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Mathematical models for growth in alligator (*Alligator mississippiensis*) embryos developing at different incubation temperatures

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ABSTRACT

A variety of model-based (growth models) and model-free (cubic splines, exponentials) equations were fitted using weighted-nonlinear least squares regression to embryonic growth data from *Alligator mississippiensis* eggs incubated at 30 and 33 °C. Goodness of fit was estimated using a χ^2 on the sum of squared, weighted residuals, and run and sign tests on the residuals. One of the growth models used (Preece & Baines, 1978) was found to be superior to the classical growth models (exponential, monomolecular, logistic, Gompertz, von Bertalanffy) and gave an adequate fit to all longitudinal measures taken from the embryonic body and embryonic mass. However, measurements taken from the head could not be fitted by growth models but were adequately fitted by weighted least squares cubic splines. Data for the stage of development were best fitted by a sum of 2 exponentials with a transition point. Comparison of the maximum growth rates and parameter values, indicated that the growth data at 30 °C could be scaled to 33 °C to multiplying the time by a scaling factor of 1.2. This is equivalent to a Q_{10} of about 1.86 or, after solving the Arrhenius equation, an $E^{\ddagger}$ of 46.9 kJmol^{-1}. This may be interpreted as indicating a common rate-limiting step in development at the 2 temperatures.

Key words: Embryo growth; *Alligator mississippiensis*; incubation temperature.

INTRODUCTION

It is widely recognised that temperature has an important influence on the embryonic development of reptiles (Deeming & Ferguson, 1991*a*, *b*). A number of important features, including the rate of embryonic development, hatchling morphology and pigment pattern are influenced by the incubation temperature. The temperature of egg incubation also determines the sex of many reptiles (Deeming & Ferguson, 1988) and also influences postembryonic variables such as hatchling and adult growth, temperature preference and behaviour. The temperature at which alligator (*Alligator mississippiensis*) eggs are incubated determines the sex of the hatchling with temperatures at or below 30 °C producing all females and temperatures of 33 °C producing all males. Pigmentation patterns of the hatchling are influenced by incubation temperature (Murray et al. 1990) as is posthatchling growth (Joanen et al. 1987). Deeming & Ferguson (1989) reported that patterns of embryonic growth of eggs incubated at 30 °C (female producing) and 33 °C (male producing) were influenced in a manner that could not be described by a simple scaling relationship. However, growth models were not fitted to these data. A variety of model-based (growth models) or model-free equations have been fitted to embryonic growth data (Ricklefs & Cullen, 1973; Ackerman, 1981; Webb et al. 1987; Whitehead et al. 1990; Leshem et al. 1991) and a number of studies of reptilian embryonic growth at different incubation temperatures have been undertaken (Holder & Bellairs, 1962; Maderson & Bellairs, 1962; Vinegar, 1973; Pieu & Dorizzi, 1981; Packard et al. 1987; Webb et al. 1987; Deeming & Ferguson, 1988, 1989; Lang et al. 1988; Whitehead et al. 1990; Leshem et al. 1991). Usually, one model-based equation has been fitted to the growth data, but the comparative fit of alternative models has not often been assessed since techniques for doing so are not widely available.

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The objective of this study was to fit growth (model-based) and other (model-free) equations to a suite of Alligator embryonic morphological growth data, so as to assess the growth of embryonic alligators developing at different incubation temperatures, test the fit of the various equations, then attempt to interpret the biological meaning of the parameter estimates.

MATERIALS AND METHODS

Experimental design

Eggs of female alligators were collected within 24 h of egg laying in June 1988 in Louisana and shipped by air freight to Manchester University. Approximately 1 d of embryonic development occurred before the eggs were incubated at 30 °C (female producing) and 33 °C (male producing) in water jacketed incubators with humidities of 100%. The temperature in the incubator was controlled to 0.1 °C and the presence of thermal gradients assessed (and eliminated from having any influence) by accurately recording the temperature at multiple points in the incubator with thermocouples calibrated with a National Standard thermometer. Starting on d 8 of incubation and continuing at 4 d intervals thereafter, sets of 5 eggs were removed at the same time from the incubator and opened. The embryo was removed, separated from its yolk and fixed as described in Deeming & Ferguson (1989). Subsequently, the embryo was staged as described in Ferguson (1985), weighed (after fixation) and 14 different morphometric length measurements made as described in Deeming & Ferguson (1989). These measurements included total length, crown–rump length, distance between limbs, length of fore limb, length of hind limb, head length, head height, head width, snout width at midpoint and at the nostrils, snout length, nape–rump length, eye length and tail length. The morphometric data were collected from the embryos before they were utilised for other investigations of the mechanisms of temperature dependent sex determination, thereby generating the maximum data from the eggs and embryos.

Statistical procedures

The curve fitting techniques and methods for assessing goodness of fit are described in the Appendix.

