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中华鳖卵孵化过程中物质和能量的动态*

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摘　要　在温、湿度分别为 30 ℃、-220 kPa 条件下孵化中华鳖(*Pelodiscus sinensis*)卵，以检测孵化过程中胚胎生长及对物质和能量的利用。孵化过程中，每隔 7 天测定卵重。孵化第 10 天始，每隔 5 天解剖 15 枚卵，分离为胚胎、卵黄和卵壳。初生幼体称重后冰冻处死，随后解剖分离为躯干、剩余卵黄和脂肪体。所有样品 65 ℃恒温干燥，用索氏抽提仪测定脂肪含量，氧弹式热量计测定能量含量，马福炉测定无机物含量。本研究中华鳖卵的平均孵化期为 45.5 天。卵重量在孵化过程中基本维持恒定，表明中华鳖卵与环境的水分交换不明显。孵化第 0～20 天、20～30 天、30～40 天和 40～45 天，胚胎分别利用新生卵内总能量的 6%、24%、59%和 4%。0～30 天，胚胎生长缓慢；30～40 天，胚胎生长最迅速；孵化末期胚胎生长减缓。胚胎发育所需的无机物来自卵黄和卵壳；卵壳提供的无机物约占初生幼体无机物总量的 13.5%。孵化过程中，干物质、脂肪和能量的转化率分别为 79.6%、58.7%和 66.5%。初生幼体的能量组成为：躯干占 71.8%，脂肪体占 19.4%，剩余卵黄占 8.8%。

关键词　中华鳖　卵　孵化　幼体　物质和能量动态

卵生爬行动物的繁殖特征（窝卵数、卵大小、产卵频率和生育力等）与亲代卵内繁殖投入密切相关。卵内繁殖投入可明确分为两部分：(1) 胚胎发育的繁殖投入，用于形成完整的幼体；(2) 亲代抚育的繁殖投入，主要以剩余卵黄形式存在于初生幼体内，用于早期的活动、维持和生长（Congdon *et al.*，1990；Ji *et al.*，1996；1997b）。孵化过程中，卵黄提供胚胎发育所需的有机物和大部分无机物，卵壳作为无机物的第二储库，提供胚胎发育所需的部分无机物（Ji *et al.*，1996；1997a；1997b；Packard *et al.*，1984；1991）。

胚胎发育过程中的物质转化和能量利用可影响爬行动物初生幼体的形态、大小和功能表现，进而影响幼体的适应性和生存机会。就特定种类而言，卵内物质和能量的转化率与孵化环境、胚胎发育能耗、幼体内剩余卵黄和脂肪体大小密切相关（Deeming *et al.*，1991；Ji *et al.*，1997a；1997b；周显青等，1998）。因而，研究胚胎发育过程中卵内物质和能量利用的动态过程，有助于理解动物的繁殖策略及其幼体对环境的适应性。

中华鳖（*Pelodiscus sinensis*）广泛分布于我国的华中、华南地区及东南亚各国（Zhao *et al.*，1993），具有显著的经济价值。鳖属极端卵生者（Shine，1983），卵产出时胚胎分化程度低，其生长几乎都在孵化过程中完成，是研究爬行动物胚胎物质和能量利用动态的极佳模式动物。

研究中华鳖胚胎生长过程的物质和能量利用可为爬行动物繁殖生态学理论提供新的证据，更为其科学养殖提供直接依据。因此，具有重要的理论意义和应用价值。

1　材料和方法

实验用鳖卵来自浙江萧山湘湖农场养鳖场。所有卵均于产出当日收集、运回杭州师范学院。经可孵性鉴别、称重、测量和编号，卵被移入内含孵化基质的塑料盒中。孵化基质的湿度设置为 -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，放置在温度设置为 30 ±0.3 ℃的生化培养箱内。鳖卵半埋于基质中，胚点朝上。每日加水，以保持孵化盒内基质湿度恒定；每日按预先设定的顺序调整孵化盒在培养箱中的位置，以减少箱内温梯度的影响。

每隔 7 天取 7 枚卵称重，直至幼体孵出。孵入第 10、15、20、25、30、35 和 40 天随机取 15 枚

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卵用电子天平称重（±0.01 mg），随后将其分离为胚胎（包括胚外膜）、卵黄和卵壳。胚胎和卵黄分别移入已知重量的玻璃皿内，卵壳则经清水冲洗、纸巾吸干，分别称出湿重（±0.1 mg）。然后，在 65 ℃烘箱中干燥至恒重，称出干重（±0.1 mg）。共孵出 27 个幼体，其中 17 个幼体在出壳 1 h 内被冰冻保存，其余幼体用于人工饲养。

