THE RESIDUES IN THE EGGS OF SQUAMATE REPTILES AT HATCHING

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ABSTRACT

The residues present in hatched eggshells of captive-bred squamates were examined and their amounts recorded. Large amounts of a semi-gelatinous fluid were typically left in the eggshell at hatching. The amount of residues, but not hatchling size, depended upon the uptake of water by the egg during incubation. In some instances egg volk was also left in the eggshell at hatching. The role of these residues in the water relations of reptile eggs have largely been ignored.

INTRODUCTION

Water is absorbed by soft-shelled eggs of squamates and turtles during incubation (Packard and Packard, 1988) but its fate in the egg is unclear. In some squamate eggs albumen mass has been reported to increase during embryonic development (Clark, 1953; Badham, 1971) from initially low levels at oviposition (Tracy and Snell, 1985). The albumen in the eggs of Amphibolorus barbatus barbatus oozed out during the hatching process (Badham, 1971). Considering that egg mass almost trebled during incubation, and that hatchling mass was 15 per cent than initial egg mass, it is clear that there was considerable loss of albumen (Badham, 1971). By contrast, Subbo Rao (1987) reported that it was allantoic fluid that oozed from eggs of the crocodilian Gavialis gangeticus at the end of incubation. The amount of albumen in crocodilian and avian eggs is initially high, diminishes during incubation and is absent at hatching (Webb, Manolis, Whitehead and Dempsey, 1987; Manolis, Webb and Dempsey, 1987; Romanoff, 1967).

Reports of fluid loss at hatching are rare (Bustard, 1966; Badham, 1971; Subbo Rao, 1987; D. Ball, personal communication; Deeming and Ferguson, 1989) despite intensive studies of the water relations of reptile eggs (M. J. Packard, Packard and Boardman, 1980; G. C. Packard, Taigen, Packard and Boardman, 1980: G. C. Packard, Packard, Boardman, Morris and Shuman, 1983; G. C. Packard, Packard and Gutzke, 1985: Packard and Packard, 1984: Morris, Packard, Boardman, Paukstis and Packard, 1983; Ackerman, Dmi'elandAr, 1985; GutzkeandPackard, 1986, 1987; Thompson, 1987). Obviously there is some confusion over the origin of this fluid but more importantly the quantities of fluid lost are unknown. This study examined the residues in the hatched eggshells from a species of lizard and four species of snake bred in captivity.

MATERIALS AND METHODS

Details of the eggs used in this study are shown in Table I. All of the eggs were kept in sealed polyethylene bags containing vermiculite and water (1:1) as an incubation medium. At the Zoological Society of London the bags were kept in a room maintained between 26°C to 28°C. At both the University of Reading, and the University of Manchester, the bags were kept in a table top box oven (Laboratory Thermal Limited, Oldham England) maintained at 27°C. The bags were opened once or twice a week to replenish the air within the bag.

The studies were carried out during three seasons (1986, 1987 and 1988). In the first season eggs from *Eublepharis* and *Elaphe o. spiloides* were incubated at Reading. After the hatchlings had emerged the mass of the eggshell and the residues in the shell were determined to the nearest 10mg. Eggs of the other species (*Hydrodynastes, Pituophis* and *Python*) were incubated at the Zoological Society of London and were collected after hatching. On return to Reading the residues and the eggshell were weighed.

In the second season five freshly-laid eggs of *Eublepharis* were collected from London and incubated at Reading. In 1988 freshly-laid eggs of *Elaphe o. obseleta* were collected from the Manchester Museum and incubated at the department of Cell and Structural Biology. Each egg was weighed to the nearest milligram at the start of incubation and every seven days thereafter upto 35 days of incubation when the mass of each egg was determined every three or four days. The mass of the hatchling was determined after emergence from the shell. Any residues were removed from the eggshell which was weighed.

Species	No.	Origin	
Lizards Gekkonidae			
Eublepharis macularius	10	The Zoological Society of London	
Snakes Colubridae			
Elaphe obsoleta spiloides	3	Ms G. Rohan-Wild	
Elaphe obsoleta obsoleta	2	The Manchester Museum	
Hydrodynastes gigas	3	The Zoological Society of London	
Pituophis melanoleucus	5	The Zoological Society of London	
Pythonidae Python molurus	3	The Zoological Society of London	

TABLE 1: Details of the number and sources of squamate reptile eggs examined.

