

EMBRYONIC USE OF ENERGY AND POST-HATCHING YOLK IN THE GRAY RAT SNAKE, *PTYAS KORROS* (COLUBRIDAE)

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Female *Ptyas korros* from a population on Dinghai, Zhoushan Islands, in eastern China, produced one clutch per breeding season. Clutch size varied from 7 to 14, and was positively correlated with female SVL. The duration of incubation at 30 ± 0.5 °C averaged 54.7 days. Dried shells from freshly laid eggs averaged 30.0% of the entire egg dry mass. Egg contents of the freshly laid egg contained higher quantities of dry material, non-polar lipids and energy than did newly hatched hatchlings. Shells from freshly laid eggs contained higher quantities of ash than did those from hatched eggs. During incubation, approximately 77% of dry material, 54% of non-polar lipids and 69% of energy in the egg contents of freshly laid eggs were transferred to the hatchling. There were inverse relationships between carcass dry mass, post-hatching yolk dry mass and fat body dry mass for hatchlings sampled immediately after hatching. Post-hatching yolk could be used to support subsequent growth of newly emerged young, as indicated by significant increases in the carcass dry mass and SVL of hatchlings during their first days of life, following the depletion of post-hatching yolk.

Key words: Colubridae, *Ptyas korros*, incubation, egg, hatchling, post-hatching

INTRODUCTION

It is a common pattern in oviparous reptiles that the nutrient provision in eggs exceeds the needs for producing a complete hatchling. While developing within eggs, embryos use yolk as the source of all organic and most inorganic nutrients, and eggshell as the additional source of some minerals (e.g. calcium). A portion of yolk, namely post-hatching yolk, may remain unutilized until the time of hatching. Post-hatching yolk may be used for maintenance metabolism and probably for soft tissue growth in some hatchling reptiles (e.g. Kraemer & Bennett, 1981; Congdon *et al.*, 1983*a,b*; Troyer, 1983, 1987; Wilhoft, 1986; Congdon, 1989; Congdon & Gibbons, 1989; Fischer *et al.*, 1991). The relative quantity of post-hatching reserves may vary considerably among reptiles that share this characteristic and, for a given species, it appears to be related to energy requirements during the first days or weeks of life (Kraemer & Bennett, 1981; Troyer, 1987). Post-hatching yolk is not a conceptually new term, but further studies are still needed to show its exact function. In several species of snake, e.g. *Elaphe carinata* (Ji *et al.*, 1997*a*), *Elaphe taeniura* (Ji *et al.*, 1999*a*) and *Dinodon rufozonatum* (Ji *et al.*, 1999*b*), we have found that nutrients in the post-hatching yolk can be transformed into the carcass of newly emerged young. As the consequence of this transformation, hatchlings increase in size (snout-vent length and tail length) during their first days of life.

Here, we present data on the gray rat snake, *Ptyas korros* (Colubridae), that is widely distributed in the

southern provinces of China (Zhao & Adler, 1993) and locally very abundant in the Zhoushan Islands (29° 32' - 31° 04' N, 121° 30' - 123° 25' E), Zhejiang, eastern China. We address the three topics: (1) the composition of freshly laid eggs and newly hatched hatchlings, (2) energy and material budgets during incubation and (3) post-hatching yolk and its contribution to the early growth of hatchlings.

MATERIALS AND METHODS

Fourteen gravid *P. korros* were collected from various localities on Dinghai, Zhoushan Islands, in late June of 1994 and 1995; five gravid females were collected from Dinghai in mid-June 1998 to increase sample size. The snout-vent length (SVL) ranged from 735 to 1000 mm, and the post-partum body mass from 98.8 to 280.9 g. The snakes were transported to our laboratory at Hangzhou Normal College and were housed individually in 80 x 80 x 80 cm wire cages until oviposition. Oviposition occurred between 22 June and 15 July. All post-partum snakes were released into the field where they were collected.

We collected, measured and weighed eggs promptly so as to avoid any uncertainty about the initial mass due to gain or loss of water, and then numbered them. Relative clutch mass was expressed as the ratio of clutch mass to the total body mass of females, including the clutch (Seigel & Ford, 1986). Sixteen eggs were considered to be abnormal because they had either a less developed shell or a condensed yolk.

