Effects of incubation temperature on growth and development of embryos of *Alligator mississippiensis*

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Summary. Eggs of *Alligator mississippiensis* were incubated at 30 °C and 33 °C throughout incubation up to hatching. Every four days several eggs were opened and the albumen, yolk and extra-embryonic fluids removed and weighed. The embryo was removed and fixed prior to being staged, weighed and measured for various morphometric criteria. Development at 33 °C was accelerated compared with 30 °C in terms of yolk and albumen utilization and embryo growth. Significant losses in yolk mass did not occur until stage 22 at 33 °C but occurred at stage 18 at 30 °C. Different patterns in growth were observed in embryos at the two temperatures at similar morphological stages: between stages 18 and 22 embryos at 33 °C were smaller (in mass and length) compared with embryos at 30 °C despite being morphologically similar. The differences in growth and physiology between embryos at 30 °C (females) and 33 °C (males) were dependent on incubation temperature but not sex. Incubation at 33 °C accelerated both growth and development in *Alligator;* initially morphogenesis was accelerated by the higher temperature but later, growth rate was accelerated.

Key words: Crocodilians – Morphometrics – Extra-embryonic fluids – Growth sex determination

Introduction

Temperature has profound effects upon the physiology of all animals. In particular, the rate of development of reptilian embryos is temperature-dependent; high temperatures (within the viable range) accelerate embryonic development compared with lower temperatures. This is usually ob-

served in the length of the incubation period, because as incubation temperature increases so hatching occurs earlier (Ewert 1979, 1985; Packard and Packard 1988). Reports of the effects of temperature on embryonic development are rarer. Embryonic growth rates at different temperatures have been reported in only a few species of turtles (Pieau and Dorizzi 1981; Packard et al. 1987), one species of snake (Vinegar 1973), a lizard (Maderson and Bellairs 1962; Holder and Bellairs 1962) and three crocodilian species (Webb et al. 1987; Lang et al. in press; Deeming and Ferguson 1988). Some data on the effects of temperature on the embryonic utilisation of yolk and albumen are known for *Crocodylus johnstoni* (Manolis et al. 1987).

This lack of data concerning the effects of temperature on embryonic growth is surprising, considering the significance of temperature in the biology of reptiles. In crocodilians alone, incubation temperature has been shown to affect sex determination (Ferguson and Joanen 1982, 1983; Webb and Smith 1984; Webb et al. 1987; Hutton 1987; Lang et al., in press), embryonic growth rates (Deeming and Ferguson 1988), pigmentation patterns of hatchlings (Deeming and Ferguson, in press a; Murray et al., in press), post-hatching thermoregulation (Lang 1987) and growth (Joanen et al. 1987). This paper presents data on the effects of incubation at 30 °C and 33 °C on the development of embryos of *Alligator mississippiensis,* using a sample of eggs different to that described in Deeming and Ferguson 1988, in press b). American alligators have temperature-dependent sex determination and these temperatures produce 100% female and male hatchlings, respectively (Ferguson and Joanen 1982, 1983). Changes in the extra-embryonic fluids, albumen and yolk are recorded throughout the incubation period, together with

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the patterns of embryonic growth using mass and morphometric measurements (Deeming and Ferguson, in press b). Morphometric criteria are used to generate equations to predict age and stage of development of embryos incubated at 30 °C and 33 °C.

Materials and methods

Eggs of *Alligator mississippiensis* were collected in 1988 from several nests of wild alligators at the Rockefeller Wildlife Refuge, Louisiana, USA, on the first or second day after laying and were immediately transported by air to Manchester, UK. On arrival at the laboratory (day 3 or 4 depending on clutch) the eggs were weighed and set in incubators (Vinden Scientific Ltd) accurate to ± 0.001 °C. Eggs were incubated at either 30 °C or 33 °C (temperatures which produce 100% females and 100% males, respectively) and clutches were randomised between incubators. Both incubators were maintained at a relative humidity as close as possible to 100%.

Starting on day 8 of incubation, and on every fourth day thereafter (time of oviposition = start of incubation), eggs were removed from the incubators. Egg mass (g), length and width (in millimetres measured using Vernier calipers to the nearest 0.1 mm) were measured and the eggs were placed on ice prior to opening. During the first three weeks of incubation the embryo was removed first and placed in 10% formal saline (a solution of 0.133 mol·l⁻¹ HCHO, 0.037 mol·l⁻¹ Na₂HPO₄ and 0.025 mol·l⁻¹ NaH₂PO₄). The sub-embryonic fluid was drained from the egg and was weighed. Yolk sac, albumen and eggshell mass were then recorded. After day 24 of incubation, allantoic fluid and amniotic fluid were removed prior to removal of the embryo and other components. On day 60 of incubation at 33 °C, and day 68 at 30 °C, embryos had withdrawn their yolk sac into the abdominal cavity (stage 28; Ferguson 1985). These embryos were weighed intact prior to removal of the yolk sac by dissection and the embryo was reweighed before fixation. The egg components were kept frozen for future analysis. Ninety eggs were measured for each temperature with a minimum sample of 4 on each day of incubation.

After fixation the embryos were staged using the tables of development presented by Ferguson (1985). The following morphometric parameters were measured (illustrated and described more fully in Deeming and Ferguson, in press b): embryo mass, total embryo length, tail length, length of the trunk (calculated), nape-to-rump length, distance between the limbs, length of the fore and hind limbs, length, height and width of the head, eye length and length and width, at the nostrils and at the midpoint, of the snout.

