CHAPTER 43

Crocodilian Eggs: A Functional Overview

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ALTHOUGH our knowledge about crocodilian eggs and embryos has advanced greatly in recent years (Ferguson 1985), some very basic aspects of egg function remain poorly understood or are at least confused in the literature. There is no simplified model of the events which occur during development. Yet with crocodilian management programmes becoming more and more involved with the collection and incubation of eggs, such a model would have obvious utility.

This chapter addresses four areas of egg and embryo function: embryo orientation within eggs; the effects of rotating eggs; opaque banding; and, the reorganization of egg contents that occurs during development. The four appear to be intimately associated with each other (Webb *et al.* 1986) and indicate a highly dynamic relationship between the embryo and egg contents. When viewed together, the results provide a simplified model of developmental events occurring in crocodilian eggs.

CROCODILIAN EGGS

The eggs of all crocodilians are large (40 to 140 g), elliptical and hard-shelled (Ferguson 1985). They contain a spherical yolk mass bounded by a thin vitelline membrane, all of which is surrounded by albumen; these contents are totally enclosed in a shell membrane and brittle shell composed mainly of calcite (Ferguson 1982, 1985).

At the time of laying, embryonic development has progressed to the 10-20 somite stage within the oviducts of the female. Crocodilian embryos at the time of laying are thus more advanced than chelonians (Ewert 1985; Miller 1985), rhynchocephalians (Moffat 1985) and birds (Romanoff 1960), but are less advanced than the majority of lizards and snakes (Hubert 1985).

The eggs used in our investigations were from Australian freshwater crocodiles *Crocodylus johnstoni*, [68.2 ± 1.4 g (SE); Webb *et al.* 1983a] and saltwater or estuarine crocodiles *Crocodylus porosus* [113.4 ± 2.6 g (SE); Webb *et al.* 1983b]. Data were collected mainly in 1983 and 1984.

EMBRYO ORIENTATION

Regardless of how crocodilian eggs become oriented at the time of laying, the embryos within them are almost invariably found on the uppermost surface of the yolk (Ferguson 1985). They attach to the shell membrane at this position within 24 hours of laying. If eggs are rotated experimentally after laying, but before the embryos attach to the shell membrane, the embryos return to the uppermost part of the yolk, where they attach and develop normally. If rotated after attachment, but while the embryos are still young (2-10 days), they remain at the attachment site beneath the yolk and die (see below; Ferguson 1985). The survival of crocodilian embryos is thus partly dependent on them attaching at the top of the egg, regardless of their orientation at laying.

At the time of egg laying, the chorion, amnion and yolk sac of crocodilian embryos has already developed (Fig. 1) and they attach the small embryo to the inside of the vitelline membrane (Fig. 2A-D). Thus when an embryo "moves" to the top of an egg in a wild nest after laying or after an experimental manipulation, the vitelline membrane must also move. The only plausible explanation is that the yolk rotates within the surrounding albumen, as in bird eggs (Romanoff and Romanoff 1949; Romanoff 1960) (Fig. 2A-D).

The ability of the crocodilian yolk to "swing" within the albumen can be readily demonstrated by inserting strings of graphite granules through the albumen and into the yolk of a fresh egg (with watchmaker's forceps), and then tilting or rotating the egg (Fig. 3). The granules also indicate that the movement is made possible by a shearing between the layers of albumen rather than between the vitelline membrane or shell membrane and the albumen. As discussed below, yolk rotation is probably caused by the influence of gravity on a density gradient through the yolk.

EGG CONTENT CHANGES

Crocodile eggs (16 *C. porosus*; 83 *C. johnstoni*) incubated at 30°C were sacrificed and the major egg contents weighed separately (see Manolis *et al.*

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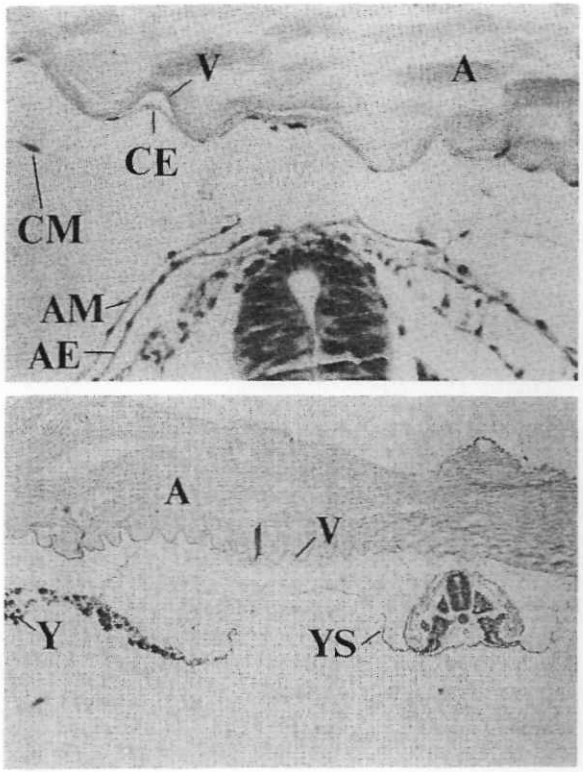