RESULTS

A summary of the results of fitting the various equations to the growth data is now given. The equations used are described in the Appendix. The

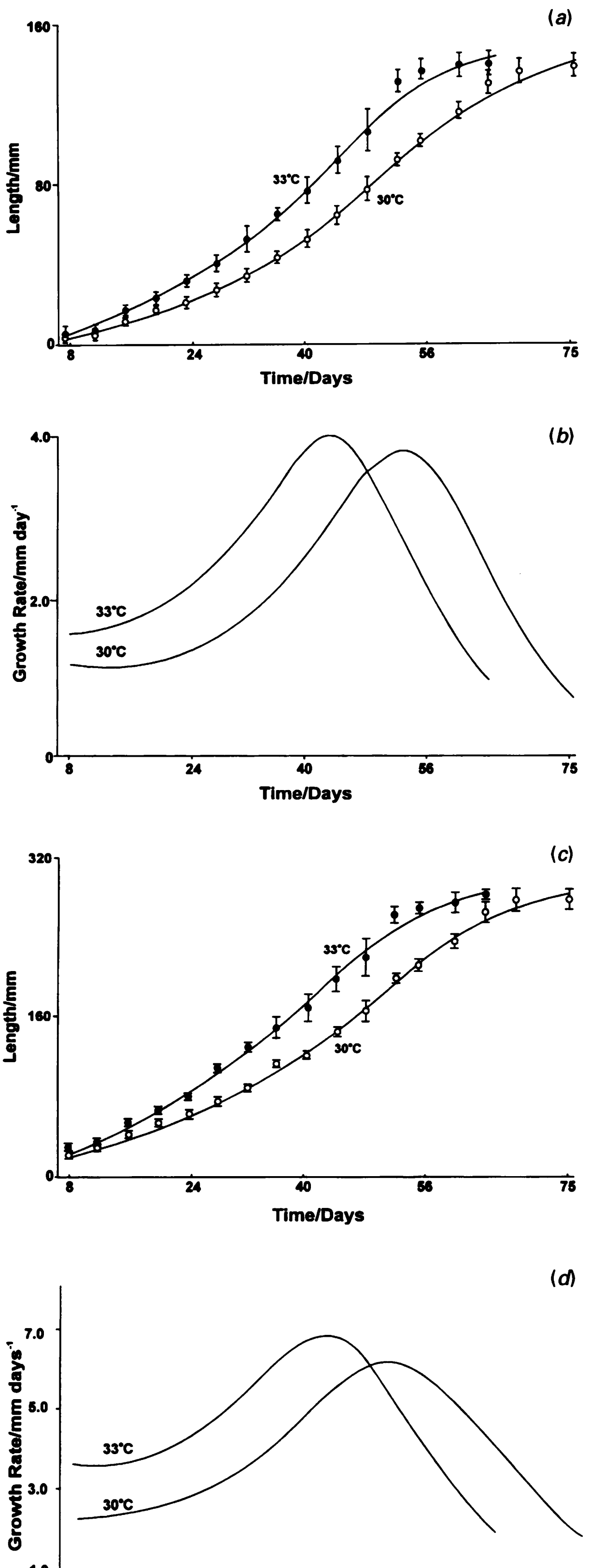

Fig. 1. Best fit growth curves for the Preece & Baines model (model 9) for total length (*a*) and trunk length (*c*). Best fit rate of growth curves are for total length (*b*) and trunk length (*d*). Open circles are for 30 °C and filled circles are for 33 °C.

Table 1. *Best-fit estimates obtained by fitting the Preece & Baines model to the given data sets*

	A (mm)		A_0 (mm)		t_0 (d)		k_0 (d^{-1})		k_1 (d^{-1})	
Data set	30	33	30	33	30	33	30	33	30	33
Nose width	7.3	8.3	5.8	6.2	58.1	46.7	0.012	0.012	0.17	0.28
Crown–rump	96.4	94.7	57.4	62.7	46.7	40.75	0.0022	0.0078	0.085	0.11
Limb width	57.4	59.3	35.5	37.0	53.8	45.6	0.0039	0.00543	0.12	0.12
Total length	294.9	292.2	193.4	203.0	52.9	45.4	0.0069	0.011	0.11	0.13
Nape–rump	84.5	83.4	61.2	63.1	53.4	44.3	0.009	0.014	0.14	0.21
Forelimb	48.6	49.9	25.1	26.7	48.7	40.4	0.0013	0.003	0.091	0.11
Snout width	13.4	13.7	8.5	10.1	53.6	48.2	0.0015	0.015	0.12	0.20
Tail	158.8	156.1	102.5	101.4	54.2	45.1	0.007	0.0091	0.13	0.13
Hindlimb	65.0	69.9	33.6	32.0	50.0	39.3	0.0011	−0.0009	0.089	0.088
Mass	49.8 (g)	59.0 (g)	24.7 (g)	29.5 (g)	64.4	54.5	0.0000	0.0000	0.1009	0.122