Data were analysed using Minitab (Ryan et al. 1985) and S.A.S. (Allen Ray 1982) statistical packages on an Amdahl 5890-300 mainframe computer. The development rate coefficient (Webb et al. 1987) was calculated for embryos at 33 °C (DRC₃₃) as follows. Staged embryos incubated at 33 °C were allocated a 'morphological age' (in days) derived from the time that each stage occurs in embryos at 30 °C. 'Morphological age' was plotted against real age (days) and this relationship was analysed using linear regression (the line was forced through zero). DRC₃₃ is equal to the slope of the regression line (Webb et al. 1987). Linear regression estimates for morphometric measurements against time and stage were calculated on untransformed, square root transformed and logₑ transformed data depending on the correlation coefficient of the regression estimate. The equations with the highest R² (i.e. the equation which explained the maximum proportion of the variation.

Table 1. Mean dimensions of all *Alligator* eggs used in the present study

	Temperature			
	30 °C		33 °C	
	Mean	SE	Mean	SE
Initial egg mass (g)	72.66	6.14	72.37	5.95
Egg length (mm)	71.43	2.81	71.41	3.18
Egg breadth (mm)	41.55	1.18	41.34	1.15
Egg volume (cm³)	64.68	5.17	63.99	5.34
Weight loss (g·day⁻¹)	0.087	0.034	0.089	0.036

ance in the data) were used (Deeming and Ferguson, in press b). Data from the two incubation temperatures were compared using GLM (S.A.S.) analysis of variance (using type III sums of squares). Principle components analysis (S.A.S.) was used to derive predictive equations for age and stage of development using a combination of 10 morphometric measurements condensed into one, PRIN (Deeming and Ferguson, in press b).

Results

Egg dimensions and contents

Mean values for initial egg mass, egg dimensions and volume are shown in Table 1. The eggs lost water throughout incubation despite the high humidity within the incubator, but the mean rates of water loss were similar at both temperatures (Table 1).

Initial egg mass could not be strictly controlled between samples and so, in order to reduce the effects of egg mass on the egg contents, data were standardised to a mean egg mass of 72.19 g (the mean value for initial mass of 600 alligator eggs transported to Manchester in 1988). Mean eggshell mass was not significantly different at the two temperatures (9.66 ± 0.91 g at 30 °C and 9.61 ± 1.05 g at 33 °C). The patterns of change in the extra-embryonic components of the eggs are shown in Fig. 1. Utilisation of albumen by the embryo during incubation (Fig. 1a) was continuous at 33 °C but at 30 °C around 6 g of albumen persisted during the middle third of the incubation period. Analysis of variance revealed that although temperature had no significant effect on albumen mass at each stage of development there was a significant difference in the patterns of utilisation at the two temperatures. At 30 °C the albumen content of the egg remained at a mean of 6 g from stage 17 to 23 compared with a mean of 10 g between stages 12 and 20 at 33 °C (Fig. 1a). Similarly changes in yolk sac mass with time were significant (Fig. 1b). Analysis of variance showed that yolk
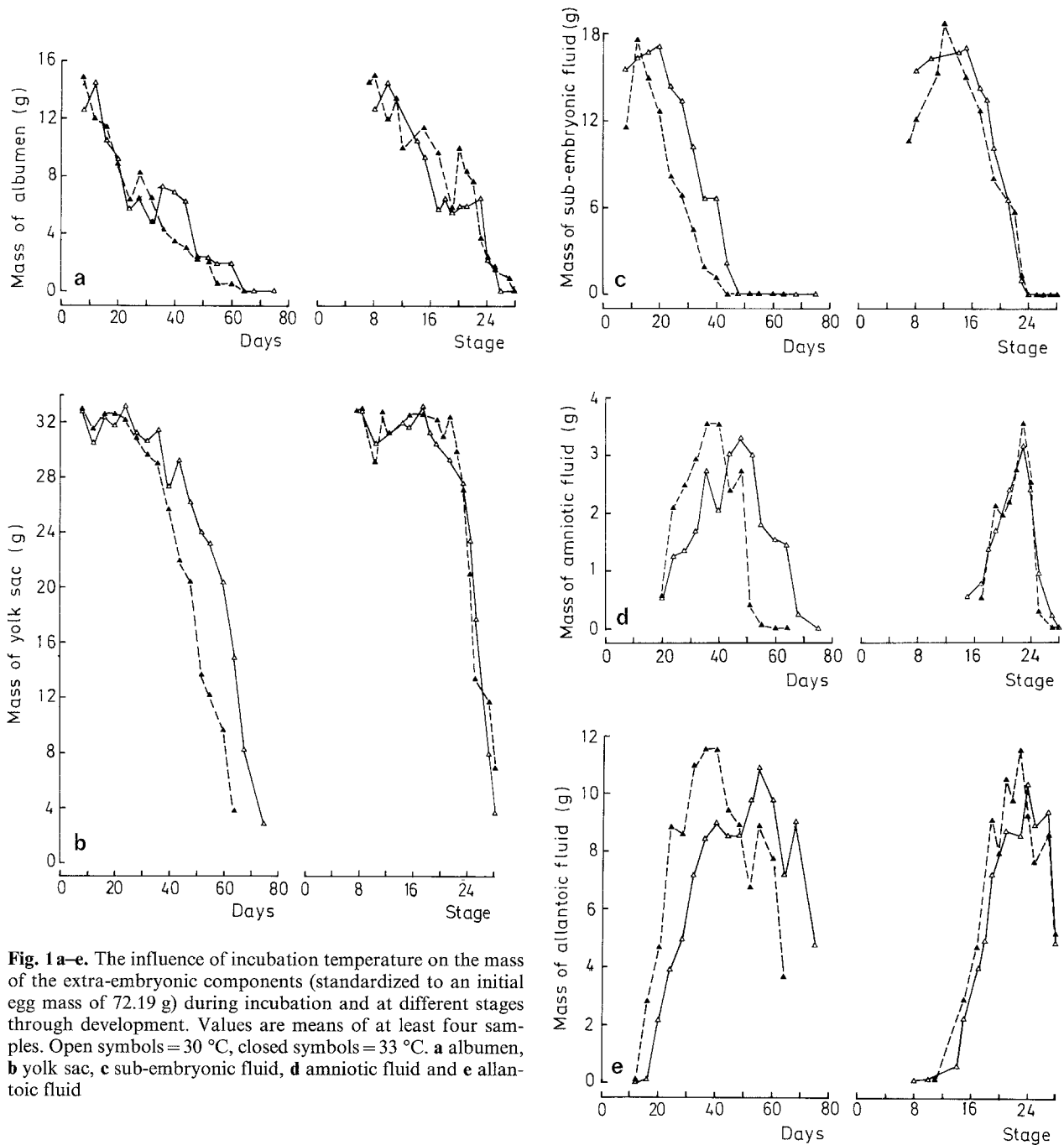
Fig. 1a–e. The influence of incubation temperature on the mass of the extra-embryonic components (standardized to an initial egg mass of 72.19 g) during incubation and at different stages through development. Values are means of at least four samples. Open symbols = 30 °C, closed symbols = 33 °C. **a** albumen, **b** yolk sac, **c** sub-embryonic fluid, **d** amniotic fluid and **e** allantoic fluid

