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# Thermal Models of TSD under Laboratory and Field Conditions

Recent studies have demonstrated a remarkable range of interactions between environmental conditions and developmental attributes and outcomes in reptilian eggs. Rate of embryonic development and length of incubation period (Ewert 1985), yolk reserves remaining at hatching (Allsteadt and Lang 1995a), hatchling size and morphology (Osgood 1978), coloration (Murray et al. 1990; Etchberger et al. 1993), posthatching behavior (Lang 1987; Burger 1991; Janzen 1993; Shine and Harlow 1996), posthatching growth rates (Joanen et al. 1987; Rhen and Lang 1995), and offspring sex (Bull 1980; Ewert and Nelson 1991; Janzen and Paukstis 1991a) may all be directly influenced by the incubation environment. These results have been established primarily 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, variable weather conditions, seasonal trends, thermal gradients within nests, and stochastic events such as rainfall, which temporarily depress nest temperatures, can all be expected to complicate the relationship between nest temperature and developmental outcome. 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 so for a reptile species (Shine and Harlow 1996). Daily fluctuations in temperature also have an impact on phenotypic attributes, including sex (Georges et al. 1994). In this chapter, the evidence in support of an independent influence of variability in temperature on developmental times and offspring sex ratios in reptiles is reviewed. The consequences for translating the results of laboratory experiments into a field context, the context in which sex-determination traits evolved, are explored. In particular, the potential of degree-hour approaches for predicting offspring sex ratios is explored, and these approaches are extended to cover cases where there is a nonlinear response of developmental rate to changes in incubation temperature. An improved algorithm for calculating the daily constant-temperature equivalent (CTE) for a natural nest is presented.

## Key Challenges

On the basis of early laboratory work, reptile nests with mean daily temperatures above the temperature-dependent sex determination (TSD) pivotal temperature, established in the laboratory, were expected to yield one sex, and nests with mean temperatures below the threshold were expected to yield the other. Results of early field studies were in broad agreement with laboratory studies—females typically emerge from hot, exposed turtle nests, males from cool, shaded nests (Bull and Vogt 1979; Morreale et al. 1982;



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Wilhoft et al. 1983) —but it was soon clear that mean nest temperature was not the best predictor of hatchling sex. For example, predominantly female hatchlings of the turtle *Emys orbicularis* emerged from nests that had a longer daily exposure to temperatures below than to temperatures above the pivotal temperature of 28.5°C (Pieau 1982), the opposite of what was expected. The mean temperature in these nests was considerably lower than the pivotal temperature, and on this basis alone should have produced only male hatchlings. In another study, hatchling sex ratios of *Chrysemys picta* were most closely related to time spent between 20.0 and 27.5°C, the upper and lower threshold temperatures, and not to mean temperature in natural nests (Schwartzkopf and Brooks 1985). In yet another study, both mean temperature and variance in temperature were required to account for sex ratio differences among nests of map turtles in the genus *Graptemys* (Bull 1985). A single mean nest temperature was inadequate as a threshold for natural nests, because the mean temperature that best discriminated between male and female nests decreased as temperatures fluctuated more widely each day.

By way of explanation, several authors have noted that because embryonic developmental rates are greater at higher temperatures than at lower temperatures (within limits), more development will occur at temperatures above the mean than below it (Bull and Vogt 1981; Pieau 1982; Mrosovsky et al. 1984a; Bull 1985). An embryo incubating under a daily sinusoidal cycle of temperature will spend 50% of its time at temperatures above the mean, but much more than 50% of development will occur during that time. It seems that the outcome of sexual differentiation depends more on the relative proportion of development taking place above and below the pivotal temperature than on the relative time spent above and below the pivotal temperature.

The first key challenge for those working on reptile sex determination in field nests was to understand precisely how mean temperature and daily variability in temperature interact to influence sexual outcomes. The relationship between temperature and sex in field nests required a concise formulation in order to translate the results of constant-temperature experiments in the laboratory to a field context.

