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PARTITIONING OF WATER WITHIN THE ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*) EGG AFTER 60 DAYS OF INCUBATION

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ABSTRACT

Eggs of *Alligator mississippiensis* were incubated up to day 60 of incubation under either dry conditions (on metal shelves, without a substrate) or wet conditions (on vermiculite). Incubation temperatures were 30°C and 33°C. Eggs on the shelves lost water during incubation; water loss from eggs on vermiculite was small, and in some cases, negligible. On day 60, after incubation at 30°C, eggs incubated on shelves had significantly less amniotic fluid and yolk sac than eggs incubated on vermiculite. In eggs incubated at 33°C, on day 60 amniotic fluid was absent and the mass of the yolk sac was smaller in eggs incubated under dry conditions. Embryo mass and the amount of allantoic fluid were unaffected by the extent of water loss from the eggs at both temperatures. The mass of allantoic fluid was correlated with the mass of the egg on day 60. Yolk sac mass at both temperatures was not correlated with egg mass on day 60 but was correlated with the water lost from the egg. These results suggest that (1) in alligator eggs, loss of water is borne primarily by the yolk sac; and (2) that retention of water in the allantois may be associated with a need to prevent a deleterious build-up of waste metabolites. This contrasts with the situation in avian eggs where water loss is borne primarily by the allantois and embryonic tissues.

INTRODUCTION

Water loss from avian eggs is a normal part of incubation (Ar and Rahn, 1980). This progressive desiccation affects differentially the water content of individual compartments within the egg as incubation proceeds. The allantoic fluid shows the first signs of dehydration. When this compartment is exhausted the volume of amniotic fluid declines (Hoyt, 1979; Simkiss, 1980; Tullett and Burton, 1982). When all of these fluid reserves are exhausted, near the end of incubation, it is the embryonic tissues which become dehydrated (Hoyt, 1979; Tullett and Burton, 1982; Davis and Ackerman, 1987; Davis, Shen and Ackerman, 1988). The yolk sac, by contrast, is unaffected by dehydration (Hoyt, 1979; Tullett and Burton, 1982).

The water relations of reptilian eggs are highly variable and are dependent upon species, eggshell structure and hydration of the incubation substrate (Packard and Packard, 1980, 1984; Packard, Packard and Boardman, 1982; Packard, 1991). Soft-shelled eggs of squamates absorb large amounts of water during incubation but where the shell structure is more complex, as in pliable-shelled turtle eggs, the exchange of water with the nesting substrate is reduced. Eggs of other turtles and crocodylians which have rigid eggshells often exhibit no net water exchange during natural incubation (Ferguson, 1985; Packard, 1991). In addition, all reptilian eggs incubated on substrates with low water potentials, which still have humidities greater than 99 per cent (Tracy, Packard and Packard, 1978), can lose water during incubation when compared with substrates with higher water potentials.

Hence, the rigid-shelled eggs of the turtle *Trionyx triungis* can lose up to 20 per cent of their initial mass during natural incubation. This has little effect upon hatchability, although higher rates of water loss are lethal (Lesham and Dmi'el, 1986). Similarly, eggs of *Crocodylus porosus* can lose or gain up to 25 per cent of their initial mass without significantly affecting hatchability (Manolis, Webb and Dempsey, 1987; Grigg, 1987).

These different water relations have significant effects on the pattern of embryonic growth and hatchling mass reptiles (reviewed by Packard, 1991). However, unlike the situation concerning avian embryos, the effects of different patterns of water exchange on the dynamic water balance of reptilian embryos and their extra-embryonic compartments during incubation are unknown. Investigations to date have revealed, however, that there are both similarities and differences in the pattern of development of birds and reptiles, particularly crocodylians (Manolis *et al.*, 1987; Deeming and Ferguson, 1989, 1991a). In particular, unlike in the fowl, large amounts of allantoic fluid are present in crocodylian eggs at hatching (Manolis *et al.*, 1987; Deeming and Ferguson, 1989). In addition, unlike in bird eggs albumen proteins do not enter the amniotic fluid during development of *Alligator mississippiensis* (Deeming and Ferguson, 1991a).

