Modelling Development of Reptile Embryos under Fluctuating Temperature Regimes

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ABSTRACT

An increase in temperature, within bounds, will accelerate development of reptile embryos, and morphogenesis can be normal over a range of temperatures despite those varying rates of development. Less well understood is the form of the relationship that best describes variation in developmental rate with temperature. In this article, we apply a linear degree.hour model, an empirical curvilinear model, a biophysical model, and a polynomial model to data on rates of embryonic development and temperature in the pig-nosed turtle *Carettochelys insculpta* from northern Australia. The curvilinear models, which have been applied with success to development of insects, describe the embryonic development of turtles well. When fluctuating temperatures extend beyond the constant temperatures that support successful incubation, the curvilinear models continue to perform well, whereas the linear model predictions fail. Sensitivity analysis indicates that under some circumstances, incubation duration may be increased by diel temperature fluctuations, independent of an influence of mean temperature. In other circumstances, incubation duration may be decreased, and in still other circumstances, diel temperature fluctuations will have no impact on incubation duration. This adds an additional dimension to our understanding of how thermal regimes can be selected or manipulated by reptiles to optimise incubation duration and the timing of offspring emergence.

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Introduction

Incubation temperature has a profound effect on developmental outcomes in reptiles. In particular, we have come to accept that an increase in temperature, within bounds, will accelerate development of reptile embryos and that morphogenesis can be normal over a range of temperatures despite the varying rates of development (Ewert 1985). Less well understood is the form of the relationship that best describes variation in developmental rate with temperature. Most studies have been conducted in laboratory experiments using constant temperatures. Much less focus has been placed on reproducing, in the laboratory, the thermal regimes that prevail in reptile nests (but see Paukstis et al. 1984; Packard et al. 1991; Georges et al. 1994). Yet daily temperature fluctuations, seasonal trends, and thermal gradients within nests can be expected to complicate the relationship between nest temperature and developmental outcomes, including developmental time. We know, for example, that developmental times of insect eggs and larvae may be affected by daily fluctuations in temperature, quite independent of the effects of average temperature (Hagstrum and Hagstrum 1970), and there is at least one instance where this is true of a reptile species (Shine and Harlow 1996).

Daily fluctuations in temperature should have no influence on developmental times over and above that of the daily mean, provided that developmental rate and temperature are approximately linearly related over the range of temperatures experienced by the eggs and provided that there is no hysteresis in the action of temperature on developmental rate (Georges et al. 1994). Early developmental models, based on these simple assumptions, were coupled with the temperature summation rule of Candolle (1855) and Reibisch (1902) to develop the notion of degree.hours widely used in the applied biological sciences under a variety of related names. However, predictions of degree.hour summation models commonly fail to match observed developmental times when temperatures range to extremes (Hagstrum and Hagstrum 1970). The assumptions of linearity in the relationship between developmental rate and temperature break down at extremes. Daily excursions into high temperatures can lead to developmental inhibition, where rates are lower than expected under linear assumptions. At the other extreme, developmental rate may approach 0 nonlinearly as temperatures decline, almost asymptotically.

A range of empirical and biophysical models has been developed to encapsulate these ideas (reviewed by Wagner et al. 1984). These models have been used to estimate developmental

times in insects, particularly those of economic importance, because developmental time is an influential parameter in insect population dynamics (Lewontin 1965). To date, these ideas have not been applied to reptilian development.

In this article, we explore the applicability of three developmental models (Sharpe and DeMichele 1977; Dallwitz and Higgins 1992) to field studies of reptilian nesting using data from the pig-nosed turtle *Carettochelys insculpta* Ramsay (1886) of the wet/dry tropics of northern Australia. This species has temperature-dependent sex determination (Webb et al. 1986; Georges 1992). During the dry season, females deposit clutches of four to 19 eggs in shallow chambers on sand banks adjacent to water (Georges and Kennett 1992). The eggs are white, hard-shelled, and almost perfectly spherical. They incubate rapidly over 50–90 d to maturation depending on date laid and incubation temperature, which rises steadily as the season progresses. Temperatures within the nests are high and fluctuate widely each day (Georges 1992). On reaching maturity, a substantial residual yolk body is internalised; the embryos then enter aestivation within the egg (Webb et al. 1986). The trigger for hatching is inundation by flooding or the first torrential rains of the wet season. This species provides a good opportunity to study nonlinear developmental models because nest temperatures vary each day well beyond the range of constant temperatures that will support successful incubation.

Material and Methods

Overall Design

Combined laboratory and field data were collected to meet the objectives of this study. The laboratory experiments were used to characterise the development of *Carettochelys insculpta* under constant temperature conditions, including the establishment of a relationship between developmental rate and temperature, within the bounds of the constant temperatures that support development. The field component provided data where temperatures often varied to extremes for part of each day and beyond those that could be used to support development in constant temperature experiments. The objective here was to optimise the parameters of nonlinear developmental models to obtain the best fit between predicted and observed developmental increments in these nests and so obtain estimates of instantaneous development rate at extreme temperatures. As a hedge against the possibility that temperatures experienced in the field during our study might not cover the full range of temperature regimes experienced by nests of *C. insculpta*, especially at the high end of the range, we supplemented the field data with cyclic temperature experiments conducted in the laboratory.

The Models

Under the degree.hours model, developmental rate, as measured by incremental change in embryonic head width (Georges et al. 1994), increases linearly as temperature (T) increases from a developmental zero (T_0). No development occurs when temperatures drop below the developmental zero.

$$\frac{ds}{dt} = A(T - T_0) \text{ for } T > T_0,$$

$$\frac{ds}{dt} = 0 \text{ for } T \leq T_0, \quad (1)$$

where A is the rate of increase (Fig. 1). The equation is constrained by the biologically realistic assumption that growth cannot be reversed ($A \geq 0$). This is a degree.hours model because developmental rate is simply proportional to temperature when temperature is measured with respect to the developmental zero (Georges et al. 1994).