The second key challenge derives from the need to model the relationship between developmental rate and incubation temperature so as to be able to estimate the period in field nests when temperature exerts its influence on sex. Sex is irreversibly influenced by temperature only during a thermosensitive period (TSP), typically the middle third of incubation (Yntema 1979; Bull and Vogt 1981; Pieau and Dorizzi 1981; Yntema and Mrosovsky 1982; Ferguson and Joanen 1983), and it is defined in terms of embryonic stages (Wibbels et al. 1991b). This TSP 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, the progression of embryonic stage, and duration of incubation become uncoupled. Estimating the middle third of incubation in terms of embryonic stage becomes problematic in field nests. Eggs have been sampled and embryos examined, either destructively (Schwartzkopf and Brooks 1985) or by candling (Beggs et al. 2000), to overcome these problems, but this may conflict with study objectives when clutch sizes are small or the study species is of conservation concern. If we are to either use temperature traces alone to estimate the TSP noninvasively or use incubation duration as a surrogate for estimating offspring sex ratios (Marcovaldi et al. 1997; Mrosovsky et al. 1999), then we require detailed knowledge of the relationship between developmental rate and incubation temperature for all temperatures experienced within the natural nests.

Obtaining such detailed knowledge has long been a focus of study in entomology (Wagner et al. 1984; Liu et al. 1995), although there are conflicting reports on the effects of fluctuating temperatures on insect development (Eubank et al. 1973). 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 ± 8°C about a mean of 26°C had no effect on incubation period for the marine turtle *Caretta caretta*, whereas fluctuations of ± 9.75°C about a mean of 23°C substantially reduced the incubation period of the Alpine skink *Bassiana duperreyi* compared to that at a constant 23°C (Shine and Harlow 1996).

The third key challenge faced by those working on sex determination in the field is to predict the likely sex ratios that result from the thermal regime experienced during the TSP, once it has been identified. Temperatures vary during the TSP on two scales—there is periodic variation on a daily scale driven by the cycle of day and night, and these periodic fluctuations track aperiodic variations on a broader temporal scale as the TSP progresses. Variation on the broader scale is caused by chance rainfall events, air temperature variation driven by weather, and seasonal trends in temperature. The daily periodic variation inflates the effective nest temperatures (Georges 1989; Georges et al. 1994) and both sources of variation can cause temperatures to move between the male-producing and female-producing

conditions during the TSP, which complicates prediction of sex rations from nest temperature measurements. The influence of daily temperature fluctuations (Georges et al. 1994) and the influence of variations on a broader time scale during the TSP (Valenzuela 2001b) may well need to be considered independently.

So there are three key practical issues for the field biologist wishing to draw upon the extensive research on TSD in the laboratory. How do we accommodate the interaction between mean nest temperature and daily variation in temperature, both of which are known to influence incubation duration and sexual outcomes? How do we noninvasively identify the period of incubation during which the embryos are influenced by the thermal environment of the nest, which in the field no longer corresponds to the middle third of development in time? Finally, how do we predict the outcome of sexual differentiation under the complex thermal conditions during the TSP, which involve aperiodic variation overlaid by periodic daily fluctuations, especially when conditions involve both the male and female domains?

## Daily Temperature Variation

### *The Degree-Hour Approach*

Early developmental models, based on linearity in the response of developmental rate to changes in temperature and no hysteresis in the action of temperature on developmental rate, were coupled with the temperature summation rule of de Candolle (1855) and Reibisch (1902) to develop the notion of degree-hours widely used in the applied biological sciences under a variety of names (cumulative temperature units, time·temperature equivalents, thermal units, cumulative heat units). Under a degree-hour model, developmental rate increases linearly as temperature ($T$) increases from a developmental zero ($T_0$). No development occurs when temperatures drop below the developmental zero.

