

孵化温度对灰鼠蛇卵孵化期、孵化成功率和孵出幼体特征的影响

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摘要: 用 4 个恒定温度 (24~ 32) 孵化灰鼠蛇卵, 检测温度对孵化期、孵化成功率和孵出幼体特征的影响。在 24~ 32 范围内, 孵化温度显著影响孵化期及孵出幼体的体长和剩余卵黄大小, 但不影响孵化成功率和孵出幼体的性别、体重、躯干重和脂肪体重。24、26、30 和 32 孵化期分别为 99.0、72.2、54.7 和 48.7 d, 24 和 26 孵出幼体的体长大于 30 和 32 孵出幼体; 24 和 32 孵出幼体内的剩余卵黄较多。不同温度下发育的胚胎对卵内物质和能量的利用有一定的差异, 但差异不显著。雌性幼体的体长、尾长和总长均大于雄性幼体, 这些两性差异与孵化温度无关。孵出幼体和新生卵内容物灰分含量无显著差异, 孵化前后卵壳灰分含量也无显著差异, 表明灰鼠蛇的卵黄可提供胚胎发育所需的所有无机物。

关键词: 游蛇科; 灰鼠蛇; 卵; 孵化温度; 孵化期; 孵化成功率; 幼体特征

Effects of Incubation Temperature on Duration of Incubation, Hatching Success, and Hatchling traits in The Gray Rat Snake, *Ptyas korros* (Colubridae)

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Abstract The gray rat snake, *Ptyas korros* (Colubridae), is a kind of popular oviparous snake in the southern provinces of China. Herein, we established an experimental protocol using the gray rate snake as the animal model to examine (1) influence of temperature on hatching success, hatching time, (2) influence of temperature on hatchling traits, (3) influence of temperature on embryonic use of energy and material.

In early July 1999, we collected 11 gravid females from a private peddler at Jiande county, Zhejiang province, east China. The females were brought to our laboratory in Hangzhou Normal College, where they were randomly assigned 1~ 2 to a 50 × 50 × 50 cm³ wire cage placed in an air-conditioned room at 28~ 30 . Food (frogs) and water were provided libitum. The animals begin to lay eggs a few days after their arrival. Cages were checked for a minimum of six times daily for the presence of eggs so that eggs could be collected, weighed measured promptly. Throughout the reproductive season, A total of 74 freshly laid eggs were collected. We randomly selected one freshly laid egg, which were then dissected and separated into eggshell and egg contents (yolk+embryo), from each of the clutches. The embryo was too small and fragile to be separated and therefore was included with the yolk. Egg contents were put into a pre-weighed dish and weighed. Eggshells were rinsed briefly in distilled water, dried by blotting with a paper towel and weighed. Egg contents and eggshells were then oven-dried to constant mass at 65 for a minimum of 24

基金项目: 浙江省自然科学基金青年人才专项基金资助项目; 浙江省 151 人才基金资助项目; 杭州市跨世纪人才基金资助项目

收稿日期: 2000-10-08; **修订日期:** 2001-05-16

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h, weighed and stored frozen at -15°C until they could be processed for determining composition. The remaining eggs were put into plastic boxes contained moist vermiculite (-220kPa , dry vermiculite : water = 1:1). The boxes were then placed into the incubators whose temperature was set at 24, 26, 30 and 32°C , respectively. As soon as possible, eggs from each clutch were equally assigned into the containers at the four above-mentioned temperatures so as to avoid the family effect. Furthermore, we moved the boxes among shelves daily according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. All containers were weighed daily and, if necessary, water was added to compensate for small evaporative losses, so that the water potential of the substrate was maintained constant.

Duration of incubation was defined as the elapse time from egg laying to hatchling emergence. Upon emergence, each hatchling was measured, weighed, killed by freezing to -15°C , and then separated into carcass, residual yolk and fat bodies. The three components were oven dried to constant mass at 65°C , weighed and preserved frozen for later determination of composition. We extracted non-polar 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. The total lipid in each hatchling was calculated as the sum of the lipids in its carcass, residual yolk and fat bodies. We determined energy density of dried samples using an adiabatic bomb calorimeter and ash (inorganic material) content in each sample using a muffle furnace at 800°C for a minimum of 8 h and then weighing the remaining ash.