This report describes an investigation of the effects of dehydration on the fluid compartments within eggs of *A. mississippiensis* on day 60 of incubation. This artificial treatment, will allow us to compare the effects of water loss in avian and crocodylian eggs. Alligator eggs incubated under dry conditions (without a substrate) were compared with eggs incubated on wet (on vermiculite) conditions.

MATERIALS AND METHODS

Eggs of *Alligator mississippiensis* were collected immediately after laying from wild nests in Louisiana, USA, and transported by air to Manchester as

described in Deeming and Ferguson (1989). On arrival in the laboratory (day 3) the eggs were weighed and set in incubators at 30°C and 33°C, accurate to 0.01°C (Vinden Scientific Ltd.), and with approximately 100 per cent air humidity. In each incubator, 10 eggs randomly were placed on the metal shelves in the incubator and 10 eggs were placed on moist vermiculite (100g of water/100g of vermiculite).

On day 60 of post-oviposition incubation (the incubation period of alligator eggs at 30°C is 75 days and at 33°C, 65 days) the eggs were removed from the incubators, weighed and candled to assess embryonic viability. Viable eggs were placed on ice for an hour prior to opening. The allantoic and amniotic fluids were then sequentially decanted from the egg and weighed; the embryo, yolk sac and albumen were then removed from the shell and all components were weighed as described by Deeming and Ferguson (1989). Data were analysed using a Minitab statistical package (Ryan, Joiner and Ryan, 1985).

RESULTS

The incubation conditions affected the water loss from the egg. Despite the high humidity, eggs placed on shelves, without any substrate, lost considerable masses during incubation (Table 1). Air spaces, formed between the shell membrane and the calcite shell, were present in almost all of the eggs at both temperatures. By contrast, those eggs on vermiculite lost much less weight (Table 1) and air spaces were very small or absent.

At 30°C, significant differences between the two groups of eggs were observed in the mass of the yolk sac and the mass of amniotic fluid which were significantly larger, and the mass of the embryo was significantly smaller in eggs incubated on vermiculite (Table 1). In eggs incubated at 33°C, only the mass of the yolk sac was significantly affected by the increased rate of weight loss (Table 1).

The mass of the yolk sac at both incubation temperatures was inversely correlated with the weight

	30°C					33°C				
	Shelf	Vermiculite		F ¹	Shelf	Vermiculite		I ²		
Number of eggs	10	10			10	9				
Initial egg mass (g)	77.94	7.66	77.79	8.55	0.00	78.05	3.78	75.23	8.81	0.85
Egg mass at 60 days (g)	73.40	7.98	77.03	8.39	0.98	72.74	4.24	72.91	8.79	0.00
% Water loss	5.90	1.75	0.96	0.64	70.52	6.83	2.10	3.10	1.44	23.15
Shell mass (g)	10.13	0.90	10.09	0.95	0.01	9.74	0.53	9.91	1.10	2.65
Albumen mass (g)	2.02	0.39	2.37	0.70	1.92	0.55	1.34	0.00	0.00	2.12
Yolk sac mass (g)	21.83	3.05	26.54	2.00	16.53	10.37	2.03	13.22	1.65	11.08
Amniotic fluid mass (g)	1.63	0.56	2.61	0.64	13.39	—	—	—	—	—
Allantoic fluid mass (g)	10.66	3.29	11.02	3.49	0.06	8.38	2.63	9.00	3.85	0.17
Residues ³ mass (g)	8.76	1.18	7.61	1.90	2.66	3.93	1.83	3.37	0.58	0.77
Embryo mass (g)	18.38	1.39	16.80	0.49	11.49	39.76	4.12	38.21	3.53	0.76

TABLE 1: Mass of *Alligator mississippiensis* eggs and their different fluid compartments on day 60 of incubation at 30°C and 33°C. Values are means and standard deviations and the results of the analysis of variance, as F-ratios (Ryan, Joiner and Ryan, 1985).

¹F = 4.38 at the 5% level and 8.18 at the 1% level with degrees of freedom of 1,19. ²F = 4.41 at the 5% level and 8.29 at the 1% level with degrees of freedom of 1,18. ³Calculated by difference.