$$\frac{ds}{dt} = A(T - T_0) \qquad \text{for } T > T_0 \qquad [1]$$

$$\frac{ds}{dt} = 0 \qquad \text{for } T \leq T_0$$

where $A$ is the rate of increase. The equation is constrained by the biologically realistic assumption that growth cannot be reversed ($A \geq 0$). This is a degree-hour model because developmental rate is simply proportional to temperature, when temperature is measured with respect to the developmental zero (i.e., $T' = T - T_0$) (Georges et al. 1994). The developmental zero can be estimated by regressing developmental rate against temperature, where developmental rate is obtained directly from embryos of sacrificed eggs incubated at a range of temperatures spanning the TSP (Georges et al. 1994), or it can be estimated by regressing the inverse of incubation period against temperature (Georges 1989; Demuth 2001)

Georges (1989) proposed that under fluctuating temperature regimes, female turtles will be produced if more than half of embryonic development occurs at temperatures above the pivotal temperature each day, and males will be produced if more than half of daily embryonic development occurs below the pivotal temperature. If the development that occurs above the pivotal temperature is the same as that which occurs below it, both sexes will be produced. The key statistic for predicting sex ratios from natural nests under this proposition is not the mean temperature or its variance, but rather the temperature above and below which half of development occurs, calculated on a daily basis. This statistic is referred to as the constant-temperature equivalent (CTE) (Georges et al. 1994). A temperature regime that fluctuates about a stationary mean with constant variance will be equivalent, in terms of phenotypic outcomes such as hatchling sex ratios, to a constant-temperature incubator set at the value of the CTE (Georges et al. 1994). The CTE can also be calculated on a daily basis for an assessment of the likely influence that day will have on offspring sex. The CTE statistic can in theory be used across a range of underlying models of development with temperature, but under linear assumptions, it is convenient to consider the CTE as the temperature above and below which half of the degree-hours occur.

A general approach to calculating the CTE when full temperature traces are available draws upon the observation that the CTE is a form of developmental median. The point temperatures obtained from the data logger are interpolated using a procedure such as a cubic spline to yield temperatures that are spaced at equal but arbitrarily small intervals for the day in question (PROC EXPAND, SAS Institute 1988). The CTE for that day is the median temperature, where the contribution of each temperature to that median is weighted by the corresponding value of developmental rate, obtained from the degree-hour model. This is readily achieved using most statistical packages, such as SAS (SAS Institute 1988).

If the daily temperature trace is modeled with a continuous equation, based perhaps on fewer daily measurements or the maximum and minimum only, then the process of

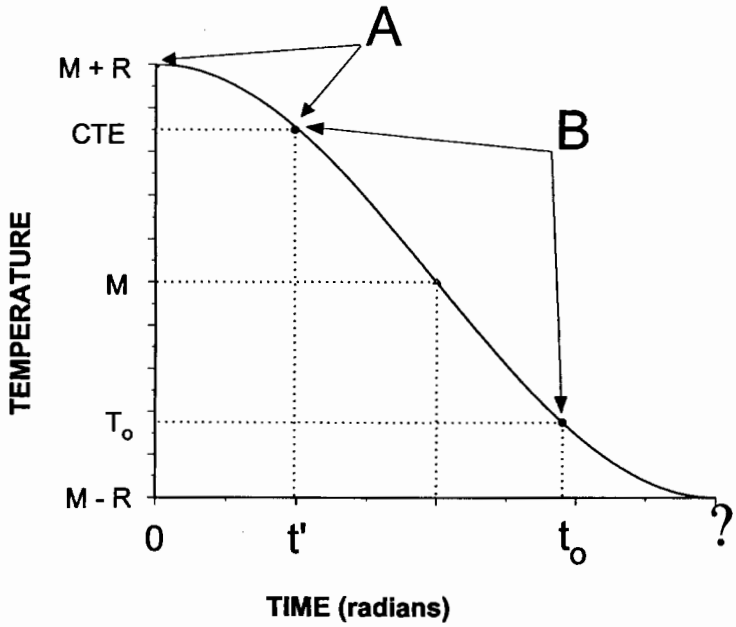


**Figure 9.1.** Segment of a sinusoidal cycle defining the parameters used in the derivation of the CTE statistic. *M*, mean nest temperature; *R*, amplitude of the daily cycle in temperature; *CTE*, temperature above and below which half of development occurs; $t'$, time at which the *CTE* is achieved (in radians); $T_0$, temperature at which development ceases; $t_0$, time at which development ceases (in radians). The *CTE* is obtained by integrating developmental rate along segments A and B of the temperature cycle, setting the two equal, and solving for temperature *T* corresponding to *CTE*.

