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Effects of Incubation Temperature on Crocodiles and the Evolution of Reptilian Oviparity¹

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SYNOPSIS. *Crocodylus porosus* is a mound-nesting crocodilian in which incubation temperature influences the rate of embryonic development, the probability that embryos will survive to hatching, post-hatching growth rates and the probability of hatchlings surviving to 2 yr of age. Similar responses have been described in *Alligator mississippiensis* (Joanen *et al.*, 1987) and *C. niloticus* (Hutton, 1987), and they reflect a suite of "non-sexual" effects of incubation temperature. Temperature-dependent sex determination allocates sex on the basis of these "non-sexual" effects. In *C. porosus*, it results in maleness being assigned to embryos with high probabilities of surviving and good potential for post-hatching growth. Within the limits of survival, effects of the moisture environment on embryological development rate and hatchling fitness seem minor relative to those of the temperature environment.

Reptilian orders have either *obligate* oviparity (chelonians, crocodilians and rhynchocephalians) or *facultative* oviparity (squamates), depending on the extent of embryonic development within the oviducts. The distinction is equally one between embryos which are buffered from thermal effects within a female's body (*facultative* oviparity) and those that are not (*obligate* oviparity). *Facultative* oviparity and internal thermal buffering may be the primitive condition within the Class Reptilia, and the "shell-less" eggs of extant squamates may reflect the original amniote egg. *Obligate* oviparity, which also exists in birds, appears to have been a specialized development, and is a blind end in the evolution of viviparity among vertebrates. The significance of thermal buffering being lost in *obligate* oviparous reptiles remains unclear.

INTRODUCTION

Incubation temperature influences the sex of crocodilians (Ferguson and Joanen, 1982, 1983; Webb and Smith, 1984; Ferguson, 1985; Hutton, 1987; Lang, 1987a; Smith, 1987; Webb *et al.*, 1987a; Yamakoshi *et al.*, 1987; Lang *et al.*, 1989) in much the same way as it does in a number of chelonians and lacertilians with temperature-dependent sex determination (TSD) (for examples see: Yntema, 1976, 1979; Bull and Vogt, 1979; Bull, 1980, 1983; Mrosovsky, 1980; Mrosovsky and Yntema, 1980; Miller and Limpus, 1981; Bull *et al.*, 1982a, b; Vogt and Bull, 1982; Vogt *et al.*, 1982; Yntema and Mrosovsky, 1982; Limpus *et al.*, 1983, 1985; McCoy *et al.*, 1983;

Wilhoft *et al.*, 1983; Hou Ling, 1985; Raynaud and Pieau, 1985; Standora and Spotila, 1985; Tokunaga, 1985, 1986; Webb *et al.*, 1986; Bull, 1987; Head *et al.*, 1987). Yet sex is only one of the embryological parameters influenced by incubation temperature, and from a fitness point of view, it may be of secondary importance.

Among crocodilians, incubation temperature affects the probability of embryos surviving (Webb and Smith, 1984; Lang *et al.*, 1989), the frequency of abnormalities among embryos and hatchlings (Webb *et al.*, 1983a, b; Ferguson, 1985), body size at hatching (Hutton, 1987; Webb *et al.*, 1987a), the weight of residual yolk at hatching (Webb *et al.*, 1987a), hatchling pigmentation patterns (Deeming and Ferguson, 1989; Lang, unpublished data), post-hatching growth rates (Hutton, 1987; Joanen *et al.*, 1987) and post-hatching patterns of thermoregulation (Lang, 1987a). These effects are independent of sex

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(Smith, 1987; Webb *et al.*, 1987a) and can be considered "non-sexual" responses to incubation temperature. Like sex they appear to be linked more proximally to embryonic metabolism and development rate than they are to temperature per se (Webb and Smith, 1984; Webb *et al.*, 1987a), and thus they can be influenced by the moisture and gaseous environments of incubation. However, these influences appear minor relative to that exerted by incubation temperature.

If there were selective advantages in allocating sex on the basis of the "non-sexual" responses to incubation temperature, TSD would provide advantages that are unavailable with genotypic sex determination (GSD) (Ferguson and Joanen, 1982, 1983; Bull, 1987; Head *et al.*, 1987; Joanen *et al.*, 1987; Smith, 1987; Webb *et al.*, 1987a), and which are consistent with the predictions of Charnov and Bull (1977). In *Alligator mississippiensis* such a situation exists; "maleness" is allocated to embryos with the greatest potential for post-hatching growth (Joanen *et al.*, 1987). A similar situation appears to exist in *Crocodylus niloticus* (Hutton, 1987), and the results we present, indicate that the relationship also exists in saltwater crocodiles (*Crocodylus porosus*).

The significance of the "non-sexual" responses of embryos to temperature may extend well beyond a plausible explanation for TSD. Crocodilian embryos are particularly sensitive to incubation temperature during the first half of development (Webb *et al.*, 1987a; Whitehead, 1987a), which is the period that most squamate embryos are retained within the maternal oviducts (Shine, 1985). Squamates may thus be buffering their embryos against temperature extremes during the most sensitive stages of embryological development.

If so, the distinction between reptiles which retain eggs to the extent of viviparity (squamates), and those that do not (chelonians, crocodilians and rhynchocephalians), may be partly related to the non-sexual effects of incubation temperature. The two groups can be referred to as having *facultative* oviparity and *obligate* oviparity respectively. Birds, which have retained a

fundamentally reptilian egg, have *obligate* oviparity.

Within the ecologically diverse reptiles and birds with *obligate* oviparity, no known species allows its embryos to develop within the oviducts to the extent found in squamates (Shine, 1985; Blackburn and Evans, 1986). As a consequence, *obligate* oviparity of the form seen in reptiles and birds today is unlikely to have given rise to *facultative* oviparity. A more plausible explanation is that *facultative* oviparity is the primitive condition, and *obligate* oviparity has been derived from a form of *facultative* oviparity.

This paper presents field and laboratory data on the incubation temperatures to which *Crocodylus porosus* embryos are exposed, and the effects they have on sex, survival and growth, both before and after hatching. The relative contributions of the temperature and moisture environment are discussed. When viewed in the context of the "non-sexual" effects of incubation temperature, TSD is demonstrated to have advantages that would be unavailable with GSD. Ways in which the amniote egg and the different modes of reptilian oviparity may have evolved are discussed. Incubation temperature has no doubt been a constraining influence on the evolution of both, but its exact role remains unclear.

SALTWATER CROCODILES—A CASE HISTORY

Crocodylus porosus nests, eggs and embryos were examined in the Northern Territory of Australia. Eggs were collected from the field at various ages and incubated under artificial conditions until hatching. Nest temperatures in the field were measured with calibrated thermometers 2–3 eggs deep, and often, a second nest temperature was taken in the mound away from the eggs. An egg from each clutch was sacrificed, and the embryo was given an age equivalent (in days) based on incubation at 30°C (MA₃₀; morphological age at 30°C [Ferguson 1985; Webb *et al.*, 1987a]). Time of nesting was approximated by correcting the MA₃₀ for nest temperature with temperature-dependent development rate coefficients (Webb *et al.*, 1987a).

Some young clutches (<8 days MA₃₀) were incubated at precise temperatures ($\pm 0.2^\circ\text{C}$) within water-jacketed incubators. Most eggs were exposed on racks without nesting media (Method B of Webb *et al.* [1987a]), but some were incubated within plastic bags (Method A of Webb *et al.* [1987a]). Large scale incubation of wild collected eggs was carried out at mean temperatures fluctuating between 31°C and 32°C , in a constant environment raising pen (Webb *et al.*, 1983c).