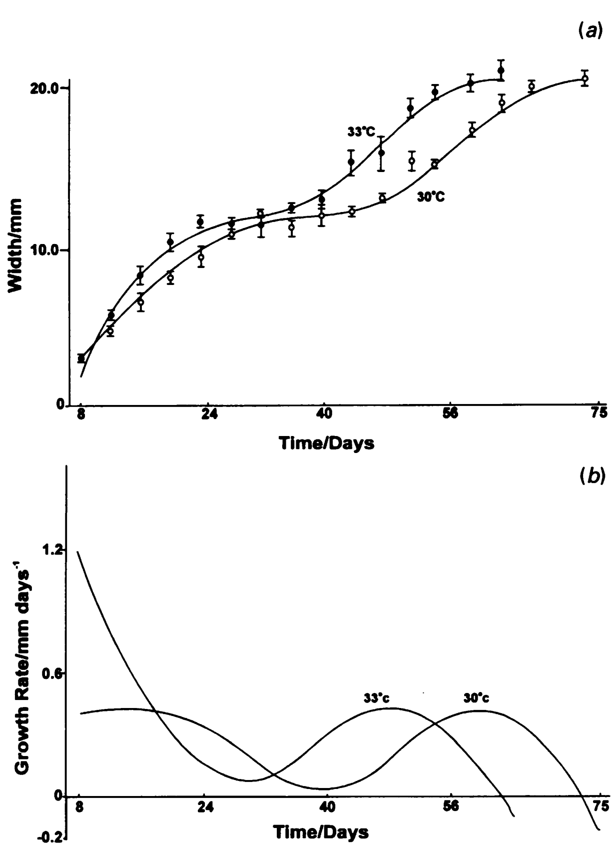

Fig. 2. Growth data for the head region. (*a*) Data for head width with the best fit piecewise least squares cubic splines with no smoothing parameter and 1 interior knot point for each 8 distinct time values. (*b*) Growth rates for head given by the best fit curves of (*a*). Open circles are for 30 °C and closed circles are for 33 °C.

alternative weighting schemes had relatively little influence on the outcome as far as goodness of fit and parameter estimates were concerned. With regard to the length data, the exponential model (model 1) and the monomolecular model (model 2) yield poor fits to the data, indicating that logarithmic growth can be rejected. The logistic (model 3), Gompertz (model 4) and 2/3 power model (model 5) yielded reasonable fits to the data. Models 6, 7 and 8 which are variants of models 3, 4 and 5 did not yield sufficient improvement in fit by the F test over models 3, 4 and 5 to justify the use of the extra parameter. The model of Preece & Baines (1978, model 10) was superior to models 1 to 8 in all cases. It passed the run and sign tests more frequently, always gave the lowest WSSQ value and the extra parameters were justified by the F test. Examples of the Preece & Baines model fitted to several data sets are shown in Figure 1 and the equation parameters for each data set are summarised in Table 1. The von Bertalanffy differential equation (model 11) was difficult to fit and was not a sufficient improvement on models 3, 4 and 5 to justify the extra computational complexity necessitated by the numerical integration. The Preece & Baines model was always superior to the von Bertalanffy equation. It was possible to use cubic splines to obtain a substantially better fit than the Preece & Baines model by increasing the number of knot points. However, this required as many as 1 knot to every 3–4 distinct times and the resulting best fit curve was no longer smooth but broke up into wavy sections in an attempt to interpolate. The data for the head measurements (head height and width at 30 and 33 °C, head length and eye length at 30 °C) were more complicated than the other data sets and could not be adequately fitted by any of the first 11 models. However, they were fitted well by splines with a low density of interior knots, usually 1 knot for every 8 distinct time values. Spline curves fitted to some of the head data sets are shown in Figure 2. The longitudinal measures of the body increased at a relatively slow rate early in incubation, reaching a peak around d 40–50 of incubation and then declined continuously thereafter. The rate of increase in eye and head length at 33 °C

Table 2. *Summary of time scale ratio at 30 and 33 °C for the data sets described in the text***

Data set	T30 (d^{-1})	T33 (d^{-1})	T30/T33	MGR30 (mm d^{-1})	MGR33 (mm d^{-1})	MGR33/MGR30	FS30 (mm)	FS33 (mm)	FS33/F30
Nose width	56	46	1.22	0.15	0.30	1.97	7.3	8.3	1.14
Crown–rump	46	38	1.21	1.72	1.97	1.15	96.4	94.7	0.98
Limb width	53	44	1.20	1.32	1.45	1.10	57.4	59.3	1.03
Total length	50	41	1.22	5.95	6.70	1.13	294.9	292.2	0.99
Nape–rump	51	44	1.16	1.80	2.30	1.28	84.5	83.4	0.99
Forelimb	48	40	1.20	1.10	1.30	1.18	48.6	49.9	1.03
Snout width	54	46	1.17	0.31	0.39	1.28	13.4	13.7	1.02
Tail	52	44	1.18	3.90	4.00	1.03	158.8	156.1	0.98
Hindlimb	50	40	1.25	1.40	1.65	1.18	65.0	69.6	1.07
Head width	59*	48*	1.23	—	—	—	20.3*	20.3*	1.00
Head height	59.8	48*	1.23	—	—	—	18.0*	18.8*	1.04
Eye length	60*	50	1.20	—	—	—	8.9*	9.0*	1.01