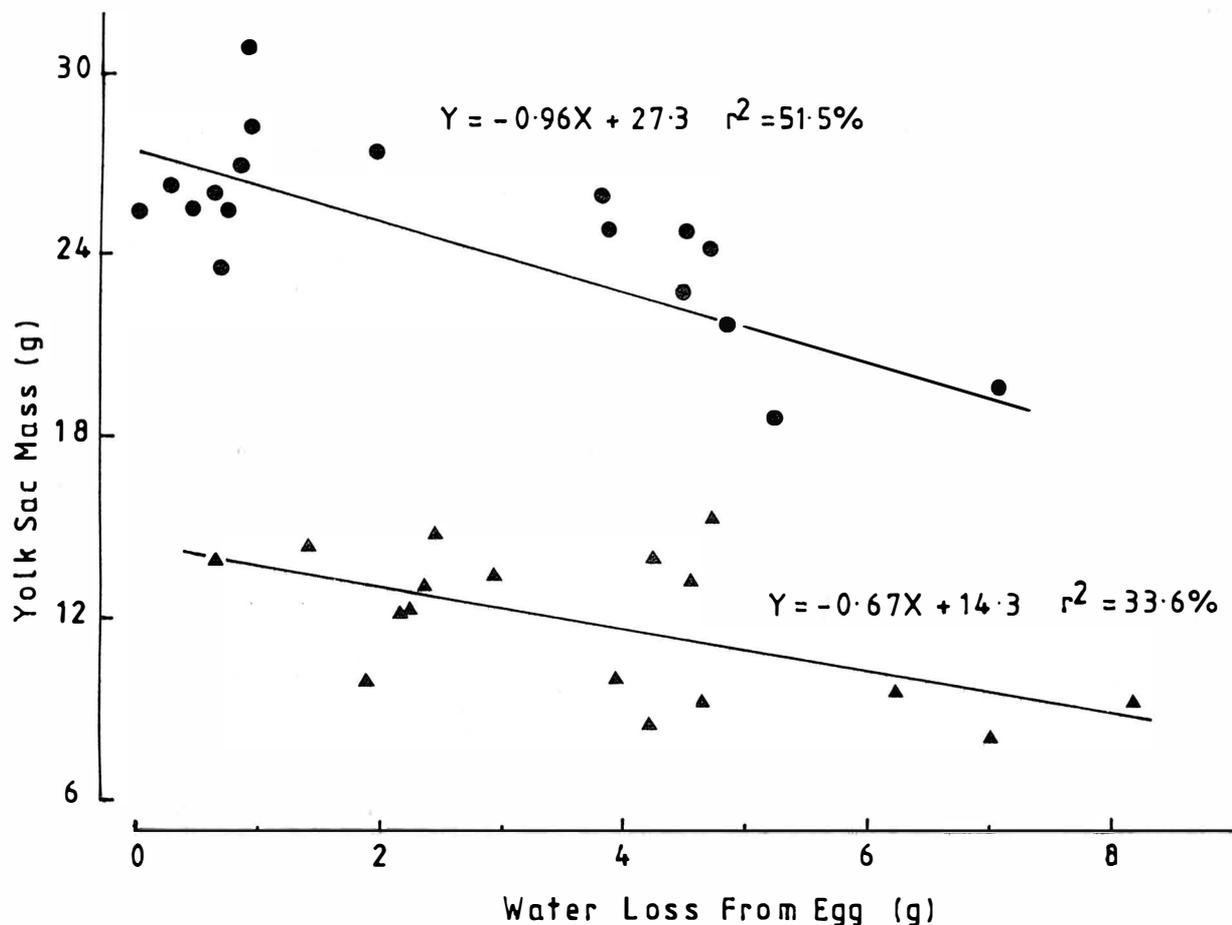


Fig. 1 The relationships between water loss from alligator (*Alligator mississippiensis*) eggs and the mass of the yolk sac by day 60 of incubation at 30°C (circles) and 33°C (triangles).

