热环境对虎斑颈槽蛇卵孵化期、孵化成功率和孵出幼体特征的影响

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摘要：用恒温（24、27、30 和 33 ℃）和波动温度（平均 26.1 ℃，范围 20.1～32.7 ℃）孵化虎斑颈槽蛇（*Rhabdophis tigrinus lateralis*）卵，检测热环境对孵化期、孵化成功率和孵出幼体的影响。孵化热环境显著影响孵化期、孵化成功率和胚胎畸形率，对孵出幼体性别无显著影响。孵化期随孵化温度升高而缩短，24、27、30 和 33 ℃的平均孵化期分别为 45.0、32.7、27.3 和 26.0 d，波动温度的平均孵化期为 37.9 d。33 ℃孵化成功率最低（16.7%），胚胎畸形率最高（100%）。孵出幼体总性比（雌性 / 雄性 = 0.6）不显著偏离 1:1。孵出幼体的尾长显示两性异形，雄性尾长大于雌性；其它被检幼体特征无显著的两性差异。24、27 和 30 ℃以及波动温度孵出幼体的所有被检指标均无显著差异。33 ℃孵出幼体的体重和个体大小小于其它热环境中孵出的幼体，并特征性地具有较小的躯干、较大的剩余卵黄。33 ℃ 中胚胎发育的能耗显著大于其它热环境中胚胎发育的能耗。33 ℃ 孵出幼体的灰分含量较低，但孵出卵卵壳较重。33 ℃孵出幼体不能运动；其它热环境中孵出的幼体在跑道上表现良好，这些幼体的不间断运动的最大距离、每分钟运动距离和每分钟停顿次数无显著的差异。结果表明，持续将虎斑颈槽蛇卵暴露在 33 ℃条件下不利于该种胚胎发育并可能对胚胎具有致死性影响，波动温度孵卵有利于拓宽存活孵化温度范围。

关键词：游蛇科；虎斑颈槽蛇；卵；孵化；孵出幼体；运动表现

The Effects of Thermal Environments on Duration of Incubation, Hatching Success and Hatchlings Traits in a Colubrid Snake, *Rhabdophis tigrinus lateralis* (Boie)

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Abstract: We incubated *Rhabdophis tigrinus lateralis* eggs laid by 12 females at constant (24, 27, 30, and 33 ℃) and fluctuating (mean = 26.1 ℃, range = 20.1～32.7 ℃) temperatures to assess the effects of thermal environments on incubation length, hatching success, and hatchling traits. Eggs were incubated at the same moisture level (2 g water / 1 g vermiculite; producing approximately-12 kPa water potential in different thermal environments in plastic containers (250mm × 180mm × 70mm) that were covered with a perforated plastic membrane. Eggs were 1/3-buried in the substrate, with the surface near the embryo exposed to air inside the container. We weighed containers daily and, if necessary, added water to the vermiculite to compensate for small evaporative losses and water absorbed by eggs. Eggs were weighed at 5-day intervals.

Upon emergence, each hatchling was weighed, examined for locomotor performance, and then killed by freezing to −15 ℃ for later studies. Because locomotor performance is highly sensitive to changes in body temperature in reptiles, we conducted trials at constant body temperature of 30 ℃. Body temperature of hatchlings was controlled by placing them in an incubator at 30 ℃. Locomotor performance was

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assessed by chasing hatchlings along a round racetrack of which the circumference was 1.92 m, which allowed vertical filming with a digital video camera. Videotapes were examined for time-specific number of stops, time-specific locomotor distance, and maximum continuous locomotor distance traveled without stopping during the entire trial.

The frozen hatchlings were later thawed for data collection. Sex of hatchlings was determined by pressing on both sides of the tail base using forceps for the presence or absence of hemipenes. SVL (snout-vent length) and tail length were measured for each hatchling. After taking the measurements, each hatchling was separated into carcass, fat bodies, and residual yolk. The three components were dried in an oven at 65 ℃ to constant mass, and then weighed. 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. We determined energy density of dried samples using an adiabatic bomb calorimeter. Ash in samples was determined using a muffle furnace at 700 C for a minimum of 12 h.