The most widely accepted nonlinear model of poikilotherm development is that of Sharpe and DeMichele (1977). They formulated a biophysical model that describes the nonlinear response of developmental rate to incubation temperature at both high and low temperatures, as well as a linear response

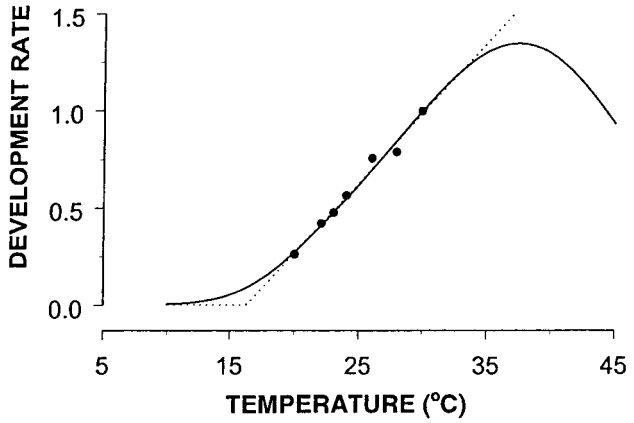

Figure 1. Sharpe-DeMichele model (*solid line*) and linear degree.hours model (*dotted line*) applied to developmental rates of the temperate-zone lizard *Bassiana duperreyi*. Data are calculated from Figure 2 of Shine and Harlow (1996). Developmental rate is incremental change in head width, expressed as a percentage of final hatchling head width per day. The linear model was fitted by least squares regression ($ds/dt = 0.07251T - 1.1818$). The Sharpe-DeMichele model was optimised at lower temperatures using developmental rates for $23.0° \pm 3.75°$C and $23.0° \pm 9.75°$C (Shine and Harlow 1996). The curvature at higher temperatures is not supported by data and is shown for illustration only. Parameters of the Sharpe-DeMichele curve are $RHO_{25} = 0.7$, $H_A = 17{,}199.9$, $T_L = 291.47$, $T_H = 311.71$, $H_L = -64{,}401$, and $H_H = 41{,}965$. Note the close agreement between the linear model and the Sharpe-DeMichele model at intermediate temperatures.

at intermediate temperatures (Fig. 1). Six fitted parameters must be estimated using nonlinear regression. A computational form of the equation more suitable for this purpose was developed by Schoolfield et al. (1981), namely,

$$\frac{ds}{dt} = \frac{\mathrm{RHO}_{25}(T/298.15)\exp\{(H_A/r)[(1/298.15) - (1/T)]\}}{1 + \exp\{(H_L/r)[(1/T_L) - (1/T)]\} + \exp\{(H_H/r)[(1/T_H) - (1/T)]\}}, \tag{2}$$

where ds/dt is developmental rate at absolute temperature T (°K), $r = 1.987$ is the universal gas constant (in calories/degree/mole), RHO_{25} is the developmental rate at 25°C (298.15 K), and T_L, H_L, T_H, H_H, and H_A are the remaining fitted parameters.

Dallwitz and Higgins (1992) describe an empirical model as an alternative to the theoretically derived model of Sharpe and DeMichele (1977; see also Dallwitz 1984). The model has five parameters and is defined by

$$\begin{aligned} \frac{ds}{dt} &= b_1 10^{-v^2(1-b_5+b_5v^2)}, \\ v &= \frac{u + e^{b_4 u}}{c_2}, \\ u &= \frac{T - b_3}{b_3 - b_2} - c_1, \\ c_1 &= \frac{1}{1 + 0.28b_4 + 0.72\ln(1 + b_4)}, \\ c_2 &= \frac{1 + b_4}{1 + 1.5b_4 + 0.39b_4^2}, \end{aligned} \tag{3}$$

where ds/dt is developmental rate expressed as a percentage per day, b_1 is the maximum developmental rate, b_2 is approximately the temperature ($b_3 > b_2$) at which ds/dt falls to $b_1/10$, b_3 is approximately the temperature at which ds/dt is a maximum, b_4 controls how sharply ds/dt approaches 0 at high temperatures, and b_5 controls the asymmetry of the relationship. The terms c_1 and c_2 allow the approximate interpretations of b_2 and b_3 given above (Dallwitz and Higgins 1992). Finally, a loosely constrained polynomial model was employed as a check against overconstraint of the form of the relationship between developmental rate and incubation temperature by the curvilinear models described above.

Field Data

Freshly laid nests of the pig-nosed turtle ($n = 25$) were located on sand banks on the Daly River in the vicinity of Oolloo Crossing (14°04′40″S, 131°15′00″E) in the Northern Territory of Australia. The sand banks were regularly inspected for turtle tracks and signs of nesting activity, and nests were located by probing the sand with a 2-mm spring steel rod (Blake 1974). On discovery, the depth of each egg was measured, and the top of each egg was marked with a pencil as it was uncovered so that it could be returned to its original orientation and position in the nest. A map in plan view was drawn outlining the position of the eggs in the horizontal plane.

Temperatures were recorded with four-wire resistive temperature device (RTD) probes fitted to a datalogger (Datataker DT500) and calibrated against a mercury thermometer certified as accurate to 0.1°C by the National Association of Testing Agencies. Three temperature probes were fitted to each nest as the eggs were replaced: one immediately below the deepest egg, one in the core of the nest, and one immediately above the shallowest egg. The probes were fitted as soon as possible after the nest was discovered, usually within 2–3 d. Temperatures in each nest were recorded at 15-min intervals, considered sufficient to encapsulate the daily thermal cycle throughout incubation.

Temperature traces for the brief period from laying until the temperature probes were inserted were determined by establishing a regression between nest temperatures and the temperatures at nest depth recorded by a standard monitoring station. The station was set up on a small sand bar used for nesting by *C. insculpta* in May, immediately after the preceding wet season. Temperatures were monitored at 15-min intervals throughout the study period, at the sand surface and at 10-cm depth intervals to 50 cm.

Eggs were removed from the nest again after they were estimated to have undergone at least two-thirds of development so as to be sure to include the thermosensitive period for sex determination. Two viable eggs from the core of each nest were retained for dissection, unless the number of viable eggs was small, in which case one egg was taken following a check of synchronous development by candling. They were opened, the embryos separated from their yolk and extra-embryonic membranes, and fixed in 10% buffered formalin. Maximum head width, including the optic capsules, was determined with a calibrated eyepiece for small embryos or vernier callipers (± 0.1 mm) after fixation. For the purposes of modelling, maximum head width was expressed as a percentage of the mean hatchling head width (13.17 mm).