summation becomes integration. Assuming a daily sinusoidal cycle with mean nest temperature *M* and range 2*R* (Figure 9.1),

$$T = R \cdot Cos(t) + M \qquad [2]$$

it is possible to solve for the temperature above and below which half of development occurs (CTE). The solution can be obtained by integrating developmental rates as they vary along the diel temperature cycle such that

$$\int_0^{t'} \frac{ds}{dt}\,dt = \int_{t'}^{t_0} \frac{ds}{dt}\,dt \qquad [3]$$

where $t'$ is the time at which the CTE is achieved and $t_0$ is the time at which temperatures drop to the developmental zero $T_0$, with time measured from the daily peak in temperature (Figure 9.1). A solution to this integral equation is given by

$$\begin{aligned} CTE &= R \cdot Cos(t') + M && R \geq 0 \\ t' &= \frac{t_0}{2} + \frac{R}{2(M - T_0)} Sin(t_0) - \frac{R}{M - T_0} Sin(t') \\ t_0 &= Cos^{-1}\left[\frac{T_0 - M}{R}\right] && \text{for } T_0 > M - R \\ t_0 &= \pi && \text{for } T_0 \leq M - R \end{aligned} \qquad [4]$$

where the second equation above is solved by application of the standard bisection method. This method relies on the intermediate value theorem, which states that any continuous function that takes on a negative value at one end of an interval and a positive value at the other must pass through zero at least once in between (Hille 1964, 155). The function $f(t')$ is continuous over the interval and

$$f(0) = \frac{t_0}{2} + \frac{R}{2(M - T_0)} Sin(t_0) = -f(t_0) \qquad [5]$$

so when the function evaluates positive at one end of the interval, it evaluates as negative at the other end. Furthermore, if a function is monotonic, decreasing or increasing, or has at most a single stationary point, it will pass through zero once only, that is, the solution will be unique. The function above is monotonic decreasing for $M > T_0$ and monotonic increasing for $M < T_0$ over this interval, provided development occurs at some time during each day, so any solution is unique. Convergence of the bisection method is slow but guaranteed. Note that this method is an improvement on that recommended by Georges (1989), because it converges to a solution for the CTE for all scenarios where temperatures drop below the developmental zero each day. An SAS program for undertaking this analysis is available from the senior author.

Whether we assume a sinusoidal cycle, or measure the daily temperature trajectory directly, the CTE can be calculated for each day, and trends in the CTE during the TSP can be examined for an assessment on the likely outcome of sexual differentiation. For turtles with a single pivotal temperature (TSD Ia), female hatchlings will emerge if the

CTE consistently exceeds the pivotal temperature for sex determination during the TSP, and male hatchlings will emerge if the CTE is consistently less than the pivotal temperature. Both sexes will emerge if the CTE consistently falls on the pivotal temperature or *may* emerge if the CTE moves through or oscillates about the pivotal temperature during the themosensitive period. This assessment would typically be done for temperature traces from the top, core, and bottom of nests, as daily temperature fluctuations are dampened with depth and can vary greatly in magnitude within single nests (Georges 1992; Demuth 2001).

