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METHODS FOR THE DETERMINATION OF THE PHYSICAL CHARACTERISTICS OF EGGS OF *ALLIGATOR MISSISSIPPIENSIS*: A COMPARISON WITH OTHER CROCODILIAN AND AVIAN EGGS

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ABSTRACT

The mass, length and breadth of 572 eggs of *Alligator mississippiensis* were measured and described as a complete sample and as subsets of 14 clutches. Egg volume and density were calculated. A multiple regression equation was generated to predict initial egg mass from egg length and breadth. A weight coefficient (K_M) was determined for alligator eggs and its value was compared both to published values for avian eggs and to values for other crocodilian eggs calculated from literature data. The value of K_M in crocodilians was higher than in avian eggs implying that the density of alligator eggs was much higher than the density of avian eggs. Egg volume in alligators was also estimated using the volume coefficient (K_V) for avian eggs but this was found not to be applicable.

INTRODUCTION

Over the past two decades the avian egg has been extensively studied and many different relationships between various egg, and incubation, parameters have been established (see Rahn and Paganelli, 1981 and Rahn, Whittow and Paganelli, 1985). Allometric relationships are observed between egg mass and incubation period (Rahn and Ar, 1974), water vapour conductance of the eggshell (Ar, Paganelli, Reeves, Greene and Rahn, 1974), surface area and density of the egg (Paganelli, Olszowka and Ar, 1974). Such allometric relationships have not been investigated in reptiles. This is surprising considering the similarity between avian eggs and those of many reptiles, particularly crocodilians which are the closest living relatives of the birds. Indeed, egg structure in birds and crocodiles is very similar (Romanoff, 1967; Ferguson, 1985; Manolis, Webb and Dempsey, 1987) but there have been only a few comparative studies. The chemical composition of crocodilian egg yolk and albumen shows both similarities and differences to that of the domestic fowl (Burley, Back, Wellington and Grigg, 1987, 1988). Eggshell conductance, to water and respiratory gases, has been found in crocodilians eggs to be two to five times greater than in avian eggs of comparable size (Packard, Taigen, Packard and Shuman, 1979; Lutz, Bentley, Harrison and Marszalek, 1980). Comparative studies of avian and reptilian eggs may prove to be important in assessing the effects of the eggshell on the physiology of the embryo and evolution of incubation conditions (Packard and Packard, 1980).

Despite such high conductances to water vapour it has been shown that air spaces in crocodilian eggs, although rare in nature and deleterious if large (Ferguson, 1982, 1985), are common in artificially incubated eggs; the loss of some water from the egg appears to be tolerated by the embryo (Manolis, *et al.*, 1987; Whitehead, 1987; Deeming and Ferguson, 1989). In common with avian eggs, which normally lose water during incubation, crocodilian eggshells are rigid and non-compliant which allows the air cavities to develop, although in contrast to avian eggs crocodilian eggs can swell under some incubation conditions (Manolis, *et al.*, 1987).

In bird eggs air spaces are normal and water loss is essential for normal development (Romanoff, 1967). It is often useful to calculate initial mass of eggs after unknown periods of incubation (Hoyt, 1979). This can be done by filling the air space but this is lethal to the embryo (Grant, Paganelli, Pettit, Whittow and Rahn, 1982). To overcome this problem, several authors have developed techniques for determining the initial mass, volume and density of avian eggs using linear dimensions (Paganelli, *et al.*, 1974; Hoyt, 1979; Rahn, Parisi and Paganelli, 1982). No such methods are available for crocodilian eggs but considering the rapidly developing interest in crocodilian eggs in the field and the laboratory (Webb, Manolis and Whitehead, 1987) such methods to determine whether eggs have lost water during incubation without killing the embryo may be useful. The aims of this study were (1) to describe methods of determining initial mass of *Alligator mississippiensis* eggs from their linear dimensions, (2) to calculate the volume and density of alligator eggs, (3) to examine these characteristics of alligator eggs from different clutches and, (4) to compare the measurements of alligator eggs with other crocodilian eggs and those of birds. It is hoped that these data will give us some indication of the variability in the dimensions of eggs within a species, between species of crocodilians and between crocodilians and birds.