All variables were tested for normality using Kolmogorov-Smirnov test and for homogeneity of variance using Bartlett's test prior to further statistical analysis, and all data met the assumption of parametric analyses. We used G-test, two-way analysis of variance (two-way ANOVA) and analysis of covariance (ANCOVA). Homogeneity of slopes was checked prior to testing for differences in adjusted means. For multiple comparisons, we used Tukey's test. Significance level was set at $\alpha = 0.05$.

Within the range from 24 to 32°C , temperature significantly affected incubation period of *P. korros* eggs, which decreased dramatically with increase in incubation temperature. The duration of incubation at 24, 26, 30, and 32°C averaged 99.0, 72.2, 54.7, and 48.7 d, respectively. On the contrast, hatching success of eggs and sex ratio and abnormality of resultant hatchlings were not influenced by temperatures involved. The hatchlings showed sexual dimorphism on snout-vent length (SVL) and tail length (TL), as indicate that females have larger SVL and TL than males, but these differences were independent of temperature treatments. Incubation temperature involved in this study could affect snout-vent length and residual yolk size of hatchlings, but did not affect hatching success and sex ratio, body mass, carcass mass and fat body mass of hatchlings. Eggs incubated at 24 and 26°C produced larger hatchlings (SVL) than did those from 30 and 32°C . Hatchlings from eggs incubated at 24 and 32°C contained more residual yolks than did those from 26 and 30°C .

Embryonic use of energy and material differed in some degrees among embryos developing at different temperatures, although the differences were statistically not significant. During incubation, approximately 74.5% ~ 79.2% dry material, 59.8% ~ 67.0% non-polar lipids and 72.2% ~ 77.9% energy in egg contents of the freshly laid egg were transferred into the hatchling. Unlike most of embryonic reptiles studied, *P. korros* embryos could obtained all inorganic matter needed for development from the yolk, as there were no significant differences in ash contents both between newly hatched hatchlings and egg contents of freshly laid eggs and between eggshells from hatched and freshly laid eggs.

Key words: Colubridae; *Ptyas korros*; egg; incubation temperature; duration of incubation; hatching success; hatchling traits

文章编号: 1000-0933 (2002) 04-0548-06 中图分类号: Q 959. 6⁺ 2 文献标识码: A

在所有影响爬行动物卵孵化和孵出幼体特征的环境因子中, 温度是最重要的因子之一^[1~4]。爬行动物卵需在一定温度范围内孵化才能孵出存活幼体, 低孵化成功率、高胚胎或幼体畸形率等指标可用于确定特定种类的存活孵化温度(viable incubation temperature)范围。然而, 即便在存活孵化温度范围内, 孵化温度亦可影响胚胎发育速率^[5]、孵化成功率和孵出幼体的形态、身体组成和功能表现等重要的幼体特征, 进而影响幼体的适应性和生存能力^[6~12]。在一些温度决定性别的种类中, 孵化温度还能决定孵出幼体的性别^[13]。因此, 野外爬行动物选择热环境适宜的巢址产卵或用人工设置的适宜热环境孵化爬行动物卵有利于孵出高适应性幼体。实验室内用多重温度组合恒温孵化爬行动物卵能精确检测孵化热环境的变化对孵出幼体的影响, 并有助于人们理解野外爬行动物巢址选择的生态学意义。

灰鼠蛇(*Ptyas korros*)广泛分布于我国华南诸省(包括香港和华东台湾)及印度、泰国和印度尼西亚等国^[14]。对灰鼠蛇卵孵化的研究目前只有零星报道^[15]。本研究在 4 个恒定温度条件下孵化灰鼠蛇卵, 旨在检测孵化温度对孵化期、孵化成功率和孵出幼体特征的影响。