### *Support for the Degree-Hour Model and CTE Statistic*

Strong support for the degree-hour model and CTE statistic stemmed from a reanalysis of the data on map turtles in the genus *Graptemys*. Bull (1985) compared the sex ratios of nests that differed with respect to mean temperature and variance in temperature; he found that nests producing females had higher means or higher variances than nests producing males. A straight line with a negative slope best discriminated between male and female nests, in contrast to the vertical line that would be expected if mean temperature alone determined hatchling sex. Reanalysis of these data using the degree-hour model generated a sloping line as the expected boundary between male-producing nests and female-producing nests (CTE on the pivotal temperature) that was remarkably close to the line produced empirically by Bull (Georges 1989). The effect noted by Bull could be explained entirely by the degree-hour model and use of the CTE statistic.

Specific experiments to address the influence of daily variation in temperature on offspring sex were even more convincing. The marine turtle *Caretta caretta* has a pivotal temperature of around 28.5°C, with 100% males produced under constant-temperature regimes of 26 and 27°C (Georges et al. 1994). When eggs of *Caretta caretta* are incubated at a mean temperature of 26°C, but with progressively increasing daily fluctuations in temperature from ± 0°C to ± 8°C, offspring sex ratios moved progressively from 100% male to 100% female (Georges et al. 1994). Daily fluctuations in temperature alone were demonstrated to influence offspring sex, independent of mean temperature. Furthermore, the sex ratios emerging from the fluctuating temperature regimes were in very close agreement with those predicted from the degree-hour model and the CTE statistic.

Other studies have been equivocal in their support for this approach. Souza and Vogt (1994) found that mean temperature in conjunction with variance and the number of hours at the pivotal temperature were the two indices that best described the sex ratio in *Podocnemis unifilis*. They rejected the CTE approach because all CTEs for their nests were found to lie below the pivotal temperature, yet many such nests produced females. Reanalysis of their data (mean and variance for each nest taken from their Figure 1B and reworked as per Georges 1989) with the degree-hour model and CTE shows that at least nine nests, including all nests with a mean temperature greater than 31°C, have a CTE exceeding the pivotal temperature of 32°C. One nest had a CTE of 33.5°C. It is difficult to tell from the paper which of the data points, in their Figure 1B, Souza and Vogt used to assess the various models of sex determination, but it appears that they may have made a mistake in the computation of the CTE.

Valenzuela et al. (1997) regarded the CTE approach as inappropriate for *Podocnemis expansa*: although the daily and overall mean temperatures observed in their study were well below the pivotal temperature and should therefore, according to their interpretation of the CTE model, produce only males, females were produced. In fact, the primary prediction of the CTE approach is that in many cases where the mean temperature is well below the pivotal temperature, females will be produced, which is in qualitative agreement with the outcomes described for *Podocnemis expansa*. Valenzuela et al. (1997) also make the observation that daily variances in temperature are typically not constant in natural nests, which they regard as a violation of the assumptions of the CTE model. However, we would argue that the CTE should be calculated separately for each day of the TSP and that trends in the CTE should be examined in relation to the pivotal temperature in the same way as one would examine trends in mean temperature if that were thought to be influential. There is no need to assume equal variances across days.

The degree-hour and CTE approach appears to have worked well in a study of the effects of constant and fluctuating incubation temperatures on sex determination in the tortoise *Gopherus polyphemus* (Demuth 2001), though the number of natural nests involved was limited. The CTEs correlated well with offpring sex ($R^2 = 0.76$), much better than did mean temperature ($R^2 = 0.139$), and Demuth used CTEs extensively in analyzing and interpreting his results.

We conclude from these observations and experiments that the degree-hour model and the CTE statistic are of value in predicting offspring sex ratios of some species when temperatures fluctuate on a daily cycle, provided we can assume linearity in the response of developmental rate to temperature change. Linearity might seem a rather unreal-

