HERPETOLOGICAL JOURNAL, Vol. 7, pp. 7-12 (1997)

UTILIZATION OF ENERGY AND NUTRIENTS IN INCUBATING EGGS AND POST-HATCHING YOLK IN A COLUBRID SNAKE, *ELAPHE CARINATA*

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This paper reports data on the mobilization of some yolk and eggshell nutrients and their incorporation into hatchlings and post-hatching yolk in an oviparous colubrid snake, *Elaphe carinata*. The incubation time at 30±0.3°C averaged 50.5 days. During incubation, pliable-shelled *E. carinata* eggs increased in wet mass. Dried shells from freshly laid eggs averaged 8.1% of the entire egg dry mass. Freshly laid eggs had significantly heavier shells than did hatched eggs with the same wet mass at oviposition. Dry mass conversion from egg contents of the freshly laid egg to hatchling averaged 81.1%. During incubation, approximately 63.7% of non-polar lipids and 72.1% of energy in egg contents of the freshly laid egg were transferred to the hatchling, with 36.3% of non-polar lipids and 27.9% of energy used for embryogenesis. Shells from freshly laid eggs had a higher level of calcium but a lower level of magnesium than did shells from hatched eggs. Fully developed embryos could obtain all magnesium from yolk but withdrew approximately 30.5% of their total calcium requirements from sources other than yolk. A few days after hatching, a decrease in post-hatching yolk dry mass was accompanied by an increase in carcass dry mass. This confirms that post-hatching yolk could be used to support early growth of hatchlings.

INTRODUCTION

Unlike viviparous reptiles that rely upon vitellogenesis and, in various degrees, placentation for embryonic nutrition (Thompson, 1977, 1981, 1982; Stewart & Castillo, 1984; Stewart, 1989; Stewart, Blackburn, Baxter & Hoffman, 1990; Stewart & Thompson, 1993; Thompson & Stewart, 1994), oviparous reptiles lay eggs in which the stored energy and material generally exceed the needs for producing a complete hatchling (Kraemer & Bennett, 1981, Troyer, 1983, 1987; Wilhoft, 1986; Congdon & Gibbons, 1989; Fischer, Mozzotti, Congdon & Gatten, 1991). It is a common pattern in oviparous reptiles that embryos use yolk as the source of all organic and most inorganic nutrients and eggshell as the additional source of specific minerals (e.g. calcium). A portion of yolk (i.e. post-hatching yolk) may remain unutilized until the time of hatching. However, egg and hatchling components and conversions of energy and/or material from egg to hatchling vary considerably among species (Ewert, 1979; Congdon, Gibbons & Greene, 1983; Congdon, Tinkle & Rosen, 1983; Wilhoft, 1986; Fischer, Mozzotti, Congdon & Gatten, 1991). The proportional amount of calcium withdrawn by the embryo from the eggshell is likewise variable, although the embryonic mobilization pattern of calcium in most oviparous reptiles seems to be consistent (Packard & Packard, 1984, 1986, 1988, 1989; Packard, Packard & Gutzke, 1984; Packard, Short, Packard & Gorell, 1984; Packard, Packard, Miller, Jones & Gutzke, 1985; Shadrix, Crotzer, McKinney & Stewart, 1994). Such variabilities should be related to several factors, e.g. nest micro-environment, costs of embryonic development, a trade-off between clutch number or clutch size and hatchling quality, etc.; thus more detailed and extensive studies of egg and hatchling components would be very helpful in our understanding of reproductive investments and reproductive strategies in oviparous reptiles.

Additionally, the function of post-hatching yolk still remains to be well addressed, although it has been known that post-hatching yolk can be used to support the hatchling's early activities (Kraemer & Bennett, 1981; Troyer, 1983; Wilhoft, 1986; Congdon & Gibbons, 1989). It can be expected that post-hatching yolk may strongly influence the values of energy and material transferred from egg to hatchling. The possible contribution of post-hatching yolk to subsequent growth of newly emerged young is, however, not clear.