Fig. 1. High power (upper) and low power (lower) views of a transverse section through the albumen (A), vitelline membrane (V) and attached *Crocodylus porosus* embryo preserved on the day of laying. Globules of yolk (Y) remain attached to the developing yolk sac tissue. The chorionic ectoderm (CE) is fused to the vitelline membrane and is separated slightly from the chorionic mesoderm (CM). The yolk sac mesoderm and endoderm (YS) and amniotic mesoderm (AM) and ectoderm (AE) are present (after Webb *et al.* 1986).

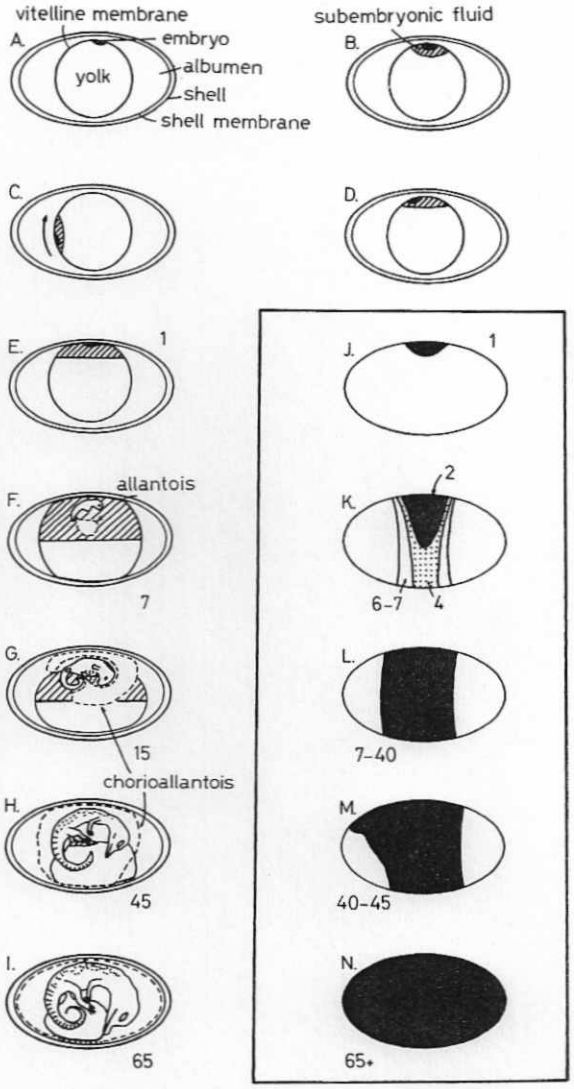

Fig. 2. The chronology of development in crocodile eggs. Numbers refer to days of incubation at 30°C for *Crocodylus porosus* and if multiplied by 0.95 approximates the situation in *C. johnstoni*. Yolk is internalised after about 90 days and pipping and hatching at 95 days. A, Oviducal egg prior to subembryonic fluid formation. B, Oviducal egg at the time of laying with <2 ml of subembryonic fluid. C, If necessary the yolk rotates within the albumen. D, Within hours of laying the embryo is on the upper surface of the yolk. E, The volume of subembryonic fluid increases at the expense of the albumen; within 24 hours the vitelline membrane and embryo adhere to the shell membrane. F, The allantois forms and as it expands subembryonic fluid formation ceases. G, The chorioallantois spreads around the midpoint axis of the egg and the majority of subembryonic fluid becomes enclosed (with yolk) within the embryonic yolk sac; hydrated albumen remains in both poles of the egg. H, The chorioallantois expands from the midpoint band around the shell membrane, separating the remaining albumen from the shell membrane. I, The chorioallantois completely surrounds the egg and the remaining albumen is dehydrated. J-N, Changes in opaque banding associated with the internal developments depicted in E-I.

