

Egg and Hatchling Components of a Viviparous Snake, *Elaphe rufodorsata*

Ji Xiang

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tively low crystallization temperatures. In contrast, the intestine and skin, which should contain the greatest number of ice nucleating bacteria, were the only samples freezing at temperatures similar to whole frogs. Thus, the present study lends support to the hypothesis that bacteria initiate spontaneous freezing of frogs in the absence of inoculative freezing.

Endogenous nucleators undoubtedly trigger freezing of frogs in the laboratory but their impact in nature is subject to debate because frogs are susceptible to inoculative freezing whenever their body temperature falls below the melting point of body fluids (Layne et al., 1990; Layne, 1991). Because moist natural substrate supercools poorly, frogs should begin freezing by inoculation at temperatures just below the melting point of water. The susceptibility of freeze tolerant animals to inoculative freezing, however, is not constant and it fails to occur in insects when the water content of plant tissue falls below 30% of the total mass of the tissue (Layne et al., 1990; Layne, 1993). It is impossible to predict if and how often similar conditions exist in the hibernacula of wood frogs since detailed observations of overwintering animals are lacking.

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Egg and Hatchling Components of a Viviparous Snake, *Elaphe rufodorsata*

Ji XIANG, Department of Biology, Hangzhou Normal College, Hangzhou 310036, Zhejiang Province, P. R. China.

Reptiles characteristically allocate the majority of their reproductive investment to eggs prior to ovulation. The energy and material allocated to each egg are first utilized for embryonic development and secondarily for the hatchling's early activity and growth (Kraemer and Bennett, 1981; Congdon et al., 1983; Troyer, 1983, 1987; Congdon and Gibbons, 1985, 1989; Wilhoft, 1986; Fischer et al., 1991; Ji, 1992). Compared to lizards and turtles, the data on egg and hatchling components of snakes are limited and have pertained primarily to oviparous snakes.

I studied *Elaphe rufodorsata*, a viviparous water snake that is common in paddy fields in Zhejiang, China. Parturition occurs in mid-summer (July–August). Clutch size varies from 7 to 24 and depends on female size. Here, I present data on egg and hatchling components of this species.

Females with SVLs >490.0 mm were collected during April–June 1993 from various localities in Dinghai (29°56'–30°11'N, 121°56'–122°20'E), Zhoushan Islands, Zhejiang Province. All the animals were transported to Hangzhou Normal College, where they were allowed to feed freely on fish and frogs. Some of the snakes were autopsied in May and June, and size and mass of yolked follicles and oviductal eggs were noted. The remaining snakes were allowed to produce their offspring in the laboratory. Infertile eggs were collected within 15 min of oviposition. Neonates were immediately measured and weighed, and then frozen. All the materials for lipid extraction and caloric determination were dried to constant mass in an oven at 65°C and dry mass was recorded. Samples of eggs (including yolked follicles, oviductal eggs, and infertile eggs) and hatchlings were then ground in a mortar with a pestle.

Caloric value was determined with a JR-2800 adiabatic bomb calorimeter (Changsha Instruments). Ash was defined as the residue remaining after combustion in the bomb calorimeter. Ash content (percentage

TABLE 1. Lipid content (%) of yolked follicles, oviductal eggs, infertile eggs, and hatchlings of *E. rufodorsata*.

	N	Mean	1 SE	Range
Yolked follicle	9	25.2	2.3	16.9–38.1
Oviductal egg	19	29.7	0.4	26.6–35.2
Infertile egg	14	29.7	0.4	25.3–31.5
Hatchling	11	21.9	0.8	17.1–26.4

of ash) was calculated as ash dry mass/sample dry mass \times 100%.

Lipids were extracted from all samples for a minimum 5.5 h using absolute ether in a Soxhlet apparatus. After extraction, samples were dried to constant mass in an oven at 65 C and weighed. Lipid content (percentage of lipids) was calculated as extracted lipid mass/sample dry mass \times 100%.

Yolked follicles, oviductal eggs, infertile eggs, and hatchlings averaged 25.5, 29.7, 29.7, and 21.9% lipids by dry mass, respectively. The differences were statistically significant (arc-sine transformation; $F_{(3,49)} = 13.3$, $P < 0.01$) for all comparisons except the comparison between oviductal eggs and infertile eggs (Tukey's test, $P > 0.05$) (Table 1).

Sample of yolked follicles, oviductal eggs, infertile eggs, and hatchlings averaged 5651, 6053, 6073, and 5337 cal/g dry mass ash uncorrected caloric value (AUCV), respectively. The corresponding samples averaged 6037, 6471, 6544, and 5946 cal/g dry mass ash free caloric value (AFCV), respectively. The differences in both AUCV and AFCV were statistically significant (AUCV- $F_{(3,39)} = 26.8$, $P < 0.01$; AFCV- $F_{(3,39)} = 17.9$, $P < 0.01$) for all comparisons except the comparison between oviductal eggs and infertile eggs (Tukey's test, $P > 0.05$) (Table 2). A strong positive correlation existed between caloric value and volume of the yolked follicle (AUCV-VOL: $r = 0.82$, $F_{(1,7)} = 13.9$, $P < 0.01$; AFCV-VOL: $r = 0.81$, $F_{(1,7)} = 13.2$, $P < 0.01$).