Eggs that obviously contained dead embryos, or which failed to hatch, were opened. The age of dead embryos allowed time of death to be assigned to either before or after collection. All hatchlings were measured, weighed and sexed by examination of the cliteropenis (Webb *et al.*, 1984a). The extent of residual yolk was gauged crudely by the extent of abdominal distension. In different clutches, high mortality before collection was associated with high mortality in the incubators, whereas low mortality before collection gave negligible mortality in the incubators (unpublished data). In the text "embryo survival" refers to the proportion of fertile eggs that survived to hatching, regardless of the age at which they were collected.

The effects of incubation temperature on post-hatching growth and survival were quantified from numbered hatchlings (scute clipped) raised at a crocodile farm. They were housed in identical outdoor concrete bays, in which cover and a radiant heat source were provided. Each hatchling was measured, weighed and sexed three times over 2 yr. These data allowed a size-age curve to be constructed for each individual, from which the size at exactly 1 and 2 yr of age could be predicted. This procedure overcame problems associated with the different real ages of individuals at each inventory.

The clutch of origin of the hatchlings was only partially controlled. The two largest clutches ($n = 35$ and 24 hatchlings) were each subdivided between three temperature treatments (29 , 30 , 33°C ; and 31 , 32 , 33°C). The remaining hatchlings came from 12 clutches (1–20 hatchlings per

clutch), each from a single temperature. The final distribution was: 29°C (16H, 6 clutches); 30°C (17H, 2C); 31°C (51H, 5C); 32°C (15H, 3C); 33°C (25H, 2C).

Unless otherwise stated, errors are ± 1 standard deviation.

NESTS AND EGGS

Crocodylus porosus nest during the annual wet season, when ambient temperatures are high and reasonably stable (Fig. 1). Females select a vegetated nest site close to permanent water, and within it construct a mound of vegetation and soil some 1.8 m long by 1.6 m wide by 0.5 m high (Webb *et al.*, 1977). They excavate a centrally situated egg chamber and lay their complete clutch within a 1 hr laying period (unpublished observations). The eggs are covered with 8–28 cm of nest material (Webb *et al.*, 1977), and they remain at the orientation at which they were laid throughout incubation (66 to at least 114 days [Kar, 1979; Magnusson, 1979a]). Females remain at or near the nest, may effect minor repairs to it (Webb *et al.*, 1977), and may attack potential predators (Webb *et al.*, 1983b). Their movements over the nest usually consolidate its structure.

Mean clutch size within 416 nests was 49.98 ± 11.43 (range 2–78 eggs), and mean egg dimensions (mean of clutch means) were: length 7.84 ± 0.41 cm ($n = 385$ clutches; range 6.57–8.95 cm); width 4.90 ± 0.24 cm ($n = 383$; range 4.18–5.50 cm); weight 109.19 ± 14.97 g ($n = 357$; range 65.4–147.0 g).

NEST TEMPERATURES

Temperatures within wild nests are affected by the siting of nests relative to the sun, exposure to wind, the incidence of rain and flooding, the heat produced by decomposition of vegetation and the heat produced as a byproduct of embryonic metabolism (Webb *et al.*, 1977, 1983b; Magnusson, 1979b; additional unpublished data). Once nests are consolidated by female activity over them, diel variation in nest temperature is reduced ($1.1 \pm 0.7^\circ\text{C}$ in one sample of nests [Magnusson, 1979b]), although temperature gradients of 1 – 3°C

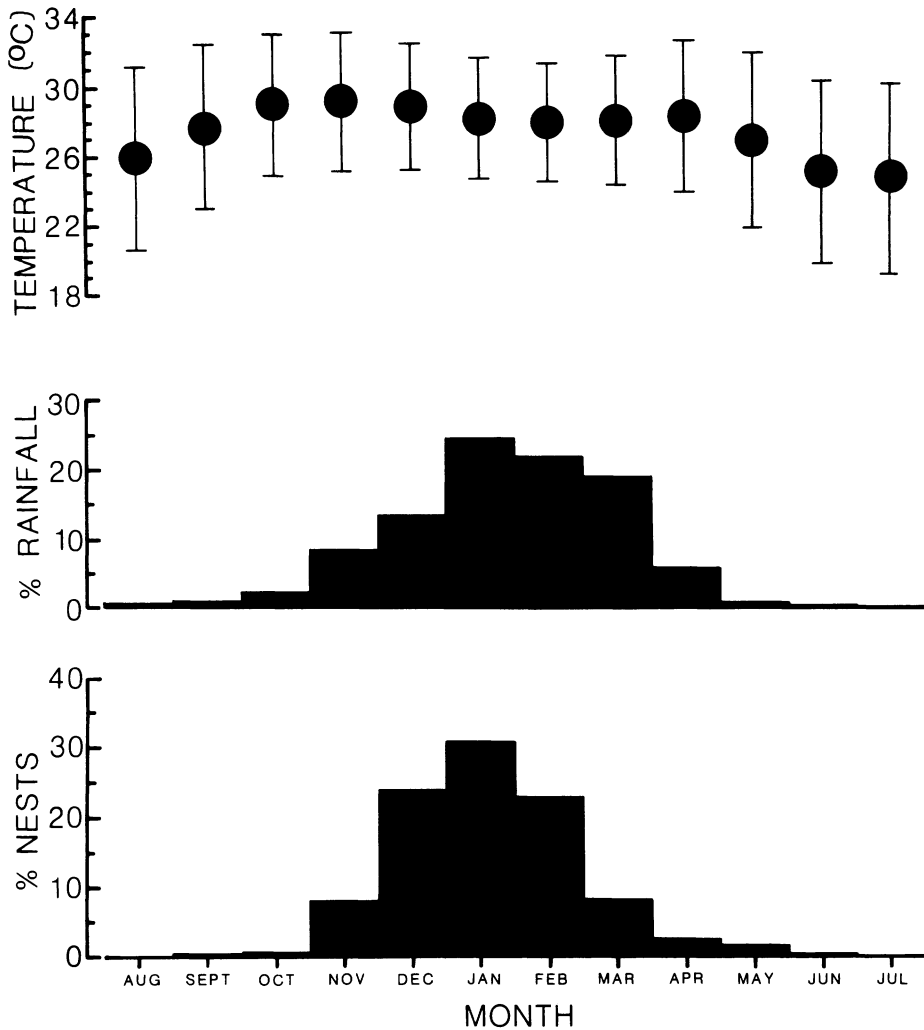


FIG. 1. Time of nesting for 492 wild *Crocodylus porosus* nests examined between 1973 and 1987 (lower), compared with average monthly rainfall (middle; total = 1,661 mm), and mean monthly maximum, mean and minimum temperatures (upper) in Darwin, Australia (1941 to 1986; Bureau of Meteorology).

exist between the top and bottom eggs (Webb *et al.*, 1977). Nest temperatures gradually follow changes in mean ambient temperature (Webb *et al.*, 1977; Magnusson, 1979b).

Mean egg temperature within 246 wild *C. porosus* nests, with embryos of various ages, was $31.65 \pm 1.94^{\circ}\text{C}$ (range 25.3 – 36.6°C). In nests with embryos between 14 and 52 days MA₃₀, the range of ages during which sex is determined (Webb *et al.*, 1987a), it was slightly less ($31.52 \pm 1.92^{\circ}\text{C}$

[range 25.3 – 36.6°C ; $n = 145$]). Nest temperatures between December and April were generally high (Fig. 2), with a marked decline at the start of the cool-dry period of the year (May to July). Independent of seasonal trends, mean nest temperature increased with embryo age (Fig. 3), which appears to reflect metabolic heat production from within the clutch (Fig. 4). That temperatures among the eggs exceeded those of the mound is inconsistent with heat of decomposition explaining the temper-

ature rise. The variation in nest temperatures between December and April (Fig. 2) reflects an interaction between rainfall (a cooling influence) and embryo age (a warming influence).