MATERIALS AND METHODS

Eggs of *Alligator mississippiensis* were collected from 14 wild nests at the Rockefeller Wildlife Refuge, Louisiana, USA. All eggs were collected 24 hours after laying (as assessed by the extent of opaque banding of the eggs [Ferguson, 1982, 1985]) and were immediately air-freighted to Manchester, UK. On arrival at the laboratory (day 3 or 4) the eggs were weighed to the nearest to 0.01g. The eggs were placed in incubators (set at 30°C and 33°C) and were used in other studies (Deeming and Ferguson, 1989). The daily rate of water loss of these eggs was less than $0.01 g.day^{-1}$ under conditions of very high humidity and irrespective of temperature (Deeming and Ferguson, 1989); for the purposes of this study the initial recording of egg mass in the laboratory was considered to be a close approximation of initial egg mass (IEM) at oviposition. During the course of the other study the maximum length (L) and maximum breadth (B) of each egg were measured using Vernier calipers to the nearest 0.01cm. All eggs lost water during incubation but only eggs with intact eggshells were used in this study.

For subsequent calculations the shape of each egg was assumed to be a true ellipsoid; the maximum length and breadth of the egg occurs at the equator of the latitudinal and longitudinal planes. Egg volume (V) was calculated using the relationship:

$$V\ (cm^3) = \frac{\pi\ L.B^2}{6} \qquad (1)$$

Initial egg density ($g.cm^{-3}$) was calculated from the measured egg mass and calculated volume. The weight coefficient (K_M) has the same units as density but it is simply a coefficient between egg mass and linear dimensions and ignores the effect of egg shape ($\pi/6$ in equation 1). K_M ($g.cm^{-3}$) was determined from eggs measured directly, and from data presented in Ferguson (1985), using the relationship:

$$K_M = \frac{\text{Initial Egg Mass}}{L.B^2} \qquad (2 — \text{Hoyt, 1979})$$

Egg volume was also calculated using the volume coefficient, $K_V = 0.509$, derived from avian eggs:

$$\text{Volume} = 0.509.L.B^2 \qquad (2 — \text{Hoyt, 1979})$$

Data were stored on a Prime mainframe computer and calculations were performed using the Minitab statistical package (Ryan, Joiner and Ryan, 1985). Multiple regression techniques were used to produce an equation to predict initial egg mass from linear dimensions (significance levels were assessed using a correlation coefficient [R^2] and an F-ratio statistic).

RESULTS

In total 572 eggs of *A. mississippiensis* from 14 clutches were weighed and measured. Mean values for measured physical dimensions, and calculated parameters, of these eggs are shown in Table 1. Both egg length (L) and breadth (B) were individually useful in predicting initial egg mass (IEM), but multiple regression analysis revealed that a better prediction for egg mass could be achieved using both linear dimensions in conjunction:

$$IEM = 11.6L + 29.7B - 134,\ R^2 = 94.8\%,\ F_{2,571} = 5248. \qquad (4)$$

	N	*S.D.*	*Range*
Initial egg mass (g)	72.80	6.55	54.89 — 91.52
Egg length (cm)	7.16	0.32	6.25 — 8.15
Egg breadth (cm)	4.15	0.12	3.83 — 4.44
Egg volume (cm^3)	64.73	5.79	49.00 — 80.88
Egg density ($g.cm^{-3}$)	1.125	0.024	1.033 — 1.298
K_M ($g.cm^{-3}$)	0.589	0.013	0.541 — 0.680
Volume — K_V (cm^3)	62.92	5.63	47.64 — 78.61

TABLE 1: Physical dimensions of 572 eggs of *Alligator mississippiensis* from 14 clutches. Initial egg mass, maximum length and maximum breadth at the equator of the egg are direct measurements. Egg volume, density and the value for the observed weight coefficient (K_M) are calculated (Hoyt, 1979). Egg volume calculated from the observed volume coefficient for avian eggs (Volume-K_V, Hoyt, 1979) is included.