** Times of maximum growth rate are T30 and T33, maximum growth rates are MGR30 and MGR33 and predicted final sizes are FS30 and FS33. Data were fitted by the Preece & Baines models (model 9) except for head data (*), which were fitted by cubic splines.

and snout length at both temperatures increased rapidly initially and then declined continuously throughout the rest of incubation. The time of maximum growth (t_{max}) occurs earlier at 33 than at 30 °C for all measures of length. The ratio of t_{max} at 33 °C to t_{max} at 30 °C is about 1.2 (mean = 1.21, S.D. = 0.17) for all length measures. The maximum growth rate (GR_{max}) is also greater at 33 than at 30 °C but the ratio of GR_{max} at the 2 temperatures was more variable than the ratio for t_{max} but also close to 1.2 (mean = 1.26, S.D. = 0.53). Table 2 summarises these ratios for the various data sets. When the asymptotes of the growth curves for each temperature were compared, the values were similar indicating that the sizes of the embryo at hatching as assessed by length were similar at the 2 temperatures. When the time points of the Preece & Baines model at 30 °C were multiplied by 1.2, the curves at 30 °C were indistinguishable from the curves at 33 °C. The cubic spline curves fitted to the data for head height and head width (Fig. 2) indicated there were 2 peaks of growth, an early one around d 20 and a later one around d 45. When the time at 30 °C is scaled by 1.2 (as described above), the spline curve superimposes on the data at 33 °C, indicating that the change in head height and head width at 33 °C is simply accelerated over that at 30 °C. The data for mass were best fitted either by the logistic model with an added constant or the Preece & Baines model. The WSSQ was similar for both models. The Preece & Baines model passed a run test at both temperatures while the logistic model with an added constant passed only at 33 °C. The rate of growth at the 2 temperatures is shown in Figure 3. Peak growth in mass is greater at 33 than at 30 °C and occurs earlier, the ratio of the times at peak growth is 1.18. However, the area under the 33 °C curve is greater than the area under the 30 °C curves as is indicated by the difference in parameter A for the 2 temperatures. The hatchlings at 30 °C had a mass of around 38 g while the hatchlings at 33 °C had a mass around 44 g. The stage data were less variable than the length data (Fig. 4). The data for stage were fitted by the following empirical exponential equation:

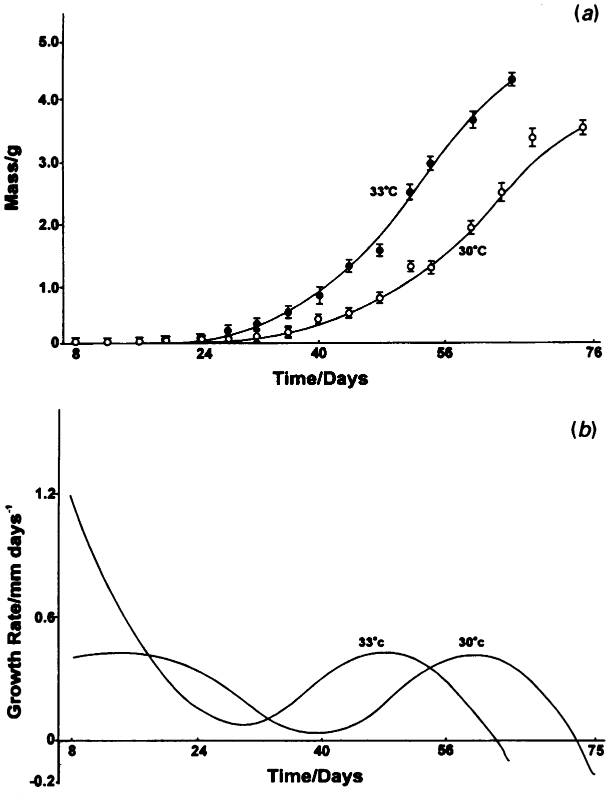

Fig. 3. Growth data for mass. Open circles are for 30 °C and filled circles are for 33 °C.

$$
\begin{aligned}
stage &= A[1-\exp(-Bt)], \quad t \leqslant E \\
&= A[1-\exp(-Bt)]+(C-A) \\
&\quad \times [1-\exp(-D(t-E))], t > E.
\end{aligned}
$$

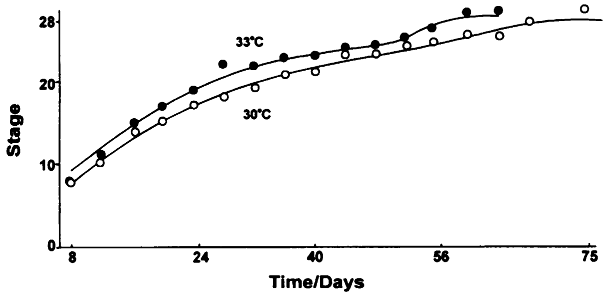

Fig. 4. Stage of development as a function of time. The best fit curves are given in the text. Open circles are for 30 °C and filled circles are for 33 °C.