All data were tested for normality using Kolmogorov-Smirnov test, and for homogeneity of variances using Bartlett's test. Log_e or arc-sine transformations were performed when necessary to satisfy the conditions for using parametric tests. We use linear regression analysis, one-and two-factor analyses of variance (ANOVA), and one- and two- factor analyses of covariance (ANCOVA), when the assumptions of parametric analyses were met. Nonparametric analyses were used when these assumptions were violated. Throughout this paper, values are presented as mean $\pm$ 1 standard error, and the significance level is set at $\alpha=0.05$.

Incubation length, hatching success and the incidence of deformed embryos were affected by incubation thermal environments, and the sex ratio of hatchlings was not. Incubation length decreased dramatically as temperature increased. The average duration of incubation at 24, 27, 30 and 33 ℃ was 45.0, 32.7, 27.3 and 26.0 d, respectively; the duration of incubation at fluctuating temperatures averaged 37.9 d. Hatching success was the lowest (16.7%) at 33 ℃, but the highest incidence (100%) of deformed embryos was recorded from eggs incubated at this temperature compared to eggs incubated in other thermal environments. The overall sex ratio of hatchlings did not differ from equality. Except for that males had longer tails than did females, all other examined hatchling traits did not differ between both sexes. Hatchlings from eggs incubated at 24 ℃, 27 ℃, 30 ℃ and fluctuating temperatures did not differ in any of the examined traits. Hatchlings from eggs incubated at 33 ℃ were lighter in mass and smaller in size than those from other incubation thermal environments, characteristically having smaller carcasses but larger residual yolks. Energy expenditure for embryonic development was higher in eggs incubated at 33 ℃ than in those incubated in other thermal environments. Hatchlings from eggs incubated at 33 ℃ contained lower quantities of ash than did those from other incubation thermal environments, but heavier hatched shells were recorded from eggs incubated at this temperature. Except for those from eggs incubated at 33 ℃, hatchlings from other incubation thermal environments did not differ in the maximum length of continuous locomotion, the locomotor distance per minute and the number of stops per minute. Taken together, our data reveal that (1) a prolonged exposure of eggs of *R. tigrinus* at 33 ℃ has an adverse and presumably lethal effect on embryonic development, and (2) the range of viable incubation temperatures can be widened when eggs are incubated at fluctuating temperatures.

Key words:Colubridae; *Rhabdophis tigrinus lateralis*; egg; incubation; hatchling; locomotor performance

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许多环境因子能影响爬行动物的胚胎发育,温度是其中最重要的因子之一。温度不仅影响爬行动物胚胎的发育速率[1~7],还影响孵出幼体的形态[6,8~10]、功能表现[11~13]、生长[11,14,15]和行为[16~20]。胚胎经历的热环境能诱导某些幼体特征永久性的变异(如性别、脊椎骨数目和一些局部形态特征),这些变异对个体的适应性可能具有长期的影响[21~25],并已见于一些有鳞类[11,13,26~30]、龟鳖类[31~33]和鳄类[34]。因此,繁殖雌体通过体温调节和巢址选择为胚胎提供适宜发育的热环境具有重要的生态学意义。由于野外繁殖雌体难被追踪、产卵巢址难被定位,要准确了解纯自然条件下的热环境对爬行动物胚胎发育的影响是困难的[35]。恒温孵卵是实验室常用的研究方法,通常用来检测特定温度的孵化成功率和孵出幼体特征。由于恒温孵卵并不代表野外卵孵化的真实情况,近年来不断有同行在野外或模拟野外环境开展爬行动物孵化生物学的研究[9,28,30,35,36]。温度因波动而不稳定及温度的季节变化是野外热环境的基本特点;然而,温度不恒定的热环境是否有益于爬行动物卵孵化有待于进一步评估。