Chapter 46). The pattern of change (Fig. 4) through incubation was similar in both species and closely parallels the situation described in turtle (Agassiz 1857; Webb *et al.* 1986) and bird eggs (Romanoff 1967). Infertile eggs (no sign of embryonic development; no opaque banding; no build up of subembryonic fluid) were used to approximate the condition which exists in the oviducts before significant embryonic development occurs (Fig. 2A), so that a chronology of developmental events could be constructed.

Within the oviducts the egg contents are initially albumen, yolk and a small mass of embryonic tissue which is probably located at the top of the yolk sphere, as in birds (Romanoff and Romanoff 1949). As development proceeds in the oviducts (Agassiz 1857), water is drawn from the water-laden albumen, presumably by the action of the ectodermal surfaces of the embryonic disc as in birds (New 1956), and is secreted beneath the embryo, on the inside of the vitelline membrane. This subembryonic fluid is appreciably lighter than the yolk (Table 1) and settles above it (Fig. 2B), beneath the embryo. There is thus a density gradient through the yolk sphere at the time of laying. If eggs are laid with the embryo displaced from the top, and hence the yolk's center of gravity displaced from its equilibrium position, then gravity could and probably does cause the yolk to rotate (Fig. 2C,D).

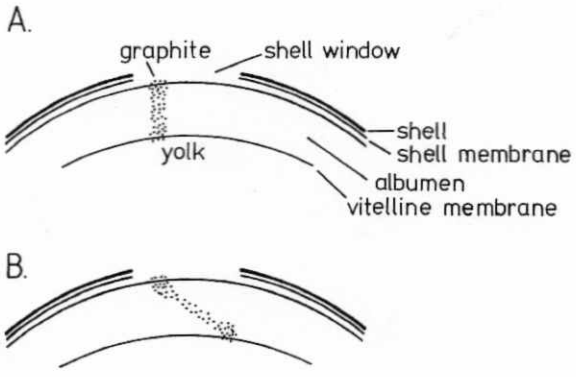

Fig. 3. Transverse section through the upper part of the minor axis of a crocodilian egg (with a shell window removed). Graphite granules inserted into the albumen and yolk (A) adopt new positions when the egg is rocked from side to side (B); the vitelline membrane and yolk move together and shearing takes place within the albumen (after Webb *et al.* 1986).

The amount of subembryonic fluid increases greatly after laying (Fig. 4) and this causes the volume within the vitelline membrane (which contains the embryo, subembryonic fluid and yolk) to increase. It occupies the space created by the dehydration of albumen (Fig. 4). The albumen immediately above the embryo is dehydrated first and the vitelline membrane and embryo adhere to the shell membrane (Fig. 2E) in that area (a layer of dehydrated albumen may be between them). The albumen between the yolk sphere and shell membrane around the minor axis of the egg is dehydrated next and the vitelline membrane attaches to the shell membrane in a band around the egg. Hydrated residual albumen becomes restricted to the poles of the egg during the first quarter of incubation.

Albumen dehydration and subembryonic fluid production peak when the allantois is expanding. Part of the allantoic membrane fuses with the chorion, creating the chorioallantois (Fig. 2G), while the remainder is within the subembryonic space. At this stage, contact between the chorioallantois and the shell membrane is restricted to the minor axis of the egg, where the albumen has been dehydrated, and where the opaque band occurs. When the chorioallantois spreads outside this band (Figs 2H,I) the remaining albumen (in the poles) is dehydrated.

Table 1. The density (g ml^{-1}) and water content of major egg components in two crocodilian species.

	C. johnstoni		*C. porosus*	
	% water	density	% water	density
Albumen	95.0	1.024	96.4	1.020
Yolk	58.1	1.058	56.4	1.042
Subembryonic fluid	98.8	1.005	—	1.004

Yolk is primarily utilized later in development (Fig. 4) as a source of nutrients for the rapidly growing embryo.