Elaphe carinata is a large sized colubrid snake that is widely distributed in the southern provinces of China (including Taiwan), northward to Henan, Shaanxi and Gansu (Zhao & Adler, 1993). Several aspects of biology and ecology of this species have been previously

examined, but little information on incubation is available other than incidental notes (see Huang & Jin, 1990). In 1994 and 1995, we incubated *E. carinata* eggs under constant temperature and humidity regimes to determine (1) conversions of energy and some nutrients from egg to hatchling during incubation, (2) sources of calcium and magnesium for embryogenesis, and (3) post-hatching yolk and its significance to the hatchling's early growth.

MATERIALS AND METHODS

Three gravid *E. carinata* were collected from the field in Baiquan, Dinghai, Zhoushan Islands (29°32'-31°04'N, 121°30'-123°25'E), Zhejiang province, eastern China, in mid-June 1994; one gravid *E. carinata* was collected from the same site in mid-June 1995. Snout-vent length (SVL) ranged from 1220 to 1275 mm, and body mass (excluding the clutch) from 548 to 569 g. The snakes were maintained individually in 800 x 800 x 800 mm wire cages until oviposition (mean=18.5 days). We obtained three clutches in 1994 and one clutch in 1995. We removed eggs from the cages, numbered, measured and weighed them within 6 hr of oviposition, and then randomly selected two eggs from each clutch and opened them. Egg contents (embryo and yolk) of the freshly laid egg were removed, placed in pre-weighed small glass dishes and weighed to the nearest 0.1 mg. Shells from freshly laid eggs were rinsed briefly, weighed to the nearest 0.1 mg, and then frozen for later analysis. All the dissected freshly-laid eggs contained a small embryo, which was too small and fragile to be sampled separately, and therefore was included with the yolk.

The remaining eggs were one-third buried on a moistened sand substrate, and incubated in four glass containers (200 x 200 x 200 mm) with ventilated covers, in a constant temperature chamber at 30±0.3°C. The incubation medium consisted of dry sand and water in a mass ratio of 4:1; water was added periodically to maintain the initial water content. We measured and weighed each incubating egg to the nearest 1 mg at weekly intervals before day 42, and at daily intervals thereafter, up to one day prior to hatching. Hatchlings were measured and weighed immediately after they left the eggs. Shells from hatched eggs were rinsed briefly, weighed to the nearest 0.1 mg, and then frozen. Ten hatchlings (1-3 from each clutch; hereafter 0-day hatchlings) were frozen immediately after hatching. The remaining 11 hatchlings (2-3 from each clutch; hereafter 7-day hatchlings) were fasted at room temperatures (26-38 °C) for seven days, and then frozen. The preserved hatchlings were later thawed, dissected and separated into carcass, yolk sac and fat bodies.

All samples for determination of non-polar lipids, ash, calories, calcium and magnesium were dried to a constant mass in an oven at 65°C, weighed, and then ground with a mortar and pestle. Non-polar lipids were extracted from all samples of egg contents, carcass, post-hatching yolk and fat bodies for a minimum of 5.5 hr using absolute ether in a Soxhlet apparatus. The mass of non-polar lipids in each sample was calculated as the difference in sample dry mass before and after extraction.

Ash and caloric values of samples of egg contents, carcass, post-hatching yolk and fat bodies were determined using a GR-3500 adiabatic bomb calorimeter (Changsha Instruments). Titrations were performed on the residue after calorimetry to correct for nitrogenous wastes. Samples of eggshells were burned in a muffle furnace at 550°C for 24 hr to determine ash mass.

Samples for calcium and magnesium determinations were weighed out into glass tubes and digested completely in hot concentrated nitric acid. Digestates were brought to volume in volumetric glassware and stored in a refrigerator until analysis for calcium and magnesium. Concentrations of the two elements in digestates were determined with a WFX-1B model atomic absorption spectrophotometer (The 2nd Beijing Optical Instruments). To check if there were any differences in the levels of calcium and magnesium between shells from freshly laid eggs and shells from hatched eggs, we took equal samples from each shell, pooled separately the samples from freshly laid eggs and hatched eggs and treated them as two different samples.