sac mass, plotted against stage of development, was significantly affected by temperature ($P < 0.05$) and the patterns of yolk utilisation were significantly different at the two temperatures. At 30 °C the size of the yolk sac diminished after stage 17 but at 33 °C this did not occur until stage 21 (Fig. 1b).

Mass of sub-embryonic fluid in the eggs was significantly affected by temperature on both the different days of incubation and the different stages of development (Fig. 1c). At 33 °C there was a rapid loss in the fluid after a short peak in mass but at 30 °C the maximum amount of sub-embryonic fluid was present for a longer period of time. Changes in the amount of amniotic fluid with time were different at the two temperatures but they occurred at equivalent stages of development (Fig. 1d). Detectable amounts of amniotic fluid were present on day 20 of incubation (stage 17 at 33 °C and stage 15 at 30 °C). Formation of allan-

Table 2. Mean values ($\pm$SD) for stage of development (Ferguson 1985) through incubation for embryos of *Alligator mississippiensis* at 30 °C and 33 °C

Day	30 °C		33 °C	
8	8.0	0.0	7.6	0.5
12	10.0	0.0	11.2	0.8
16	14.0	0.0	15.0	0.0
20	15.0	0.0	17.0	0.0
24	17.0	0.0	19.2	0.4
28	18.0	0.0	21.8	0.4
32	19.0	0.0	22.0	0.0
36	21.0	0.0	23.0	0.0
40	21.0	0.0	23.0	0.0
44	23.0	0.0	24.0	0.0
48	23.0	0.0	24.0	0.0
52	24.0	0.0	25.0	0.0
55	24.0	0.0	26.2	1.1
60	25.0	0.0	27.9	0.3
64	25.0	0.0	28.0	0.0
68	26.6	0.9	—	—
75	28.0	0.0	—	—

toic fluid occurred earlier in incubation and at an earlier stage of development in eggs at 33 °C compared with eggs at 30 °C (Fig. 1e). Substantial amounts of allantoic fluid were present at the end of incubation but this oozed out and was lost during hatching.

Embryo growth

Development of *Alligator* embryos, as assessed by stage, was accelerated by incubation at 33 °C compared with 30 °C (Table 2). DRC_{33} was 1.209 ± 0.019. Stage 28 (equivalent to hatchling) was attained by day 60 at 33 °C and by day 68 at 30 °C. Hatching occured by 64 days at 33 °C but at 30 °C embryos were terminated at 75 days of incubation when they were close to hatching. Embryos grew exponentially (as measured by mass after fixation) up to hatching at both temperatures but the rate of growth was slower at 30 °C than at 33 °C (Fig. 2). For a long period of incubation (days 24 to 55) embryo mass at 30 °C was 50% of that at 33 °C and incubation at the lower temperature retarded growth by 8 days. Temperature did not significantly affect growth at equivalent stages although analysis of variance showed that the patterns of growth at the two temperatures were different (Fig. 2). Between stages 18 and 22 embryos at 33 °C were smaller than at 30 °C but showed rapid growth between stages 22 and 25. At 30 °C growth was more sustained with maximal growth occurring during stages 23 to 27.

This pattern was repeated for most of the morphometric parameters measured. Embryonic
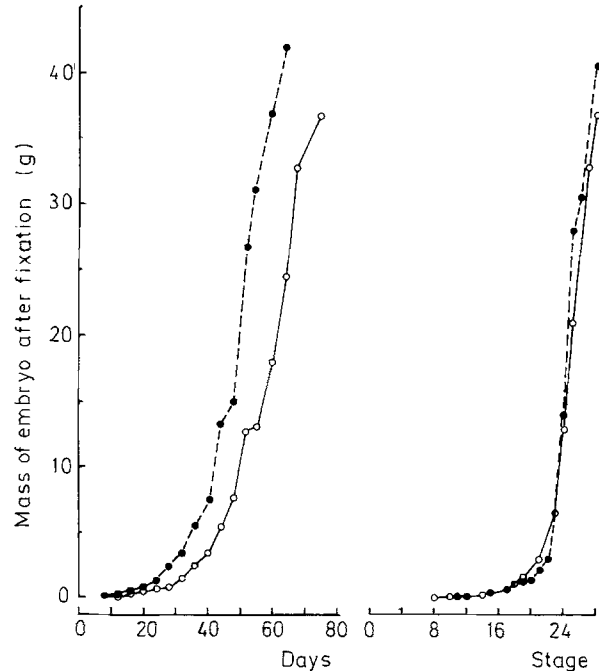