There was a wide variation in initial egg mass in the eggs sampled; the range of nearly 37g in the sample (Table 1) was reflected by a range of 20g difference in mean egg mass between clutches (Fig. 1). There was greater variation in egg length within the sample and between individual clutches than was observed for egg breadth (Fig. 1). Generally, both egg length and breadth increased with increasing egg mass although breadth was better correlated with egg mass for each clutch (Fig. 1).

Egg volume was closely correlated with initial egg mass (Fig. 2). There was a range of densities within the sample (Table 1), but there was no correlation with

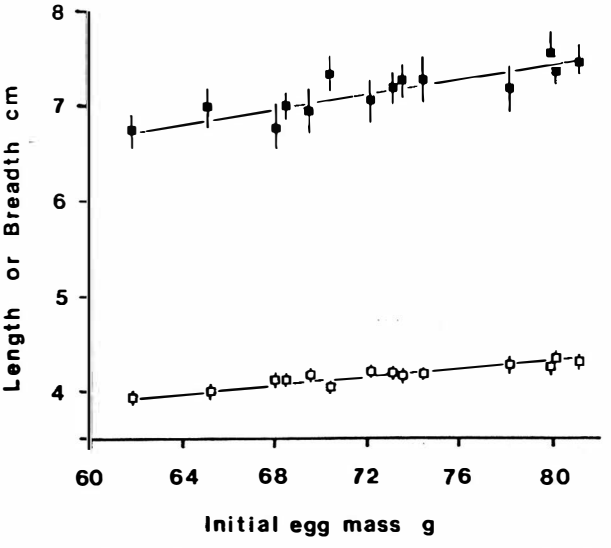

Fig. 1 The relationship between mass and length (closed symbols) and breadth (open symbols) of eggs of *Alligator mississippiensis* from 14 different clutches. Values are means ± S.D. and lines are fitted by eye.

initial egg mass between clutches. The coefficient of variation (Hoyt, 1979) around the mean value for K_M in the sample of eggs was only 2.2%. Egg volume, calculated using the mean K_V for avian eggs underestimated the values for egg volume calculated from egg shape (Table 1).

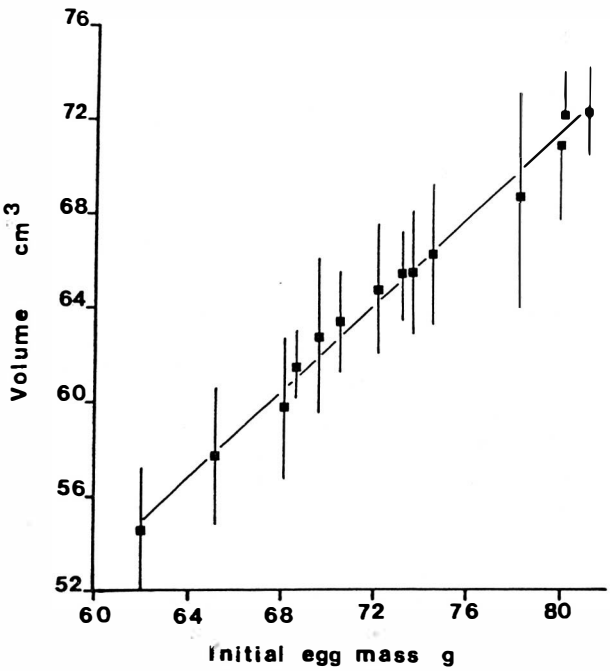

Fig. 2 The relationship between egg mass and volume for alligator eggs from 14 clutches. Values are means ± S.D. and lines are fitted by eye.