SEX, SURVIVAL AND DEFORMITIES

The sex ratio of hatchlings from wild nests containing embryos older than 52 days MA₃₀, reflects the relationship between "spot" nest temperatures measured in the field and sex (Fig. 5; lower). Clearly, high and low nest temperatures are associated with a preponderance of females, whereas a peak of males comes from nests between 31.1 and 32.0°C. Results from constant temperature incubation in the laboratory (Fig. 6), closely parallel those from the wild.

The primary cause of embryo mortality in wild *Crocodylus porosus* nests is flooding, and it could well account for 50% of all eggs laid in northern Australia (Webb *et al.*, 1977, 1983b, 1984b). In contrast, only 0.9% of wild eggs examined ($n = 12,241$) had signs of desiccation (air spaces). Predation of eggs containing living embryos occurs even more rarely (Webb *et al.*, 1983b, 1984b), although varanid lizards are attracted to nests with rotting eggs (Magnusson, 1982). Unfavorable incubation temperatures are perhaps the second most important cause of embryo mortality (Webb *et al.*, 1983b). In a sample of 173 clutches (Fig. 5; middle), very high nest temperatures ($>35.1^{\circ}\text{C}$) occurred rarely, but induced a variety of deformities (Fig. 5; upper). Nest temperatures between 33.1 and 35.0°C, and between 28.1 and 29.0°C both resulted in higher mortality than those between 29.1 and 33.0°C.

In the laboratory, incubation at 34°C was only attempted once. Nine of 11 eggs produced deformed embryos, which died during incubation, and two eggs contained living, apparently normal embryos when the eggs were opened. Eleven eggs from the same clutch were incubated at 28°C, and 8 of them produced premature but otherwise normal hatchlings; 1 died before hatching and 2 at the stage of hatching. Although based on small sample sizes, the

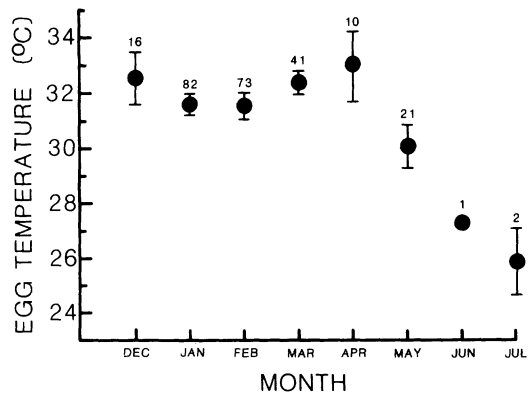


FIG. 2. Egg temperatures within 246 wild *Crocodylus porosus* nests, subdivided according to the month in which they were measured. Horizontal lines delimit two standard errors on either side of the means (circles); non-overlap signifies significant differences at approximately the 5% level. Numbers are the sample sizes.

laboratory observations are consistent with the field results (Fig. 5).

TOTAL INCUBATION PERIOD

In addition to the date of nesting relative to peak rainfall, the probability of mortality due to flooding is a function of the time that eggs remain in the nest before hatching. Laboratory results (Fig. 7) indicate that total incubation time is highly correlated with incubation temperature, regardless of whether eggs are incubated in bags (Method A) or on racks (Method B). A similar relationship exists in the field (Webb *et al.*, 1983b, d), but it has not been quantified precisely. Switch experiments, in which eggs are incubated at one temperature and then switched to another, indicate that the major effect of temperature on total incubation time occurs during the first half of embryonic development (Fig. 8).

RESIDUAL YOLK

Excluding maintenance costs, the size of an embryo at hatching will depend largely on how much of the yolk has been converted to tissue and how much has been retained as residual yolk (Webb *et al.*, 1987a). In 124 hatchlings from eggs incubated at different constant temperatures

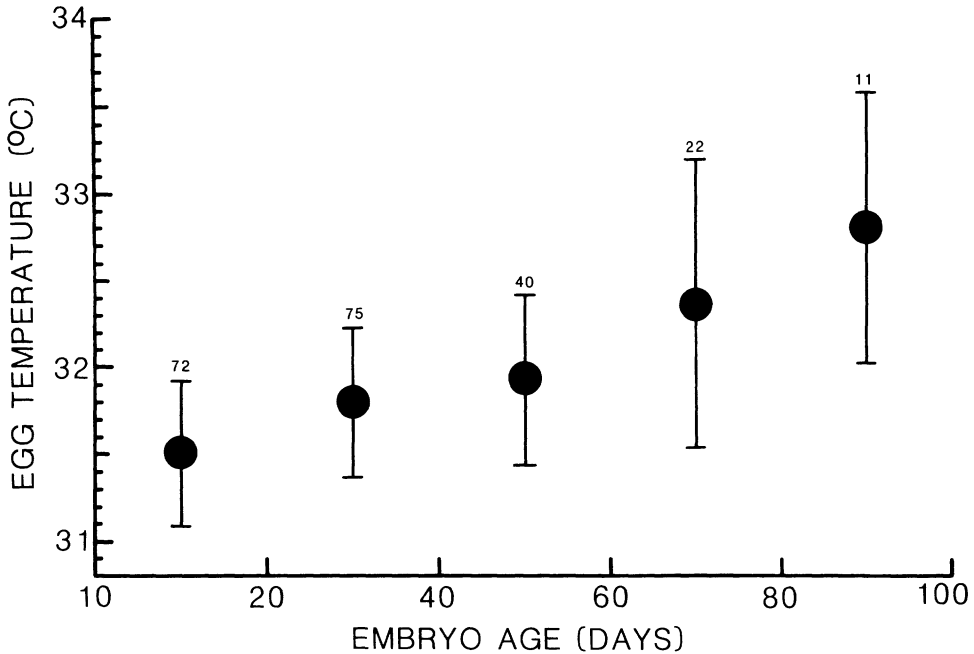


FIG. 3. Egg temperatures within 220 wild *Crocodylus porosus* nests (December to April; the warmer months), subdivided on the basis of embryo age (30°C age equivalent; MA_{30}). Horizontal lines delimit two standard errors on either side of the means (circles). Numbers are the sample sizes.

(Table 1), two (1 each at 29°C and 33°C) were given a residual yolk code of 1, because they hatched prematurely, before the residual yolk was fully internalised. Of the remaining 122, at 30°C, 6% of animals had abundant yolk, whereas at 32°C, 73% did (Table 1). However, no consistent trend was present at the other temperatures.

HATCHLING SIZE

Multiple regression analysis with the sample of 122 apparently normal hatchlings indicated that egg weight (transformed in various ways) explained a maximum of 38.6% of the variation in head length, 40.3% of the variation in snout-vent length and 81.3% of the variation in body weight (hatchling plus residual yolk). Clearly, hatchling size is determined by egg size. Incubation temperature explained none of the additional variation in head or snout-vent lengths, but did explain an additional 2.3% ($P < 0.0001$) of the variation in body weight. The temperature coefficient (1.22 ± 0.30 [SE]) indicated a little over 1 g increase in body weight

(hatchling + residual yolk) per 1°C increase in incubation temperature.

POST-HATCHING SURVIVAL AND GROWTH

Crocodylus porosus hatchlings raised on crocodile farms usually start feeding and growing within two weeks of hatching, although some individuals are reluctant to feed, become emaciated, and eventually die of starvation. Among the 122 animals incubated at constant temperature (Table 1), survival to 2 yr of age was highly correlated with incubation temperature (Fig. 9), and was thus correlated with sex.