Fig. 2. Growth of the alligator embryo, measured as mass after fixation, through time at two incubation temperatures. Embryo mass at different incubation stages is shown. Open symbols = 30 °C, closed symbols = 33 °C
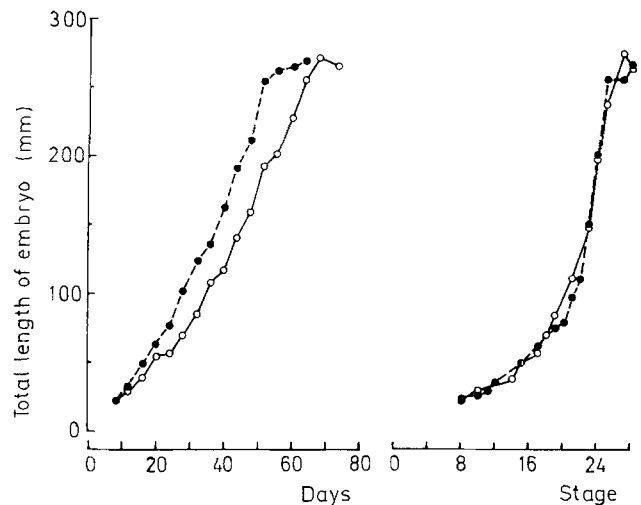

Fig. 3. The influence of temperature on growth of the alligator embryo, measured as total length, plotted against time and different stages of development. Open symbols = 30 °C, closed symbols = 33 °C

growth through time, as measured by linear dimensions, was enhanced by increased incubation temperature but the patterns of growth at equivalent morphological stages were different at the two temperatures. Total length of the embryo increased through time at a faster rate at 33 °C compared with embryos at 30 °C but growth was not continuous (Fig. 3). There was a distinct plateau in embryo
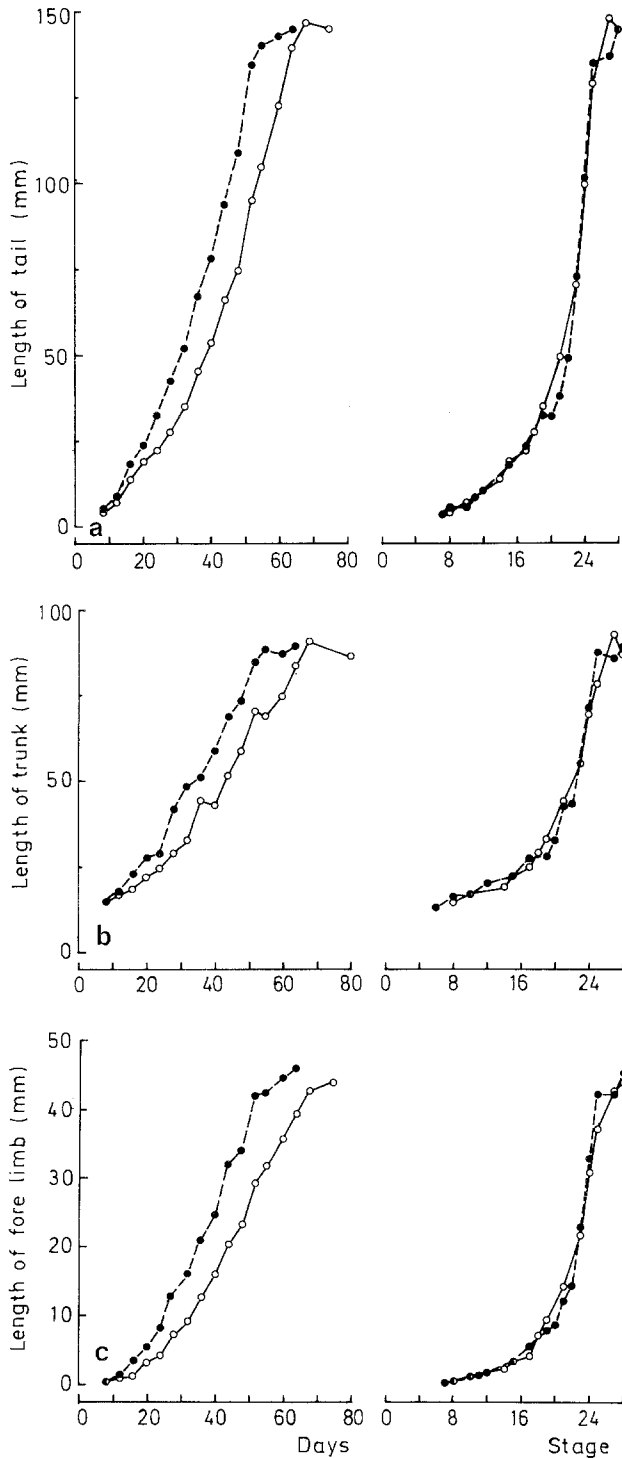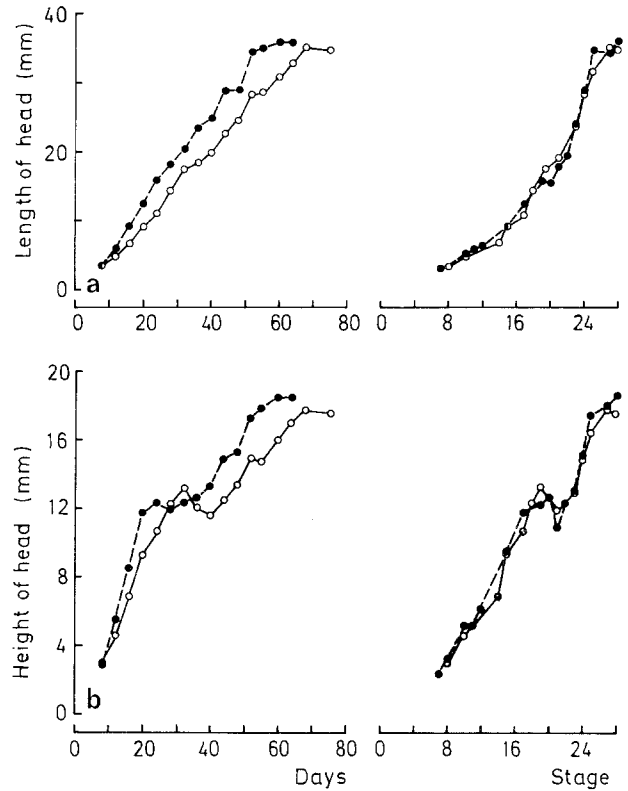
Fig. 5a, b. Growth of the head of the alligator embryo as affected by temperature and plotted against time and stage of development. Open symbols = 30 °C, closed symbols = 33 °C; a length of head and b height of the head