冰冻幼体以后被解冻，解剖分离成躯干、剩余卵黄和脂肪体。幼体三组分在 65 ℃烘箱中干燥 48 h 至恒重，称出干重（0.1 mg）。胚胎（第 10 和 15 d 的胚胎小，未能测定其脂肪含量）、卵黄和幼体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。上述材料的能量用 GR-3500 氧弹式热量计（长沙仪器厂造）测定。灰分含量用马福炉在 800 ℃条件下焚烧至少 8 h 测得。

所有数据在作进一步统计分析前，用 Kolmogorov-Simirnov 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。用协方差分析（ANCOVA）、方差分析（one-way ANOVA）处理和比较数据。描述性统计值用平均值 ±标准差表示，显著性水平设置为 $\alpha = 0.05$。

2 结 果

30 ℃条件下中华鳖卵的平均孵化期为 45.5 ± 1.1 天（$n = 27$）。在整个孵化期，跟踪测定的 7 枚卵重量基本维持恒定（one-way ANOVA：$F_{(6,42)} = 0.01$，$P > 0.99$）。本实验随机取出用于解剖的各组卵平均重量无显著差异（one-way ANOVA：$F_{(7,99)} = 1.10$，$P > 0.36$）。

图 1：A，B 显示：孵化过程中，卵黄重量和能量随孵化时间增加而减少（one-way ANOVA：重量，$F_{(7,99)} = 99.0$，$P < 0.0001$；能量，$F_{(7,99)} = 101.1$，$P < 0.0001$），胚胎重量和能量随时间增加而增加（ANOVA：重量，$F_{(7,99)} = 326.2$，$P < 0.0001$；能量，$F_{(7,99)} = 271.3$，$P < 0.0001$）。孵化最初 20 d，胚胎仅动用了约 6 %的卵内能量；孵化第 20～30 d，胚胎动用了约 24 %的卵内能量；孵化的第 30～40 d，胚胎动用约 59 %的卵内能量；孵化末期（40～45 d），胚胎动用了约 4 %的卵内能量。卵黄非极性脂肪随孵化时间的增加而减少（one-way ANOVA：$F_{(7,99)} = 109.0$，$P < 0.0001$），胚胎非极性脂肪随孵化时间的增加而升高（one-way ANOVA：$F_{(5,70)} = 112.5$，$P < 0.0001$）（图 1：C）。

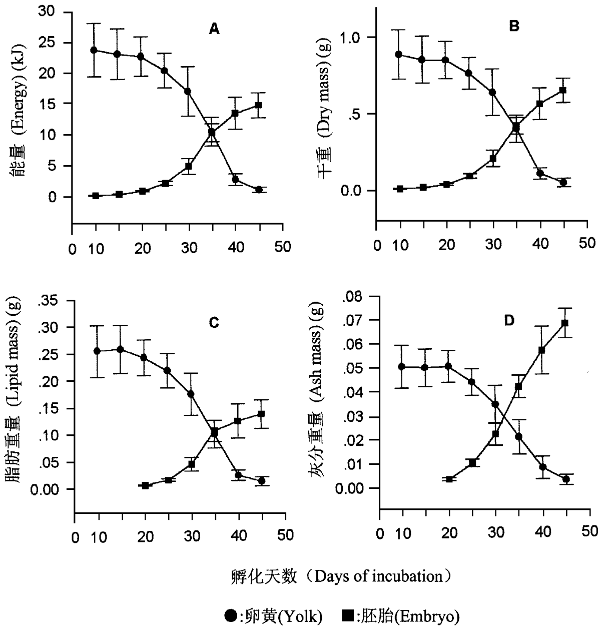

图 1 中华鳖胚胎发育过程中卵内物质和能量的动态

Fig. 1 Dynamics of material and energy during embryonic development in the Soft-shelled turtle

图中纵向短线表示标准差（Vertical lines in the figure represent ± *SD* of means of data. $n = 15$）

卵黄灰分随孵化时间的增加而减少（one-way ANOVA：$F_{(7,99)} = 91.0$，$P < 0.0001$），胚胎灰分随孵化时间的增加而增加（one-way ANOVA：$F_{(5,70)} = 272.0$，$P < 0.0001$）。第 0～30 d 孵化期内，胚胎灰分增加较少，仅占胚胎发育所需灰分的 30 %，胚胎灰分增加量与卵黄灰分减少量基本持平。孵化 30 d 后，胚胎灰分含量快速增加，胚胎发育所需灰分的 70 %在孵化 30 d 后吸取，胚胎灰分递增量明显高于卵黄灰分递减量（图 1：D）。