DISCUSSION

Egg size varies between female alligators; each clutch has different characteristics. Some clutches show a large variation in egg dimensions, others do not. Mean egg breadth between clutches is more uniform than mean length which may indicate some kind of limitation on egg breadth by the oviduct which may not apply to the length of the egg. These differences may be related to clutch size or maternal age but in the present study no clear relationships between clutch size and egg mass could be found. Further study is required to establish the factors which determine the size of eggs in individual clutches.

The initial mass of avian eggs can be calculated (Hoyt, 1979) or can be determined by filling the air space with water (Grant, *et al.*, 1982). It is difficult to repeat such a method in crocodilian eggs because formation of air spaces is not uniform: they can occur within the albumen, between the chorio-allantois and shell membrane, or between the shell membrane and the calcitic shell (Ferguson, 1985; Whitehead, 1987). The methods described in this study allow a value for initial mass to be allocated to alligator eggs from unknown incubation conditions. Such a technique has applications both in the field and in the laboratory in assessing whether the egg has lost water during incubation, though it is not applicable when the egg has swollen and cracked (Manolis, *et al.*, 1987; Grigg, 1987). Knowledge of initial egg mass is useful in assessing the amount of the albumen and yolk in the egg and in converting egg contents into hatchling. Important relationships between egg mass and the metabolic rate of the embryo, incubation period, water vapour conductance and egg surface area in birds (Rahn, *et al.*, 1974; Rahn and Ar, 1974; Ar, *et al.*, 1974; Paganelli, *et al.*, 1974) may also apply in crocodilians and other reptiles but further study is required.

Values for K_M calculated for eggs of various crocodilians (data from Ferguson [1985]) are shown in Table 2. K_M derived from alligator eggs in the present study (Table 1) is lower than that derived from data presented by Ferguson (1985). The reason for this discrepancy may lie with the size and sources of the data. Ferguson (1985) presents a mean value for egg mass and dimensions from several sources collected over many years from different geographic locations both in relation to *Alligator* and other species listed in Table 2. Data for alligator eggs in the present study are

Species	*L*	*B*	*IEM*	K_M
Alligator mississippiensis	7.4	4.3	84	0.614
Alligator sinensis	6.8	3.4	52	0.662
Caiman crocodilus crocodilus	6.5	4.0	59	0.567
Caiman crocodilus yacare	6.8	4.2	75	0.625
Caiman latirostris	6.6	4.6	84	0.601
Paleosuchus palpebrosus	6.6	4.2	69	0.593
Crocodylus johnstoni	6.6	4.2	68	0.584
Crocodylus niloticus	7.5	4.8	110	0.637
Crocodylus novaeguinae	7.6	4.3	85	0.605
Crocodylus palustris	7.5	4.6	84	0.529
Crocodylus porosus	7.7	5.2	113	0.543
Osteolaemus tetraspis tetraspis	6.3	3.7	52	0.603

TABLE 2: Mean values for egg length (L), breadth (B) and initial mass (IEM) from a variety of crocodilians (Ferguson, 1985). Observed weight coefficient is calculated from the relationship: $K_M = IEM/L.B^2$ (Hoyt, 1979).

from a single geographic location, supplied at the same time in the nesting season of one particular year. It is, therefore, likely that these eggs were laid by similar sized females and are more likely to be similar to each other than data from other populations sampled at different times and in different years.