In the laboratory experiments, embryos inspected at two points in incubation were used to estimate development rate. Using embryo size at a single point in incubation to estimate developmental rate in the field studies assumes that the index of size, in this case maximum head width, is 0 at the time of laying. Of course, the embryo does not have a measurable head at the time of laying or indeed for several days later (10–14 d at 30°C), but the extrapolated relationship between head width and time passes close to 0 (0.5 mm). This indicates that no great distortion will result from using embryo head width at a single time late in incubation as an indication of overall rate of development.

Laboratory Data

Eggs were collected from the Daly River between Claravale Crossing (14°20′S, 131°34′E) and inflow of Jinduckin Creek (14°07′S, 131°17′E). They were transported by boat to Oolloo Crossing and then either by road to Darwin Airport, and by air to the University of Canberra, or by road to the Douglas-Daly Research Farm (Department of Primary Industries and Fisheries).

One clutch of nine eggs (CAI97_140), for which the laying date was known, was selected to reveal the progress of growth with time at a constant temperature (see also Beggs et al. 2000). The clutch was laid on August 18, 1997, collected on August 19, 1997, and allocated to a refrigerated incubator (Thermoline model RI-170) set at 30°C constant temperature on August 21, 1997. The eggs were buried in beds of moist vermiculite (four parts water to three parts vermiculite by weight) in 500-mL circular plastic food containers fitted with lids. Water potentials were not measured, but water content was kept relatively constant by monitoring the weight of the eggs, substratum, and container and adding water as necessary to maintain a constant weight. This was seldom necessary. Temperatures were recorded initially with four-wire RTD probes fitted to a datalogger (Datataker DT500) to ensure constancy of temperatures throughout the 24-h cycle and then twice daily with mercury thermometers (± 0.1°C). Datalogger probes and the thermometers were placed in close proximity to the eggs and had been calibrated. One egg was opened on 32, 34, 38, 44, 48, 54, 60, 70, and 78 d incubation corresponding to Yntema stages 18–26 (Yntema 1968; Beggs et al. 2000). Data for stages 12–17 and additional data for stages 24–26 were obtained opportunistically from other eggs incubated at 30°C as part of other experiments. Candling (Beggs et al. 2000) was applied to eggs in stages 24–26 to determine the time to yolk internalization after head width was estimated to have achieved its maximum. Early-stage embryos were killed by chilling the eggs to less than 5°C for a minimum of 36 h. Late-stage embryos were killed by intracranial injection of pentabarbitone. The embryos were then processed as in the field component of the study. Change in maximum head width was calculated as the index to developmental rate (percent of total development per day).

In an experiment to measure variation in developmental rate with temperature, eggs were initially placed in 30°C incubators (Thermoline RI-170). After 1 wk they were candled to determine viability and embryonic stage (Beggs et al. 2000). Eggs younger than Yntema's stage 8 were transferred to incubators set at 24.5°, 26.0°, 28.0°, 30.0°, 32.0°, 34.0°, and 36.0°C. Temperatures were again monitored as above. Eggs from each clutch at each temperature were opened at two points during incubation spanning Yntema stages 17–22, which is the thermosensitive period for sex determination in *C. insculpta* (Young et al. 2004).

On the basis of the results obtained in the constant temperature experiments, fluctuating regimes were selected to include likely low-temperature inhibition (26° ± 6° and 25° ± 7°C), high-temperature inhibition (30° ± 6° and 31° ± 6°, 31.5° ± 6.5°, and 32° ± 7°C), and no inhibition (28° ± 4° and 29° ± 5°C). Eggs under each treatment (typically one clutch only) were incubated according to the protocols established by Georges et al. (1994). The daily mean, minimum, and maximum temperatures of the eggs could be tightly controlled. Standard deviations for the daily mean ranged across experiments from 0.08° to 0.23°C, for the maximum from 0.08° to 0.22°C, and for the daily minimums from 0.07° to 0.18°C ($n = 23$ d).

Eggs from each experiment were opened at intervals to be sure to include the middle third of development determined by candling (Beggs et al. 2000). Two eggs were opened on each occasion and the embryos compared for consistency of size. Where they disagreed, a third egg was opened, and data from the aberrant embryo was discarded.

Fitting the Degree.Hour Model

Parameters of the degree.hour model were obtained by regressing developmental rate at 26.0°, 28.0°, 30.0°, 32.0°, and 34.0°C against incubation temperature in a simple linear regression. Embryos incubated at 24.5° and 36°C did not survive to hatching, and although some developmental increments were available, they were not included in the analysis. The linear regression was extrapolated to obtain the theoretical developmental zero T_0.

Fitting the Nonlinear Models

Temperatures that cause the embryo to die when held constant throughout incubation can nevertheless support development provided that exposure is for a limited period each day (Headlee 1941; Harries 1943; Lin et al. 1954; Fitch and Fitch 1967; Phelps and Burrows 1969; Luo and Li 1993; Morales-Ramos and Cate 1993). Constant temperature experiments alone may therefore not yield the necessary data at high or low temperatures to permit good estimates of the parameters T_H, H_H, T_L, or H_L of the Sharpe-DeMichele model or parameters b_4 and b_5 of the Dallwitz-Higgins model. To adequately characterise the nonlinear models, developmental rates are required for the high and low temperatures that support development during brief exposures but that yield low or no survivorship when held constant. These data were provided by field nests whose temperatures varied to extremes, supplemented by the cyclical temperature experiments in the laboratory as described above.

Point temperatures taken at 15-min intervals in each temperature trace were interpolated using cubic splines (PROC EXPAND; SAS Institute 1988) to yield temperatures that were evenly spaced at equal but arbitrarily small intervals ($\Delta t = 7 \times 10^{-4}$ d). In a variation of the approach of Dallwitz and Higgins (1992), initial parameter values were selected, and de-