In 1994 and 1995, a total of 39 freshly laid eggs (1-4 from each clutch) were opened at laying. Each dissected freshly laid egg contained a small embryo which was too small and fragile to be separated and therefore was included with the yolk. Egg contents (embryo plus yolk) of the dissected freshly laid eggs were removed

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and weighed. Shells were rinsed briefly in distilled water, dried by blotting with a paper towel and weighed. Egg contents and shells were then dried to constant mass in an oven at 65 °C, weighed and stored frozen at -15 °C until they could be processed for determining composition. None of the eggs collected in 1998 was opened at laying.

We incubated 129 eggs in covered plastic containers that were placed in an incubator inside which the temperature was set at 30±0.8 °C. The containers contained known amounts of vermiculite and distilled water to produce approximately -12 kPa (2 g water/1 g vermiculite) water potential (Lin & Ji, 1998). Eggs were one-third buried in the substrate. We moved containers daily between shelves in the incubator according to a predetermined schedule to minimize any effects of thermal gradients inside the incubator. Incubation temperatures in close proximity to the eggs were monitored twice daily using a digital thermometer. We weighed containers every other day and, if necessary, mixed distilled water evenly into the substrate to compensate for water absorbed by the eggs and for the small losses due to evaporation. We weighed incubating eggs at weekly intervals before day 42 and at daily intervals thereafter. The duration of incubation was defined as the time elapsed from egg laying to hatchling emergence.

Upon emergence, each hatchling was measured and weighed. Body measurements included snout-vent length (SVL), tail length (TL) and body mass. Shells from hatched eggs were processed following the procedures described above for those from freshly laid eggs. Deformed hatchlings were excluded from further analyses.

Thirty-seven hatchlings (1-3 from each clutch; hereafter 0-day hatchlings) were sacrificed on the day of hatching, 30 (1-3 from each clutch; hereafter 7-day hatchlings) 7 days after hatching, and 28 (0-2 from each clutch; hereafter 14-day hatchlings) 14 days after hatching. Both 7- and 14-day hatchlings were fasted at room temperatures (24-36 °C) before they were sacrificed. The hatchlings were dissected and separated into carcass, yolk sac (if present) and fat bodies. The three components of the hatchling were oven dried to constant mass at 65 °C, weighed and then ground with a mortar and pestle.

We extracted non-polar lipids from dried samples of egg contents, carcass, post-hatching yolk and fat bodies in a Soxhlet apparatus for a minimum of 5.5 hr using absolute ether. The amount of lipids in a sample was determined by subtracting the lipid-free dry mass from the total sample dry mass. We determined the energy density of egg contents, carcass, post-hatching yolk and fat bodies using a GR-3500 adiabatic bomb calorimeter (Changsha Instruments). We determined the ash content of eggshells by burning samples in a muffle furnace at 600 °C for a minimum of 12 hr and weighing the remaining ash.

To avoid pseudoreplication, data from a single clutch were blocked prior to further statistical analysis and then were tested for normality using the Kolmogorov-Smirnov test, and for homogeneity of variance using Bartlett's test. Log_e transformation was performed when necessary to achieve normality. We compared the measured variables of dissected freshly laid eggs and hatched eggs using analysis of covariance (ANCOVA) with total egg wet mass at oviposition as the covariate. We tested for differences in carcass dry mass, SVL and TL among 0-day, 7-day, and 14-day hatchlings using ANCOVA with total egg wet mass at oviposition as the covariate. The homogeneity of slopes was checked prior to testing for differences in the adjusted means. A four-factor (dry masses of hatchling, carcass, post-hatching yolk and fat body entered as factors) partial correlation analysis was performed on 0-day hatchlings to test the relationships between these variables. Descriptive statistics are presented as mean ± 1 standard error.

RESULTS

Female *P. korros* laid pliable-shelled eggs. Clutch size in our sample averaged 10.2±0.4 (range 5-14, *N*=19), and was positively correlated with maternal SVL ($r^2=0.49$, $F_{1,17}=16.01$, $P<0.001$). Relative clutch mass averaged 0.317±0.011 (range 0.23-0.440, *N*=19). Clutch means (*N*=19) for the wet mass and length and width of freshly laid eggs averaged 9.0±0.3 (range 6.6-10.9) g, 38.1±0.7 (range 33.5-43.5) mm and 19.0±0.3 (range 16.6-20.9) mm, respectively. Dried shells from freshly laid eggs averaged 30.0% of the entire egg dry mass (Table 1).