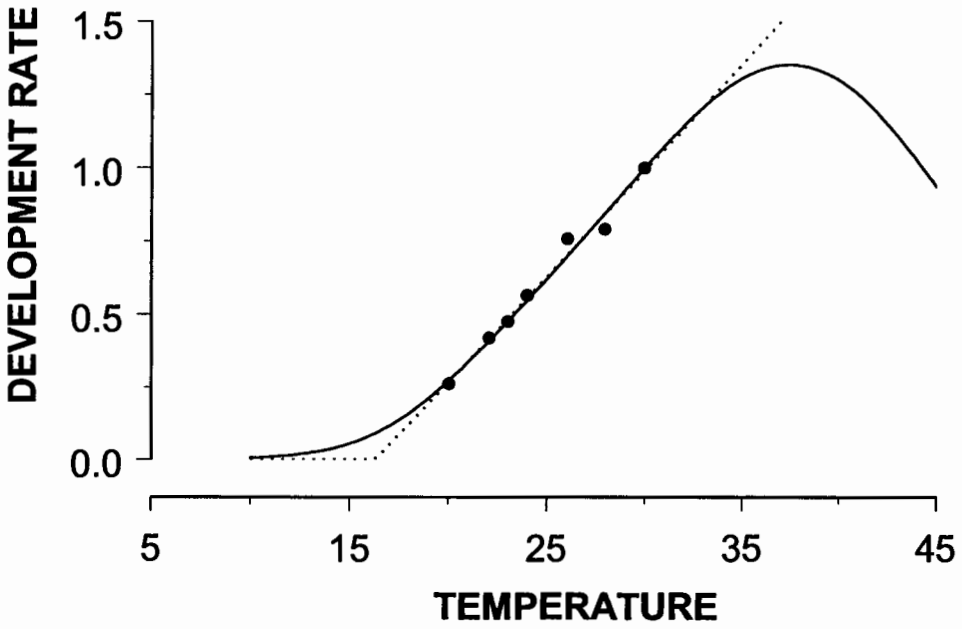


**Figure 9.2.** The Sharpe-DeMichelle model (solid line) and linear degree-hours model (broken line) applied to developmental rates of the temperate-zone lizard *Bassiana duperreyi*. Data are calculated from Figure 2 of Shine and Harlow (1996). Development rate is incremental change in head width, expressed as a percentage of final hatchling head width, per day. The linear model was fitted by least squares regression ($ds/dt = 0.07251\ T - 1.1818$). The Sharpe-DeMichelle model was optimized at lower temperatures using development rates for 23.0 ± 3.75°C and 23.0 ± 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-DeMichelle curve are $RHO_{25} = 0.7$, $H_A = 17199.9$, $T_L = 291.47$, $T_H = 311.71$, $H_L = -64401$, and $H_H = 41965$. Note the close agreement between the linear model and the Sharpe-DeMichelle model at intermediate temperatures.

istic assumption, and it certainly will not be true of all species in all circumstances, but there are indications that it is a reasonable assumption for a wide range of natural scenarios. These indications derive from an unlikely source, the study of nonlinear responses of developmental rate to change in temperature.

### *Why Expect Linearity?*

The most widely accepted model of poikilotherm development is that of Sharpe and DeMichele (1977). They extended the work of Eyring (1935), Johnson and Lewin (1946), and Hultin (1955) to formulate 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 at intermediate temperatures (Figure 9.2). At low temperatures, developmental rate does not decrease linearly to the developmental zero, but rather decelerates to approach zero almost asymptotically. At high temperatures, developmental rate does not increase linearly without limit. Rather, it achieves a maximum, establishing an optimum temperature for development (at least in terms of rates). It then drops precipitously as temperatures rise above the optimum and enzymatic deactivation comes to dominate over the tendency for exponential increase with temperature (Figure 9.2).

Despite its overall curvilinearity, a triumph of the Sharpe-DeMichelle model is its explanation of a strong linear response at intermediate temperatures (Figure 9.2), establishing the validity of the degree-hour concept and the CTE approach in the midtemperature region. At low temperatures, developmental rates are greater than anticipated on the basis of a solely exponential relationship (the Eyring equation) because of progressive activation of enzymes with increasing temperature. At high temperatures, developmental rates are lower than would be expected because of the progressive deactivation of enzymes with increasing temperature. These two compensating effects cause a linearization of the Eyring equation at midrange temperatures (Sharpe and DeMichele 1977).