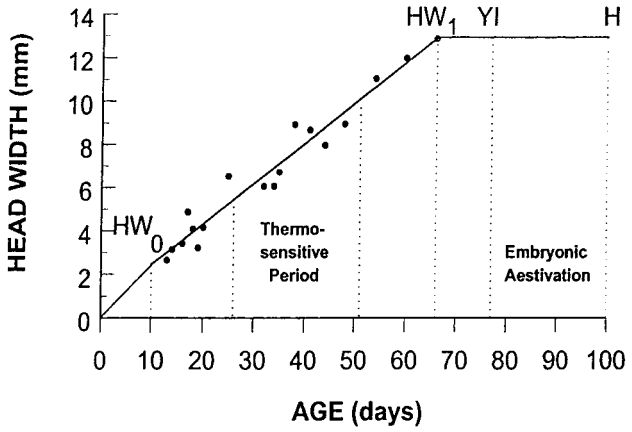

Figure 2. Phases in the development of *Carettochelys insculpta* at 30°C (after Webb et al. 1986; Beggs et al. 2000). Abbreviations are as follows: HW_0 = maximum head width at stage 10; HW_1 = mean maximum head width for hatchlings (13.17 ± 0.035 mm, $n = 150$); YI = point at which residual yolk is internalised in preparation for hatching; H = point at which hatching occurs in response to environmental stimuli. The thermosensitive period is the middle third of incubation (Young et al. 2004). Note that head width of the embryo increases linearly through incubation to a maximum that occurs 10 d before yolk internalisation. Once yolk is internalised, the embryo is ready to hatch, so this point is considered the terminal point of incubation.

velopmental rate was integrated over each of several temperature traces to obtain estimates of the development increment that accompanies each trace. Each such estimate was then matched to an observed development increment, and the parameters of the model were adjusted iteratively using nonlinear techniques (PROC NLIN; SAS Institute 1988) to minimise the squared deviations between the two, that is, to minimise

$$\sum_{k=1}^{k=K} w_k \left(S_k - \sum_{t=1}^{t=t'} \frac{ds}{dt} \Delta t \right)^2, \tag{4}$$

where S_k is the observed percentage of total development that occurred after the embryo traversed temperature trace k of the K traces; ds/dt is the developmental rate as a function of temperature (percent of development per day); t is time measured in discrete but arbitrarily small increments ($1 \ldots t'$); t' is the time increment corresponding to S_k; Δt is the size of the time increments (in days), usually small; and w_k are weightings given to each trace to reflect their precision. Because all traces were of similar duration, these weightings were set to 1.

Initial values $b_1 = 2$, $b_2 = 25$, $b_3 = 33$, $b_4 = 1$, and $b_5 = 1$ were chosen as good first estimates of the parameters for the Dallwitz-Higgins model, given the intuitive interpretations of these parameters provided by Dallwitz and Higgins (1992). Choice of initial values that resulted in convergence for the Sharpe-DeMichelle model was more difficult. An initial value of RHO_{25} was estimated from the linear model. The remaining five parameters were adjusted manually, after an initial examination of how each parameter adjusted the location and shape of the curve, to obtain a rough agreement between observed and predicted values. The parameters of the resulting preliminary curve were used as initial estimates in the nonlinear modelling used to optimise the curve using PROC NLIN. A grid search did not yield any evidence that the final solution had resulted from a local suboptimal minimum. The polynomial model was fitted using initial parameters within the following bounds: $30 < b_1 < 100$, $-30 < b_2 < 30$, $-3 < b_3 < 3$, $-.3 < b_4 < .3$, $-0.03 < b_5 < 0.03$ in a grid search (PROC NLIN; SAS Institute 1988).

Relative performance of the linear and nonlinear models was judged by examination of the residuals and comparison of Akaike's Information Criterion (AIC; Akaike 1973; Sakamoto et al. 1986). SAS PROC NLIN does not calculate the AIC statistic, which was computed using the approximation

$$\mathrm{AIC_c} = n \ln\left(\frac{\mathrm{SSE}}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1},$$

where n is the number of data points (38), k is the number of fitted parameters plus one for the estimated mean, and SSE is the error sums of squares (Burnham and Anderson 2000; Motulsky and Christopolous 2003). The last term in the above equation is a correction for small sample sizes ($n < 40$). The relative "probability" that the model with the smallest $\mathrm{AIC_c}$ is the correct selection between models is given by

$$w = \frac{e^{-0.5\Delta \mathrm{AIC_c}}}{1 + e^{-0.5\Delta \mathrm{AIC_c}}},$$

where $\Delta\mathrm{AIC_c}$ is the difference in $\mathrm{AIC_c}$ scores. This scales the difference in $\mathrm{AIC_c}$ scores to a range between 0 and 1, which is easier to interpret. The statistic w is sometimes referred to as an Akaike weight.

Comparison of models using the AIC criterion requires that the models are applied to the same data set. The development of the linear degree.hours model outlined above follows the normal practice of fitting the line to constant temperature developmental rates only. The AIC statistic for the linear model was calculated in two ways: first by fitting the linear model based on constant temperatures only to the full data set and second by using nonlinear modelling (PROC NLIN) to fit the best-case linear model to the full data set. Throughout this article, developmental time is used synonymously with incubation period, the latter term being used more commonly in reference to reptilian development.

Results

Development versus Time

Several phases in the development of *Carettochelys insculpta* could be identified (Fig. 2), influenced in part by our choice of maximum head width as the index to embryo size. There was an early phase from time of laying to stage 10 (10–14 d at 30°C) before the formation of a discrete head that can be measured (ca. 2 mm). There was an intermediate phase during which head width increases linearly to a maximum at stage 24 (66 d at 30°C). This includes the thermosensitive period for sex determination, approximately spanning the middle third of incubation (26–52 d at 30°C; stages 17–22). Strong linearity in the relationship between head width and time over most of development confirms that change in head width was a good choice as an index of developmental rate. Maximum head width, that is, hatchling head width (mean 13.17 mm), was achieved by stage 24. There was a maturation phase at the end of development during which the head did not increase in width but qualitative attributes of the embryo continued to change (Beggs et al. 2000), including the progressive internalization of yolk. This phase was of 10-d duration at 30°C. Finally, there was a period of embryonic aestivation of variable duration (estimated 59 d to yolk exhaustion at 30°C; Webb et al. 1986), terminated by us in the laboratory by immersion in water, in the field presumably by flooding or torrential rain.

Developmental Rate versus Temperature

Developmental rate of the embryos of *C. insculpta* varied linearly with incubation temperature to good approximation in the range of 26°–34°C (data shown in Fig. 3). Regression of developmental rate (percent of total development per day) against temperature (°C) yielded

$$\frac{ds}{dt} = 0.2984T - 7.39 \text{ for } T > T_0,$$

$$\frac{ds}{dt} = 0 \text{ for } T \leq T_0, \quad (5)$$

($R^2 = 0.958$, $P < 0.01$, $n = 5$). The developmental zero for *C. insculpta* was estimated from this relationship to be 24.8° ± 1.04°C (Fig. 3). The AIC_c for this model when fitted to the full data set was 313 ($n = 38$, $k = 2$; $R^2 = 0.908$). A linear model optimised for the full data set using PROC NLIN yielded an AIC_c of 278 ($n = 38$, $k = 2$; $R^2 = 0.963$).