OPAQUE BANDING

The pattern of opaque banding on the shell (Fig. 2J-N) has been described previously (Webb *et al.* 1983a,b), and is similar to that on the eggs of *Alligator mississippiensis* (Ferguson 1982, 1985) and perhaps all crocodilians. The opacity is caused by changes in the optical properties of the shell following loss of water from it, and by structural changes that may also be associated with dehydration, but are more likely to arise from the dissolution of calcium

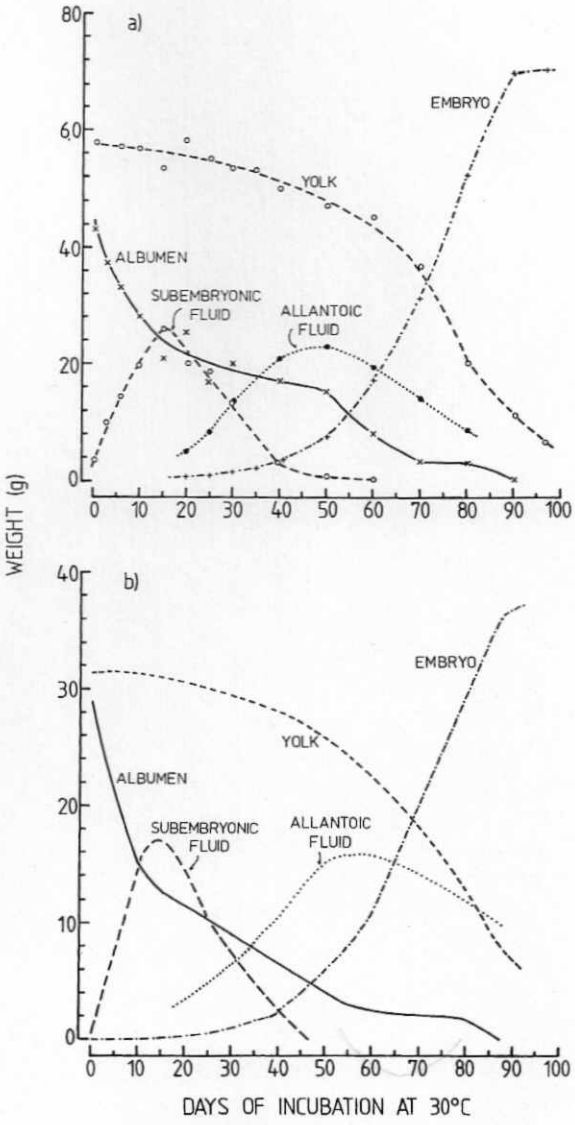

Fig. 4. Changes in the weights of egg components with progressive development of *Crocodylus porosus* (a) and *C. johnstoni* (b) eggs incubated at 30°C. *Crocodylus porosus* eggs (N = 16) were from a single clutch (121.7 ± 0.55 g (SE), N = 46). *Crocodylus johnstoni* eggs (N = 83) were from 16 clutches and the weights of components were scaled to the mean egg weight (68.2 ± 1.4 g (SE); Webb *et al.* 1983a) (after Webb *et al.* 1986).

carbonate crystals (Ferguson 1982). As in turtles, one of the functions is probably enhanced gas exchange through the opaque areas (Thompson 1985; see Whitehead Chapter 47).

Based on the correlation between egg content changes and the pattern of banding (Fig. 2), it seems likely that the optical and structural changes in the shell and shell membrane that cause the opacity are linked to intrinsic rather than extrinsic dehydration.

When the albumen above the embryo is being dehydrated, the opaque patch forms at the site in which the embryo will attach (Fig. 2J). Initially a thin layer of partly dehydrated albumen remains there, between the shell membrane and the vitelline membrane, but this rapidly disappears. The dehydration of albumen appears to be simply extended to the adjoining shell membrane area.

As albumen is dehydrated around the minor axis of the egg, so the opacity spreads in an identical pattern (Fig. 2K), well before the chorioallantois spreads. Later, the chorioallantois surrounds the inside surface of the opaque band area. It is when the chorioallantois eventually expands outside that banded area, along the inner surface of the shell membrane towards the egg poles (Fig. 2H), that the remaining albumen is dehydrated and the volume of allantoic fluid increases (Fig. 4). The opacity mirrors the lateral spread of this dehydrating influence (Fig. 2M) and the egg shell becomes completely opaque (Fig. 2N).

The pattern of opaque banding described occurs in air fully saturated with water vapour, even if droplets of free water are present on the shell surface. However, if crocodilian eggs containing live embryos are partly or completely submerged, the shell does not go opaque in the region in contact with water (as in turtle eggs; Ewert 1979). Immersion in water may permit bulk water flow through liquid filled pores in the shell, exceeding the capacity of intrinsic mechanisms to remove water from the shell membrane or shell.

EFFECTS OF EGG ROTATION

The results of an experiment in which *C. johnstoni* eggs were rotated (Table 2), are explicable in terms of the developmental model given above.