Fig. 4a–c. Growth of various parts of the body of the alligator embryo as affected by incubation temperature plotted against time and different stages of development. Open symbols = 30 °C, closed symbols = 33 °C; a length of the tail, b length of the trunk and c length of the forelimb

length which was achieved well before hatching. Embryo length at equivalent stages showed a similar pattern to mass; at 33 °C embryos at stages 19 to 22 were smaller than embryos at 30 °C but grew

faster during later stages of development (Fig. 3). These patterns of growth were also present in the tail (Fig. 4a), the nape to rump length, distance between the limbs, trunk length (Fig. 4b), and the limbs (length of forelimb is shown in Fig. 4c).

Growth of the head, as measured by length (Fig. 5a) showed similar patterns to total embryo length but changes in the height and width of the head were different. At both temperatures, increases in the height of the head showed a distinct plateau around 12 mm for 15 days (stages 17 to 22), before growth reached another plateau prior to hatching (Fig. 5b). The changes in head width (not illustrated) were similar with a plateau at 11.5 mm, but for only 8 days (stages 19 to 23). Eye length increased to a plateau prior to hatching but showed a different pattern from stage to stage of development (Fig. 6). Eye length showed a plateau at similar stages of development to head height and width but during this period the eye was larger in embryos at 30 °C compared with 33 °C. The snout was measurable from stage 17 of incubation (20 days at 33 °C and 24 days at 30 °C) and increased in length up to hatching (Table 3). The width of the snout showed a plateau during a simi-
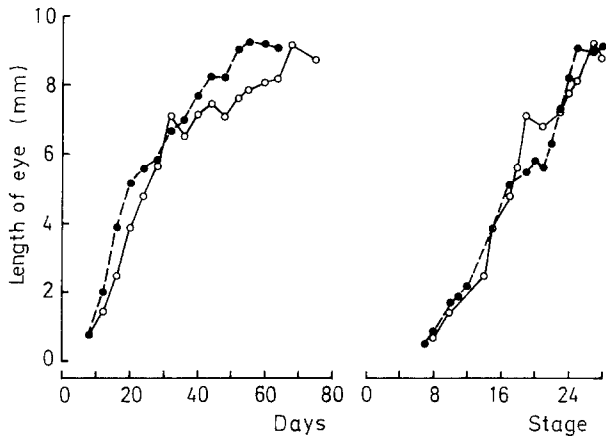
Fig. 6. The effects of temperature on growth of the eye of alligator embryos during incubation and at different stages of development. Open symbols = 30 °C, closed symbols = 33 °C
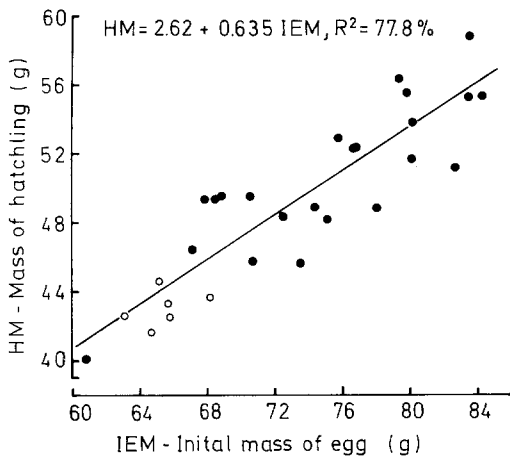

Fig. 7. The relationship between mass of alligator hatchlings and the initial mass of the egg. Open symbols = 30 °C, closed symbols = 33 °C
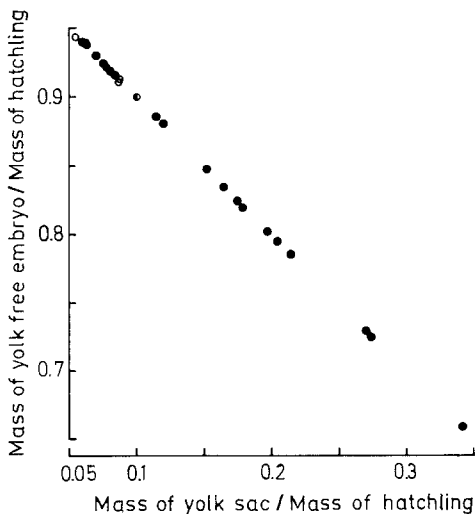