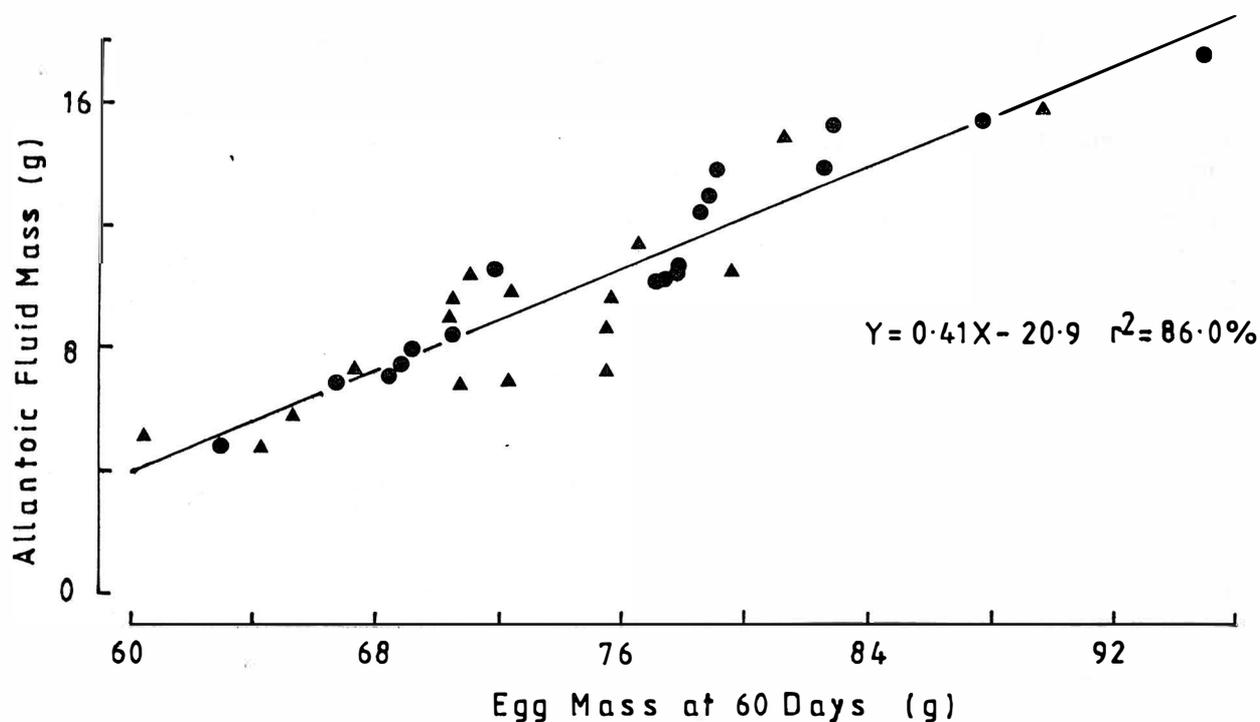


Fig. 2 The relationship between egg mass on day 60 of incubation and the mass of the allantoic fluid for alligator eggs incubated at 30°C (circles) and 33°C (triangles). Regression analysis (Ryan, Joiner and Ryan, 1985) revealed that the relationship was very similar at both temperatures and the line is a regression estimate for the combined data.

lost from the egg during incubation (Fig. 1). There was no significant correlation between mass of the yolk sac and egg mass on day 60 at either or both temperatures (not illustrated). The mass of allantoic fluid in eggs incubated at both 30°C and 33°C was not correlated with the rate of water loss from the egg at both incubation temperatures (not illustrated). By contrast, the mass of the egg on day 60 of incubation, irrespective of temperature, was correlated with the mass of allantoic fluid: larger eggs had more allantoic fluid (Fig. 2).

DISCUSSION

Embryos from eggs incubated at 30°C are at an earlier stage of development and are smaller than those at 33°C; the patterns of yolk and albumen utilisation and fluid formation are sufficiently similar at the two temperatures (Deeming and Ferguson, 1989). Therefore, in this analysis the data for embryos at 30°C were considered to be equivalent to embryos some 8-10 days earlier in incubation at 33°C. Hence, comparisons could be made both between embryos at different temperatures, and embryos at different stages of development.

Loss of water from alligator eggs affected the water relations of the different fluid compartments of the egg; the effects of dehydration were borne first by the amniotic fluid and then by the yolk sac. The allantoic fluid and embryo remained unaffected. This contrasts with eggs of the fowl: normal dehydration of the egg is borne by the allantois, amniotic fluid and by embryonic tissues whereas the yolk sac is unaffected (Hoyt, 1979; Tullett and Burton, 1982; Davis and Ackerman, 1987; Davis *et al.*, 1988). The high water content of the yolk sac is considered to be important in the process of yolk-sac retraction (Tullett and Burton, 1982). In dehydrated alligator eggs, the effects of a reduced water content of the yolk sac upon yolk sac retraction are unclear.