The parameter estimates for 30 °C were $A = 26.3$ stage units, $B = 0.043\ \text{d}^{-1}$, $C = 28$ stage units, $D = 0.19\ \text{d}^{-1}$ and $E = 58.0$ d. The parameter estimates for 33 °C were $A = 26.1$ stage units, $B = 0.055\ \text{d}^{-1}$, $C = 28$ stage units, $D = 0.061\ \text{d}^{-1}$ and $E = 51.6$ d.

DISCUSSION

Growth effects of temperature

The influence of incubation temperature on the rate of reptilian embryonic development is well known (Deeming & Ferguson, 1991*a*). Indeed, the rate of change of all the measures that we undertook increased with an increase in incubation temperature. Most of the longitudinal measures and embryonic stage increased by a time scaling factor of 1.2 from 30 to 33 °C. This will be clear from the results summarised in Table 2. The relationship between growth and differentiation of *Alligator mississippiensis* embryos does not appear to be altered by changes in incubation temperature as has been reported for *Crocodylus johnstoni* embryos (Whitehead et al. 1990). Some of the head measures did not scale simply by a time scaling factor of 1.2 between 30 and 33 °C and the patterns of growth appeared different. Although the pattern of mass change with temperature also scaled by about 1.2, the asymptote of the growth curve was slightly greater at 33 than at 30 °C, that is, hatchlings were heavier at the higher temperature but they were no bigger as measured by length. This may be related to the observation (Deeming & Ferguson, 1989) that embryos at 33 °C utilise more yolk components (beginning at an earlier stage of development) and consequently hatch with a smaller absorbed abdominal yolk reserve than those at 30 °C. The 33 °C embryos grew fastest as hatchlings and as adults (Joanen et al. 1987), perhaps indicating an increased food conversion efficiency.

That head growth does not scale precisely from 30 to 33 °C is interesting. First, embryonic head growth shows much more variation among different crocodilian species than many other embryonic length measurements (Deeming & Ferguson, 1990). Secondly, head growth data are heavily influenced by morphological differences in the flexure of the brain at different stages of development. The hind brain flexure straightens out with progressive embryonic development, decreasing head height and increasing head length. It is interesting that, in malformed embryos caused by abnormally high incubation temperatures (⩾ 35 °C), the brain case appears to ossify earlier than the hind brain flexture reduces, so resulting in an abnormal hind brain bump on the head (Ferguson, 1985). A similar asynchrony between cranial ossification and brain morphogenesis may explain the differing head growth curves observed at the normal incubation temperatures of 30 and 33 °C in this study. The temperature of egg incubation has been postulated to affect the embryonic and adult characteristics of growth, pigmentation, preferred thermoregulatory temperature, behaviour and other characters as discussed in the introduction, by influencing the maturation of the hypothalamus and the pulsatile release of its releasing hormones (Deeming & Ferguson, 1988).

That incubation temperature affects head growth differently at female producing (30 °C) and male producing (33 °C) temperatures strengthens the necessity for a careful analysis of brain and neuroendocrine development and function at these 2 incubation temperatures. Deeming & Ferguson (1989) used principle component analysis to suggest that there was not a simple scaling relationship for growth of alligator embryos incubated at 30 and 33 °C. The present analysis indicates that there is actually a simple scaling relationship for all but a few measures when assessed separately. The difference in the 2 conclusions may lie in the contribution of mass to the principal components analysis and in the fact that the present study used a more realistic nonlinear regression approach to modelling. The influence of incubation temperature on hatchling mass of reptiles is not well defined. Webb & Cooper-Preston (1989) reported no influence of incubation temperature on *Crocodylus porosus* hatchling mass. Whitehead et al. (1990) reported that hatchling mass was inversely related to incubation temperature for *C. porosus* and *C. johnstoni*, while Gutzke & Packard (1987), Gutzke et al. (1987) and Packard & Packard (1987) reported similar findings for hatchlings of several turtle and snake species. Our result is just the opposite. Heavier hatchlings occurred at higher incubation temperatures but these did not necessarily have larger length measurements. It may well be that different species respond differently to temperature or that there are other factors operating of which we are not cognizant. One such factor may be initial egg mass. Deeming & Ferguson (1989) showed that hatchling mass, but not various length measurements, was influenced by initial egg mass. How incubation temperature and initial egg mass interact is unknown. Murray et al. (1990) reported that the development of pigmentation patterns of *A. mississippiensis* embryos was influenced by incubation temperature. The size of embryos in which pigmentation first appeared increased with increase in temperature. Since size and stage scale in the same way at the temperatures studied, this suggests that pigmentation pattern is influenced by other factors such as the rate of chemical production and diffusion at the higher temperature resulting in a different pattern of pigmentation (2 more stripes at 33 than at 30 °C).