虎斑颈槽蛇见于中国大部分省区[37],是浙江省常见蛇类之一。有关该种繁殖和卵孵化的研究工作不多,零星数据主要见于各省动物志(如《浙江动物志-两栖类、爬行类》和《安徽两栖爬行动物志》)。赵群等曾用单一温度(30 ℃)孵化该种蛇卵,报道卵内物质和能量的利用率[38]。本研究中,作者用模拟野外热环境的波动温度和 24~33 ℃范围内 4 个恒温条件孵化虎斑颈槽蛇卵,研究温度对孵化期、孵化成功率、卵内物质和能量利用和孵出幼体特征的影响。

1 材料和方法

研究用的 12 条虎斑颈槽蛇于 2000 年 5 月中旬捕自浙江建德寿昌。动物带回杭州实验室,关养在专用蛇笼(0.6×0.6×0.5 m^3)内。动物在笼内能自由饮水和取食足量供应的泽蛙(*Rana limnocharis*),接受自然光照。虎斑颈槽蛇年产单窝卵,12 条雌体于 5 月 23 日~6 月 4 日间各产一窝卵。为避免吸水或失水导致卵初始重量变化,所有卵均在产后数分钟内被收集、称重和编号。

卵经可孵性鉴别后,移入 190×220 mm^2 内含潮湿蛭石(vermiculite)、加盖、通气的塑料盒内,蛭石湿度恒定为 −12 kPa(干蛭石重∶水 = 1∶2)。装载恒温孵化卵的塑料盒放置在 24、27、30、33 ℃(±0.3 ℃)的生化培养箱内。装载波动温度孵化卵的塑料盒放置在预置于室外围栏地下的玻璃缸(0.8m×0.5m×0.3m)内,围栏上覆石棉瓦,玻璃缸上覆草被。图 1 显示 2000 年 5 月 23 日~7 月 11 日波动温度孵化卵经历的热环境,期间每日最低、最高和平均温度的平均值分别为 24.0(SE = 0.3,$range$ = 20.1~28.4)、28.2(SE = 0.4,$range$ = 22.7~32.7)和 26.1(SE = 0.3,$range$=21.3~30.5)℃。波动温度孵化卵经历的最低温度(20.1 ℃)低于最低恒温孵化温度(24 ℃),最高温度(32.7 ℃)与最高恒温孵化温度(33 ℃)相近。由于部分 30 ℃孵化卵用于研究孵化过程中的物质和能量收支,每窝有 6~7 枚卵孵入该温度;其余同窝的卵尽可能均匀地孵在不同的热环境中。卵的 1/3 被埋在蛭石中,胚胎位置向上。每日将塑料盒内的水分调整到原有量,保持湿度恒定;每日按照预先设定的顺序调整塑料盒在生化培养箱中的位置,以减少箱内可能存在的温度梯度影响。每隔 5 日称恒温孵化卵的重量;每日 4 次(06∶00、12∶00、18∶00、22∶00)用精度为 0.1 ℃ 的 WNY-150A 型电子点温计(上海医用仪器厂)记录室外玻璃缸内的温度。

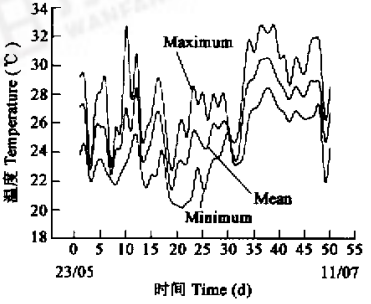

图 1 虎斑颈槽蛇卵波动温度孵化的热环境

Fig. 1 The thermal environments in which *Rhabdophis tigrinus* eggs were incubated

图中线条显示每日最高、平均温度和最低温度 Lines in the figure show daily maximum, average and minimum temperatures