1 材料和方法

怀卵灰鼠蛇于 1999 年 6 月中旬购自浙江建德, 动物带回杭州后被关养在 50 × 50 × 50 cm³ 的专用蛇笼中, 提供食物(黑斑蛙(*Pelophylax nigromaculata*)、泽蛙(*Fejervarya limnocharis*))和饮水。为避免因失水而导致初始卵重的误差, 所有卵均在产后 1 h 内被称重、测量和编号。卵经可孵性鉴别后, 随机从每窝取 1 枚卵, 解剖分离成卵壳和卵内容物(卵黄+ 胚胎)。新生卵内胚胎极小、不易分离, 卵壳和卵内容物在 65 ℃ 烘箱中干燥至恒重, 称其重量。干燥样品冰冻保存, 待以后做成分分析。其余卵移入内含潮湿孵化基质的塑料盒中, 卵的 1/3 埋入基质中。孵化基质湿度设置为 - 220 kPa, 由干蛭石(vermiculite) 水= 1 : 1 配合而成。孵化盒用穿孔的塑料薄膜覆盖, 分别放置 24、26、30、32 ± 0.3 ℃ 的 LRH-250G 生化培养箱(广东医疗器械厂)内。每日补充孵化基质散失的水分, 以保持湿度恒定; 每日按预先设定的顺序调整孵化盒在培养箱中的位置, 以减少箱内可能存在的温梯度的影响。

幼体孵出 1 h 内即被称重, 随后冰冻保存。冰冻幼体以后被解冻, 解剖分离成躯干、剩余卵黄和脂肪体。幼体 3 组分在 65 ℃ 烘箱中干燥至恒重后, 称其干重。卵黄和幼体躯干中的非极性脂肪用索氏脂肪抽提仪在 55 ℃ 条件下抽提 5.5 h 测得, 分析纯乙醚作抽提溶剂。用 GR-3500 型弹式热量计(长沙仪器厂)测定能量, 灰分用马福炉在 800 ℃ 条件下焚烧 8 h 测得。

所有数据在作进一步统计分析前, 用 Kolmogorov-Smirnov 和 Bartlett(Statistica 统计软件包)分别检验正态性和方差的同质性。经检验, 原始数据无须转化即能用于参数统计。用 G 检验、协方差分析(ANCOVA)、双因素方差分析(双向 ANOVA)、Tukey's 检验等处理和比较相应的数据。初始卵重作为所有 ANCOVA 的协变量。比较矫正平均值前, 检验斜率的均一性。描述性统计值用平均值 ± 标准误表示, 显著性水平设置为 $\alpha = 0.05$ 。

2 结果

孵化温度显著影响灰鼠蛇的孵化期(ANOVA, $F_{3,45} = 1068.41, P < 0.0001$)。24~ 26 ℃, 孵化期平均缩短 26.8 d; 26~ 30 ℃, 孵化期平均缩短 17.5 d; 30~ 32 ℃, 孵化期平均缩短 6.0 d(表 1)。24~ 32 ℃ 范围内, 孵化温度对孵化成功率($G = 4.31, df = 3, P > 0.10$)、孵出幼体性别($G = 5.46, df = 3, P > 0.10$)和畸形率($G = 4.79, df = 3, P > 0.10$)无显著的影响。孵出幼体总性比不偏离 1:1($G = 0.05, df = 1, P > 0.90$)。

孵出幼体 SVL、TL、总长的两性差异显著, 雌性幼体大于雄性幼体(表 2)。孵化温度显著影响幼体的 SVL 和剩余卵黄干重, 24 ℃ 和 26 ℃ 孵出幼体的 SVL 大于 30 ℃ 和 32 ℃ 孵出幼体, 24 ℃ 和 32 ℃ 孵出幼体的剩余卵黄大于 26 ℃ 和 30 ℃ 孵出幼体(表 2)。

表 1 孵化温度对灰鼠蛇孵化期、孵化成功率及孵出幼体性比和畸形率的影响

Table 1 The effects of incubation temperatures on incubation length, hatching success and sex ratio and abnormality of hatchlings in *Ptyas korros*