Eggs gained mass over the course of incubation due to absorption of water and, one day prior to hatching, weighed approximately 133% (107-195%) of egg mass at oviposition. Fourteen eggs failed to hatch following incubation. Hence, the hatching success was 87% (112/129). The clutch mean for duration of incubation averaged 54.7±0.6 (range 50.5-57.2, *N*=19) days. Three hatchlings were abnormal. The clutch means (*N*=19) for wet body mass, SVL and TL of newly emerged hatchlings averaged 6.36±0.19 g (range=4.55 -7.32), 224.7 ± 1.9 mm (range 205.7-249.5) and 94.4±1.1 mm (range 82.7-101.5), respectively.

Egg contents of the dissected freshly laid eggs contained higher quantities of dry material, non-polar lipids and energy than did 0-day hatchlings (Table 1). Shells from freshly laid eggs contained higher quantities of ash than did those from hatched eggs (Table 1). During incubation, approximately 77% of dry mass, 54% of non-polar lipids and 69% of energy in egg contents of the freshly laid egg were transferred to the hatchling (Table 1).

The hatchlings sampled on 7 days after hatching had significantly larger carcasses than did 0-day (ANCOVA; $F_{1,35}=6.05$, $P<0.02$) and 14-day hatchlings (ANCOVA; $F_{1,33}=4.49$, $P<0.04$) (Table 2). We did not

TABLE 1. Composition and F values of ANCOVA for *Ptyas korros* freshly laid and hatched eggs. Data from a single clutch are blocked and are expressed as adjusted means \pm 1 standard error, with total egg wet mass as the covariate. $N=14$ (clutches). All mass units are in g, and energy units KJ. Symbols immediately after F values represent significance levels: NS $P>0.05$, * $P<0.05$, *** $P<0.001$.

	Freshly laid egg	Hatched egg	$F_{1,25}$
	Egg contents	Total hatchling	
Wet mass	6.95 \pm 0.04	6.04 \pm 0.09	69.41 ***
Dry mass	2.06 \pm 0.03	1.59 \pm 0.03	92.78 ***
Water	4.89 \pm 0.06	4.54 \pm 0.15	3.73 NS
Non-polar lipids	0.61 \pm 0.01	0.33 \pm 0.01	230.95 ***
Energy	49.5 \pm 0.8	34.4 \pm 0.7	199.61 ***
	Eggshell	Eggshell	
Dry mass	0.88 \pm 0.02	0.84 \pm 0.01	3.08 NS
Organic mass	0.69 \pm 0.02	0.66 \pm 0.01	1.41 NS
Ash mass	0.195 \pm 0.005	0.178 \pm 0.005	6.22 *

find a significant difference in the mean carcass dry mass between 0- and 14-day hatchlings (ANCOVA; $F_{1,33}=0.09$, $P=0.767$; Table 2). A partial correlation analysis on 0-day hatchlings showed inverse relationships between carcass dry mass, post-hatching yolk dry mass and fat body dry mass (carcass vs post-hatching yolk: $r=-0.97$; carcass vs fat bodies: $r=-0.80$; post-hatching yolk vs fat bodies: $r=-0.74$; all $P<0.001$).

The hatchlings sampled on 7 (ANCOVA; $F_{1,35}=30.01$, $P<0.0001$) and 14 days (ANCOVA; $F_{1,33}=48.97$, $P<0.0001$) after hatching had significantly larger SVLs than did 0-day hatchlings (Table 2). There was no significant difference in the mean SVL between 7- and 14-day hatchlings (ANCOVA; $F_{1,33}=1.64$, $P=0.210$; Table 2).

DISCUSSION

Numerous studies have indicated that conversion of nutrients and energy from egg to hatchling during the course of incubation or embryogenesis vary amongst reptiles (e.g. Ewert, 1979; Congdon *et al.*, 1983a,b; Wilhoft, 1986; Fischer *et al.*, 1991; Ji *et al.*, 1996, 1997a,b; Zhao *et al.*, 1997). However, inter-specific comparisons should be made with caution, because incubation conditions may influence material and energy budgets during incubation, and different methods by which investigators use to incubate eggs might make data uncomparable. Our experience with incubating eggs of squamate reptiles is that the conversion efficiencies of dry material, non-polar lipids and energy during incubation vary among species whose eggs

TABLE 2. Size, mass and components of *Ptyas korros* hatchlings sacrificed immediately after hatching ($N=19$ clutches) and on 7 ($N=19$ clutches) and 14 days ($N=17$ clutches) after hatching. Data from a single clutch are blocked and are expressed as means \pm 1 standard error. All mass units are in g, and length units mm.