Yolked follicles, oviductal eggs, infertile eggs, and hatchlings averaged 6.5, 6.3, 7.2, and 10.3% ash by dry mass, respectively. The differences were statistically significant ($F_{(3,39)} = 35.7$, $P < 0.01$) for all comparisons except the comparison between yolked follicles and oviductal eggs (Tukey's test, $P > 0.05$) (Table 3).

As in other reptiles (e.g., Ji, 1992; Ji and Zheng, *in press*), caloric values reported here were correlated with lipid content and/or ash content of the samples. The maximum caloric value occurred in the sample

TABLE 3. Ash contents (%) of yolked follicles, oviductal eggs, infertile eggs, and hatchlings of *E. rufodorsata*.

	N	Mean	1 SE	Range
Yolked follicle	9	6.5	0.4	4.8–8.8
Oviductal egg	12	6.3	0.1	5.5–7.0
Infertile egg	10	7.2	0.1	6.3–8.8
Hatchling	12	10.3	0.3	9.2–12.0

having the highest lipid level, and for ash uncorrected caloric value, the lowest ash level. The positive correlation between caloric value and volume of the yolked follicle indicated that lipids were stored at a more rapid rate than other nutrients during vitellogenesis.

Ash allocated to each egg can be utilized for embryonic development. In this study, total ash present in developing eggs increased as the eggs developed, and reached a peak in infertile eggs. This suggests that oviductal eggs of *E. rufodorsata* steadily obtained ash from the mother, and implies that there might be a similarity in ash storage pattern between oviparous and viviparous reptiles. In the wall lizard, *Gekko japonicus*, a typical oviparous species based on its position in the oviparous-viviparous continuum, I observed that the yolk of the newly-laid egg had much higher level of ash than the oviductal egg (Ji, 1992).

The data presented here only demonstrate the major components of *E. rufodorsata* eggs and hatchlings. I cannot use these data to demonstrate the proportion of the egg energy and material recovered from the hatchling, because I do not know a specific oviductal egg within which a hatchling completes its embryonic development. Previous studies have showed that (1) the cost of embryonic development in snakes is the lowest amongst reptiles (Dmi'el, 1970; Black et al., 1984; Vleck and Hoyt, 1992; Thompson, 1993) and (2) embryos of some reptiles do not rely exclusively on ash or minerals in their yolk (Packard et al., 1984a, b, 1985; Packard and Packard, 1988, 1989; Ji, 1992). To demonstrate the cost of embryonic development in *E. rufodorsata* and the possibility that an embryo may obtain a portion of ash from a source other than the yolk, I need further experimental data which should contain information on a specific oviductal egg.

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TABLE 2. Caloric values (cal/g dry mass) of yolked follicles, oviductal eggs, infertile eggs, and hatchlings of *E. rufodorsata*.

	N	Ash uncorrected caloric value			Ash free caloric value		
		Mean	1 SE	Range	Mean	1 SE	Range
Yolked follicle	9	5651.4	143.0	5057.5–6355.2	6036.8	147.6	5463.4–6848.3
Oviductal egg	12	6065.3	34.1	5844.1–6240.1	6471.4	37.1	6281.0–6686.1
Infertile egg	10	6073.0	34.5	5860.0–6244.0	6544.9	37.3	6299.0–6722.6
Hatchling	12	5337.2	46.2	5020.8–5556.8	5946.2	46.7	5759.1–6162.6

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On the Presence of Overlap During the Development of the Pectoral Girdle of *Colostethus subpunctatus* (Amphibia: Anura) and its Relevance in the Classification of the Dendrobatidae

MOISES KAPLAN,¹ School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0118, USA.

Pectoral girdle architecture has been an important character in the systematics of the anuran family Dendrobatidae. Because its members have a firmisternal condition, the family Dendrobatidae has been considered to be closely related to ranids (Boulenger, 1882; Griffiths, 1963; Duellman and Trueb, 1986; Ford, 1989; Ford and Cannatella, 1993). However, some authors (e.g., Noble, 1922, 1926; Lynch, 1971) have suggested that the firmisternal condition of dendrobatids and ranids is nonhomologous.

The varying opinions of diverse authors (e.g., Noble, 1926; Griffiths, 1959; Lynch, 1971; Ford, 1989) regarding the homology of firmisterny in ranids and dendrobatids are related to a dispute about one developmental character—viz., the overlap of the girdle halves. Noble (1926) reported that in the dendrobatid, *Colostethus subpunctatus*, the girdle halves overlap during development. This observation, coupled with the absence of girdle overlap during development in ranids, led Noble (1926) to conclude that the firmisterny was derived independently from different arciferal taxa in ranids and dendrobatids. In contrast to Noble, Griffiths (1959) and Lynch (1971) reported that overlap did not occur during the development of *C. subpunctatus*. Ford (1989) noted further that developmental overlap is absent in all dendrobatid species. The lack of observed differences between the ranid

¹ Present Address: Sección de Herpetología, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-153, 04510, México D.F., México.