The observation that growth at 30 °C can be scaled to 33 °C by a time scaling factor of 1.2 is of some interest. Webb et al. (1987) reported a similar, though slightly higher, value for change in a different measure of growth, the developmental rate coefficient (DRC_{33}) between 30 and 33 °C for *C. porosus*. The data of Whitehead et al. (1990) for the growth constant (from the logistic growth equation) of *C. johnstoni* embryos indicate a value of 1.25 between 30 and 33 °C for wet embryonic mass. The data of Leshem et al. (1991) for the logistic growth constants of embryos of the turtle, *Trionyx triunguis* indicate a value of 1.2 to 1.23 between 30 and 33 °C for both wet and dry embryonic mass. Thus there appears to be a similarity in the sensitivity of embryonic growth to temperature changes over the range of 30 and 33 °C for at least several reptilian species. Influences of temperature on biological rates are often described using Q_{10} as a measure of the influence. The Q_{10} relationship is derived (Nobel, 1991) from the Arrhenius equation

$$k(T) = A\exp(-E^{\ddagger}/RT)$$

where $k(T)$ is a rate constant, T is the temperature (°K), A is the pre-exponential or frequency factor which is only slightly dependant on T, R is the gas constant (8.31 $\mathrm{kJmol^{-1}}$) and $E^{\ddagger}$ is the free energy of activation. When the Arrhenius equation for a rate constant k_1 at one temperature T_1 is divided by the equation for a rate constant k_2 at a second temperature T_2 we have

$$\ln\left(\frac{k_1}{k_2}\right) = \frac{E^{\ddagger}}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right).$$

If the ratio of the 2 rates is 1.2 for 30 and 33 °C as we observed for most of our measurements, then $E^{\ddagger} = 46.9\ \mathrm{kJmol^{-1}}$. It appears that incubation time changes with incubation temperature as if there is a rate-limiting step or reaction for the growth process. An $E^{\ddagger}$ of 46.9 $\mathrm{kJmol^{-1}}$ is equivalent to a Q_{10} of 1.86. However, the scaling factor is not linear with change in incubation temperature. While rates at 30 °C can be scaled to 33 °C by multiplying by 1.2, the scaling factors (to 33 °C) at 32, 31, 29 and 28 °C are 1.06, 1.13, 1.28 and 1.34, respectively. These scaling factors are quite similar to those reported as developmental rate coefficients (DRC) for *C. porosus* and *C. johnstoni* (Webb et al. 1987; Whitehead et al. 1990). Thus $E^{\ddagger}$ is similar for all 3 species, indicating that the sensitivity of developmental rate to change in incubation temperature is similar among the species. It was suggested, on the basis of the differences in the times of hatching between the 3 crocodilian species at 30 and 33 °C (Deeming & Ferguson, 1989, 1990), that *A. mississippiensis* embryonic development is less sensitive to incubation temperature because of the more northern and thus cooler environment of the alligator. Although alligator eggs do have a shorter incubation period and a smaller difference in hatching time between 30 and 33 °C than the 2 crocodile eggs, this is not due to a change in sensitivity but rather to a shift of the entire curve relating incubation time to incubation temperature. It may also reflect a difference in the mechanism triggering hatching. Additionally, despite the similar scaling factors among the species, the pattern of growth in *A. mississippiensis*, *C. porosus* and *C. johnstoni* is quite different. For example, the alligator hatchling is longer and lighter than the 2 crocodilian hatchlings (Deeming & Ferguson, 1990). This reflects the importance of other (e.g. genetic) factors in the growth patterning process.

Concerning the fitting of growth curve models

The main handicap with growth studies is the relatively featureless nature of the growth curve and, as with many biological experiments, the small signal-to-noise ratio, which we take as the ratio of the mean to the standard error of the mean (Parzen, 1960). This generally means that parameter estimates obtained from biological experiments are poorer than those obtained from more physical or chemical experiments. It is desirable to use as few parameters as possible when describing growth because, as the number of parameters increases, the less well determined they become. Data can be fitted either by model-free equations or by model-based equations. Model-free equations such as polynomials or cubic spline are too flexible and, if so desired, can be made to pass through every single data point. Thus such equations may be very useful for interpolation (e.g. calibration) but have little or no biological relevance. Model-based equations are based on an underlying growth model, usually the differential form of some growth equation and may be preferred for this reason. It is easy to measure a variety of different morphological variables during the course of growth and it is no surprise that much effort has been expended attempting to derive mathematical models of growth processes. However, Feller (1946) has pointed out that a large number of such models can give comparable goodness of fit due to the lack of information in featureless, symmetric sigmoid growth curves. The allometric relationship has often been invoked to support power law growth models (von Bertalanffy, 1957) and the relationship between classical sigmoidal models and the von Bertalanffy model was clarified by Richards (1959) who showed that the classical models were special cases of the von Bertalanffy model. However, in some cases (e.g. human adolescent growth spurts), more complicated and subtle models were required to describe growth adequately (Marubini et al. 1972; Preece & Baines, 1978).