Of the 81 survivors, mean snout-vent length (SVL) after two years of age was highly variable, but trends with both incubation temperature and sex were apparent (Fig. 10; upper). Among females alone, mean size after two years increased with increasing incubation temperature between 29°C and 33°C. Although sample sizes of males were small, their mean size was elevated above the general relationship for females. Multiple regression analysis, predicting SVL from both sex (coded as 1 and

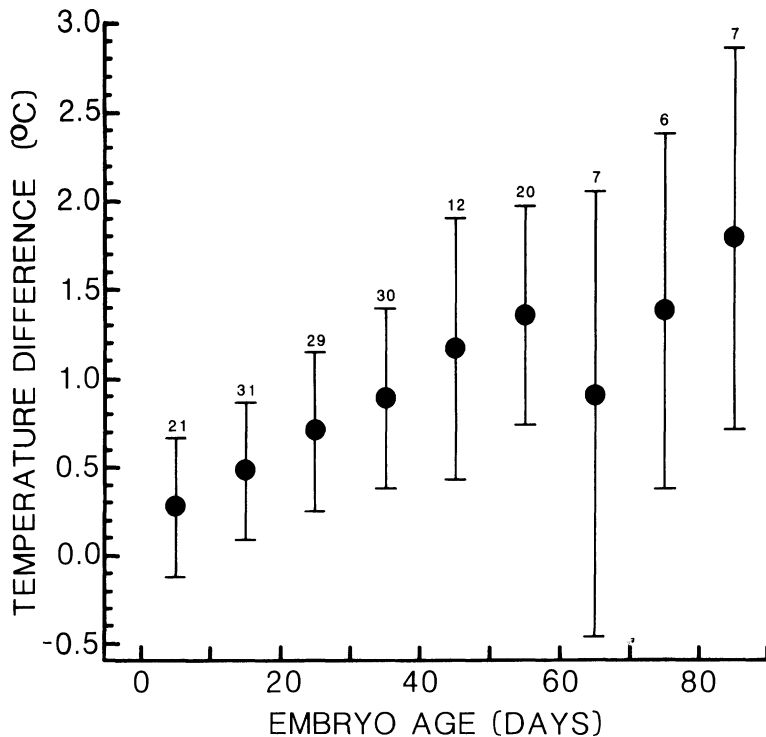


FIG. 4. The difference between nest temperatures measured among the eggs and away from the eggs, as a function of embryo age (30°C age equivalent; MA_{30}), in 163 wild *Crocodylus porosus* nests. Metabolic heat raises egg temperature during incubation. Horizontal lines delimit two standard errors on either side of the means (circles). Numbers are the sample sizes.

0) and temperature, explained 14.4% ($P = 0.003$) of the total variation in SVL: temperature independently explained 8.2% ($P = 0.009$) and sex 6.2%, although it was outside the 5% rejection level ($P = 0.071$).

"NON-SEXUAL" TEMPERATURE EFFECTS

The effects of incubation temperature on crocodilians have usually been studied in conjunction with research into TSD, and it is only recently that they have been considered independent entities (Webb and Smith, 1984; Joanen *et al.*, 1987; Lang, 1987a; Smith, 1987; Webb *et al.*, 1987a). The effects remain poorly understood, and the mechanism through which they operate is essentially unknown.

Incubation temperature affects the size of hatchlings. In *Crocodylus johnstoni*, low temperature incubation gives relatively heavy hatchlings with little residual yolk, whereas high temperature incubation gives relatively light hatchlings with a lot of

residual yolk (Webb *et al.*, 1987a). In *C. niloticus* (Hutton, 1984, 1987) short animals with abundant residual yolk come from the highest incubation temperatures (34°C). Trends in this direction were detected with *C. porosus* at 30°C and 32°C, but they were not consistent over the full temperature range. The extent to which size at hatching and/or the amount of residual yolk with which embryos hatch affects fitness is unclear.

In contrast to size at hatching, post-hatching survival of *C. porosus* in captivity was related to incubation temperature: maximum survival occurred at 32°C (87%) and minimum survival at 29.0°C (44%) (Fig. 9). In *A. mississippiensis*, survival under captive raising conditions is generally high and stable over the incubation temperature range 29.4–32.8°C (Joanen *et al.*, 1987). Mean size of *C. porosus* after 2 yr increased with increasing incubation temperature, and there was an additional tendency for

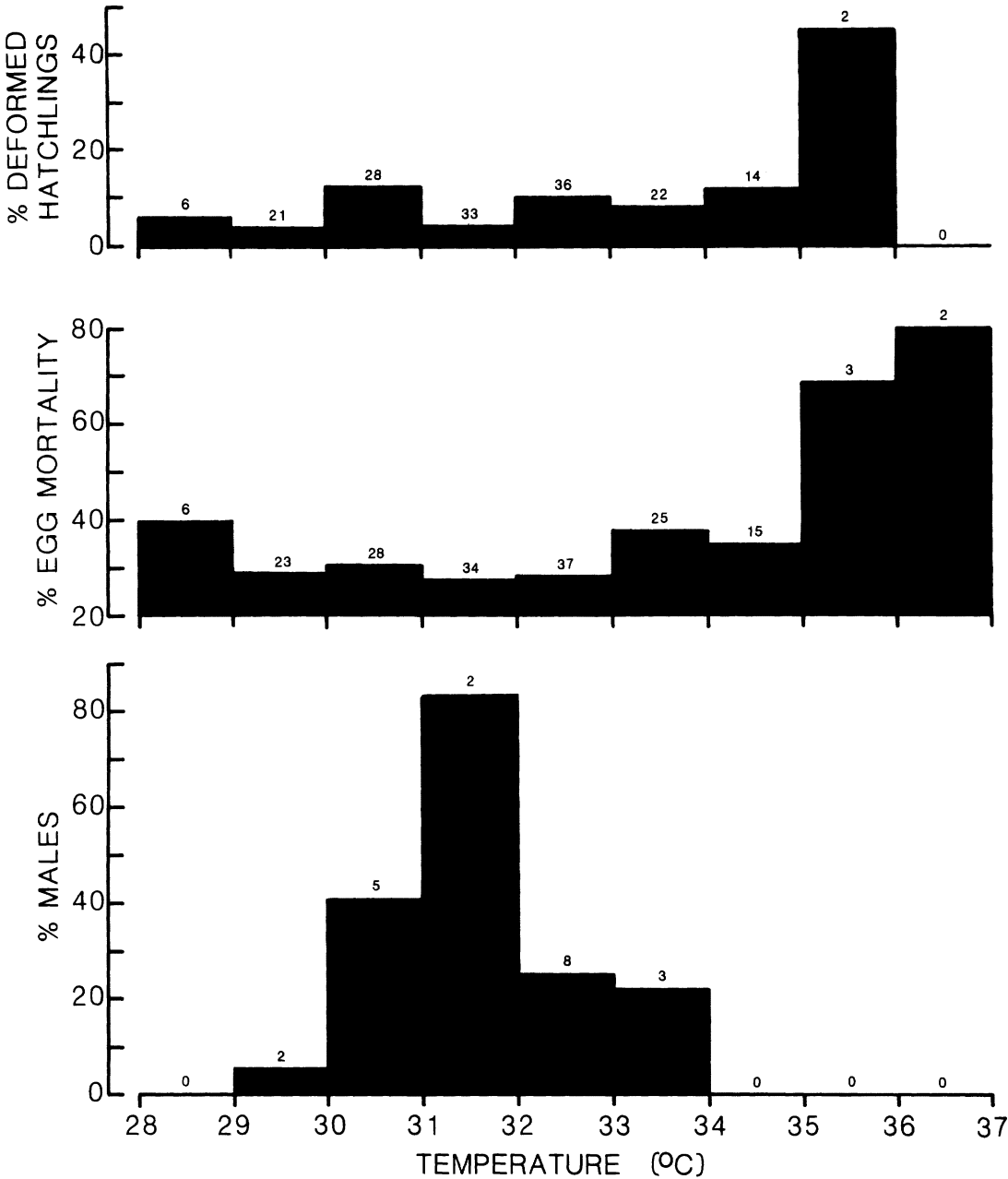


FIG. 5. The relationship between *Crocodylus porosus* nest temperatures (measured by spot nest temperature 2–3 eggs deep in each nest) and the sex of hatchlings in nests collected after sex was determined (lower), the mortality of fertile eggs in nests collected at various ages (middle) and the incidence of obvious deformities among hatchlings from nests collected at various ages (upper). For quantifying sex, only clutches collected from the field when older than 52 days MA₅₀ were used. The high baseline level of mortality is attributable mainly to flooding. Numbers refer to the number of clutches used to calculate a mean value for any 1°C temperature range.