Because all components of egg contents of freshly laid eggs and 0-day hatchlings were highly correlated with total egg wet mass at oviposition and because there were intra-clutch variations in egg size (we will report data elsewhere), we compared all means using analysis of covariance (ANCOVA) with total egg wet mass at oviposition as the covariate which was a significant source of variation in all analyses. The

TABLE 1. Components and F values of ANCOVA for eight freshly laid *Elaphe carinata* eggs and ten 0-day hatchlings (including post-hatching yolk and fat bodies). Data were expressed as adjusted mean±1SE, with total egg wet mass at oviposition as the covariate. Symbols immediately after F values represent significance levels: NS $P>0.05$, * $P<0.05$, ** $P<0.01$, and *** $P<0.001$.

	Freshly laid egg	Hatched egg	F
	Egg contents	Total hatchlings	
Wet mass (g)	34.8±0.2	25.7±0.2	956.40***
Dry mass(g)	8.41±0.1	6.82±0.07	169.20***
Water (g)	26.4±0.2	18.9±0.2	949.82***
Organic mass (g)	7.72±0.1	6.04±0.07	217.28***
Ash mass (mg)	687.0±23.1	780.1±25.0	6.48*
Non-polar lipids(g)	2.59±0.06	1.65±0.04	160.64***
Calcium (mg)	93.8±3.6	135.0±4.5	37.84***
Magnesium (mg)	32.9±1.3	31.6±1.0	0.61^{NS}
Energy (Kcal)	50.9±0.5	36.7±0.6	288.18***
	Eggshell	Eggshell	
Dry mass (mg)	739.4±16.8	642.2±11.1	22.49***
Organic mass (mg)	534.8±11.8	489.0±7.9	10.07**
Ash mass (mg)	204.6±5.3	153.2±3.4	64.23***

homogeneity of slopes was checked prior to testing for differences in the adjusted means. The levels of calcium and magnesium in the egg contents of freshly laid eggs and posthatching yolks, and the level of non-polar lipids of 0-day hatchlings and 7-day hatchlings, were compared by analysis of variance (ANOVA); percentage data were arc-sine transformed before the ANOVA. We used a partial correlation analysis to test the relationships between carcass dry mass, posthatching yolk dry mass and fat-body dry mass. Descriptive statistics are presented as mean±1SE.

RESULTS

Female *E. carinata* laid pliable-shelled eggs. Clutch size in our sample averaged 7.5±1.2 (range 5-10, n=4). Freshly laid eggs averaged 36.2±1.3 g (range 26.3-45.1, n=30) wet mass, 58.8±1.5 mm (range 49.4-77.2, n=30) length and 30.8±0.7 mm (range 24.4-36.0, n=30) width. During incubation, eggs increased in wet mass and, one day prior to hatching, weighed 128.3±1.4% (range 117.5-136.5, n=21) of total egg wet mass at oviposition. The incubation time averaged 50.5±0.1 days (range 50.0-51.0, n=21). Hatchlings at hatching averaged 26.2±1.2 g (range 18.4-33.1, n=21) wet mass, 361.1±8.3 mm (range 308.3-418.5, n=21) SVL and 83.2±3.3 mm (range 62.1-103.3, n=21) tail length.

Egg contents of freshly laid eggs averaged 75.9% water by mass (Table 1). Egg contents of freshly laid eggs averaged 91.8% organic material, 8.2% ash, 30.8% non-polar lipids, 1.12% calcium and 0.39% magnesium by dry mass (Table 1). Shell from freshly laid eggs averaged 8.1% of total egg dry mass, and 72.3% organic material and 27.7% ash by dry mass (Table 1). Shells from freshly laid eggs had a higher level of calcium (12.07%) but a lower level of magnesium (0.83%) than did shells from hatched eggs (calcium: 7.88%; magnesium: 0.98%).