The parameters of the Sharpe-DeMichelle model are presumably subject to selection, and for many species, an advantage of extending the midrange linear region would be to ensure coincident development of eggs at different

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depths within a nest. This is because mean temperature varies little with depth (Collis-George et al. 1968, 14), and mean temperature under linear assumptions is the prime determinant of developmental rate. In shallow-nesting species, eggs experience roughly the same mean temperatures, but quite different thermal ranges, each day (Georges 1989; Demuth 2001). In the presence of a nonlinear response of developmental rate to change in temperature, differing daily thermal ranges among eggs would lead to hatching asynchrony. A linear response across the range of temperatures experienced by embryos in natural nests is therefore of likely advantage, and the linearization of the response of developmental rate to midrange temperatures, as described by Sharpe and DeMichelle, would be subject to positive selection. It also means that for many species, and under many circumstances in the field, the degree-hour model and the CTE will yield good estimates of sex ratios for nests.

### *Nonlinear Models and the CTE Statistic*

Notwithstanding the broad applicability of the degree-hour model, there are circumstances where it will fail. Linear relationships apply to good approximation within the limits of constant temperatures that support successful development for some species (Georges et al. 1994; Shine and Harlow 1996), but not all (Muth 1980; Yadava 1980). For these latter species, the degree-hour approach is not appropriate. Also, for many shallow-nesting species, nest temperatures will commonly vary to extremes, well beyond the intermediate linear region of the Sharpe-DeMichelle model, for some period of each day. In many poikilotherms, temperatures that result in embryo death when held constant throughout incubation can readily support development, provided the embryo is exposed to those temperatures for only a limited period each day (Dallwitz and Higgins 1992; Morales-Ramos and Cate 1993; Demuth 2001; Valenzuela 2001b). For example, temperatures in 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) (Georges and Doody, unpubl. data). These brief daily exposures to more extreme temperatures were tolerated and supported development to successful hatching. Again, because the relationship between developmental rate and temperature cannot be expected to be linear at extremes, and because many embryos are experiencing these extremes briefly each day, the CTE statistic calculated using the degree-hour approach can be expected to perform poorly in predicting the outcome of sexual differentiation for embryos in such nests.

The approach to take under these circumstances involves first estimating the parameters of the nonlinear relationship between instantaneous developmental rate and incubation temperature within and beyond the range of temperatures that support successful development when held constant, then applying summation to estimate the proportion of development that occurs above and below the pivotal temperature each day to estimate the CTE.

A computational formula is available for the Sharpe-DeMichelle model (Schoolfield et al. 1981), but the model is more demanding of data than the simpler degree-hour model. Estimating its parameters will usually require estimates of developmental rate for temperatures outside the range of the constant temperatures that support successful development, that is, beyond the constant-temperature survival thresholds (Georges et al. 2005). The necessary data typically cannot be obtained by constant-temperature experiments alone.

### *Survival Thresholds*

Three temperature zones can be identified with respect to reptile development (Figure 9.3). There is a central zone defined by those constant temperatures that support successful incubation (between $T_2$ and $T_3$ of Figure 9.3, often referred to as the critical thermal minimum and the critical thermal maximum, respectively (Ewert 1979)). There are the two absolute lethal limits ($T_1$ and $T_4$), beyond which even brief daily exposure to temperature extremes causes embryonic death. There are the sublethal temperature zones ($T_1 - < T < T_2$ and $T_3 - < T < T_4$) where the embryo can tolerate exposures of short duration each day, and where a positive correlation exists at each temperature between mortality and duration of daily exposure.