The Sharpe-DeMichele relationship was fitted to the constant temperature data (PROC NLIN, method = MARQUARDT), the fluctuating temperature data obtained in the laboratory, and the data collected from natural nests to yield the equation shown in Figure 3. The coefficients of the curve of best fit were $RHO_{25} = 0.7547$, $H_A = 29{,}672.8$, $T_L = 297.0$, $H_L = -81{,}317.6$, $T_H = 308.7$, $H_H = 161{,}196$. A plot of residuals indicated a well-specified model and an adequate fit. Residual sums of squares were 2,998 ($AIC_c = 242$, $n = 38$, $k = 7$; $R^2 = 0.986$), a considerable improvement over the 19,135 ($\Delta AIC_c = 67.0$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using constant temperature data only and over the 7,655 ($\Delta AIC_c = 32.2$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using the full data set.

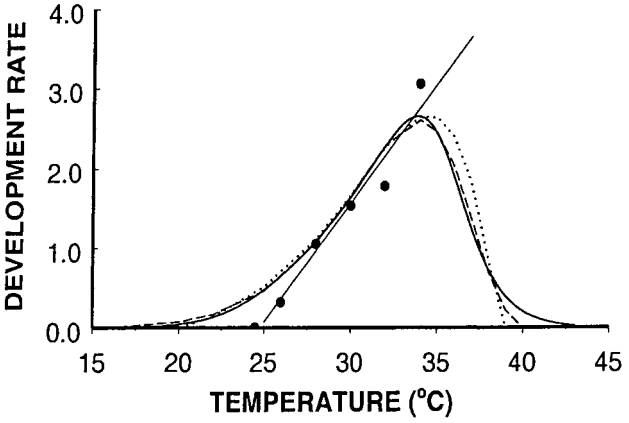

Figure 3. Developmental rate curves of best fit to known development increments for eggs of *Carettochelys insculpta* incubated under constant and fluctuating temperature regimes in the laboratory and regimes in natural nests. *Solid curve*, Sharpe-DeMichele model; *dashed curve*, Dallwitz-Higgins model; *dotted curve*, unconstrained polynomial. The solid straight line represents the linear model. Developmental rate is incremental change in head width, expressed as a percentage of final hatchling head width per day. Note that only data for the constant temperatures can be shown, and as such, the data shown are a small part of those used to derive the models. All three curvilinear models broadly agree.

The corresponding Dallwitz-Higgins model obtained using the same data (PROC NLIN, method = DUD) is shown in Figure 3, again supported by a satisfactory distribution of residuals. The coefficients of the curve of best fit were $b_1 = 2.5969$, $b_2 = 23.1947$, $b_3 = 33.8855$, $b_4 = 2.5829$, $b_5 = 0.00523$. Residual sums of squares were 3,096 ($AIC_c = 243$, $n = 38$, $k = 6$; $R^2 = 0.985$), again a considerable improvement over the 19,135 ($\Delta AIC_c = 66.8$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using constant temperature data only and over the 7,655 ($\Delta AIC_c = 32.0$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using the full data set. The Dallwitz-Higgins model showed no significant improvement over the Sharpe-DeMichele model ($\Delta AIC_c = 0.20$, $w = 0.52$, $n = 38$, $k = 2$).

As a check against potential constraint of form by the Sharpe-DeMichele and Dallwitz-Higgins models, a loosely constrained sixth-order polynomial was fitted to the data also (PROC NLIN, method = DUD; Fig. 3). The coefficients were $b_1 = 58.44$, $b_2 = -14.165$, $b_3 = 1.3124$, $b_4 = -5.9009 \times 10^{-2}$, $b_5 = 1.2961 \times 10^{-3}$, $b_6 = -1.1094 \times 10^{-5}$. Residual sums of squares

were 3,026 ($AIC_c = 242$, $n = 38$, $k = 6$; $R^2 = 0.985$), again a considerable improvement over the 19,135 ($\Delta AIC_c = 66.7$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using constant temperature data only and over the 7,655 ($\Delta AIC_c = 31.9$, $w = 1.00$, $n = 38$, $k = 2$) for the linear model established using the full data set. The polynomial model showed no significant improvement over the Sharpe-DeMichele model ($\Delta AIC_c = 0.35$, $w = 0.54$, $n = 38$, $k = 2$) or the Dallwitz-Higgins model ($\Delta AIC_c = 0.15$, $w = 0.52$, $n = 38$, $k = 2$). Clearly, the form of the polynomial of best fit indicates that the Sharpe-DeMichele and Dallwitz-Higgins models are appropriately specified. Indeed, all three relationships are remarkably similar (Fig. 3).

Observed and predicted development increments for the four models applied to the cyclic laboratory experiments (Table 1) and the field nests (Table 2) also provide an indication of the relative performance of the models. All four models provided adequate predictions for at least some nests with intermediate temperatures, that is, when temperatures remained within the linear region of each model (26°–34°C). When temperatures varied beyond this intermediate range for a part of each day (hot or cold eggs), the predictions of the linear degree.hours model were no longer adequate, except in one instance, and superior predictions arose from the nonlinear models. There was no practical difference between the predictions of the polynomial, Sharpe-DeMichele, and Dallwitz-Higgins models.

Model Responses

Response of overall developmental rate to changes in mean temperature and daily fluctuations in temperature under the degree.hour and Sharpe-DeMichele models is illustrated in Figure 4. Developmental rate is shown as a deviation (percent of total development per day) from that which would have occurred if temperature had been held constant at the mean. In so doing, Figure 4 illustrates the impact of the daily fluctuations, corrected for mean temperature, on developmental rate.