比较新生卵和初生幼体的成分发现：初生幼体的干重、脂肪含量、能量均明显低于新生卵内容物（卵黄 + 卵清 + 胚胎）；幼体灰分含量则显著高于新生卵内容物；孵后卵壳的干重和灰分含量明显低于新生卵卵壳（表 1）。

30 ℃孵化条件下，中华鳖卵的干物质、脂肪和能量转化到初生幼体的比率分别为 79.6 %、58.7 %和 66.5 %。能量在幼体内的分配为：躯干 71.8 %，脂肪体 19.4 %，剩余卵黄 8.8 %；脂肪在幼体中的分配为：躯干 39.6 %，脂肪体 50.2 %，剩余卵黄 10.2 %。初生幼体内剩余卵黄干重占新生卵内容物干重的 6 %，占幼体干重的 7 %。

表 1　中华鳖新生卵 (n = 27) 和初生幼体 (n = 17) 组成成分比较

Table 1　A comparison of composition between 27 freshly laid eggs and 17 newly emerged hatchlings of Soft-shelled turtle

	新生卵 Freshly laid eggs		已孵卵 Hatched eggs		ANCOVA: $F_{(1,41)}$	
	卵内容物 (Egg contents)	卵壳 (Egg shell)	初生幼体 (Hatchlings)	卵壳 (Egg shell)		
干重 (mg) (Dry body mass)	933.6 ±61.0	533.8 ±39.7	742.7 ±66.1	482.6 ±43.3	93.32***	15.77**
非极性脂肪 (mg) (Non-polar lipid)	269.0 ±26.8	-	158.0 ±31.3	-	134.03***	-
灰分重量 (mg) (Ash mass)	65.1 ±8.4	288.1 ±26.8	75.3 ±7.3	258.2 ±25.2	17.11**	13.19**
能量 (kJ) (Energy)	25.01 ±1.61	-	16.62 ±1.77	-	233.49***	-

注 (Note) : 数据用矫正平均值 ±标准差表示, 新生卵重为协变量。F 值后的符号代表显著性水平 (Data are expressed as adjusted mean ±SD, with freshly laid egg mass as the covariate. Symbols immediately after F values represent significant level)

** $P < 0.001$　　*** $P < 0.0001$

3 讨　论

在爬行动物卵生—卵胎生连续谱中, 龟鳖类、鳄类和有鳞类壁虎科动物接近卵生一端 (Shine, 1983)。中华鳖新生卵内无明显可见胚胎, 表明胚胎分化程度较低, 属极端卵生类型。中华鳖胚胎在前 1/2 孵化期生长缓慢, 与其它极端卵生爬行动物相似, 如尼罗鳖 (*Trionyx triunguis*) (Leshem *et al*., 1991) 和美洲鳖 (*T. spiniferus*) (Packard *et al*., 1991)。中华鳖胚胎生长类型不同于许多有鳞类爬行动物, 有鳞类动物受精卵在母体输卵管内滞留时间较长, 孵化过程中胚胎缓慢增长期较短 (约为孵化期的前 1/3 或更短) (*Agkistrodon piscivorus*, Fischer *et al*., 1994; *Eumeces fasciatus*, Shadrix *et al*., 1994; *Sceloporus aeneus*, Guillette, 1982)。因此, 产后卵内胚胎发育缓慢期长短在很大程度上与受精卵在母体输卵管内滞留时间有关。

爬行动物胚胎发育过程中物质和能量转化率有显著的种间差异 (Ji *et al*., 1997a; 1997b), 并受卵内外环境因素的影响 (Deeming *et al*., 1991)。因此, 比较孵化过程中卵内物质和能量收支时应注意孵化条件的一致性, 以便得出有价值的比较结果。与在相似条件下孵化的其它爬行动物相比较, 中华鳖脂肪转化率与大部分龟鳖类 (50 %～60 %) 接近 (Congdon *et al*., 1983; Nagle *et al*., 1998; Wilhoft, 1986), 低于鳄类 (70 % 以上) (Fischer *et al*., 1991)。有鳞类脂肪转化率的种间差异则较大 (40 %～75 %) (Ji *et al*., 1996; 1997a; 1997b; 赵群等, 1997)。中华鳖的能量转化率与眼镜蛇 (*Naja naja atra*) 接近 (69 %) (Ji *et al*., 1997a), 高于蛇鳄龟 (*Chelydra serpentina*) (60 %) (Wilhoft, 1986) 和虎斑游蛇 (*Rhabdophis tigrinus lateralis*) (37 %) (赵群等, 1997)。