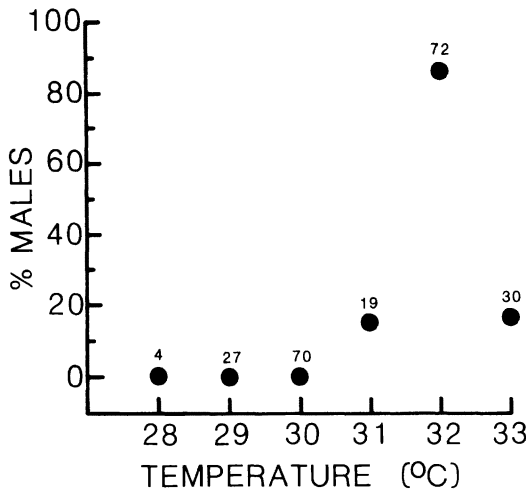


FIG. 6. The relationship between constant incubation temperature and sex for *Crocodylus porosus* embryos incubated in the laboratory for the whole of their development. Numbers are sample sizes. Data are the pooled results of Methods A and B incubation (from Webb *et al.* [1987a]).

males to exceed the sizes of females (Fig. 10). Among females, the maximum size was attained by animals from 33°C (47 cm SVL) and the minimum size by animals from 29°C (37 cm SVL). Within the incubation temperature range at which *C. porosus* embryo survival was generally high (29.1–33.0°C), better post-hatching performance came from a more restricted range (32.0–33.0°C).

In *Crocodylus niloticus* hatchlings incubated at 34°C outgrew those from 28°C and 31°C within three months, even though they were shorter at hatching (Hutton, 1987). Post-hatching growth rates of *A. mississippiensis* are affected by incubation temperature (Joanen *et al.*, 1987), but maximum growth of females and males occurs in animals incubated at temperatures within the middle of the range that produces both sexes (30.6°C and 31.7°C respectively) (Fig. 11).

There are thus data for *Crocodylus porosus*, *C. niloticus* and *Alligator mississippiensis* which indicate incubation temperature is exerting significant effects on post-hatching growth and/or survival. Given that clutch effects were not completely con-

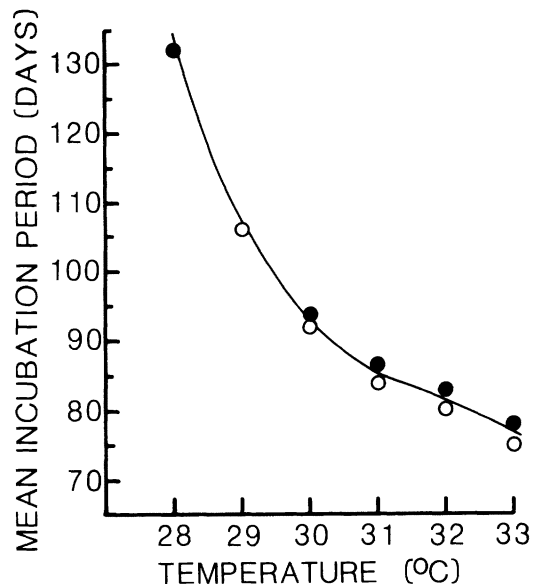


FIG. 7. The relationship between constant incubation temperature in the laboratory and total incubation period (to pipping of eggs) for *Crocodylus porosus* eggs incubated on racks (open circles) and in plastic bags (closed circles). Data are means from Webb *et al.* (1987a). The line is a polynomial, least squares regression.

trolled and that raising strategies differed greatly, at this stage we attach little significance to the different forms of the relationship (Fig. 10 *vs.* Fig. 11). However, it is clearly an area which merits experimentation under tightly controlled conditions.

"SEXUAL" TEMPERATURE EFFECTS AND TSD

The mechanism through which incubation temperature influences sex within any crocodilian species remains unknown (Raynaud and Pieau, 1985; Standora and Spotila, 1985; Gutzke, 1987; Head *et al.*, 1987). However, the rate at which embryos develop is as highly correlated with sex as is incubation temperature itself (Webb *et al.*, 1987a), and thus by independently influencing development rate, there are avenues through which the moisture (Gutzke and Paukstis, 1983) and gaseous environments of incubation could influence sex.

The non-sexual effects of temperature,

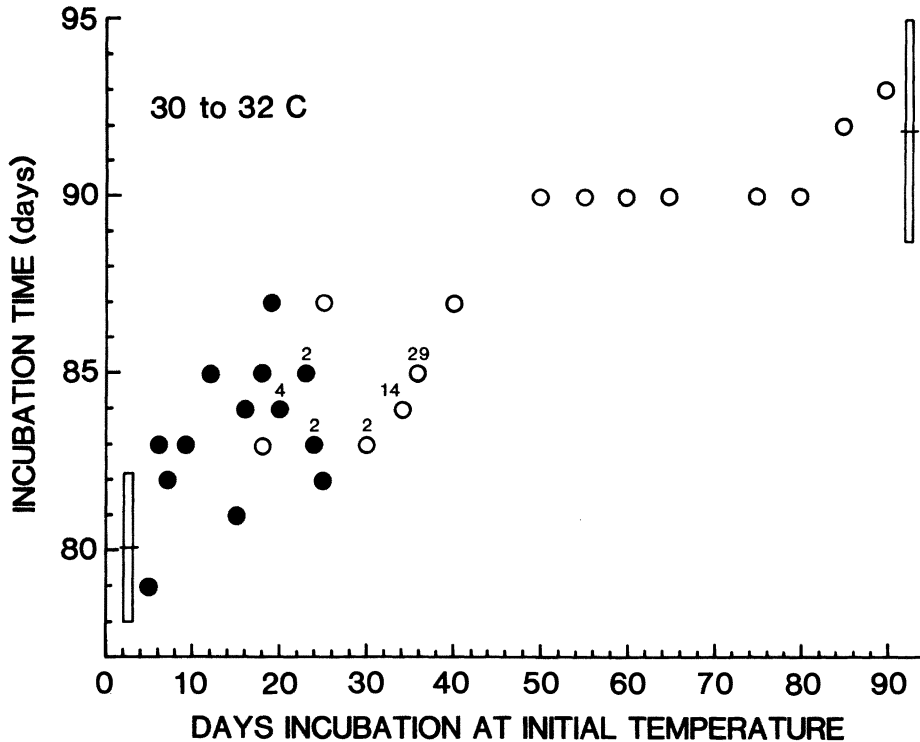


FIG. 8. The relationship between embryo age, and the effect of temperature on total incubation time in *Crocodylus porosus*. Eggs were incubated at 30°C for varying periods and switched to 32°C for the remainder of incubation. Closed and open circles are males and females respectively. Vertical bars are the mean incubation times (\pm SD) at constant temperatures of 30°C and 32°C (reprinted from Webb *et al.* [1987a]).

like sex, are predictable from embryonic development rate *before* sex is allocated. In *Crocodylus porosus* the sex determining mechanism allocates “maleness” to embryos developing at incubation temperatures around 32°C; these have the lowest probability of survival and growth being compromised. As adults the maximum size of *C. porosus* (both sexes) is highly variable (Webb *et al.*, 1978). Males (4–6 m) are much larger than females (2.2–3 m) (Webb *et al.*, 1978, 1984b), and they engage in territorial conflicts with other large males (Lang, 1987b; unpublished data). Small or deformed males are unlikely to be able to maintain a territory and breed successfully, yet each year a great range of different sized females have their eggs fertilised and nest successfully (even though female *C. porosus* are themselves remarkably intolerant of other females [Lang, 1987b]).