温度 () Temperature	孵化卵数 Incubated eggs	孵化期 (d) Duration of incubation	孵化成功率 (%) Hatching success	性比 (/) Sex ratio	畸形率 (%) Abnormality
24	12	99.0 ± 0.7 (96.5 ~ 102.0)	75.0 (9/12)	2/7	8.3 (1/12)
26	15	72.2 ± 0.4 (70.0 ~ 74.8)	86.7 (13/15)	6/7	6.7 (1/15)
30	21	54.7 ± 0.4 (50.2 ~ 58.0)	90.5 (19/21)	12/7	4.8 (1/21)
32	15	48.7 ± 0.4 (47.6 ~ 50.2)	53.3 (8/15)	4/4	20.0 (3/15)

新生卵卵壳干重分别占卵干、湿重的 30.5% 和 10.6%，孵出卵卵壳干重占卵初始重的 9.6% ~ 10.1%；新生卵和孵出卵卵壳灰分的百分含量分别为 22.3% 和 22.4% ~ 24.0% (表 3)。24~ 32 范围内，温度对孵出幼体的各项成分测定指标无显著的影响，新生卵内容物的灰分含量与孵出幼体无显著差异，新生卵卵壳的干重和灰分含量与孵出卵无显著差异 (表 3)。温度对胚胎利用卵内物质和能量有一定程度的影响，但差异不显著。孵化过程中，卵内容物的干物质、脂肪和能量转化率分别为 74.5% ~ 79.2%、59.8% ~ 67.0%、72.2% ~ 77.9% (表 3)。

表 2 孵化温度和性别对灰鼠蛇孵出幼体形态表型特征的影响

Table 2 Morphological phenotypes of *Ptyas korros* hatchlings, according to sex and incubation temperature

幼体特征 Hatchling trait		温度 () Temperature				影响 Effects		
		24	26	30	32	Sex <i>F</i> _{1,40}	Temperature <i>F</i> _{3,40}	S × T <i>F</i> _{1,40}
入孵卵重 (g)	F	8.9 ± 2.4	10.1 ± 0.4	8.7 ± 0.4	8.2 ± 0.2			
Initial egg mass	M	9.2 ± 0.5	9.4 ± 0.5	8.9 ± 0.5	9.7 ± 0.3			
体长 (mm)	F	231.5 ± 12.5	247.5 ± 3.2	229.0 ± 5.1	230.0 ± 7.3	6.69*	4.21*	0.85 ns
Snout-vent length	M	232.9 ± 1.8	237.6 ± 3.4	219.5 ± 3.7	222.0 ± 5.2	<i>F</i> > <i>M</i>	24 ^{ab} , 26 ^a , 30 ^b , 32 ^{ab}	
尾长 (mm)	F	96.0 ± 9.0	102.0 ± 1.6	94.7 ± 2.5	95.0 ± 4.5	8.02**	0.41 ns	0.95 ns
Tail length	M	92.1 ± 2.5	94.4 ± 1.8	94.1 ± 2.7	90.8 ± 2.8	<i>F</i> > <i>M</i>		
总长 (mm)	F	327.5 ± 21.5	349.5 ± 3.9	323.8 ± 7.4	325.0 ± 11.4	8.51**	2.42 ns	0.45 ns
Total length	M	325.0 ± 3.9	332.0 ± 4.9	313.6 ± 6.2	312.8 ± 7.3	<i>F</i> > <i>M</i>		
幼体湿重 (g)	F	6.3 ± 1.5	7.0 ± 0.2	6.4 ± 0.3	6.0 ± 0.1	1.28 ns	1.34 ns	0.18 ns
Wet body mass	M	6.3 ± 0.3	6.4 ± 0.3	6.3 ± 0.4	6.9 ± 0.2			
幼体干重 (g)	F	1.68 ± 0.47	1.81 ± 0.08	1.62 ± 0.10	1.56 ± 0.07	0.23 ns	0.63 ns	0.20 ns
Dry body mass	M	1.67 ± 0.10	1.71 ± 0.07	1.65 ± 0.12	1.84 ± 0.13			
躯干 (g)	F	1.16 ± 0.25	1.31 ± 0.07	1.18 ± 0.05	1.04 ± 0.06	0.07 ns	1.17 ns	0.05 ns
Carcass	M	1.16 ± 0.06	1.23 ± 0.04	1.20 ± 0.07	1.18 ± 0.13			
剩余卵黄 (g)	F	0.31 ± 0.12	0.20 ± 0.01	0.21 ± 0.04	0.28 ± 0.04	0.55 ns	6.26**	0.70 ns
Residual yolk	M	0.27 ± 0.05	0.22 ± 0.03	0.23 ± 0.05	0.44 ± 0.12		24 ^{ab} , 26 ^b , 30 ^b , 32 ^a	
脂肪体 (g)	F	0.22 ± 0.10	0.29 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	3.08 ns	0.47 ns	1.55 ns
Fat bodies	M	0.24 ± 0.01	0.26 ± 0.02	0.22 ± 0.02	0.22 ± 0.02			