Fig. 8. The relationship between yolk-free embryo mass and residual yolk sac mass expressed as a ratio of hatchling mass. Open symbols = 30 °C, closed symbols = 33 °C

Table 3. Length and width at the mid-point, (mean in mm ± SD) of the snout through incubation for embryos of *Alligator mississippiensis* at 30 °C and 33 °C

Day	Length				Width at mid-snout			
	30 °C		33 °C		30 °C		33 °C	
20	–	–	2.34	0.15	–	–	2.82	0.34
24	2.70	0.41	4.64	0.38	3.24	0.06	4.16	0.26
28	4.30	0.12	5.96	0.06	3.76	0.06	4.08	0.15
32	4.98	0.21	7.10	0.29	4.16	0.32	4.68	0.58
36	6.10	0.17	8.96	0.27	4.00	0.16	6.34	0.31
40	7.30	0.31	9.58	0.15	4.84	0.23	7.06	0.51
44	7.96	0.49	11.16	0.50	5.90	0.35	8.86	0.71
48	9.42	0.39	11.32	1.03	6.78	0.44	9.36	1.07
52	10.86	0.30	13.72	0.60	8.56	0.36	11.70	0.73
55	11.02	0.30	14.42	0.42	8.64	0.27	12.60	0.24
60	11.98	0.39	15.13	0.53	9.90	0.59	13.08	0.63
64	13.24	0.64	15.63	0.99	11.50	0.69	13.34	0.85
68	14.04	0.51	–	–	12.44	0.65	–	–
75	14.30	0.40	–	–	12.50	0.49	–	–

lar period of incubation as head height and width (width at the mid-point of the snout is shown in Table 3) before recommencing growth. Growth of the snout followed the patterns exhibited by other dimensions of the embryo.

The effects of egg mass on embryo size

Data for all embryos at stages 23 to 28 of development were examined for any indication of egg size affecting embryo mass or embryo morphometrics. Embryos at earlier stages were considered to be too small to be affected by egg size. Regression estimates for fixed embryo mass against initial egg mass did not show any effect until the final stage of development (stage 28) but the correlation coefficient was only 22.3%. There was no significant relationship between egg length and embryo length; at stage 28 the correlation coefficient was 7.4%.

Hatchling mass (all embryos at stage 28) was related to initial egg mass (Fig. 7) and on average constituted 66.76 ± 3.55% of the initial mass of the egg. There was a clear relationship between embryo mass and yolk sac mass; larger embryos had smaller yolk sacs (Fig. 8). Fixation also reduced embryo mass (Fig. 9).

Use of morphometrics to predict embryonic development

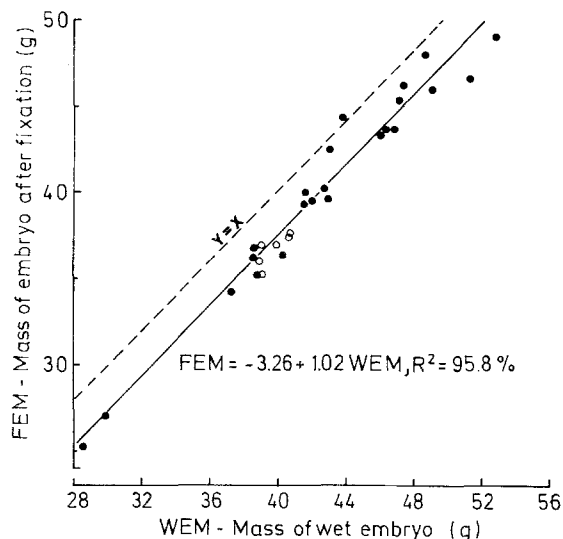Principle component analysis applied to morphometric data against days of incubation showed that

Fig. 9. The effects of fixation upon the mass of stage 28 embryos. Open symbols = 30 °C, closed symbols = 33 °C
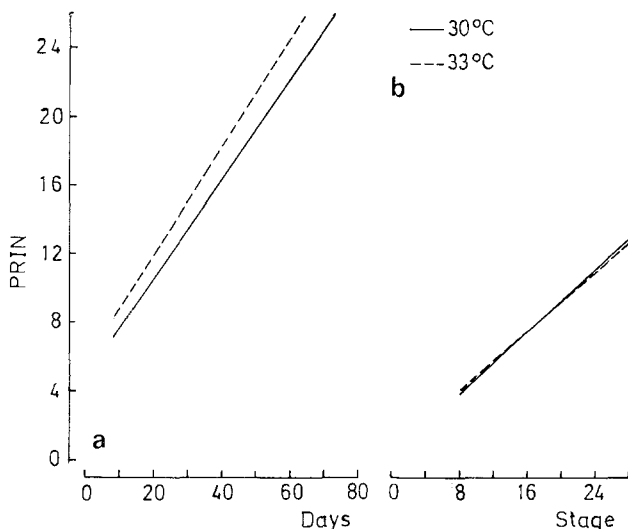

Fig. 10. The effect of incubation temperature on the relationships between PRIN and developmental time (plot a), and stage of development (plot b)

temperature had a significant effect upon the relationship between time and PRIN (Fig. 10a):

At 30 °C:
$PRIN = 0.310.Days + 5.87$; $R^2 = 96.6\%$,
At 33 °C:
$PRIN = 0.284.Days + 4.97$; $R^2 = 98.5\%$.

By contrast, values for PRIN versus stage of development generated for both temperatures were similar to each other (Fig. 10b):

At 30 °C:
$PRIN = 0.419.Stage + 0.793$; $R^2 = 97.1\%$,
At 33 °C:
$PRIN = 0.451.Stage + 0.216$; $R^2 = 98.4\%$.