There thus seem to be growth advantages in *C. porosus* having TSD rather than GSD, and they are consistent with the predictions of Charnov and Bull (1977), Head *et al.* (1987), Joanen *et al.* (1987), Smith (1987) and Webb *et al.* (1987a).

The allocation of “femaleness” at an incubation temperature of 33.0°C, which proved to give both high survival (Fig. 9) and high post-hatching growth rates (Fig. 10), is superficially inconsistent with this hypothesis. However, our observation may be an artefact induced by constant-temperature incubation, where the “expected” increase in temperature does not occur (incubators were continually adjusted to compensate for metabolic heat production). Metabolic heat production in wild nests increases nest temperature by 1–2°C during development (Fig. 4), and the allocation of sex in a nest incubating at 33°C

TABLE 1. Mean hatchling size among 122 *Crocodylus porosus* hatchlings was largely unaffected by incubation temperature.*

Temp. (°C)	n	HL (cm)	SVL (m)	Weight (g)	% abundant yolk
29	15	4.2	13.3	63.8	53
30	17	4.3	13.5	68.4	6
31	51	4.2	13.4	66.3	47
32	15	4.3	13.6	67.9	73
33	24	4.3	13.5	69.6	50

* Measurements have been standardised to the mean egg weight for the sample (105.5 g). The increase in hatchling weight (hatchling + residual yolk) was significant, but reflects in part the weight of residual yolk. HL = head length, SVL = snout-vent length, weight = hatchling + residual yolk, % abundant yolk = the percentage of the sample which, on the basis of abdomen appearance, had abundant yolk.

midway through development, must be consistent with the potential fitness of embryos coming from a nest at 34–35°C at the end of development where survival is compromised.

For the relationship between TSD and post-hatching performance to be correct, the “non-sexual” effects of temperature need to be induced prior to or concurrent with the allocation of sex; that is, during the first half of incubation (see also Bull [1987]). This is the period in which development rate is highly temperature dependent, and it is also the period in which incubation temperature exerts a major influence on total incubation time (Fig. 8).

In summary, hatchling fitness varies profoundly with incubation temperature, independent of sex, and quite probably independent of the sex determining mechanism. TSD allows that variation to be exploited differently by the two sexes, giving advantages that would be unavailable with GSD. That these may be related to body size is suggested by the largest reptiles (crocodilians and sea turtles) having exclusively TSD. No extant crocodilians have sex chromosomes (Cohen and Gans, 1970), and the group as a whole may be committed to TSD (Ferguson, 1985); but this is not the case in chelonians (Bull, 1983). Given that GSD is widespread among the invertebrate and vertebrate ancestors of

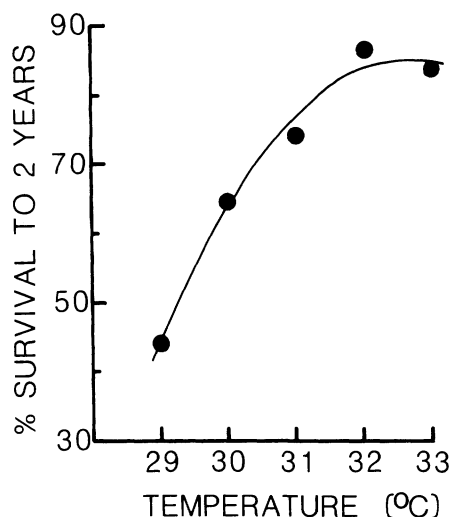


FIG. 9. The relationship between constant incubation temperature in the laboratory and survival to 2 yr of age for *Crocodylus porosus* hatchlings raised on a crocodile farm (hatching details are in Table 1). The line is a polynomial, least squares regression.

reptiles, TSD would seem to be a derived rather than a primitive trait in reptiles.

TEMPERATURE VERSUS MOISTURE EFFECTS

Successful development of any crocodilian embryo depends on there being an adequate moisture, temperature and gaseous environment for incubation. Many studies emphasise the effects of the moisture environment on hatchling fitness (for example see: Packard *et al.* [1981], Gutzke *et al.* [1987]), whereas we have clearly emphasised the temperature environment. Some believe that the moisture, temperature and gaseous environments act synergistically (Miller, 1985) such that no one parameter can be considered more important than any other. As explained below, we consider moisture and temperature to play equivalent roles in setting the survival limits within which most embryos will develop. However, within those limits effects of the moisture environment appear minor relative to those of the temperature environment.

With *Crocodylus porosus* in Australia and Papua New Guinea (Cox, 1985), desiccation of eggs is rare and flooding is common,