幼体孵出数分钟后即被收集、测量和称重,随后测定幼体的运动表现。测定开始前 30 min,将幼体移入温度设置为 30 ℃的生化培养箱内,使其体温达到 30 ℃。幼体运动表现在周长为 1.92 m 的环形跑道内测定进行,跑道底面粗糙、间隔 240 mm 有标记线。测定时由一

人强烈驱赶（但不接触和推动）幼体[11]，另一人用摄像机追踪拍摄动物的运动表现，每一幼体的拍摄时间为 90 s。用 3 项指标显示动物的运动表现，即单位时间运动距离、单位时间停顿次数和最大持续运动距离（不间断持续运动的最大距离）。

测定完毕后将幼体移入 −20 ℃冰柜。冰冻幼体以后被解冻，鉴别性别（雄性有明显的半阴茎），解剖分离成躯干、剩余卵黄和脂肪体。分离出的幼体 3 组分和孵出卵卵壳在 65 ℃烘箱中干燥至恒重，分别称其干重。样品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 测定，分析纯乙醚作抽提溶剂。样品中的能量用 WGR-1 氧弹仪（长沙仪器厂造）测定，灰分含量用马福炉在 700 ℃焚烧 12 h 测定。

数据在作进一步统计检验前，用 Kolmogorov-Smirnov 和 Bartlett 分别检验其正态性和方差同质性（Statistica 统计软件包）。经检验，部分数据需经 $\mathrm{Log_e}$ 和 arc-sine 转化才符合参数统计的条件。用 G-检验、线性回归、单向（one-way）和双向（two-way）协方差分析（ANCOVA）、单向和双向方差分析（ANOVA）和 Tukey's 多重比较等处理相应的数据。入孵卵重为所有 ANCOVA 的协变量。比较矫正平均值前，检验斜率的均一性。文中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

2.1　恒温孵化卵的重量变化

不同热环境中孵化卵的初始重量（入孵卵重）无显著差异（ANOVA，$F_{4,60} = 1.01$，$P = 0.412$）。恒温孵化卵的初始重量亦无显著差异（ANOVA，$F_{3,50} = 1.03$，$P = 0.386$），卵从孵化环境中净吸水导致重量增加（图 2）。温度显著影响恒温孵化卵的重量变化，高温孵化卵的吸水速率大于低温孵化卵，孵化后期各温度孵化卵均减重（图 2）。孵化温度对终末卵重量有显著的影响（ANCOVA，$F_{3,40} = 8.81$，$P < 0.0001$）；30 ℃孵化卵的终末重量显著小于其它温度孵化卵（Tukey's test，all $P < 0.03$），其它温度孵化卵的终末重量无显著的差异（Tukey's test，all $P > 0.538$）（图 2）。

2.2　孵化期、孵化成功率及孵出幼体的性比和畸形率

两性孵化期（$\mathrm{Log_e}$ transformation，two-way ANOVA）和孵化成功率（G-test）无显著差异（both $P > 0.75$），故数据被合并。温度显著影响虎斑颈槽蛇的孵化期（$F_{4,55} = 313.99$，$P < 0.0001$）。孵化期随孵化温度升高而缩短，27 ℃孵化期比 24 ℃平均缩短 12.3 d，30 ℃孵化期比 27 ℃平均缩短 5.4 d，33 ℃孵化期平均缩短 1.3 d（表 1）。波动温度孵化卵的孵化期介于 24 ℃和 27 ℃孵化卵之间（表 1）。

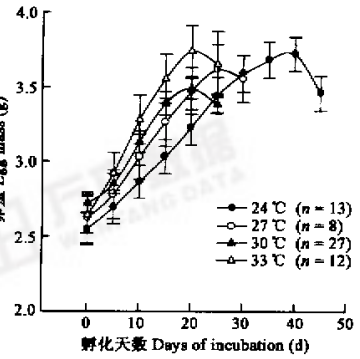

图 2　不同孵化热环境中虎斑颈槽蛇卵重量的变化

Fig. 2　Temporal changes in mass of *Rhabdophis tigrinus* eggs incubated in different thermal environments