Table 4. Development rate coefficients at 33 °C (DRC_{33}) and the difference in days in incubation period at 30 ° and 33 °C for four species of crocodilian ($DRC_{30} = 1.000$)

Species	DRC_{33}	Days	Reference
Alligator mississippiensis	1.209[a]	8	Joanen et al. 1987
Crocodylus palustris	1.256	11	Lang et al., in press
Crocodylus porosus	1.280	15	Webb et al. 1987
Crocodylus johnstoni	1.329	22	Webb et al. 1987

[a] Present study

Discussion

Effects of incubation temperature on growth and morphogenesis

Growth of *Alligator* embryos through time was significantly affected by incubation temperature, confirming previous reports that increasing incubation temperature accelerates the growth rate of reptilian embryos (Vinegar 1973; Deeming and Ferguson 1988). Morphological stages are often used to assess rate of development, and increasing incubation temperature accelerates development (Pieau and Dorizzi 1981; Webb et al. 1987; Lang et al., in press). Incubation temperature also had significant effects on morphogenesis of *Alligator* embryos which was accelerated at 33 °C, with each stage of development occurring earlier during incubation than at 30 °C. The development rate coefficient at 33 °C (DRC_{33}; Webb et al. 1987) recorded for embryos of *A. mississippiensis* was lower than has been recorded for other crocodilian species (Table 4). There is a clear correlation between DRC_{33} and the differences in the length of the incubation period at 30 °C and 33 °C. Temperature has less effect upon the rate of embryonic development in *A. mississippiensis* than in *Crocodylus* species (Table 4).

Incubation at 33 °C appeared to increase the rate of morphogenesis at the expense of increase in the size of embryos; at 30 °C growth and morphogenesis showed a closer association. At particular stages of development differences in the size of embryos were observed. During stages 19 to 22 embryos at 30 °C were heavier than those embryos at 33 °C but after stage 22 growth rates of embryos at 33 °C were increased rapidly up to stage 25. This pattern was reflected in both mass and many of the linear dimensions recorded. At stage 20 the eyes of embryos at 30 °C were larger than in embryos at 33 °C despite the similarity in overall morphology. Morphogenesis at the expense of growth has been observed in the embryonic gonad of *A. mississippiensis* (Deeming and Ferguson

1988). At 33 °C there is rapid differentiation of the medulla of the embryonic gonad and by 34 days of incubation there are distinct signs of testicular organisation; at 30 °C cellular organisation of the gonad to form an ovary is not apparent until after day 47 (Deeming and Ferguson 1988). The pattern of pigmentation in hatchlings of *A. mississippiensis* is also affected by incubation temperature (Deeming and Ferguson, in press a). The pigmentation pattern becomes apparent at stage 22 when melanin is deposited in the skin (Ferguson 1985). At 33 °C pigmentation is observed on day 36 of incubation compared with day 44 at 30 °C (Murray et al., in press).

Incubation temperature significantly affected extra-embryonic components and therefore, by implication, embryonic physiology. Previously only one report has detailed the changes in the extra-embryonic fluids of a reptile egg (*Crocodylus johnstoni*, Manolis et al. 1987) but the effects of temperature were not extensively studied. Examination of the changes in the extra-embryonic fluids through time and at different stages of development in *A. mississippiensis* has revealed interesting effects of temperature. The processes of fluid formation and utilisation are more rapid at 33 °C. The amount of yolk did not begin to decline until stage 22 of development in eggs at 33 °C, compared with stage 18 at 30 °C. This reflects the reduced mass of embryos at 33 °C between these stages. Patterns of albumen utilization through developmental time vary according to incubation temperature (Manolis et al. 1987), but in addition there were differences at different stages of development. The transfer of water from the albumen to the sub-embryonic fluid (Ewert 1979; Manolis et al. 1987) continued up to stage 17 at 30 °C but had stopped by stage 12 at 33 °C. The rapid decline in albumen mass occurred after stage 20 at 33 °C and after stage 23 at 30 °C. The amounts of sub-embryonic fluid increased and declined rapidly at 33 °C but maximum levels were prolonged at 30 °C. In *C. johnstoni* the maximum amount of sub-embryonic fluid occurred earlier at 33 °C compared with 30 °C but occurred at similar stages of development (Manolis et al. 1987). In both *A. mississippiensis* and *C. johnstoni*, at 30 °C and 33 °C, loss of sub-embryonic fluid occurred at stage 24 (Manolis et al. 1987). Formation of allantoic fluid was accelerated at 33 °C, occurring both at an earlier time and an earlier stage, mirroring the decline in sub-embryonic fluid. Later in development the amount of allantoic fluid in eggs at the two temperatures was similar.

In summary, incubation at 33 °C accelerates both differentiation and growth. The rates of utilisation of albumen and yolk and formation of the extra-embryonic fluids are also accelerated. These observations reflect different physiological processes of the embryo at different incubation temperatures: development of *Alligator* embryos at 33 °C is not simply scaled up from development at 30 °C, the patterns are different. These results highlight the importance of incubation temperature in the development of reptilian embryos. Although staging embryos may be useful for standardizing development (Miller 1985; Ferguson 1985, 1987) the effects of temperature must be appreciated in any experimental design.