Under the degree.hour model, overall development rate is

Table 1: Comparison of actual development increments with those predicted from temperature traces by the four models in cyclic laboratory experiments on eggs of the pig-nosed turtle (*Carettochelys insculpta*)

Regime			Embryo Attributes					Model Predictions			
Nominal	Actual	Class	Initial Yntema Stage	Initial Head Width (mm)	Final Yntema Stage	Final Head Width (mm)	Observed Development (%)	Degree. Hour (%)[a]	Polynomial (%)[b]	Dallwitz-Higgins (%)[c]	Sharpe-DeMichele (%)[d]
25° ± 7°C	24.66 (15.74–32.56)	Low	18	6.8	23	10.2	25.5 (23.4–27.8)	26.5	33.2	31.4	30.9
26° ± 6°C	26.08 (19.84–32.02)	Low	18	7.3	24	12.7	41.3 (37.0–45.0)	30.6	38.8	36.6	36.0
28° ± 4°C	28.00 (23.82–32.21)	Med	21	7.7	25	13.2	42.0 (38.6–45.8)	36.7	47.9	44.1	44.1
29° ± 5°C	29.16 (24.09–34.15)	Med	18	6.5	23	11.2	35.5 (32.6–38.7)	30.5	37.5	34.6	34.8
30° ± 6°C	30.09 (23.64–36.36)	High	17	6.1	23	10.7	34.4 (31.6–37.5)	32.0	34.3	29.9	29.7
31° ± 6°C	31.02 (25.12–36.96)	High	15	4.8	22	9.3	33.9	39.7	39.1	33.4	33.0
31.5° ± 6.5°C	31.55 (24.97–38.00)	High	15	5.4	23	9.4	30.8	47.1	39.0	31.8	31.4
32° ± 7°C	32.05 (24.92–39.02)	High	14	4.9	22	9.3	33.6	56.0	36.0	30.6	31.1

Note. Comparison uses each of the degree.hour, Dallwitz-Higgins, Sharpe-DeMichele, and unconstrained polynomial models. Actual temperatures given are the mean daily mean, the mean daily minimum, and the mean daily maximum. Class indicates whether the daily trace transgresses the extremes of sublethal temperatures: low = low-temperature enhancement likely; med = primarily within the linear region; high = high-temperature inhibition likely. Development is embryo head width expressed as a percentage of final head width (mean 13.17 mm). The range given in parentheses below observed development increments is derived from the observed range in final embryo sizes. Residual sums of squares (see footnotes) are calculated from data in this table only, with overall model residuals given in parentheses (total sums of squares = 208,810). For 31° ± 6°C, 31.5° ± 6.5°C, and 32° ± 7°C, $n = 3$.

[a] Residual sum of squares = 2,683 (10,680).

[b] Residual sum of squares = 467 (3,026).

[c] Residual sum of squares = 171 (3,096).

[d] Residual sum of squares = 164 (2,998).

Table 2: Comparison of actual development increments with those predicted from temperature traces by the four models for nests of the pig-nosed turtle (*Carettochelys insculpta*)

			Embryo Attributes			Model Predictions			
Nest	Class	Date Laid	Yntema Stage	Head Width (mm)	Observed Development (%)	Degree Hour (%)[a]	Polynomial (%)[b]	Dallwitz-Higgins (%)[c]	Sharpe-DeMichele (%)[d]
CI98_007	Low	July 15	17 (50 d)	6.42	48.7 (44.7–53.1)	26.1	39.3	37.3	37.9
CI98_009	Low	July 18	18 (45 d)	7.52	57.1 (52.4–62.2)	40.4	49.9	48.6	48.8
CI98_036	Low	August 23	17 (36 d)	5.50	41.8 (39.3–45.5)	36.7	43.4	42.3	42.5
CI98_017	Low/med	July 19	20 (38 d)	8.74	66.3 (60.9–72.2)	60.9	64.8	64.0	64.1
CI98_015	Low/high	July 19	19 (38 d)	8.53	64.8 (59.5–70.5)	56.3	61.1	60.1	60.5
CI98_021	Low/med	July 22	18 (41 d)	7.07	53.7 (49.2–58.4)	64.3	48.1	47.1	47.2
CI98_006	Med	July 15	22 (48 d)	9.63	73.1 (67.1–79.6)	60.57	67.8	66.6	66.7
CI98_004	Med	July 14	20 (49 d)	7.77	59.0 (54.2–64.2)	46.2	55.9	54.3	54.7
CI98_001	Med	July 11	20 (42 d)	8.26	62.7 (57.5–68.2)	50.2	57.3	56.3	56.4
CI98_003	Med	July 12	19 (41 d)	7.75	58.8 (54.0–64.0)	50.5	56.4	55.2	55.3
CI98_013	Med	July 19	19 (38 d)	7.77	59.0 (54.2–64.2)	52.4	57.8	57.0	57.1
CI98_002	Med	July 12	22 (41 d)	9.19	69.8 (64.0–76.0)	68.3	72.2	71.3	71.5
CI98_034	Med	August 21	17 (31 d)	5.06	38.4 (35.2–41.8)	40.7	45.3	44.6	44.7
CI98_039	Med	August 24	20 (40 d)	7.31	55.5 (50.9–60.4)	51.8	57.5	56.4	56.5
CI98_064	Med	September 9	20 (35 d)	7.01	53.2 (48.8–57.9)	62.7	63.2	62.6	62.5
CI98_051	Med	September 2	20 (34 d)	7.71	58.5 (53.7–63.7)	62.0	58.7	58.4	58.3
CI98_014	Low/high	July 19	20 (38 d)	8.53	64.8 (59.5–70.5)	59.6	63.2	62.3	62.4
CI98_016	Low/high	July 19	20 (38 d)	8.74	66.3 (60.9–72.2)	58.9	62.7	61.8	61.9
CI98_038	Low/high	August 24	22 (43 d)	7.81	59.3 (54.4–64.5)	66.3	58.7	56.8	56.7
CI98_050	High	September 2	20 (34 d)	7.61	57.8 (53.0–62.9)	78.7	61.6	61.2	61.1
CI98_045	High	August 30	21 (37 d)	8.61	65.4 (60.0–71.2)	83.0	72.4	69.9	69.5
CI98_044	High	August 30	21.5 (37 d)	8.31	63.1 (57.9–68.7)	83.0	72.4	69.9	69.5
CI98_060	High	September 6	22 (35 d)	8.86	67.3 (62.7–73.2)	77.4	71.2	69.1	68.8

Table 2 (*Continued*)

			Embryo Attributes			Model Predictions			
Nest	Class	Date Laid	Yntema Stage	Head Width (mm)	Observed Development (%)	Degree Hour (%)[a]	Polynomial (%)[b]	Dallwitz-Higgins (%)[c]	Sharpe-DeMichele (%)[d]
CI98_053	High	September 3	20.5 (33 d)	7.61	57.8 (53.0–62.9)	79.7	62.5	60.0	59.6
CI98_052	High	September 2	19 (34 d)	6.76	51.3 (47.1–55.8)	60.4	56.4	56.0	55.9

Note. Comparison uses each of the degree.hour, Dallwitz-Higgins, Sharpe-DeMichele, and unconstrained polynomial models. Class indicates whether the daily trace transgresses the extremes of sublethal temperatures: low = low-temperature enhancement likely; med = primarily within the linear region; high = high-temperature inhibition likely. Development is embryo head width expressed as a percentage of final head width (13.17 mm). The range given in parentheses below observed development is derived from the observed range in final embryo head sizes. Residual sums of squares (see footnotes) are calculated from data in this table only, with overall model residuals given in parentheses (total sums of squares = 208,810).