These differences between the alligator and the fowl may be related to differences in the pattern of nitrogen excretion: urea in the alligator (Clark, Shannon and Siskin, 1957; Manolis *et al.*, 1987; Deeming and Ferguson, unpublished observations) and uric acid in the fowl (Romanoff, 1967). During normal incubation as the fowl egg becomes dehydrated, water is lost from the allantois causing uric acid to crystallise and precipitate out of solution (Romanoff, 1967). By contrast, crocodilian embryos, in common with all other reptilian embryos (Vleck, 1991), produce urea as their major nitrogenous waste product. Alligator embryos in this study showed that during the later stages of development (stages 25-28) progressive dehydration of the egg leads to loss of water from the amniotic fluid and then the yolk sac; the volume of the allantoic fluid is maintained. Presumably, this prevents a deleterious increase in the concentration of urea within allantoic fluid. Although turtle embryos are tolerant of high concentrations of urea in their plasma (Packard and Packard, 1989) a threshold of toxicity must exist. If alligator embryos possess similar levels of tolerance to urea, loss of water from embryonic tissues, as is the case in the chick embryo

(Davis and Ackerman, 1987; Davis *et al.*, 1988), may lead to a deleterious build-up of this toxic metabolite which would inhibit normal metabolism and threaten survival to hatching.

In eggs of the snake *Elaphe obsoleta*, the amount of residual allantoic fluid is affected by the amount of water absorbed during incubation whereas live hatchling mass is unaffected (Deeming, 1989). Yolk sac mass was not determined in the above study but other reports show that dehydration of eggs during incubation appears not to affect the mass of the yolk sac in other reptiles. In pliable-shelled eggs of turtles, substrate water potential affects the rates of embryonic growth and yolk utilisation (Packard, Packard, Boardman, Morris and Shuman, 1983; Morris, Packard, Boardman, Paukstis, and Packard, 1983) but not the degree of hydration of the hatchling or residual yolk. For example, eggs of the turtle *Terrapene ornata* (initial mass of 10.5g) gain 0.6g of water on a wet substrate (-150kPa) but lose 1.8g of water on a dry substrate (-800kPa) (Packard, Packard and Gutzke, 1985). Both yolk-free hatchling, and residual yolk sac masses are reduced in eggs from the dry substrate but the percentage water content of the hatchling and yolk are unaffected by substrate water potential (Packard *et al.*, 1985). Similarly, in parchment-shelled eggs of squamates, hatchling mass is unaffected by substrate water potential (Gutzke and Packard, 1987; Packard and Packard, 1987; Plummer and Snell, 1988) but in these reptiles, egg size is important. Small lizard eggs (<1g) normally produce hatchlings that are larger than the initial mass of the egg (Deeming and Ferguson, 1991b).

Water loss from crocodilian eggs from natural nests has been considered to be abnormal (Ferguson, 1985) but under artificial incubation conditions it is common and, within limits, does not appear to compromise embryonic survival (Manolis *et al.*, 1987; Webb, Beal, Manolis and Dempsey, 1987; Whitehead, 1987). Hence, air spaces are, in all probability, experimental artifacts but they have proved useful in assessing the gaseous environment within the egg (Whitehead, 1987).

The percentage of water lost during incubation at 30°C was smaller than incubation at 33°C. This may be an experimental artifact but it may indicate more profound effects of temperature on water loss from these eggs. Temperature has direct effects upon the water exchange of turtle and snake eggs: as incubation temperature increases less water is exchanged between the egg and its environment (Gutzke and Packard, 1987; Packard, Packard, Miller and Boardman, 1987; Gutzke, Packard, Packard and Boardman, 1987). In addition, incubation temperature significantly affects the rate of development of alligator embryos and formation of sub-embryonic fluid is more rapid in alligator eggs incubated at 33°C, compared with eggs at 30°C (Deeming and Ferguson, 1989). It may be possible, therefore, that the pattern of water exchange of the eggs is dependent upon the stage of development of the embryo, and by association, upon incubation temperature. For example, at 33°C removal of water from the albumen by the embryo to form sub-embryonic fluid may sequester water and prevent its

loss across the shell during the first few days of incubation, thereby reducing the overall loss of water during incubation.

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