The approach used in this paper has been to estimate best fit parameters using established methods for model discrimination and parameter estimation (Bardsley et al. 1986, 1987; Bardsley & McGinlay, 1987, 1989) and then to use the parameters to represent the growth process. In other words, if a model fits well, then any conclusions drawn about the growth process from the best fit parameters are likely to be fairly sensible as long as the number of parameters estimated is small. However, it must be remembered that the best fit curve is merely an empirical equation giving an acceptable smoothing of the experimental data set. A variety of growth curves have been utilised to describe reptilian embryonic growth (Dmi'el, 1970; Ricklefs & Cullen, 1973; Ackerman, 1981; Webb et al. 1987; Whitehead et al. 1990; Leshem et al. 1991) and posthatching growth (Brisbin, 1990) but tended toward the use of logistic and exponential models. Ricklefs (1967) described a method of fitting several growth equations using linear regression and/or visual fitting to growth data and this technique has been applied to embryonic reptile data (Ackerman, 1981; Whitehead et al. 1990) but does not yield good parameter estimation or estimation of comparative goodness of fit. Leshem et al. (1991) utilised nonlinear regression techniques but investigated only a few models and did not investigate weighting.

Many growth trajectories are simple monotonically increasing sigmoid types so an autonomous differential equation of the form

$$\frac{dS}{dt} = f(S)$$

can be used, where $S(t)$ is size, length, weight, etc. as a function of time t and $f(S)$ is some unknown function of size only. The traditional models used for this purpose are reviewed by Richards (1959) and Draper & Smith (1981) while Savageaux (1979) has supported long established arguments for allometric types of relationships, such as those proposed by von Bertalanffy (1957), in order to justify power law expressions for $f(S)$.

Of course there are many departures from sigmoid growth laws. The original way to handle such complications was simply to fit sums of logistic or other types of equations, but the simplicity of the differential formulation is lost when this is resorted to. Attempts to make the differential equations more versatile were induced by Preece & Baines (1978). Their approach is valuable for calculating growth rates and predicting final sizes where the departure from sigmoid behaviour is only slight. When the departure from sigmoid behaviour is marked, such as when there are abrupt growth spurts or even turning points, it is probably best to use piecewise cubic splines as advocated for instance by Largo et al. (1978) and Silverman (1985), because the differential equation may well be nonautomomous, i.e. of the form

$$\frac{dS}{dt} = g(S, t).$$

Whether there is anything to be gained from fitting growth curves to any particular data set will depend upon the nature of the data, the ability to assess goodness of fit and parameter redundancy, and the use to be made of the best-fit parameters.

CONCLUSIONS

We have investigated weighted nonlinear least square regression to embryonic growth data from *Alligator mississippiensis* eggs incubated at 30 and 33 °C using computer programs (GCFIT, CALCURVE and QNFIT) from the SIMFIT package to fit 12 models in sequence and compare goodness of fit by χ^2 and run tests (Swed & Eisenhart, 1943) and graphical analysis. The data were weighted in a variety of ways, including constant weighting, using a smoothed representation of sample standard deviation as a function of time or mean size, assuming constant relative error and weighting with actual standard errors. Weighting schemes can often be critical in nonlinear regression but, in the present study, we discovered that the weighting scheme had very little influence on the outcome.

All the data for growth, except the data from the head region, were best fitted by the model of Preece & Baines (1978). This model was first used for human adolescent growth and was clearly superior to the classic deterministic growth models for estimating the asymptote (i.e. final predicted size) and maximum growth rates. However in order to fit data for head growth or stage we had to resort to model free empirical curve fitting.

A finding of outstanding interest was that almost all the fits at the lower temperature become indistinguishable from the fits at the higher temperature when the time at the lower temperature was corrected by a factor estimated to be equal to 1.2. This suggests that a common temperature dependent rate limiting step controls all the growth features in the species investigated.

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APPENDIX: STATISTICAL PROCEDURES AND CURVE FITTING

The SIMFIT package

SIMFIT is a computer package written by one of us (W.G.B.) for simulation, curve fitting, statistical analysis and graph plotting in the life sciences (Bardsley et al. 1986, 1987, 1989; Bardsley & McGinlay 1989). This package is freely available on request. The programs take in measurements y_i made at design points x_i, where the response is supposed to result from a deterministic model $f(x, \Theta)$ and random additive variation ϵ_i as in

$$y_i = f(x_i, \Theta) + \epsilon_i.$$

The best fit parameters are then obtained by minimising the sum of weighted squared residuals WSSQ given by

$$WSSQ = \sum_{i=1}^{n} \left(\frac{y_i - f(x_i, \Theta)}{s_i}\right)^2$$

where the s_i are weighting factors. If the model is correct and the random errors ϵ_i are uncorrelated and normally distributed with zero mean and s.d.s s_i then the parameter estimates would be maximum likelihood estimates (Edwards, 1972).