数据用平均值±标准误表示，图中显示样本含量 Data are expressed as means ± standard error. Sample sizes are indicated in the figures

孵化热环境显著影响虎斑颈槽蛇孵化成功率（$G = 11.05$，$\mathrm{d}f = 4$，$P < 0.03$）和孵出幼体的畸形率（$G = 37.52$，$\mathrm{d}f = 4$，$P < 0.001$），33 ℃孵化卵孵化成功率最低、孵出幼体的畸形率最大（表 1）。孵化热环境对孵出幼体性别无显著影响（$G = 1.95$，$\mathrm{d}f = 4$，$P > 0.50$），孵出幼体总性（♀♀/♂♂ = 39/26）比不显著偏离 1∶1（$G = 2.62$，$\mathrm{d}f = 1$，$P > 0.10$）（表 1）。

2.3　孵出幼体的大小、重量、含能量和组成成分

双向 ANCOVA 显示两性孵出幼体尾长有显著差异（$F_{1,49} = 9.07$，$P < 0.004$），雄性幼体尾长大于雌性幼体；其它幼体特征无显著的两性差异（all $P > 0.05$）。表 2 显示不同热环境中孵出幼体的大小、重量和组分。24 ℃、27 ℃、30 ℃和波动温度孵出幼体的体长、尾长以及所有其它被检测的幼体指标（包括孵出卵卵壳干重）均无显著差异（表 2）。33 ℃孵出幼体的湿重、能量以及躯干的干重和灰分含量显著小于其它热环境孵出的幼体，33 ℃孵出幼体的剩余卵黄干重和灰

分含量则大于其它热环境孵出的幼体(表 2)。33 ℃孵出幼体的脂肪体和脂肪含量小于 24～30 ℃孵出幼体的对应数值,与波动温度孵出幼体的脂肪体和脂肪含量无显著差异(表 2)。33 ℃孵出幼体的灰分含量显著小于 30 ℃孵出幼体,与其它热环境孵出幼体的灰分含量无显著差异(表 2)。33 ℃孵出卵卵壳干重比其它热环境的孵出卵卵壳重(表 2)。

表 1 孵化热环境对虎斑颈槽蛇孵化期、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 thermal environments on incubation length, hatching success, and sex ratio and abnormality of hatchlings in *Rhabdophis tigrinus*

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	13	45.0±0.7(40.8～50.0)	100 (13/13)	10/3	0 (0/13)
27	8	32.7±0.4(31.2～34.7)	100 (8/8)	6/2	0 (0/8)
30	27	27.3±0.2 (25.6～29.5)	96.3 (26/27)	12/14	3.7 (1/27)
33	12	26.0	16.7 (2/12)	5/2	100 (12/12)
F†	11	37.9±0.6 (34.8～41.7)	100 (11/11)	6/5	0 (0/11)

* 性比数据包括后期死亡个体 Data on sex ratio including individuals that died at the late stage of incubation

† 波动温度 Fluctuating temperature

2.4 孵化热环境对孵出幼体运动表现的影响

33 ℃孵出幼体即便在强烈驱赶下亦不能运动,对应数据不被用于进一步统计分析。ANOVA 显示,其它热环境中孵出幼体的单位时间运动距离($F_{3,42}=0.51$, $P=0.679$)、最大持续运动距离($F_{3,42}=1.71$, $P=0.180$)和单位时间停顿次数($F_{3,42}=0.69$, $P=0.613$)无显著的差异(表 3)。