Nineteen freshly laid eggs, which had no opaque band development, were either rotated (N = 6) or left as controls (N = 13). In all cases when the eggs were subsequently opened, the embryos were alive and had attached at the top of the egg. In the six rotated eggs, the yolk had presumably swung within the albumen, bringing the embryo to the top before attachment and opaque banding (within 12 hours of being rotated).

Forty-two eggs with embryos between 2 and 10 days of age (at 30°C incubation) were either rotated (N = 9) or left as controls (N = 33). When examined 13 to 16 days later, all the rotated eggs contained embryos attached at the bottom of the egg, surrounded by yolk, and all the embryos were dead; based on the sizes of the dead embryos, they had lived for 2-8 days [mean = 4.8 ± 1.6 days (SD)] in their new position. In contrast, the controls developed successfully and hatched (one died at approximately 45 days of age).

Clearly, once embryos attach to the shell membrane, they are unable to reposition themselves within the yolk, and in the absence of subembryonic fluid (which percolates through the yolk material to the top of the vitelline membrane in rotated eggs) and a developed allantois, they die. Embryo orientation at the top of the egg is essential for embryo survival.

When two eggs containing 13 day old embryos were rotated, both remained attached at the bottom of the egg, but one died (three days after rotation) and the other was alive and apparently normal after 10 days. Its developing, fluid-filled allantois had floated up through the yolk and was protruding into the subembryonic fluid. Similarly, a rotated 18 day old embryo was alive at the bottom of the egg with its allantois upward when opened after 17 days. Six

Table 2. Results of rotating *Crocodylus johnstoni* eggs. The uppermost point of each egg was marked in the nest and they were rotated (about the major axis) relative to that mark. Eggs were rotated before (B) or after (A) embryo attachment as determined by opening an egg from the clutch used. Controls were not rotated. Incubation temperatures were between 30.0°C and 31.5°C. N = number of eggs in the sample. * = position assumed; ** = death probably unrelated to experimental treatment; *** = possibly related to experimental treatment.

Embryo age incubation started (days)	N	Rotation (degrees)	Days until opened	Embryo position	% Alive
0.5B	13	control	17-33	top	100
0.5B	6	45-225	6	top	100
2-10A	33	control	hatched	top*	97**
2-9A	9	180	13-16	bottom	0
13-18A	3	180	10-21	bottom	67
18A	6	180	hatched	bottom*	83***

other eggs rotated when the embryos were 18 days of age developed through to the hatching stage (one died after the yolk had been internalized immediately prior to hatching).

Together, these results suggest that rotation of eggs is lethal if it occurs after embryo attachment (> 1 day) but before the respiratory and excretory functions of the allantois are adequately developed (around 13 days).

DISCUSSION

The generalized model of development described here requires further study, but in its present form is consistent with the available information on crocodilian eggs and embryos (Ferguson 1985). Movement of fluids within the egg during development appears to be intimately associated with both embryo orientation and opaque banding. The model is also consistent with most observations on the development of embryos within large, hard-shelled, chelonian eggs (Agassiz 1857; Ewert 1985). The spherical eggs of the pitted-shell turtle *Carettochelys insculpta* [33.7 ± 0.9 g (SE); Webb *et al.* 1985], developed as the model would predict for a spherical crocodile egg. [In spherical eggs the shell opacity ("chalking") and albumen dehydration proceed as a field from the top to the bottom of the egg rather than as a band spreading from the midline].

Yolk rotation appears essential for normal embryonic development, and we suspect that variation in both the water content and quantity of albumen supplied to the eggs of different crocodilian and chelonian species may be constrained by the need to facilitate yolk rotation. For example, marine, freshwater and terrestrial chelonians supply their ova (yolk) with similar proportions of albumen (Ewert 1979, 1985), the bulk of which is a water supply, even though water availability in the three environments differs greatly. This is surprising, because many turtle eggs can absorb appreciable amounts of water after laying (Packard and Packard 1984) and a less hydrated albumen would seem consistent with water economy in some species.

However, yolk rotation requires the ovum to be suspended in a low friction medium at the time of laying. The water-laden albumen provides that medium, and for mechanical purposes alone, a minimum surrounding of thin, watery albumen may be needed for embryo survival, regardless of the water and chemical constituents of albumen needed for later embryonic development.

From a management viewpoint, maintaining the correct orientation of eggs is essential if they are handled between about one and fifteen days. Before that time, the embryo is able to orient to the top regardless of egg position, and after that time the expanded allantois appears capable of adequately providing for respiration and excretion, even though the embryo is at the bottom of the yolk.

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