[a] Residual sum of squares = 3,284.5 (10,680).

[b] Residual sum of squares = 564.2 (3,026).

[c] Residual sum of squares = 620.3 (3,096).

[d] Residual sum of squares = 582.9 (2,998).

unaffected by daily fluctuations in temperature, provided that the fluctuations do not carry temperatures below the developmental zero of 24.8°C. For example, for a regime with a mean of 30°C, fluctuations of up to ±5°C have no influence on overall developmental rate and therefore incubation period. In contrast, fluctuations greater than ±6°C about a mean of 30°C carry temperatures below the developmental zero and cause a deviation between the developmental rate anticipated on consideration of the mean and that observed under the fluctuating regime. The effect of this is to yield developmental rates that are greater than, and incubation periods that are less than, those expected on consideration of the mean temperature alone (Fig. 4*A*).

The response of overall developmental rate to variation in mean temperature and variability in temperature under the Sharpe-DeMichele model is much more complex (Fig. 4*B*). For some mean temperatures, overall developmental rate increases above that of the mean temperature as daily fluctuations increase (traces for means of 23°–26°C; Fig. 4*B*). For others, overall developmental rates decrease in response to increasing fluctuations (traces for means of 32°–33°C). For regimes with a mean of 30°C, the fluctuations have a negligible effect on developmental rates until the fluctuations exceed ±5°C, after which overall developmental rates decline. For regimes with a mean of 28°C, there is a negligible effect for fluctuations of ±0°–2°C, substantially enhanced developmental rates for fluctuations in the range of ±4°–8°C, and retarded developmental rates when the daily fluctuations exceed ±9°C. While many of these regimes would not occur in tropical northern Australia, they are not beyond the bounds of possibility in the temperate zones where nest temperatures have been recorded to vary by up to ±9°C (Pieau 1982).

Discussion

Linear relationships between developmental rate and incubation temperature apply to good approximation in laboratory experiments using constant temperatures in some species (Georges et al. 1994; Shine and Harlow 1996) but not others (Muth 1980; Yadava 1980). Regardless of how well we model these relationships using constant temperature experiments, there will be many cases when such modelling will be insufficient to adequately describe development and incubation duration in field nests. Eggs of many shallow-nesting reptile species will experience temperatures that vary beyond the range that supports successful incubation when held constant for some period of each day. In this study, temperatures in surviving nests of *Carettochelys insculpta* varied from 18° to 45°C, well beyond the range of constant temperatures that will support successful incubation (26°–34°C). Modelling development requires that we incorporate rates during these relatively brief daily exposures to more extreme sublethal temperatures. The nonlinear approaches adopted in this article, drawing from the literature on development of insects (Sharpe and DeMichele 1977; Dallwitz 1984; Dallwitz and Higgins 1992), incorporate such rates and outperformed the linear degree.hours model in describing the relationship between developmental rate and incubation temperature in *C. insculpta*.

The influence of fluctuating temperatures on insect developmental times has long been a focus of study in entomology (reviewed by Wagner et al. 1984; Liu et al. 1995), primarily because of the importance of developmental time for the population dynamics of insects of economic importance. The literature contains conflicting reports on the effects of fluctuating temperatures on insect development (Eubank et al. 1973). Some studies have shown that insect developmental times decrease

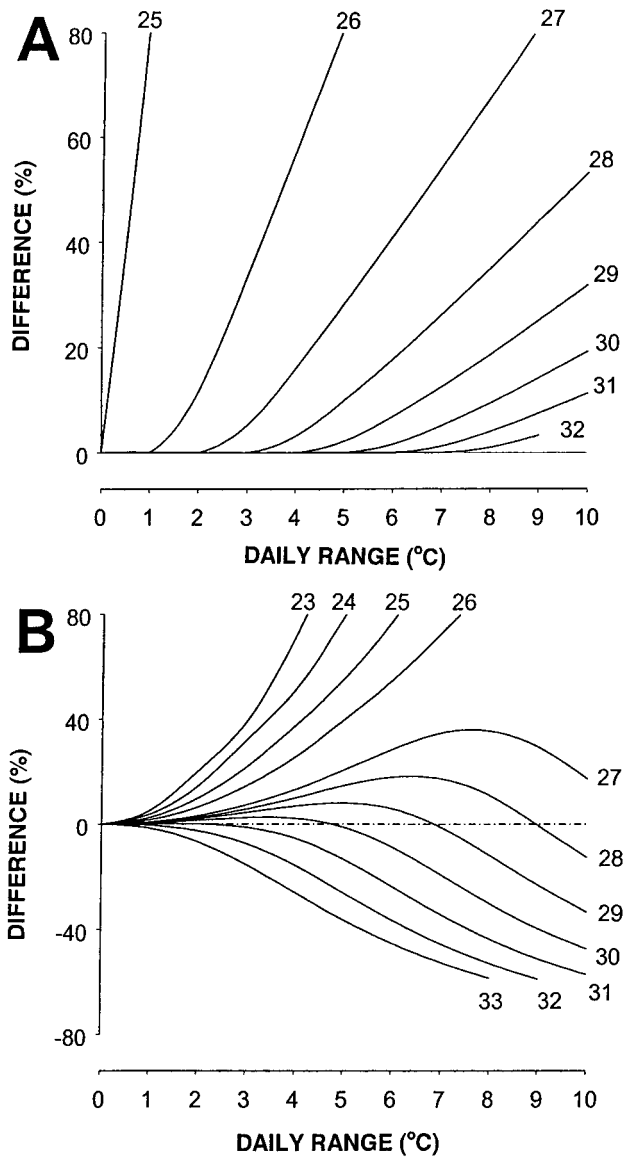

Figure 4. Sensitivity analyses showing the difference in developmental rate (percent of total development per day) for a fluctuating temperature regime from that which would occur if temperatures were held constant at the mean. *A*, Degree.hour model; *B*, Sharpe-DeMichele model. Daily range is expressed as $\pm$R; that is, a value of $\pm$5 equates to a range of 10°C. Under the degree.hour model, fluctuations can only have no effect or increase developmental rate relative to the mean. The responses under the Sharpe-DeMichele model are much more complex.