中华鳖胚胎孵化早期动用能量少, 孵化中后期动用能量多, 孵化末期动用能量又减少。这种胚胎动用能量的类型也反映在胚胎重量的动态变化中, 孵化第 30、35 和 40 d, 胚胎重量分别为初生幼体的 30 %、60 %和 87 %。中华鳖胚胎在孵化末期能量动用少, 与不少龟鳖类孵化末期胚胎耗氧量 (即代谢能耗) 不再升高甚至下降的结果相吻合, 这种胚胎代谢能耗的下降与幼体孵出的同步化有关 (Thompson, 1989; 王培潮等, 1990)。

爬行动物缺乏孵卵、育雏和哺乳等高等羊膜动物的产后亲代抚育, 初生幼体内的剩余卵黄是其亲代抚育的一种主要表现形式。剩余卵黄能用于幼体的早期维持和组织生长, 从而提高幼体的生存机会。因此, 对特定种类而言, 剩余卵黄在初生幼体中所占的比例与幼体早期生存密切相关。剩余卵黄大小存在种的特异性, 中华鳖幼体内剩余卵黄所占的比例 (7 %) 明显低于已见报道的其它爬行动物, 如: 密西鳄 (*Alligator mississippiensis*) (40 %) (Fischer *et al*., 1991)、王锦蛇 (*Elaphe carinata*) (25 %) (Ji *et al*., 1997b)、乌龟 (*Chinemys reevesii*) (18 %; 由文辉等, 1994)、蛇鳄龟 (11 %; Wilhoft, 1986) 和海龟 (*Caretta caretta*) (12 %; Kraemer *et al*., 1981)。

中华鳖初生幼体内的无机物含量大于新生卵内容物, 孵出卵卵壳灰分含量明显低于新生卵卵壳,

表明胚胎发育所需的无机物不仅来自卵黄，还来自卵壳。由文辉等（1992）观察孵化前后中华鳖卵壳微观结构的差异，也证明胚胎从卵壳动用无机物。卵壳作为胚胎所需无机物第二来源的现象在卵生羊膜动物中非常普遍（Dunn *et al*., 1977；Ji *et al*., 1997a；1997b；Packard *et al*., 1984；1991；Shadrix *et al*., 1994）。

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外 文 摘 要 (Abstract)

DYNAMICS OF MATERIAL AND ENERGY DURING INCUBATION IN THE SOFT-SHELLED TURTLE (PELODISCUS SINENSIS) *

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We incubated a total of 132 *Pelodiscus sinensis* eggs at 30 ℃ using wet vermiculite as the incubation substrate, of which the moisture was kept constant at -220 kPa water potential. We paid particular attention to the growth trajectory of embryo and embryonic use of material and energy during incubation. Eggs were weighed at 7-day intervals to test for temporal changes in egg mass. From the tenth day of incubation, we opened 15 eggs at 5-day intervals and separated them into shell, embryo and yolk. The three egg components were oven dried to constant mass at 65 ℃, weighed and preserved frozen for later determination of composition. Upon emergence, size (carapace length and width) and mass were measured on each hatchling. Hatchlings (n = 17) were then killed by freezing to -15 ℃ 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 ℃, 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 ℃ for a minimum of 8 h and then weighing the remaining ash. The incubating eggs did not show significant temporal changes in mass over the course of incubation, suggesting that mass gain or loss due to exchanges of water between incubating eggs and their surroundings was negligible in this study. The incubation length averaged 45.5 days. At the stage of 0～20, 20～30, 30～40 and 40～45 days of incubation, the developing embryos mobilized approximately 6 %, 24 %, 59 % and 4 % of the total egg energy in the yolk of the freshly laid egg, respectively. Embryos grew slowly during the first 30 days and last 5 days of incubation, as indicated by the small increase in embryo mass and the low rate of embryonic mobilization of energy. The maximum embryonic growth occurred at the stage of 30～40 days of incubation. The pattern that embryos grew slowly in the last days of incubation was much similar to that seen in some other turtles, presumably resulting from the synchronized emergence of hatchlings. Embryos used both yolk and eggshell as the sources of inorganic material for development, because total ash in newly emerged hatchling exceeded that in the yolk of the freshly laid egg. This claim could be further substantiated by the fact that shells from hatched eggs were lighter in mass and contained less quantities of ash than those from freshly laid eggs. During incubation, approximately 79.6 % dry material, 58.7 % non-polar lipids and 66.5 % energy in egg contents of the freshly laid egg were transferred into the hatchling. Of all energy in the newly emerged hatchling, 71.8 % was in the carcass, 19.4 % in fat bodies and 8.8 % in the residual yolk.

Key words Soft-shelled turtle (*Pelodiscus sinensis*), Egg, Incubation, Hatchling, Material and energy dynamic

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