3 讨论

虎斑颈槽蛇产柔性卵[38],孵化卵与孵化环境之间进行水分交换净吸水导致重量增加。卵吸水速率与孵化热环境有着显著的相关性,低温孵化卵吸水速率低于高温孵化卵(图 2)。不同的吸水速率和净吸水量引起孵化卵内水环境的差异。24～30 ℃恒温孵化卵的孵化成功率($G=2.02$, $df=2$, $P>0.25$)、胚胎畸形率($G=1.14$, $df=2$, $P>0.25$)以及孵出幼体的大小、重量、组分和运动表现均无显著的差异(表 1～3),这些结果表明卵内水环境一定程度的差异对虎斑颈槽蛇孵化卵和孵出幼体无重要的影响。小型柔性卵孵化早期对水环境的变化极为敏感,卵在这一时期失水会导致胚胎死亡[2, 5, 43]。虎斑颈槽蛇卵早期失水会形成表面凹陷,严重的凹陷是不可恢复的,表面具有显著凹陷痕迹的卵通常不能进一步孵化,属于不可孵卵。

孵化热环境显著影响虎斑颈槽蛇卵的孵化期、孵化成功率和孵出幼体的畸形率(表 1)。低温孵卵导致胚胎发育减缓或停滞。提高孵化温度能加速胚胎发育,缩短卵孵化期,但极端高温能导致胚胎死亡率和畸形率上升[1, 6, 13, 43]。持续将卵暴露在临界低温以下或临界高温以上的温度中会导致胚胎死亡。本研究中最高恒温孵化温度(33 ℃)下孵化成功率最低(16.7%)、孵出幼体畸形率最高(100%),表明畸形率和死亡率是关联的,持续将卵暴露在高温(33 ℃或更高的温度)中不利于胚胎发育并可导致胚胎死亡。

爬行动物母体的卵内繁殖投入可以分为两个部分:一部分用于胚胎发育,形成完整的幼体;另一部分以剩余卵黄存在,作为母体亲代抚育(parental care)的一种形式,可视为母体持续的繁殖投入[1, 2, 6, 39～41, 47, 48]。剩余卵黄除了能用于维持幼体的早期活动外[46～49],还能用于躯干的早期生长[1, 2, 6, 39～41]。剩余卵黄大小有显著的种间差异,这与各种亲代抚育的强度不同有关。同种特别是同一种群剩余卵黄大小的差异在很大程度上则反映了胚胎发育过程中卵黄利用率的差异。孵化温度能显著影响卵黄利用率,有鳞类爬行动物的共同特点是,高温孵卵卵黄利用较差,剩余卵黄较多、躯干发育较差(小)[1, 2, 6, 13, 44, 45]。这一结果也见于高温孵化的虎斑颈槽蛇卵(表 2)。本研究中,33 ℃孵出幼体剩余卵黄多,躯干干重小、发育较差(表 2)。其它孵化热环境(24 ℃、27 ℃、30 ℃和波动温度)中胚胎对卵黄利用较为充分,剩余卵黄较小,幼体躯干发育较好(较重)(表 2)。

孵化热环境显著影响虎斑颈槽蛇胚胎发育的物质和能量消耗。33 ℃中胚胎发育的能耗显著大于其它

热环境中的孵化卵,脂肪含量显著小于 24～30 ℃恒温条件孵出的幼体(表 2)。33 ℃孵出幼体剩余卵黄虽然较多且可能用于躯干的生长,但这些幼体的总干重较小、脂肪含量较低且能量均低于其它热环境孵出的幼体(表 2),因而难以凭动用剩余卵黄内的物质和能量达到其它热环境孵出幼体的躯干发育水平。

表 2　孵化热环境对虎斑颈槽蛇孵出幼体大小、重量和组分的影响

Table 2　The effects of incubation thermal environments on size, mass and composition of hatchling *Rhabdophis tigrinus*