Weighting factors

We fitted the data using $s_i = 1$ (unweighted, constant variance), s_i, proportional to best fit function or proportional to mean response at each fixed x (constant relative error), $s_i =$ the sample s.d.s or s_i calculated by fitting smooth curves to the s.d.s as a function of time or mean response. Programs MAKFIL, EDITFL and EXFIT were used for these transformations, but the best fit parameters and WSSQ values were insensitive to the alternative weighting schemes. Program FTEST was used to explore whether addition of extra parameters could be justified by an excess variance test.

Models fitted

Models 1 to 10 below were fitted by program GCFIT. This program selects starting estimates, fits the models, calculates maximum growth rates and goodness of fit statistics, displays graphs, then creates a table containing all the necessary statistics to compare goodness of fit with the alternative models. Model 11 was fitted by program QNFIT using Gear's method with an explicit Jacobian to integrate the differential equation numerically. Model 12 was fitted by program CALCURVE.

1. The exponential model (2 parameters)

$$\frac{dS}{dt} = kS$$

$$S(t) = S(0)\exp(kt)$$

2. The monomolecular model (3 parameters)

$$A = S(\infty)$$

$$B = 1 - S(0)/A$$

$$\frac{dS}{dt} = k(A - S)$$

$$S(t) = A[1 - B\exp(-kt)]$$

3. The logistic model (3 parameters)

$$A = S(\infty)$$
$$B = A/S(0) - 1$$
$$\frac{dS}{dt} = kS(A-S)/A$$
$$S(t) = \frac{A}{1 + B\exp(-kt)}$$

4. The Gompertz model (3 parameters)

$$A = S(\infty)$$
$$B = \log[A/S(0)]$$
$$\frac{dS}{dt} = kS\log(A/S)$$
$$S(t) = A\exp[-B\exp(-kt)]$$

5. The von Bertalanffy 2/3 model (3 parameters)

$$A^{1/3} = \eta/\kappa$$
$$A = S(\infty)$$
$$B = \eta/\kappa - S(0)^{1/3}$$
$$k = \kappa/3$$
$$\frac{dS}{dt} = \eta S^{2/3} - \kappa S$$
$$S(t) = [A^{1/3} - B\exp(-kt)]^3$$

6. The logistic model with a constant term (4 parameters)

$$f(t) = S(t) - C$$
$$A = f(\infty)$$
$$B = A/f(0) - 1$$
$$\frac{df}{dt} = \frac{dS}{dt}$$
$$= kf(A-f)/A$$
$$S(t) = \frac{A}{1 + B\exp(-kt)} + C$$

7. The Gompertz model with a constant term (4 parameters)

$$f(t) = S(t) - C$$
$$A = f(\infty)$$
$$B = \log[A/f(0)]$$
$$\frac{df}{dt} = \frac{dS}{dt}$$
$$= kf\log(A/f)$$
$$S(t) = A\exp[-B\exp(-kt)] + C$$

8. The von Bertalanffy 2/3 model with a constant term (4 parameters)

$$f(t) = S(t) - C$$
$$A^{1/3} = \eta/\kappa$$
$$A = f(\infty)$$
$$B = \eta/\kappa - f(0)^{1/3}$$
$$k = \kappa/3$$
$$\frac{df}{dt} = \frac{dS}{dt}$$
$$= \eta f^{2/3} - \kappa f$$
$$S(t) = [A^{1/3} - B\exp(-kt)]^3 + C$$

9. The von Bertalanffy variable m (i.e. Richards) model (4 parameters)

$$A^{1-m} = \eta/\kappa$$
$$A = S(\infty)$$
$$B = \eta/\kappa - S(0)^{1-m}$$
$$k = \kappa^{1-m}$$
$$\frac{dS}{dt} = \eta S^m - \kappa S$$
$$S(t) = [A^{1-m} - B\exp(-kt)]^{1/(1-m)}$$

10. The first model of Preece & Baines (5 parameters)

$$A = S(\infty)$$
$$A_0 = S(t_0)$$
$$f(t) = \exp[k_0(t - t_0)] + \exp[k_1(t - t_0)]$$
$$S(t) = A - \frac{2(A - A_0)}{f(t)}$$

11. The von Bertalanffy differential equation (5 parameters)

$$\beta > \alpha$$
$$\eta > 0$$
$$\kappa > 0$$
$$S(\infty)^{(\beta-\alpha)} = \eta/\kappa$$
$$\frac{dS}{dt} = \eta S^\alpha - \kappa S^\beta$$
$$S(t) = \int_0^t \frac{dS}{dt}\,du.$$

This model involves a numerical integration and the constant of integration is also a parameter to be estimated.

12. Weighted least squares smoothing splines

This model is a piecewise cubic spline curve with an algorithm for choosing knot positions until satisfactory fit is achieved. After curve-fitting the best-fit equation is defined by a set of knot positions and coefficients.