0.01 (0.22–0.48)	0.29 ± 0.01 (0.23–0.36)	0.060 ± 0.002 (0.042–0.089)
30	−220	30	73.9 ± 4.1 (41.6–127.2)	0.85 ± 0.05 (0.41–1.54)	0.31 ± 0.01 (0.19–0.48)	0.28 ± 0.01 (0.21–0.38)	0.060 ± 0.002 (0.042–0.089)
30	0	37	74.6 ± 3.4 (45.1–124.1)	0.86 ± 0.05 (0.40–1.54)	0.32 ± 0.01 (0.22–0.49)	0.29 ± 0.01 (0.21–0.42)	0.058 ± 0.002 (0.041–0.080)
32	−220	28	67.0 ± 2.8 (45.2–102.4)	0.76 ± 0.04 (0.42–1.30)	0.29 ± 0.01 (0.21–0.40)	0.27 ± 0.01 (0.217–0.365)	0.060 ± 0.002 (0.041–0.078)
32	0	23	64.5 ± 3.0 (45.6–100.2)	0.73 ± 0.03 (0.47–1.09)	0.29 ± 0.01 (0.22–0.41)	0.27 ± 0.01 (0.229–0.375)	0.057 ± 0.002 (0.043–0.071)
Effects *F*	Temperature		2.752* $24^b, 26^a, 30^a, 32^b$	1.174^{NS}	3.560* $24^b, 26^a, 30^a, 32^b$	3.788* $24^a, 26^{ab}, 30^b, 32^b$	4.334** $24^a, 26^{ab}, 30^b, 32^{ab}$
	Moisture		0.756^{NS}	0.264^{NS}	0.017^{NS}	0.028^{NS}	0.811^{NS}
	Interaction		0.372^{NS}	0.201^{NS}	0.950^{NS}	0.083^{NS}	1.331^{NS}

eggs of different reptiles do not necessarily respond to differences in hydric environments in the same manner.

The Effects of Incubation Temperature.—Compared with substrate moisture, incubation temperature pervasively influenced egg incubation of *N. n. atra*. Overall, incubation temperature influenced water uptake by eggs during the course of incubation, hatching success, embryonic use of energy and nutrients during incubation, and size, mass, and composition of hatchlings.

Significant differences in length and mass were found among hatchlings from eggs incubated at different temperatures, and hatchlings from eggs incubated at moderate temperatures (26–30°C) were larger and heavier than those from eggs incubated at lower (24°C) and higher (32°C) temperatures. These findings are somewhat consistent with those reported for other reptiles (e.g., Gutzke et al., 1987; Packard et al., 1989; Burger, 1990; van Damme et al., 1992; Ji and Braña, 1999; Braña and Ji, 2000). The larger hatchling size was apparently associated with the greater carcass dry mass, as indicated by hatchlings from eggs incubated at 26 and 30°C (Tables 2–3). It is worth noting that embryos at 26 and 30°C also consumed less energy to complete development. The lower energy expenditure for embryogenesis could be the main reason why eggs incubated at 26 and 30°C produced larger and heavier hatchlings. The length (snout–vent length plus tail length) at hatching alone may not be a good indicator for judging hatchling quality, because smaller hatchlings with greater residual yolk may increase their size during their first days after hatching by transferring resources in the residual yolk to the carcass. The reverse relationship between residual yolk mass and carcass mass and the contribution of residual yolk to subsequent growth of newly emerged young have been found in several species of snakes, for example, *Elaphe carinata* (Ji et al., 1997a), *E. taeniura* (Ji et al., 1999b), *Dinodon rufozonatum* (Ji et al., 1999c), and *Ptyas korros* (Ji and Sun, 2000).

Our data provide evidence for the thermal dependence of embryonic use of inorganic material. Larger hatchlings, which characteristically had heavier carcasses, from eggs incubated at 26 and 30°C contained more inorganic material than did smaller hatchlings from eggs incubated at 24 and 32°C, mainly because these larger hatchlings contained more inorganic material from the shell. Our conclusion can be supported by the fact that *N. n. atra* embryos use eggshell

as the secondary source of inorganic material but do not accumulate inorganic material from the shell in the yolk (Ji et al., 1997b, 1999a). The smaller hatchling size, which was mainly induced by the higher energy expenditure for embryonic development at the two more extreme incubation temperatures in this study, explains why there was less ash in the hatchlings from eggs incubated at 24 and 32°C (Table 4).