幼体大小、重量和组分 Hatchling size, mass and composition	温度(℃) Temperature					*F* 值及多重比较结果 *F* values and results of multiple comparisons
	24	27	30	33	*F*	
	N = 13	*N* = 8	*N* = 26	*N* = 7	*N* = 11	
入孵卵重量 (g)	2.55±0.10	2.62±0.17	2.72±0.06	2.78±0.10	2.57±0.10	1.01 ns
Initial egg mass	1.95～3.36	1.90～3.35	2.17～3.38	2.42～3.23	2.26～3.20	
体长† (mm)	145.9±2.3	152.0±2.6	150.8±1.0	—	146.3±2.8	2.37 ns
Snout-vent length	126.0～157.0	141.0～163.0	139.0～163.0		126.0～163.0	
尾长† (mm)	35.5±0.7	38.4±0.7	36.8±0.6	—	36.0±1.6	1.09 ns
Tail length	31.0～40.0	35.0～44.0	32.0～43.0		24.0～44.0	
幼体湿重† (g)	2.17±0.11	2.24±0.13	2.22±0.05	1.87±0.13	2.07±0.10	6.93***
Wet body mass	1.31～2.80	1.75～2.74	1.75～2.74	1.26～2.09	1.57～2.80	24^{a},27^{a},30^{a},33^{b},F^{a}
幼体干重† (mg)	491.5±23.2	515.0±39.1	535.1±13.5	487.1±28.7	483.9±22.8	2.76*
Dry body mass	351.4～641.4	345.4～666.3	377.9～690.1	393.7～555.1	374.8～615.4	24^{ab},27^{ab},30^{a},33^{b},F^{ab}
躯干干重 (mg)	357.7±15.8	370.7±22.1	352.7±5.6	226.4±25.2	348.4±17.0	11.05***
Carcass dry mass	256.5～454.5	279.9～454.7	294.8～400.3	102.6～292.1	269.1～444.3	24^{a},27^{a},30^{a},33^{b},F^{a}
剩余卵黄干重† (mg)	93.7±10.9	93.8±13.8	136.7±9.5	252.8±45.1	97.8±9.5	9.20***
Residual yolk dry mass	37.2～144.4	29.9～152.8	34.9～241.8	142.7～481.0	43.9～148.4	24^{b},27^{b},30^{b},33^{a},F^{b}
脂肪体干重† (mg)	40.1±3.4	50.4±6.1	45.6±1.8	25.3±3.6	34.0±3.6	6.10***
Fatbody dry mass	22.0～59.3	35.0～80.4	35.3～75.3	13.5～39.5	9.7～51.1	24^{abc},27^{a},30^{ab},33^{c},F^{bc}
幼体能量† (KJ)	11.5±0.6	12.0±0.9	12.3±0.3	11.0±0.7	11.1±0.5	3.53*
Hatchling energy	8.1～15.4	8.0～15.8	8.4～16.2	8.7～12.3	8.9～14.4	24^{a},27^{a},30^{a},33^{b},F^{a}
幼体脂肪† (mg)	97.9±4.6	102.9±8.8	101.9±28.8	85.5±4.5	85.3±4.2	4.64**
Hatchling lipids	74.8～133.9	72.2～143.5	76.6～134.6	70.0～102.5	65.5～108.2	24^{ab},27^{a},30^{ab},33^{c},F^{bc}
幼体灰分† (mg)	61.3±2.3	64.3±4.5	67.6±1.8	61.2±3.2	61.6±3.2	3.22*
Hatchling ash	45.0～76.3	44.3～80.4	51.4～93.5	47.8～71.1	46.6～81.4	24^{ab},27^{ab},30^{a},33^{b},F^{ab}
躯干灰分 (mg)	52.5±2.1	55.6±3.6	54.1±1.2	34.3±4.0	52.4±2.8	9.10***
Carcass ash	37.5～63.8	42.1～70.0	45.6～76.7	16.9～45.1	39.5～70.8	24^{a},27^{a},30^{a},33^{b},F^{a}
剩余卵黄灰分† (mg)	8.7±1.1	8.9±1.4	13.5±1.0	26.9±4.3	9.2±1.0	10.09***
Residual yolk ash	2.6～15.1	2.2～14.2	4.5～23.0	14.4～47.2	3.2～13.7	24^{b},27^{b},30^{b},33^{a},F^{b}
孵出卵壳干重† (mg)	59.1±2.6	60.7±4.6	60.9±1.4	74.4±4.7	58.0±1.6	7.11***
Hatched eggshell dry mass	44.0～83.8	41.9～75.4	50.8～75.0	64.0～93.7	52.0～67.5	24^{b},27^{b},30^{b},33^{a},F^{b}