Species	Egg Number	Shell	Fluids	Yolk	
Eublepharis macularius	Al	0.28	1.01		*
	Λ2	0.34	1.18		*
	B1	0.23	0.48		*
	B2	0.33	0.50		*
	CI	0.32	0.38	—	*
Elaphe obsoleta spiloides	Al	1.97	4.44	_	*
	A2	1.76	1.16	2.63	*
	A3	1.92	2.68	_	*
Hydrodynastes gigas	Λ2		3.49	15.14	
	A3		3.99	10.94	
	A4		1.25	13.57	
Pituophis melanoleucus	Al	2.95	1.75		
	A2	2.71	1.69		
	A3	3.16	3.12	_	
	$\Lambda 4$	3.34	0.58	15.97	
	A5	3.45	1.62		
Python molurus	Al	10.09	26.54	25.25	
	A2	11.28	46.17	0.67	
	A3	19.91	43.98	0.77	

TABLE 2: The mass (grams) of the shell, residual fluids and residual yolk after the hatchling reptile has emerged from the egg.* = Eggs incubated in Reading; other eggs incubated at the Zoological Society of London.

RESULTS

In the first season, neither the initial egg mass nor hatchling mass were available. The fresh mass of the hatched eggshell and the residues are shown in Table 2. Varying amounts of a semi-gelatinous fluid were found in eggshells of all the species examined. No details of possible leakage from the egg prior to collection were known. There were substantial amounts of yolk remaining in several snake eggs (Table 2).

Data collected from the *Eublepharis* eggs studied in the second season are shown in Fig. 1 and Table 3. The eggs increased in mass throughout incubation though after day 35 there was an increase in the rate of water uptake. Final egg mass, recorded prior to hatching, was almost twice that recorded at the start of incubation (Fig. 1). After hatching (day 55) the average mass of the eggshell was 0.23g. As data was unavailable from oviposited eggs, initial eggshell mass was assumed to be the same as at the end of incubation. The total mass of the residues in the eggs was determined by difference (Table 3). Hatchling mass did not differ significantly from the initial calculated egg contents (t = -1.70, p>0.05, two sample student's t test).

Eggs of *Elaphe o. obseleta* likewise increased continuously throughout incubation; the first egg doubled in mass (after 57 days of incubation)

Variable	Eublepharis		Elaphe	Elaphe
	Х	s.e.	1	2
nitial egg mass.	3.198	0.164	15.95	15.56
Final egg mass.	5.805	0.275	33.72	23.51
Shell mass.	0.230	0.016	2.33	1.29
Hatchling mass.	2.849	0.061	11.66	12.77
Residual mass at hatch.	2.725	0.249	19.73	9.76
nitial egg contents.	2.968	0.157	13.62	14.27

TABLE 3: Data from five *Eublepharis* eggs and two eggs of *Elaphe obseleta obseleta* at the beginning and end of incubation. Values for *Eublepharis* are means (X) in grams and standard errors (s.e.). Values for *Elaphe* are for the two individual eggs.



Fig. 1 The mass of five eggs of the gecko *Eublepharis macularius* during incubation. Values are means (grams) with standard error bars.

compared to the second egg which increased by half of its initial mass (Table 3). Despite this difference in water uptake hatchling mass was similar and less than the initial egg contents (85.6 per cent of initial egg mass, egg 1; 89.5 per cent, egg 2). The 10g difference in final egg mass was accounted for by the egg residues after hatchling emergence (Table 3).

DISCUSSION

Much residual material was present in the eggshells examined in the present study. Comparison of the residues found in eggs of *Eublepharis* in 1986, with those in 1987, highlighted a major problem in experimental technique. In 1986 neither the mass of the eggs prior to hatching, nor the mass of the hatchling, were recorded. Data collected in 1987 were more extensive and large amounts of residues were lost at hatching. It is clear that data collected in 1986 underestimated the actual anount of the residues present at the end of incubation in this species and it is likely that this result applies to all of the speciesshown in Table 2.