The 0-day hatchlings averaged 73.5% water by mass (Table 1). These hatchlings averaged 88.6% organic material, 11.4% ash, 24.4% non-polar lipids, 1.98% calcium and 0.46% magnesium by dry mass (Table 1). Shells from hatched eggs averaged 76.1% organic material and 25.9% ash by dry mass (Table 1).

The 0-day hatchlings contained lower quantities of total dry mass, organic mass, non-polar lipids and energy, but higher quantities of calcium and ash than did egg contents of freshly laid eggs (Table 1). There was no significant difference in the quantity of magnesium between egg contents of freshly laid eggs and 0-day hatchlings (Table 1). Shells from freshly laid eggs had higher quantities of total dry mass, organic mass and ash than did shells from hatched eggs (Table 1). There were no significant differences in the levels of calcium and magnesium between egg contents (calcium: 1.12±0.04%, range 0.89-1.30%, n=8; magnesium: 0.39±0.02%, range 0.32-0.47%, n=8) of the freshly laid egg and post-hatching yolks at hatching (calcium: 1.01±0.04%, range 0.83-1.27, n=10; magnesium: 0.44±0.02%, range 0.36-0.55, n=10) (calcium: $F_{1,16}$=1.92, P>0.05; magnesium: $F_{1,16}$=2.63, P>0.05).

During incubation, 81.1% of dry mass, 63.7% of non-polar lipids and 72.1% of energy in egg contents of the freshly laid egg were transferred to the hatchling, with 18.9% of dry mass, 36.3% of non-polar lipids and 27.9% of energy used for embryogenesis (Table 1). Fully developed embryos could obtain all magnesium from the yolk but withdrew 30.5% of their total calcium requirements from sources other than yolk (Table 1).

The 7-day hatchlings had significantly heavier carcasses than did 0-day hatchlings with the same total hatchling wet mass at hatching ($F_{1,18}$=104.01, P<0.001) (Table 2). In 0-day hatchlings, we found that there was a strong negative correlation between post-hatching yolk dry mass and carcass dry mass when holding total hatchling dry mass and fat-body dry mass constant (r=-0.99, t=17.19, df=6, P<0.001), there was no significant correlation between fat-body dry mass and carcass dry mass when holding total hatchling dry mass and post-hatching yolk dry mass constant (r=0.37, t=0.98, df=6, P>0.05) and there was no significant correlation between fat-body dry mass and post-hatching yolk dry mass when holding total hatchling dry mass and carcass dry mass constant (r=-0.56, t=1.66, df=6, P>0.05) (Table 2). When data from 0- and 7-day hatchlings were pooled, we again found that there was a strong negative correlation between posthatching yolk dry mass and carcass dry mass when holding total hatchling dry mass and fat-body dry mass constant (r=-0.98, t=20.30, df=17, P<0.001), there was no significant correlation between fat-body dry mass and carcass dry mass when holding total hatchling dry mass and post-hatching yolk dry mass constant (r=0.24, t=1.02, df=17, P>0.05) and there was no significant correlation between fat-body dry mass and post-hatching yolk dry mass when holding total hatchling dry mass and carcass dry mass constant (r=-0.35, t=1.54, df=17, P>0.05) (Table 2). 7-day hatchlings (21.6±0.6%, range 18.6-25.1%, n=11) had a lower level of non-polar lipids than did 0-day hatchlings (24.4±0.3%, range 22.1-25.7%, n=10) ($F_{1,19}$=16.82, P<0.001).

TABLE 2. Data, expressed as mean±1SE, based on ten 0-day hatchlings and eleven 7-day hatchlings of *Elaphe carinata*. All mass units are in g.