under fluctuating temperatures compared with constant temperature regimes with the same mean, and others have shown them to increase, while others have found no effect of temperature fluctuations at all (Hagstrum and Hagstrum 1970; Morris and Fulton 1970; Hagstrum and Milliken 1991). Similar conflicting results are available in the more limited literature on reptile development under fluctuating regimes. For example, Georges et al. (1994) found that fluctuations of up to $\pm$8°C about a mean of 26°C had no effect on incubation period for the marine turtle *Caretta caretta*, whereas fluctuations of $\pm$9.75°C about a mean of 23°C substantially reduced the incubation period of the alpine skink *Bassiana duperreyi* compared with that at a constant 23°C (Shine and Harlow 1996).

Even under the linear assumptions of the degree.hour model, the mathematical nuances are such that under some circumstances, incubation will be reduced, and under others, it will be unaffected by diel fluctuations in temperature. Which outcome is observed depends on the relative positions of M and T_0 (and so the species under consideration) and the magnitude of R. When temperatures do not transgress below the developmental zero each day ($T_0 \leq M - R$), the accelerated development at temperatures above the mean each day is perfectly balanced by the retarded development that occurs below the mean. Incubation period should not be affected by the fluctuations (Fig. 4*A*), a prediction in agreement with the outcomes of experiments on the marine turtle *C. caretta* (Georges et al. 1994). When the fluctuations do carry temperatures below the developmental zero ($T_0 > M - R$), the accelerated development at temperatures above the mean is no longer balanced by the retarded development at temperatures below the mean because negative rates of development are not possible. Because of the unbalanced compensation, wide fluctuations universally accelerate development, and reduce incubation period, compared with that expected from mean temperature (Fig. 4*A*). Retardation of development by daily fluctuations in temperature is not a prediction of the degree.hour model.

When curvilinearity of the Sharpe-DeMichele and Dallwitz-Higgins models are introduced, many more possibilities exist (Fig. 4*B*). The complex behaviour arises through the interplay of two competing influences. The first is unbalanced compensation at low temperatures, as occurred in the degree.hour model, leading to accelerated development compared with that predicted from the mean. The second is high-temperature inhibition, where developmental rates are retarded during the brief daily excursions of temperature above those that could be tolerated if held constant. For regimes with mean temperatures of 23°–26°C, the first influence dominates, and apart from a near asymptotic approach of developmental rates to 0 as temperatures decline, the curves behave in similar fashion to those of the degree.hour model. For regimes with means in the high range of 32°–33°C, daily excursions into high temperatures that retard development dominate, and overall developmental rate is reduced below expectation on consideration of the mean. At 30°C, the two influences initially balance out, and overall developmental rate and incubation period are unaffected until the fluctuations become sufficiently extreme for high-temperature inhibition to dominate. These varied potential outcomes cover the range of observed outcomes in the

literature and may explain the apparently conflicting results identified by Eubank et al. (1973).

As with insects, incubation period is an important parameter in reptilian life history. Females need to lay their eggs at a time dictated by the constraints on accumulation of resources. They must be laid at a time when seasonal temperatures are conducive to the mobilisation of those resources for vitellogenesis, ovulation, and the deposition of oviducal contributions to the egg and are conducive to female nesting activity. The offspring must hatch at a time conducive to their survival and growth. In addition to providing the time required for development, incubation period provides the link between these often competing requirements of mother and offspring. The model prediction that incubation period is influenced by diel fluctuations in nest temperatures, over and above the influence of mean temperature, adds an additional dimension to nest site selection in reptiles. This additional dimension applies under the linear model when temperatures drop below the developmental zero each day, as is likely to occur in nests laid at high latitudes or altitudes. It applies under the Sharpe-DeMichele and Dallwitz-Higgins models under a wider range of scenarios. Optimal incubation duration can be achieved with many combinations of mean temperature and daily variability in temperature. Shallow-nesting reptiles may preferentially seek out highly variable locations over those that are simply warm on average to optimise incubation duration (Shine and Harlow 1996). Indeed, many burrowing reptiles do this when they choose to nest in shallow excavations rather than deep in their burrows, despite mean soil temperatures being similar at different depths (below the first few centimetres; Hillel 1982).

Knowledge of the relationship between developmental rate and temperature is particularly important for reptiles with temperature-dependent sex determination (Bull 1980; Ewert and Nelson 1991; Janzen and Paukstis 1991). First, sex is irreversibly influenced by temperature only during a thermosensitive period, typically the middle third of incubation (Yntema 1979; Bull and Vogt 1981; Pieau and Dorizzi 1981; Yntema and Mrosovsky 1982; Ferguson and Joanen 1983; Bull 1987; Webb et al. 1987), defined in terms of embryonic stages (Wibbles et al. 1991). This thermosensitive period is readily identified under constant conditions because it coincides with the middle third of incubation in both time and embryonic stage of development. However, when nest temperatures are subject to wide diel fluctuations, seasonal shifts, and abrupt changes brought about by rainfall, the middle third of development measured as embryonic stage (Yntema 1968) will not correspond to the middle third in time. The two become uncoupled when incubation temperature varies daily about a nonstationary mean, as it commonly does in natural reptile nests, and estimation of the middle third of incubation in terms of embryonic stage becomes problematic. Sampling eggs and examining embryos destructively (Schwartzkopf and Brooks 1985) or by candling (Beggs et al. 2000) have been used to overcome these problems but may conflict with study objectives when clutch sizes are small or the study species is of conservation concern. If we are to estimate the thermosensitive period noninvasively, using temperature traces alone, then we require the detailed knowledge of the relationship between developmental rate and incubation temperature that the modelling in this article provides.

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