双向 ANCOVA (入孵卵重为协变量) 的 *F* 值: *F*: 雌性幼体; *M*: 雄性幼体 ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ 。不同上标的平均值差异显著 $a > b$ *F* values correspond to single effects and factor interactions in two-way ANCOVA (with initial egg mass as the covariate). *F*: female; *M*: male ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ 。Means with different superscripts differ significantly. $a > b$

3 讨论

在 24~ 32 的恒定温度范围内，孵化温度主要影响灰鼠蛇卵的孵化期和孵出幼体的体长和剩余卵黄大小。24 和 26 孵出幼体的 SVL 较大，这与其他爬行动物中发现的较低或温和温度中孵出较大的幼体的结论一致^[8-11]。在 24~ 32 范围内，孵化温度影响胚胎对卵黄的利用，低温 (24) 和高温 (32) 孵出

幼体中剩余卵黄大于温和温度孵出幼体。剩余卵黄大小与胚胎发育过程中物质和能量的转化及能量消耗等密切相关。灰鼠蛇卵在高温下(32℃)孵化,孵出幼体内的剩余卵黄较大,这一结果与高温孵化爬行动物卵导致卵黄利用不充分的结果是一致的^[10, 16~18]。24℃孵出的灰鼠蛇幼体内有较多的剩余卵黄可能与胚胎在较低温度中发育能耗较小有关。

在实验温度范围内,孵化温度对幼体的其它特征,如幼体湿重、干重、躯干和脂肪体重量无显著的影响(表2)。此外,对卵孵化成功率、胚胎对卵内物质和能量的利用及幼体畸形率也没有明显的影响(表1)。总体而言,24~32℃范围内恒定温度对灰鼠蛇孵化卵和孵出幼体特征的影响是微弱的。因此,该范围温度均适合孵化灰鼠蛇卵。由于在适宜温度范围内较高的孵化温度能缩短孵化期,使得幼体能较早孵出并在当年越冬季节开始前有较长的生长期,野外亲体选择平均温度相对较高的巢址产卵可能具有重要的生态学意义。此外,在确保孵出幼体正常且发育良好的前提下,用相对较高的温度孵卵使孵化期缩短,还能缩短孵化卵暴露在不利生物或非生物因子作用的时间,有效地提高胚胎发育的成功率。

表3 灰鼠蛇孵出卵和新生卵的主要成分比较和 ANCOVA 分析的 F 值

Table 3 Major components and F values of ANCOVA for *Ptyas korros* hatched eggs and freshly laid eggs