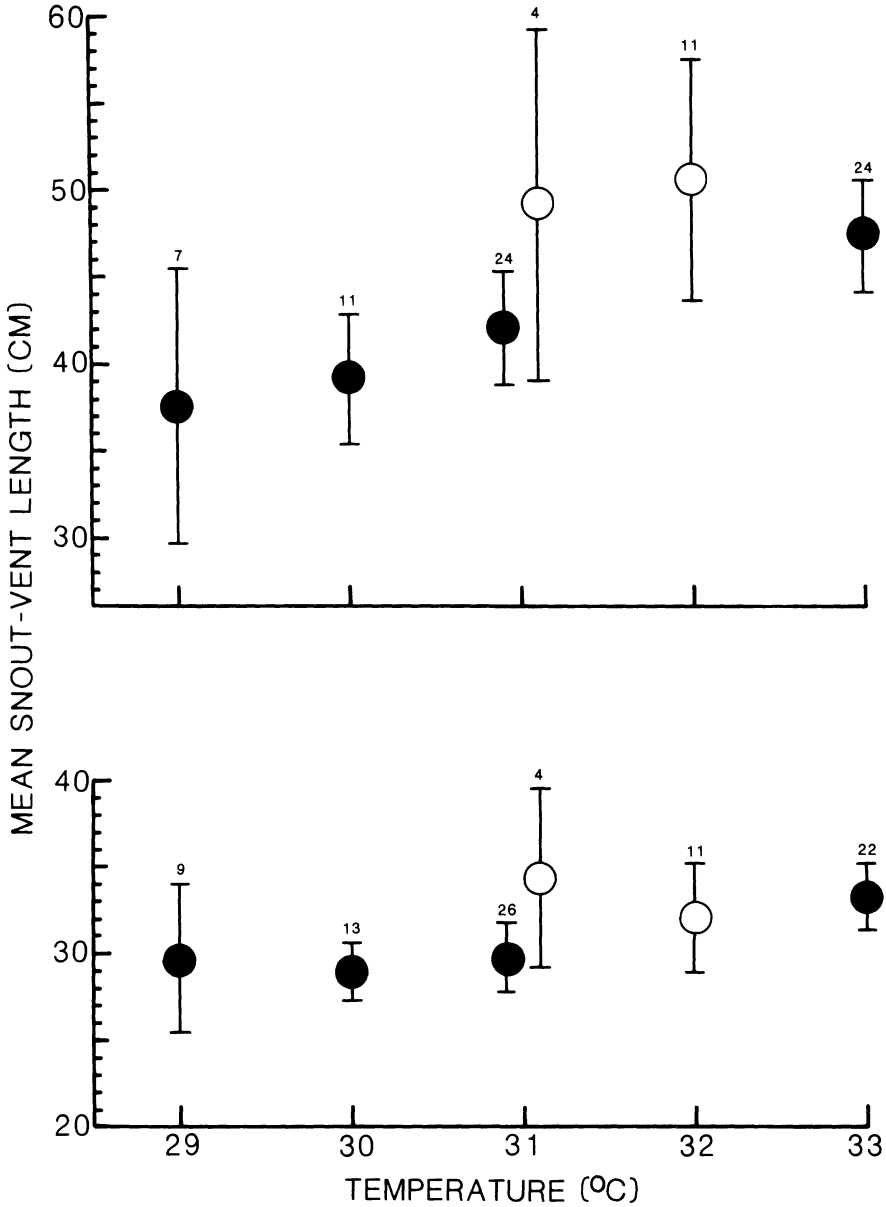


FIG. 10. The relationship between constant incubation temperature in the laboratory and mean snout-vent length at 1 yr (lower) and 2 yr (upper) of age for *Crocodylus porosus* hatchlings, from mixed clutches, raised on a crocodile farm. Horizontal lines indicate 2 SEs on either side of the mean for males (open circles) and females (closed circles). Numbers are sample sizes.

but both these extremes of the moisture environment are lethal. Incubation temperature has similar lethal limits (Fig. 5). Gas exchange within nest mounds varies with the structure of the mound (Lutz and Dunbar-Cooper, 1984) and probably with the frequency of rainfall and flooding

(Whitehead, 1987a, b). Critical limits occur in flooded nests, and they appear to occur in nests containing a high proportion of mud; there are a few data with which to fully evaluate them.

As selection can be expected to act rapidly on females which consistently choose

nests with a temperature, moisture or gaseous environment in which embryonic survival is compromised, it seems likely that all three parameters are intimately involved in maintaining the nesting strategy (mound or hole) and delimiting the period of the year in which nesting takes place. For many crocodilians, the nesting period corresponds to the only time of the year during which adequate moisture and temperature conditions are synchronised, given the constraints of hole or mound nesting. The relative roles played by moisture and temperature in delimiting time of nesting probably fluctuate greatly among different species nesting in different habitats, in different geographic regions.

Within these broad survival limits, incubation temperature exerts a profound effect on the rate at which embryos develop, which in turn influences the fitness and sex of crocodilian embryos (Webb *et al.*, 1987a). Effects of the moisture (Gutzke *et al.*, 1987) and gaseous environments (Ackerman, 1980) appear to reflect the same underlying effects of development rate (Miller, 1985; Webb *et al.*, 1987a). Two lines of evidence suggest that temperature can be validly considered the primary environmental variable affecting development rate, and thus fitness and sex.

Firstly, total incubation time is highly correlated with developmental rate and for example, it can be used to predict sex ratios with more precision than can be achieved from spot nest temperatures (Webb and Smith, 1984; Smith, 1987). Whitehead (1987a, b and unpublished data) quantified the extent to which total incubation time within 17 wild *Crocodylus johnstoni* nests (69–82 days), could be explained by the temperature, moisture and gaseous environments within those nests. Fifty-eight percent of the variation in total incubation time was attributable to nest temperature (range 27–35°C), whereas none could be explained (regardless of transformations or varying orders of entry into the analyses) by either the levels of oxygen in the nest throughout incubation (PO₂ range: 118–151 torr initially, dropping to 71–144 torr at hatching), nor the extent to which eggs lost or gained water during incubation

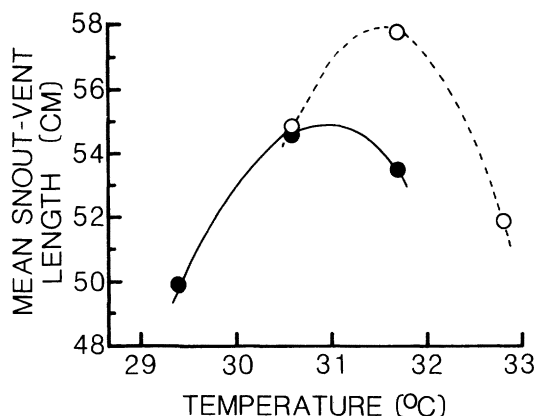


FIG. 11. The relationship between constant incubation temperature in the laboratory and mean snout-vent length at 2 yr of age for male (open circles) and female (closed circles) *Alligator mississippiensis*. Data are from Joanen *et al.* (1987), with total lengths converted to snout-vent lengths (measured to the back of the cloaca) with the formulae in Chabreck and Joanen (1979).

(range +2.3% to -8.1% of initial egg weight).

A further example, with turtle embryos, is provided by Gutzke *et al.* (1987). Eggs were incubated in wet (-150 kPa water potential), moist (-300 kPa) and dry (-1,100 kPa) substrates at three temperatures (22, 27 and 32°C). Survival was compromised in the dry substrate, but the range of temperatures tested was within survival limits. Mean incubation time varied from 42.8 days at 32°C to 98.6 days at 22°C, a change of 130% attributable to temperature. In contrast, no effect of substrate moisture was demonstrated at 22°C and 27°C, and only an 11% increase in incubation time occurred at 32°C (41.0 days in the dry to 45.4 in the wet substrate). However, even this was partly attributable to egg temperature, as egg temperatures increased in the dry substrates relative to the wet ones, because of greater retention of metabolic heat (Gutzke *et al.*, 1987).

The second line of evidence is the degree to which embryos are buffered from the vagaries of the nest environment. Growth and differentiation is the net result of a suite of biochemical reactions, all of which are temperature dependent to varying

degrees (Romanoff, 1967). The egg shell and its contents provide little insulation, and as a consequence, nest temperatures exert a profound effect at the tissue level. In contrast, the shell and shell membrane are partial barriers to water and gas exchange respectively (Whitehead, 1987*a*, *b*), and the embryos have homeostatic mechanisms which provide additional buffering. For example, up to 20% weight loss from *Crocodylus johnstoni* eggs (Manolis *et al.*, 1987; Whitehead, 1987*a*, *b*) and at least 11% from *Carettochelys insculpta* eggs (Webb *et al.*, 1986) have little effect on the embryos; the water loss is compensated for by exchanges of fluids within egg compartments.

In summary, there are critical levels of the temperature, moisture and gaseous environments at which embryonic survival is compromised. But within those limits, development rate and the effects of incubation environment on fitness and sex appear to be largely dependent on temperature. More subtle influences of the moisture environment exist, but they need to be demonstrated with rigid control of temperature. It seems likely that the relationship between fitness and moisture within survival limits is a plateau rather than a peaked normal distribution (Grigg, 1987).

INCUBATION TEMPERATURE AND THE EVOLUTION OF THE REPTILE EGG

It seems reasonable to assume that the provision of adequate incubation temperatures has always been a constraint with which reptiles have had to contend, and it seems equally likely that incubation temperature effects, such as those described in crocodilians, are more widespread among extant reptiles. However, we found that our consideration of the role that incubation temperature may have played in the evolution of reptilian modes of reproduction, was confounded by anomalies in the classical view of how reptilian oviparity evolved.