The Range of Suitable Incubation Temperatures.—It is not suitable to incubate *N. n. atra* eggs at 24°C, because eggs incubated at the temperature exhibited the highest embryo mortality, had an extremely long duration of incubation (approximately three months), and produced the smallest hatchlings. The highest embryo mortality at 24°C suggests that this temperature presumably is near the lower threshold for incubating *N. n. atra* eggs. Taking approximately three months to complete embryonic development means newborns will emerge later than mid-October, which is approximately one month prior to the onset of winter dormancy of *N. n. atra* in Zhejiang (late November; Huang and Jin, 1990). Because newly emerged *N. n. atra* young need at least 1–3 weeks to absorb residual yolks, and before that period they do not eat (X. Ji, unpubl. data), a prolonged exposure of embryos at 24°C means reduced opportunity to grow during the first active season of hatchlings. In addition, smaller hatchlings from eggs incubated at 24°C might have lower fitness when compared with larger ones from eggs incubated at warmer temperatures, as numerous studies have indicated that smaller hatchlings have lower survival in their first active season and winter (Ferguson and Bohlen, 1978; Fox, 1978; Ferguson and Fox, 1984; van Damme et al., 1992). Based on our data, we may reasonably conclude that the northern limit of the distribution of *N. n. atra* may be partly influenced by incubation temperatures in addition to thermal stress in the winter, as suggested by Burger (1991a).

It is worth noting that an increase in incubation temperature from 30 to 32°C only shortens the incubation period by about four days. This advantage of the shortened incubation length could not be very great, whereas eggs incubated at 32°C exhibited noticeably higher embryo mortality, and produced more deformed hatchlings and smaller hatchlings, than did eggs incubated at 30°C (Table 1). These observations suggest that 32°C is near the upper threshold for incubating *N. n. atra* eggs and the cost of laying eggs in sites with unfavorably high temperatures will be high.

An increase in incubation temperature from 26 to 30°C shortens the incubation period by about 20 days. This difference in incubation duration could be ecologically important for *N. n. atra* eggs incubating in nature. Unfortunately, we did not test the effects of incubation temperature on posthatching performance of hatchlings; therefore, the optimal incubation temperature remains unclear. However, it is clear that the range of temperatures suitable for incubating *N. n. atra* eggs is quite narrow, extending approximately from 26 to 30°C. This suggests that females in nature may increase their reproductive benefits if they select nest sites with suitable thermal conditions for incubating eggs.

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Geographic and Annual Variation in Life-History Traits in a Temperate Zone Australian Skink

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ABSTRACT.—**Life-history theory seeks to explain patterns of variation between species or populations of the same species. Studies of squamates in general, and lizards in particular, have assumed a prominent place in the understanding of such variation. However, to date, there have been surprisingly few studies of the Scincidae, a major squamate taxon. We investigated geographic and interannual variation in life-history traits in two populations of the Tasmanian spotted snow skink, *Niveoscincus ocellatus*, living at the climatic extremes of the species' distribution. Within each population, there were no interannual or intersexual differences in adult body size. However, mature individuals from a cold subalpine/alpine site were significantly larger at maturity, and had a larger maximum body size than mature individuals from a warmer coastal site. These findings are consistent with current predictions of the proximate effect of the thermal environment on lizard growth and size and age at maturity. In both populations, female fecundity was size-related. Litter size did not vary between years at either site, but, contrary to expectations, females from the cold site had the same or higher size-adjusted reproductive output as those from the warm coastal site. We suggest that resource availability is high at both sites and that a high reproductive output by females from the cold site does not confer a significantly higher survival risk than a lower reproductive commitment. Offspring were largest at the cold site, which is consistent with variation in offspring size of other widespread species and may occur because of strong selective pressures on early survival and growth at the cold site.**

Life-history tactics were defined by Stearns (1976) as a series of coadapted traits "designed by natural selection to solve ecological problems." An understanding of the life history of a species, and more important of annual or geographic variation, is crucial to an understanding of its ecology, to its adaptation to a particular environment, and ultimately to what factors may limit its distribution and reproductive output. Lizards exhibit a great diversity of life histories (Tinkle et al., 1970; Ballinger, 1983; Dunham et al., 1988), and the challenge remains to explain the geographic variation observed within many species that occupy wide geographic, altitudinal or climatic ranges. Although numerous medium or long-term field-based studies of lizard life histories (e.g., Ballinger, 1973, 1983; Ferguson et al., 1980; Dunham, 1982; Jones and Ballinger, 1987; Jones et al., 1987; Grant and Dunham, 1990; Howland, 1992; Tinkle et al., 1993), and more recently a series of experimental approaches (e.g., Sinervo and Adolph, 1989; Sinervo, 1990a,b; Sinervo et al., 1992; Ferguson and Talent, 1993), have contributed to our understanding of the evolution of lizard life history, these have largely been based on North American phrynosomatid species, particularly oviparous examples. Thus, our knowledge of

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