The data from different crocodilians show that, like birds (Hoyt, 1979), K_M varies between species and is not related to egg length, breadth or mass. The values for K_M for 12 species of crocodilian (mean = 0.597, range = 0.529-0.662) are, however, higher than those for 26 species of bird (mean = 0.548, range = 0.527-0.597; Hoyt, 1979) and very much higher than values for eight species of emydid turtles (mean = 0.526, range = 0.304-0.612; calculated from Ewert [1979]). Therefore, for any given set of egg dimensions, crocodilian eggs are heavier, and turtle eggs are lighter, than bird eggs. As K_M ignores egg shape, this suggests that differences between avian and crocodilian eggs are not due to their different shapes (all crocodilian eggs are ellipsoid; Ferguson, 1985) but due to their density. Alligator eggs have a mean density (1.125 $g.cm^{-3}$) higher than that for many bird eggs (mean = 1.073, range = 1.055-1.104; Rahn, *et al.*, 1982). The density of crocodilian albumen is lower than that for avian eggs but crocodilian yolk has a greater density than avian yolk (Manolis, *et al.*, 1987). Differences in total egg density between birds and crocodilians may lie in the relative densities of the egg contents or the density the eggshell. The extent to which these factors contribute to the observed differences in egg density and K_M is yet to be determined.

The value of K_V for avian eggs underestimated the volume of crocodilian eggs but by a constant amount ($2cm^3$). K_V for crocodilian eggs must, therefore, be higher than that for bird eggs. Egg volume is greater for any set of linear dimensions in crocodilians. The volume of alligator eggs was not, however, determined empirically in the present study, and K_V cannot be calculated in this study but may have a value of 0.524 ($\pi/6$).

In conclusion, differences in mass, volume and density occur between avian and crocodilian eggs. The allometric relationships between egg mass, various incubation parameters and shell characteristics (Rahn, *et al.*, 1974; Rahn and Ar, 1974; Ar, *et al.*, 1974; Paganelli, *et al.*, 1974) may well be present in crocodilians, turtles and squamates. However, these three types of reptiles differ greatly in their eggshell structure and incubation requirements (Packard and Packard, 1980, 1988; Ewert, 1985; Ferguson, 1985) and therefore, any attempts to derive allometric relationships for reptiles as a whole (as has been accomplished for birds) may mask important patterns for each type of reptile.

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PALMATE NEWT PREDATION ON COMMON FROG, *RANA TEMPORARIA*, AND COMMON TOAD, *BUFO BUFO*, TADPOLES

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ABSTRACT

In a series of laboratory experiments, male palmate newts that had no previous experience of anuran tadpoles as potential prey were conditioned for five days to small worms, common frog tadpoles, common toad tadpoles or a 50:50 mixture of frog+toad tadpoles. During three experiments, conditioned newts were offered 1) a 50:50 mixture of frog+toad tadpoles 2) only frog tadpoles or 3) only toad tadpoles.

The results showed that palmate newts with no previous experience of either frog or toad tadpoles very quickly learnt to distinguish between them and take only frog tadpoles. This was supported by the results of a fourth experiment using male palmate newts from a pond that contained both tadpole species. Common toad tadpoles were almost totally rejected.

The conclusion is, that common frog tadpoles gain no long term protection against predation from palmate newts through associating with common toad tadpoles.

INTRODUCTION

Three species of newt occur in Great Britain, the warty newt (*Triturus cristatus*), smooth newt (*T. vulgaris*) and palmate newt (*T. helveticus*). All three species are voracious predators and are known to take a wide range of aquatic invertebrates (Avery, 1968; Griffiths, 1986). In addition, smooth newts are also known to take frog tadpoles (Cooke, 1974) but, like palmate newts, reject toad tadpoles (Cooke, 1974; Griffiths, 1986) whilst warty newts will take both frog and toad tadpoles (Cooke, 1974; Heusser, 1971).

Unlike frog eggs and tadpoles, those of toads are generally thought to be unpalatable to many potential predators (Licht, 1968; Wassersug, 1971). The difference in palatability between common frog (*Rana temporaria*) and common toad (*Bufo Bufo*) tadpoles presents an interesting question: In ponds where both tadpole species occur together, do frog tadpoles gain any protection against predation by newts due to the presence of toad tadpoles?

This paper reports the results of a series of laboratory experiments designed to investigate tadpole predation by palmate newts.