It has long been accepted that reptiles evolved from amphibian-like ancestors (Romer, 1970; Archer and Molnar, 1984) and that their success in the terrestrial

environment was in part attributable to their refinement of oviparity: the development of the amniote egg which allowed reptiles to nest away from water (Packard, 1966; Packard *et al.*, 1977; Archer and Molnar, 1984). Viviparity among reptiles is usually viewed as being derived from oviparity, through increasing retention of eggs in the oviduct, in many cases a response to adverse thermal conditions (see review by Shine, 1985). The general picture is that oviparity allowed reptiles to overcome the constraints of the moisture environment, and later, viviparity the constraints of the thermal environment.

Egg-retention in squamates is really a sub-category of oviparity, regardless of the existing levels of placentation (Packard *et al.*, 1977; Yaron, 1985). It is fundamentally a period in which "shell-less" amniote eggs develop in the oviducts, for varying periods. Squamates can also be considered to have *facultative* oviparity; most species retain eggs in the oviducts for half the total period of embryonic development, and a fifth of all species retain eggs until development is complete (Shine, 1985). The extent of retention is labile within the group, and selection has increased and decreased it frequently and independently, often in response to cold and hot climates respectively (Shine, 1985). If post-hatching growth of squamates, like that of crocodilians, is affected by incubation temperature, then it is likely that the effects are induced during the first half of development, when egg retention allows females to exert some control over the thermal environment.

In contrast to *facultative* oviparity, chelonians, rhynchocephalians and crocodilians have *obligate* oviparity. Birds, which have an almost identical egg and share a common ancestry with reptiles, also have *obligate* oviparity. Eggs are laid when embryos are at an early stage of development (well before the allantois is formed), and that stage is remarkably consistent within any particular group. Most important, *obligate* oviparous reptiles and birds are a diverse assemblage of different sized aquatic, terrestrial and aerial vertebrates, existing in a wide range of climates. All the

factors which have been hypothesised as leading to viviparity in *facultative* oviparous reptiles (Shine, 1985), should apply to *obligate* oviparous reptiles and birds—yet in not one species is there significant retention of eggs. For example, *Sphenodon punctatus* exists in an intensely cold region, and has a 13–15 month total incubation period; yet egg-laying still occurs when embryos are at a gastrula stage (Moffat, 1985). Penguins are *obligate* oviparous birds which incubate eggs in an intensely cold environment. They would not have their “flight” compromised by retaining eggs, and if they were *facultative* oviparous reptiles, they would be prime candidates for “egg-retention” and viviparity under current theory (Shine, 1985); yet no such developments seem to have taken place (Blackburn and Evans, 1986).

If it is assumed that selection has at some time tried to move *obligate* oviparous reptiles and birds in the direction of egg retention, one can only conclude that it has been a consistent and total failure. Notwithstanding the fact that this could be explained in terms of the many costs and benefits of retaining eggs (Blackburn and Evans, 1986), the most plausible explanation is that it is a lethal pathway; each time it has been attempted, embryonic survival has been compromised. If so, *obligate* oviparity of the form seen in reptiles and birds today could never have given rise to *facultative* oviparity nor to viviparity.

The possibility that *facultative* oviparity is the primitive condition among reptiles does not appear to have been considered (Shine, 1985) until now. The first reptiles, the captorhinomorphs, were small terrestrial animals with a body form, posture and niche remarkably similar to today's lizards (Archer and Molnar, 1984). Based on heart structure, crocodiles and birds evolved from an animal similar to small extant lizards (Webb, 1979). We suggest that the captorhinomorphs also mirrored extant lizards in having *facultative* oviparity, and that the varying levels of placentation seen in squamates today (Yaron, 1985) have a long antiquity.

The suggestion raises the question of whether the extraembryonic membranes

of amniote embryos evolved in an oviduct or an external egg. An oviducal origin has not been considered seriously (Szarski, 1968), yet it is the most plausible explanation. Survival in an external reptilian egg is dependent on the extraembryonic membranes being in place, so it is likely that they evolved first (Szarski, 1968). The “shell-less” eggs of squamates may in fact reflect the original amniote egg with the original disposition of extraembryonic membranes: the yolk sac surrounding the non-cellular food supply; the allantois storing waste products and facilitating gas exchange; the amnion enclosing the embryo within a fluid-filled sac and buffering it from mechanical shock; and the chorion, which completely surrounds the embryo and yolk sac; thereby, containing in a single egg unit a series of separate chambers with different densities (Webb *et al.*, 1987b, c).

Facultative oviparity also seems to be an ideal reproductive system within which to refine reptilian oviparity. It would allow the amniote embryo to be modified in an environment controlled by the female, while simultaneously (but independently), selection could pursue the oviparous packaging for life outside the oviducts.

Obligate oviparity is best explained as being derived from *facultative* oviparity, specifically to meet the needs of voiding amniote embryos at an early stage of development. But it required a number of embryological specialisations. For example, abundant albumen was needed for the immediate supplies of subembryonic fluid (to store excretory wastes) and to facilitate yolk rotation (Webb *et al.* 1987b, c). The timing of egg-laying with respect to embryo attachment to the shell membrane needed to be synchronised precisely. If attachment occurs before laying, embryos are unable to rotate to the uppermost part of the egg, and in large eggs they die. In the absence of an allantois, their position at the top is needed for gas exchange and access to subembryonic fluid (Webb *et al.*, 1987b, c). These and a suite of other specialisations could have evolved gradually in a *facultative* oviparous system. However, once integrated into an *obligate* oviparous system,

even minor deviations in the direction of increased egg retention in the oviducts could prove fatal.

A variety of costs associated with females retaining eggs (Shine, 1985; Blackburn and Evans, 1986) may have stimulated selection for *obligate* oviparity, but there were almost certainly significant advantages obtained by freeing development from the female. Selection for increased egg size, hatchling size, adult size and perhaps even body form are likely ones; the ability to combine large clutch sizes, with large embryos and free-swimming hatchlings, and perhaps ornamented hatchlings. Even in *facultative* oviparous reptiles, embryos must be maintained above the yolk, which could well preclude having large clutches of large eggs unless they are aligned longitudinally in the oviducts (such as in snakes).

If *obligate* oviparity was derived in the fashion suggested, then adaptations of the external egg for water (the shell) and gas exchange (the shell membrane) would have been achieved before the embryonic stage at laying was reduced. A significant problem remaining may have been the exposure of embryos to the vagaries of the external thermal environment during the period when they are most sensitive to the effects of incubation temperature. This could be a basic cost of *obligate* oviparity, outweighed by advantages accrued from other sources. That *obligate* oviparity is a mechanism for avoiding high maternal body temperatures (Anderson *et al.*, 1987) may apply to birds, but is not a compelling argument for reptiles. However, if post-hatching growth is enhanced by fluctuating incubation temperatures (relative to stable ones), which is suggested if Garnett and Murray's (1986) data on *C. porosus* growth are re-analysed with respect to the age at which eggs were collected, there may in fact be advantages in large, thermally-stable, aquatic reptiles voiding their eggs into a more labile thermal environment.

In summary, we believe the amniote egg evolved in the oviducts of a reptilian ancestor, rather than in the external egg. It evolved in an environment in which the effects of incubation temperature could be moderated by a female's behaviour. This

led to *facultative* oviparity in the first reptiles, where again temperature effects were controlled by the female. *Obligate* oviparity arose as a specialised development to reduce greatly the time eggs were retained in females. Incubation temperature effects became more dependent on the external ambient environment, although this was not necessarily a disadvantage.

The scenario would see the potential for viviparity being present in the earliest reptiles, which is consistent with some ichthyosaurs being viviparous (Romer, 1945). It would also suggest that primitive stages in the development of amniote eggs are likely to be found in amphibians with varying degrees of internal development of embryos, rather than in those with specialised external eggs alone.

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