	0-day hatchling	7-day hatchling	14-day hatchling
<i>Initial egg mass</i>	8.8 \pm 0.3	8.3 \pm 0.3	8.1 \pm 0.3
<i>Hatchling wet mass</i>			
- at hatching	6.3 \pm 0.2	5.9 \pm 0.3	6.0 \pm 0.3
- 7 or 14 days after hatching	-	5.6 \pm 0.3	5.4 \pm 0.2
<i>Hatchling SVL</i>			
- at hatchling	223.3 \pm 3.0	212.9 \pm 2.9	210.6 \pm 1.9
- 7 or 14 days after hatching	-	237.0 \pm 2.8	238.4 \pm 3.0
<i>Hatchling TL</i>			
- at hatching	93.3 \pm 1.6	90.8 \pm 2.0	89.6 \pm 1.9
- 7 or 14 days after hatching	-	91.4 \pm 2.8	92.4 \pm 2.4
<i>Hatchling dry mass</i>	1.63 \pm 0.07	1.35 \pm 0.06	1.18 \pm 0.0
- carcass	1.18 \pm 0.04	1.21 \pm 0.05	1.10 \pm 0.05
- yolk sac	0.22 \pm 0.03	0.004 \pm 0.001	0
- fat bodies	0.23 \pm 0.02	0.13 \pm 0.01	0.08 \pm 0.01
<i>% water of hatchling</i>	74.3 \pm 0.6	75.5 \pm 0.8	76.6 \pm 0.4

TABLE 3. A comparison of the conversion efficiencies of dry mass, non-polar lipids and energy between *Ptyas korros* and other squamate reptiles whose eggs were incubated under similar conditions to those described in this paper.

Species	Conversion efficiency (%)			Duration of incubation (days)	Data sources
	Dry mass	Non-polar lipids	Energy		
Scincidae					
<i>Eumeces chinensis</i>	66	44	62	22.6	Ji <i>et al.</i> (1996)
Colubridae					
<i>Ptyas korros</i>	77	54	69	54.7	This study
<i>Elaphe carinata</i>	81	64	72	50.5	Ji <i>et al.</i> (1997a)
<i>Elaphe taeniura</i>	85	75	81	54.9	Ji <i>et al.</i> (1999a)
<i>Dinodon rufozonatum</i>	81	70	79	45.8	Ji <i>et al.</i> (1999b)
<i>Rhabdophis tigrinus lateralis</i>	70	37	61	29.3	Zhao <i>et al.</i> (1997)
<i>Zaocys dhumnade</i>	76	63	70	48.3	Ji (unpubl. data)
Elapidae					
<i>Naja naja atra</i>	75	64	69	50.7	Ji <i>et al.</i> (1997b)

are incubated under similar conditions to those described in this paper (Table 3). The variations could, of course, be partly due to inter-specific differences in costs of embryonic development and incubation length (Dmi'el, 1970; Vleck & Hoyt, 1991). For example, a species having higher costs of embryonic development and longer incubation length can be expected to have a lower conversion efficiency of energy. However, the differences in the measured conversion efficiencies can also be greatly dependent on egg and hatchling characteristics that may differ considerably among species.

The inverse relationships between post-hatching yolk dry mass, carcass dry mass and fat body dry mass in 0-day hatchlings suggest trade-offs among the three hatchling components. The increase in body size (SVL) during the first post-hatching week represents growth due mainly to the transformation of nutrients in the post-hatching yolk into the carcass. This conclusion is supported by the fact that newly emerged young increase in carcass mass following the depletion of post-hatching yolks. The same pattern has been found in other snakes, e.g. *Elaphe carinata* (Ji *et al.*, 1997a), *Elaphe taeniura* (Ji *et al.*, 1999a) and *Dinodon rufozonatum* (Ji *et al.*, 1999b), indicating that post-hatching yolk can be used for tissue growth in these snakes. The apparently greater carcass dry mass of 7-day hatchlings and the similarity in the mean carcass dry mass between 0- and 14-day hatchlings suggest a pattern of carcass dry mass increasing during the first week and decreasing thereafter. Such a pattern is more evident in *E. carinata* (Ji *et al.* 1997a) and *E. taeniura* (Ji *et al.*, 1999a), whose post-hatching yolks are relatively larger and are not completely exhausted one week after hatching.

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