数据用平均值±标准误和范围表示。上标为"† "的变量用单向 ANCOVA 比较,入孵卵重量为协变量。其余变量用单向 ANOVA 比较。上标不同的热环境间差异显著,a > b > c Data are expressed as mean±SE and range. Variables with the superscript of "† " are compared with one-way ANCOVA, using initial egg mass as the covariate. The remaining variables are compared with one-way ANOVA. The thermal environments with different superscripts differ significantly, a > b > c

有鳞类爬行动物胚胎发育所需的无机物主要来源于卵黄和卵壳,卵黄不贮积从卵壳获得的无机物,孵出幼体中的灰分主要分布在剩余卵黄和躯干中,胚胎因从卵壳动用部分无机物导致卵壳重量下降[1, 2, 6, 38～42, 50, 51]。33 ℃孵出幼体剩余卵黄灰分含量较高与剩余卵黄较大有关(表 2)。33 ℃孵出卵壳干重较大表明高温下胚胎对卵壳无机物的利用程度较低,使得 33 ℃孵出幼体的躯干灰分含量低于其它热环境孵出的幼体(表 2)。

24～30 ℃范围内的恒温条件均能成功孵化虎斑颈槽蛇卵,但用此范围内相对较高的温度孵卵有利于该种卵孵化。例如,24 ℃的孵化成功率和孵出幼体特征与 27 ℃和 30 ℃无显著差异,但孵化期较长,意味着

孵出幼体当年越冬前生长期短。越冬前生长期与幼体当年生长和积累越冬能量有关，并能影响幼体的越冬存活率。

表 3 孵化热环境对虎斑颈槽蛇孵出幼体运动表现的影响

Table 3 The effects of incubation thermal environments on locomotor performance of hatchling *Rhabdophis tigrinus*

孵化温度 (℃) Incubation temperature	样本含量 *N*	单位时间停顿次数 (stops · min^{-1}) Time-specific number of stops	单位时间运动距离 (m · min^{-1}) Time-specific locomotor distance	最大持续运动距离 (m) Maximum continuous locomotor distance
24	10	3.1±0.3 1～5	4.2±0.4 2.2～6.4	1.4±0.2 0.5～2.5
27	5	2.8±0.4 2～4	3.9±0.8 2.3～6.6	1.2±0.2 0.7～1.6
30	22	2.6±0.2 0～5	4.6±0.3 2.7～8.4	1.7±0.1 0.5～3.1
F	9	3.0±0.4 1～4	3.9±0.6 2.1～7.2	1.4±0.2 0.9～2.4

波动温度的最高温度达到 32.7 ℃(图 1)，与最高恒温孵化温度(33 ℃)非常接近，但波动温度下孵出幼体无一畸形。这一结果表明虎斑颈槽蛇孵化卵具有短期耐受足以导致胚胎畸形或死亡的高温环境。波动温度孵化卵的孵化成功率以及孵出幼体特征与 24～30 ℃恒温孵化卵无显著的差异，表明波动孵化温度并不显著优于 24～30 ℃恒温孵化温度。然而，用波动温度孵化虎斑颈槽蛇卵有利于拓宽存活孵化的温度范围，这对于虎斑颈槽蛇在野外多变的环境中提高孵化成功率、最终强化其繁殖成功率有着重要意义。由于本项研究未饲养孵出幼体，波动温度孵化对其后续生长的影响待于进一步的研究。

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