A previous examination of embryonic growth patterns revealed that *A. mississippiensis* grew differently to *Crocodylus porosus* and *C. johnstoni* (Deeming and Ferguson, in press b). Compared with two species of *Crocodylus*, *Alligator* embryos developed very quickly sustaining a rapid rate of growth throughout incubation and emerging as long but light hatchlings (Deeming and Ferguson, in press b). In *Crocodylus* hatchlings were long but heavier, having spent more time in the egg (Deeming and Ferguson, in press b). The low DRC_{33} recorded in this study confirms that development is less temperature-dependent in *Alligator* than in other crocodilians. This may be important in the biology of *A. mississippiensis* which occurs at the northernmost limit for crocodilians (Groombridge 1987). It hibernates during the cool winter months and hatchlings have only a few weeks in order to feed before hibernation. Selection has acted upon alligator populations to produce embryos which develop rapidly (Deeming and Ferguson, in press b). Interestingly, the rate of embryonic development in different populations of turtles also shows much less dependence on incubation temperature depending on latitude (Ewert 1985; Legler 1985).

Hatching, egg size and hatchling mass

Embryo mass from stages 23 to 28 did not show any significant relationship to either egg mass or egg length, contrary to reports for other species of crocodilian (Webb et al. 1983a, 1983b, 1987). By contrast, the mass of hatchling alligators (embryo plus abdominal yolk sac) was related to initial egg mass. In birds, egg size begins to affect embryo size after day 16 of incubation and the effect is very clear at hatching (Burton and Tullett 1985). The results of the present study may reflect the small variation in sizes between samples and further investigation is required to document fully the

effects of initial egg mass on the patterns of embryonic growth in crocodilians.

The ratio of hatchling mass to initial egg mass in *Alligator* was similar to that reported for preococial birds, several lizards and turtles but lower than that reported for another species of crocodilian, other turtles and squamates (Ewert 1979). Yolk-free hatchling mass was inversely related to abdominal yolk mass and at 30 °C relatively more yolk had been converted into tissue compared with hatchlings from eggs at 33 °C. This confirms a previous report which showed that the energetic requirements of the crocodilian embryo are greater at lower incubation temperatures: more yolk is utilised during the prolonged incubation period and embryo mass is greater at hatching (Manolis et al. 1987). The amount of abdominal yolk is related to incubation length in other reptiles. In some turtles, as the nest substrate become progressively drier the incubation period is shorter and hatchlings contain more abdominal yolk (Morris et al. 1983).

During hatching of *Alligator* embryos allantoic fluid was lost from the egg, confirming reports of fluid loss from hatching eggs of the gharial (*Gavialis gangeticus*) and some squamate reptiles (Bustard 1966; Badham 1971; Subbo Rao 1987; Deeming, in press). At hatching, the amount of allantoic fluid present in crocodilian eggs (Manolis et al. 1987), and probably those of most other reptiles, is relatively large but its significance has been largely ignored (Deeming, in press). In crocodilian embryos urea is the main nitrogenous excretory product: it is stored in the allantoic fluid (Manolis et al. 1987). By contrast, avian embryos convert urea into uric acid (Romanoff 1967) which is left as solid deposits in the eggshell after hatching; the water in allantoic fluid is absorbed prior to hatching. Crocodilians appear then to leave a solution of their nitrogenous waste in the eggshell at hatching (Deeming, in press). The amount of allantoic fluid that would have oozed from the egg would probably have been greater if it had not lost water during incubation. The extent of water lost from eggs in the present study was probably abnormal (Ferguson 1985), but the effects of excessive loss of water (>15% of initial egg mass) on embryonic physiology have not been examined in crocodilians (Manolis et al. 1987).

The use of morphometrics to predict embryonic age and stage

Recent studies of embryonic development in crocodilians have often relied upon field preservation of intact eggs (Webb and Manolis 1987). Many studies have described staging criteria and growth of embryos fixed in formal saline (Magnusson and Taylor 1980; Webb et al. 1983a, 1983b; Ferguson and Joanen 1982, 1983; Ferguson 1985, 1987; Deeming and Ferguson 1988, in press b) but some studies describe fresh embryo mass (Manolis et al. 1987). Fixation causes a significant loss of water from tissue leading to shrinkage of the embryo. Future studies of embryonic development must take shrinkage associated with fixation into account, particularly in comparative studies.

Incubation temperature significantly affects the development rate of *Alligator* embryos. Predictive equations for embryonic age and stage, generated for embryos at 30 °C and 33 °C, are useful in laboratory studies but equations generated from pooled data from a different sample of eggs incubated at a variety of temperatures (Deeming and Ferguson, in press b) remain more useful for field studies. The incubation temperature of a nest is not accurately reflected by a single temperature recording on any particular day; reptile nests increase in temperature throughout incubation, but temperature may also reflect the climate (Packard and Packard 1988). Equations using a mean incubation temperature are more useful in allocating an age to embryos in nests. Equations to predict stage of development of embryos are less affected by incubation temperature and are of particular value in assigning a stage to a particular embryo, particularly if the investigator has a poor knowledge of embryology.

Embryo growth and sex determination

The two temperatures in this study were chosen because they produce hatchlings of only one sex; in *A. mississippiensis* eggs at 30 °C produce 100% females, eggs at 33 °C 100% males (Ferguson and Joanen 1982, 1983). This study has demonstrated periods during which embryos incubated at 30 °C and 33 °C show differences in size and physiology: interestingly these correspond to important times for sex determination (Deeming and Ferguson 1988). Differential growth of particular types of embryonic tissue has been suggested to be important in the mechanism of temperature dependent sex determination. In mammals, male embryos grow faster than females from the earliest stages (Cattanach et al. 1988) and differences in the rate of growth of the embryonic gonad have been suggested to be important in the sex determining mechanism (Mittwoch 1985). In addition, differential growth of the adrenal-kidney complex relative

to the gonad at different incubation temperatures has been suggested to be part of the mechanism of temperature-dependent sex determination in *C. johnstoni* (Webb and Smith 1984). Whether the differences in embryonic growth between 30° C and 33 °C are a cause or an effect of sex determination remains unclear (Deeming and Ferguson 1988, in press a).

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