	0-day hatchling	7-day hatchling
hatchling wet mass at hatching	26.7±1.9	25.7±1.70
hatchling wet mass 7 days after hatching	-	25.0±1.70
decrease in wet mass	-	0.70±0.12
hatchling dry mass	7.08±0.46	6.30±0.43
carcass	4.12±0.27	4.74±0.25
yolk	1.75±0.14	0.51±0.05
fat bodies	1.21±0.10	1.05±0.11
% water of hatchling	73.5±0.30	74.8±0.20

DISCUSSION

The majority of oviparous squamates lay pliable-shelled eggs (Packard & Hirsch, 1989), but the proportional amount of shell in total egg dry mass and the level of calcification of shell are quite different among squamates (Ji, unpubl. obs.). Additionally, a few squamates (e.g. some geckos) lay rigid-shelled eggs, although the structure of their egg shells is actually quite different from that of crocodilians and some turtles that also lay rigid-shelled eggs (Packard & Hirsch, 1989; Packard & DeMarco, 1991). We are presently not very certain if a pliable-rigid shelled egg continuum, in turtles (Lamb & Congdon, 1985; Congdon & Gibbons, 1985), also exists in squamates. Thus more detailed studies of eggshells of squamates would be of great value in substantiating this claim. Because data on ash content of eggshell (Lamb & Congdon, 1985) and the proportional amount of shell in total egg dry mass (Congdon & Gibbons, 1985) have been used to classify turtle eggs, we have paid attention to these data in squamates. Similar to that reported for pliable-shelled eggs of other squamates (Packard & Packard, 1988), *E. carinata* eggs increased in wet mass and swelled during incubation because of a net gain of water absorbed from the incubation environment. However, we are unable to demonstrate the relative contributions of water vapour exchange (Kam & Ackerman, 1990) and liquid water transportation (Packard & Packard, 1988) to egg water uptake.

Because *E. carinata* embryos at the start of incubation have only a negligible body size, we can reasonably consider the transference of energy and material from egg to hatchling during incubation as an approximation to the transference overall. This makes it possible to compare our data with those for other oviparous reptiles with freshly laid eggs containing very small embryos, or near the oviparous end in the oviparity-viviparity continuum (Shine, 1983). *E. carinata* exhibited relatively high conversions of energy and material from egg to hatchling. Dry mass conversion from egg contents of the freshly laid egg to hatchling in *E. carinata* (81.1%) was greater than the values reported for the American alligator (79%; Fischer, Mazzotti, Congdon & Gatten, 1991) the chicken turtle (72%; Congdon, Gibbons & Greene, 1983) and the painted turtle (72%; Ewert, 1979). The proportion of non-polar lipids transferred from egg to hatchling in *E. carinata* (63.7%) was less than the value reported for the American alligator (74.3%; Fischer, Mazzotti, Congdon & Gatten, 1991), but greater than the values reported for some turtles and lizards (50-60%; Congdon, Tinkle & Rosen, 1983; Wilhoft, 1986; Ji, 1992).

The above comparisons indicate that conversions of materials from egg to hatchling vary considerably among species; however, any general explanations of these variabilities are unknown at this time because of the lack of data. We feel that data from studies of parental reproductive investment, embryonic metabolism and ecology of neonates would be very valuable. For example, it is well known that the costs of embryonic development vary considerably among reptiles (Dmi'el, 1970; Black, Birchard, Schuett & Black, 1984; Vleck & Hoyt, 1991), parental investment in each offspring should be related to its survivorship (Congdon & Gibbons, 1989; Fischer, Mazzotti, Congdon & Gatten, 1991) and incubation conditions have an impact on resultant hatchlings (Gutzke & Packard, 1987; Packard, 1991). Additionally, we recommend the use of data from caloric determinations, because neither dry mass conversion efficiency nor lipid conversion efficiency provides enough information on the costs of embryogenesis and/or the exact level of parental investment in each egg. Samples of eggs and hatchlings with different lipid and ash contents might differ considerably in energy density (Ji, 1992, 1995).