	幼体 Hatchlings				卵内容物 (n = 11) Egg contents		F _{4,54}
	24 (n = 9)	26 (n = 13)	30 (n = 19)	32 (n = 8)			
湿重 (g) Wet mass	6.21 ^b ± 0.06	6.23 ^b ± 0.07	6.51 ^b ± 0.12	6.51 ^b ± 0.04	7.32 ^a ± 0.08	17.13	***
干重 (g) Dry mass	1.64 ^b ± 0.05	1.61 ^b ± 0.04	1.67 ^b ± 0.04	1.71 ^b ± 0.04	2.16 ^a ± 0.04	25.38	***
脂肪重 (g) NPL lipid mass	0.397 ^b ± 0.008	0.399 ^b ± 0.012	0.373 ^b ± 0.013	0.418 ^b ± 0.008	0.624 ^a ± 0.015	58.13	***
灰分 (g) Ash mass	0.195 ± 0.007	0.194 ± 0.006	0.197 ± 0.010	0.207 ± 0.009	0.189 ± 0.005	0.78	ns
能量 (kJ) Energy	38.7 ^b ± 1.1	38.1 ^b ± 1.0	37.6 ^b ± 0.7	40.6 ^b ± 0.9	52.1 ^a ± 0.9	39.02	***
卵壳干重 (g) Shell dry mass	0.89 ± 0.02	0.90 ± 0.02	0.91 ± 0.02	0.86 ± 0.02	0.95 ± 0.02	1.76	ns
卵壳灰分 (g) Shell ash mass	0.205 ± 0.007	0.216 ± 0.008	0.204 ± 0.007	0.201 ± 0.004	0.212 ± 0.005	0.66	ns

数据用矫正平均值 ± 标准误表示,初始卵重(设置为 9 g)为协变量, F 值后的符号代表显著性水平: ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ 。不同上标的矫正平均值差异显著 (Tukey's test, $\alpha = 0.05$)。Data are expressed as adjusted means ± SE, with initial egg mass (set at 9 g) as the covariate. Symbols immediately after F values represent significant level: ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Adjusted means with different superscripts are statistically different (Tukey's test, $\alpha = 0.05$)

灰鼠蛇孵出的雌性幼体体长、尾长和总长均大于雄性幼体,显示出孵出幼体形态的两性差异,这些特征的两性差异显然与孵化温度无关(表2)。爬行动物中普遍存在个体大小和局部形态特征的两性异形,一些爬行动物形态特征的两性异形在初生幼体中就已出现,如北草蜥 (*Takydromus septentrionalis*)^[19]和地中海岩蜥 (*Podarcis muralis*)^[10]。

灰鼠蛇幼体中灰分含量与新生卵没有差异,卵壳孵化前后灰分含量没有差异, Ji 等^[20]虽然在 30℃ 孵出幼体中检测到了卵壳孵化前后灰分含量的差异,但这种差异是非常细微的。这些都说明灰鼠蛇胚胎发育所需的无机物几乎全部来自卵黄。这与绝大多数爬行动物将卵壳作为无机物的第二储备库,并在胚胎发育过程中从中获得相当可观的无机物的现象不同^[21~25]。由此可见,爬行动物从卵壳中动用无机物的量存在种间差异,这种差异可能与卵壳结构和成分以及无机物在卵黄和卵壳之间的分配等种间差异有关。

参考文献

- [1] Booth D T and Thompson M B. A comparison of reptilian eggs with those of megapode birds. In: Deeming, D. C. and Ferguson, M. W. J. eds. *Egg Incubation, Its Effect on Embryonic Development in Birds and Reptiles*. Cambridge: Cambridge University Press, 1991. 325~344.