Residual yolk was easily recognised in eggs examined in the present study. It was surprising that such large amounts could be left in the eggshell. Normally, the initial mass of the yolk determines hatchling mass and larger hatchlings have better rates of survival (Tracy, 1982). The significance of the ability to leave residual yolk in the eggshell and its effects upon the size of the hatchling and its potential survival are not clear.

Although the present study was limited in its scope it highlights our poor understanding of the developmental physiology of reptile eggs. Unfortunately, this study cannot confirm the origin of the semi-gelatinous fluid in these eggs but examination of the literature suggests that it is allantoic fluid. Two reports have led to confusion over the role of albumen in squamate eggs (Clark, 1953; Badham, 1971). The albumen content of eggs of Amphibolorus (Badham, 1971) and Coluber constrictor (Clark, 1953) increased during incubation. The urea content of albumen in Coluber eggs increased during development and the fluid was seen as a major excretory store (Clark, 1953). In birds the normal storage compartment for nitrogenous waste (uric acid) is the allantoic fluid. Protein was present in the albumen of Amphibolorus eggs (Badham, 1971) but it only constituted less than 0.5 per cent of the total mass of the fluid measured. By contrast, albumen in eggs of birds (Romanoff, 1967) and crocodilians (Burley, Black, Wellington and Grigg, 1987) contains much more protein. Fresh albumen in reptile eggs is gel-like (Ewert, 1985; Ferguson, 1985) whereas allantoic fluid is much less viscous (Moffat, 1985) resembling thin albumen of birds eggs. The residual fluid in squamate eggs was similar in appearance to allantoic fluid in eggs of *Alligator mississippiensis*; large amounts of allantoic fluid are present in these eggs at the end of incubation and it oozes from the egg during hatching (Deeming and Ferguson, 1989). Therefore, I agree with the suggestion of Packard and Packard (1988) that Badham (1971) and Clark (1953) mistook allantoic fluid in lizard eggs for albumen.

In both *Eublepharis* and *Elaphe* increases in egg mass during incubation were reflected in the amount of residues (allantoic fluid) at hatch and not in hatchling mass. Hatchling mass was similar to, or less than, the mass of the initial eggs contents. Python hatchlings weigh less than both the initial and final egg mass (Black, Birchard, Schuett and Black, 1984). Similarly, hatchlings of Amphibolorus weighed less than the initial mass of the eggs despite a three-fold increase in egg mass during incubation (Badham, 1971). It is interesting to note that crocodilian eggs also ooze allantoic fluid at hatching although they do not take up additional water from the nest substrate during incubation (Subbo Rao, 1987; Deeming and Ferguson, 1989). Eggs of Crocodylus johnstoni have around 10g of allantoic fluid present at the end of incubation (Manolis et al., 1987) but all other fluids in the egg had disappeared. Unfortunately the fate of the allantoic fluid was not reported but as the combined masses of the hatchling and eggshell constituted only 72-76 per cent of initial egg mass it is likely that the fluid was lost from the egg at hatching.

Although many studies have examined the water relations of squamate eggs (Badham, 1971; Tracv, 1980; M. J. Packard, Packard, Miller, Jones and Gutzke, 1985; M. J. Packard et al., 1980; Muth, 1981; Black, et al., 1984; Gutzke and Packard, 1987; Packard and Packard, 1987) verv few studies have examined the changes in the individual components of the egg during incubation. For example, various studies have shown that the urea content of chelonian and squamate eggs increases during incubation (Packard and Packard, 1983, 1987; G. C. Packard et al., 1983; G. C. Packard, Packard and Boardman, 1984) but whole eggs were homogenised and the location of the urea was unknown. By contrast. Manolis et al. (1987) showed that urea increased in eggs of Crocodylus during incubation and that it was only present in large quantities in allantoic fluid. Indeed water absorbed during incubation may play an important role in diluting the large amounts of urea in reptile eggs allowing the embryo to develop for a longer period (Packard et al., 1984). The toxic allantoic fluid would be released from the egg at hatching.

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