The pattern of use of the shell as a secondary source of calcium for development by *E. carinata* embryos is the same as that in other oviparous squamates (Packard, Packard & Gutzke, 1984; Packard, Packard, Miller, Jones & Gutzke, 1985; Packard & Packard, 1988; Shadrix, Crotzer, McKinney & Stewart, 1994), turtles (Packard, Short, Packard, & Gorell, 1984; Packard & Packard, 1986) and the American alligator (Packard & Packard, 1989). The level of calcium withdrawn by *E. carinata* embryos (30.5%) from sources other than yolk was much less than the values reported for crocodilians and turtles (50-80%; Bustard, Jenkins & Simkiss, 1969; Jenkins, 1975; Packard & Packard, 1984, 1989). Among squamates, it was also less than the value reported for the skink *Eumeces fasciatus* (39%; Shadrix Crotzer, McKinney & Stewart, 1994), but greater than the value reported for the colubrid snake *Coluber contrictor* (20%; Packard, Packard & Gutzke, 1984). The differences presumably reflect the interspecific differences in eggshell structure and/or allocation of minerals between eggshell and yolk.

Studies of magnesium metabolism in embryonic oviparous reptiles have been extremely limited. As in the American alligator (Packard & Packard, 1989), *E. carinata* embryos obtain all magnesium for their development from the yolk. The result that shells from hatched eggs were lower in calcium level but higher in magnesium level than shells from freshly laid eggs implied that developing embryos selectively withdrew calcium from the shell, providing additional evidence which demonstrated that it was not necessary for *E. carinata* embryos to withdraw magnesium from the eggshell.

Although we did not sample at different stages of incubation, the fact that there were no significant differences in calcium and magnesium levels between egg contents of the freshly laid egg and posthatching yolk at hatching might suggest that the two minerals were not accumulated by the developing *E. carinata* embryos in the yolk. The fact also implied that the embryonic calcium mobilization pattern in *E. carinata*

was similar to that seen in most other oviparous reptiles (Packard, Packard & Gutzke, 1984; Packard, Short, Packard, & Gorell, 1984; Packard, Packard, Miller, Jones & Gutzke, 1985; Packard & Packard, 1986; Packard & Packard 1988; Shadrix, Crotzer, McKinney & Stewart, 1994).

One of the most interesting findings in this study was that a decrease in post-hatching yolk dry mass was accompanied by an increase in carcass dry mass a few days after hatching. This finding indicates that at least a portion of nutrients in the post-hatching yolk can be transferred to the carcass. This finding also indicates that posthatching yolk is not only one of the energy sources for newly emergered young, as suggested by some investigators (e.g. Congdon, 1989; Congdon & Gibbons, 1989; Fischer, Mozzotti, Congdon & Gatten, 1991), but also plays an important role in the hatchling's early growth. Post-hatching yolk, together with fat-bodies, in the hatchling was thought to be one (parental investment in care) of the two components of pre-ovulatory parental investment (parental investment in embryogenesis and parental investment in care; Congdon, 1989), but whether the two components are constant characteristics for a specific species remains unclear. Some investigators (Packard, 1991; Vleck, 1991) pointed out that the two components in oviparous reptiles vary reciprocally in response to the incubation conditions. Thus, for future studies, it could be very interesting to test the differences in carcass dry mass, post-hatching yolk dry mass and fat-body dry mass of hatchlings from different incubation conditions. Compared to post-hatching yolk, fat-bodies in *E. carnata* hatchlings seem to be used mainly for hatchling maintenance. An obvious decrease in the level of non-polar lipids in 7-day hatchlings primarily reflects a decrease of storage lipids.

ACKNOWLEDGEMENTS

We thank X. Z. Zheng for assistance both in the field and in the laboratory and F. Braña, Mª de los Angeles Marcos Garcia and V. Perez-Mellado for hosting and helping XJ during his research stay in Spain. We also thank two anonymous reviewers for useful comments. This work was supported by a grant from the National Natural Science Foundation of China to XJ (NNSFC-39270124). A grant from Paos' Foundation supported XJ while writing this paper in Spain.

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Accepted: 5.6.96