- [2] Congdon J D and Gibbons J W. Turtle eggs: their ecology and evolution. In: Gibbons, J. W. ed *Life History and Ecology of the Slider Turtle* Washington, D. C.: Smithsonian Institution Press, 1990 109~ 123
- [3] Deeming D C and Ferguson M W J. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: Deeming, D. C. and M. W. J. Ferguson eds *Egg Incubation, Its Effect on Embryonic Development in Birds and Reptiles* Cambridge: Cambridge University Press, 1991. 147~ 171.
- [4] Packard G C and Packard M J. The physiological ecology of reptilian eggs and embryos. In: Gans, C. and Huey, R. B. eds *Biology of the Reptilia* vol 16 New York: A. Liss, 1988 523~ 605
- [5] Booth D T. Incubation of turtle eggs at different temperatures: do embryos compensate for temperature during development? *Physiol Zool* 1998, **71**: 23~ 26
- [6] Allstead J and Lang J W. Incubation temperature affects body size and energy reserves of hatchling American alligators (*Alligator mississippiensis*). *Physiol Zool*, 1995, **68**: 76~ 97.
- [7] Burger J. Effects of incubation temperature on behavior of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *J. Herpetol*, 1990, **24**: 158~ 63
- [8] Gutzke W H N, Packard G C, Packard M J, et al. Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica*, 1987, **43**: 393~ 404
- [9] Van Damme R, Bauwens D, Braña F. et al. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica*, 1992, **48**: 220~ 228
- [10] Ji X and Braña F. The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comp. Biochem. Physiol*, 1999, **124A**: 205~ 213
- [11] Lin Z H (林植华), Ji X (计翔) The effects of thermal and hydric environments on incubating eggs and resultant hatchlings of the grass lizard, *Takydramus septentrionalis*. *Zool Res* (in Chinese) (*动物学研究*), 1998, **19** (6): 439~ 445
- [12] Braña F, Ji X. Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J. Exp. Zool*, 2000, **286**: 422~ 433
- [13] Bull J J. Sex determination in reptiles. *Quart Rev. Biol*, 1980, **55**: 3~ 21.
- [14] Zhao E M and Adler K. Herpetology of China. Published by Society for the study of Amphibians and Reptiles Oxford, Ohio, USA, 1993 255~ 256
- [15] Huang M H (黄美华) Reptilia. In: Huang M H (黄美华), Jin Y L (金贻郎) and Cai C M (蔡春沐) eds *Fauna of Zhejiang (Amphibia and Reptilia)* (in Chinese). Hangzhou: Zhejiang Science and Technology press, 1990 227~ 229.
- [16] Beuchat C A. Temperature effects during gestation in a viviparous lizard. *J. Theor. Biol*, 1988, **13**: 135~ 142
- [17] Phillips J A, Garel A, Packard G C, et al. Influence of moisture and temperature on eggs and embryos of green iguanas (*Iguana iguana*). *Herpetologica*, 1990, **46**: 238~ 245.
- [18] Phillips J A and Packard G C. Influence of temperature and moisture on eggs and embryos of the white-throated Savanna monitor *Varanus albigularis*: implications for conservation. *Biol. Conserv.*, 1994, **69**: 131~ 136
- [19] Zhang Y P (张永普), Ji X (计翔). Ontogenetic changes of sexual dimorphism in head size and food habit in grass lizard, *Takydramus septentrionalis*. *Zool Res* (in Chinese) (*动物学研究*), 2000, **21** (3): 181~ 186
- [20] Ji X and Sun P Y. Embryonic use of energy and post-hatchling yolk in the gray rat snake, *Ptyas korros* (Colubridae). *Herpetol. J.*, 2000, **10**: 13~ 17.
- [21] Ji X, Sun P Y, Fu S Y, et al. Incubation and utilization of energy and material during embryonic development in the cobra *Naja naja atra*. *J. Herpetol*, 1997, **31**: 302~ 306
- [22] Ji X, Fu S Y, Zhang H S, et al. Material and energy budget during incubation in a Chinese skink, *Eumeces chinensis*. *Amphibia-Reptilia*, 1996, **17**: 209~ 216
- [23] Packard M J and Packard G C. Comparative aspects of calcium metabolism in embryonic reptiles and birds. In: Seymour, R. S. ed *Respiration and Metabolism of Embryonic Vertebrates* Dordrecht, The Netherlands: Junk, 1984 155~ 179.
- [24] Packard M J and Packard G C. Sources of calcium, magnesium, and phosphorus for embryonic softshell turtles (*Trionyx xiphiferus*). *J. Exp. Zool*, 1991, **258**: 151~ 157.
- [25] Shadrix C A, Crotzer D R, Shauna L M, et al. Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces fasciatus*. *Copeia* 1994, **1994**: 493~ 498.