The position of these zones in relation to the Sharpe-DeMichele curve or Dallwitz-Higgins (1992) alternative (Figure 9.3) will determine how successful constant-temperature data are in supporting parameter estimates for the models. For example, constant temperatures will support incubation of eggs of the marine turtle *Caretta caretta* (Georges et al. 1994) and the lizard *Bassiana duperreyi* (Shine and Harlow 1996) only in the linear region of the Sharpe-DeMichele model. Data in support of estimates of parameters governing curvilinearity at high and low temperatures cannot be obtained from constant-temperature experiments on these species. In contrast, high-temperature inhibition is clearly evident in constant-temperature experiments conducted on eggs of the lizards *Dipsosaurus dorsalis* (Muth 1980) and *Physignathus lesueurii* (Harlow 2001). At the other extreme, higher developmental rates occur at 22°C in the eggs of the freshwater turtle *Kachuga dhongoka* than would be expected

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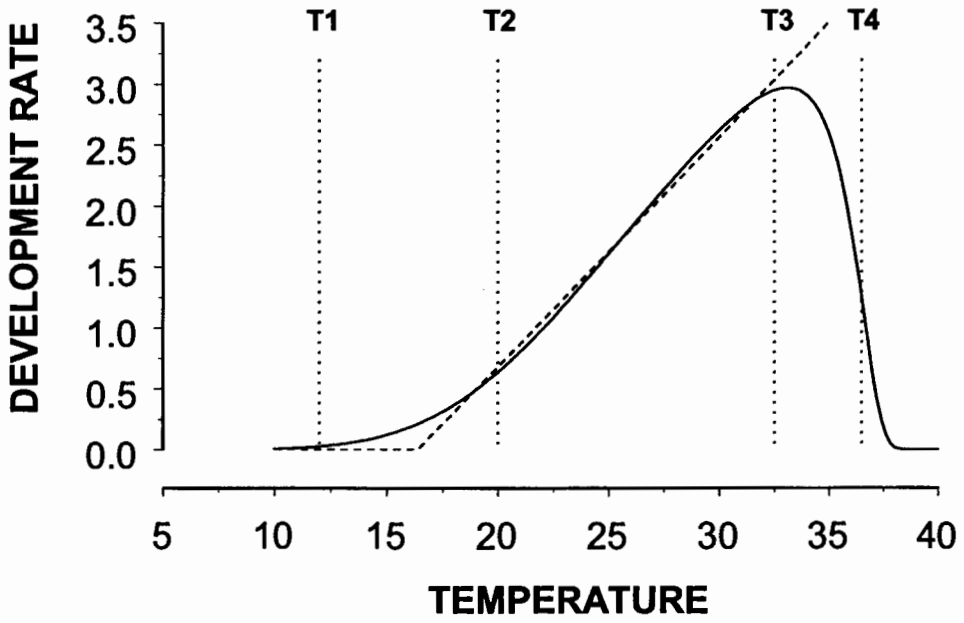


**Figure 9.3.** The Dallwitz-Higgins model (1992) (solid line) and the linear degree-hours model (broken line) applied to hypothetical data. Developmental rate is incremental change in head width, expressed as a percentage of final hatchling head width, per day. Note the close agreement between the linear model and the Dallwitz-Higgins model at intermediate temperatures, similar to that of the Sharpe-DeMichelle model of Figure 9.2. $T_1$ and $T_4$ are the lower and upper absolute lethal limits, outside which even brief exposure causes embryo death or gross abnormality. $T_2$ and $T_3$ are the constant-temperature lethal limits, outside which a temperature held constant throughout incubation will cause embryo death or gross abnormality. Temperatures in the sublethal ranges $T_1$–$T_2$ and $T_3$–$T_4$ will support embryonic development, provided exposure is for a part of each day only, but the duration of exposure that can be tolerated will decline as one moves to extremes.

from consideration of the linear relationship between developmental rate and temperature in the range 25–33°C (reworked from the data of Yadava 1980). For these species, estimation of high or low temperature parameters (respectively) of the curvilinear models may be possible from constant-temperature experiments alone.

In most cases, parameters of the Sharpe-DeMichelle model will have to be estimated by the method of Dallwitz and Higgins (1992). They use data from both constant and fluctuating temperature regimes to estimate the developmental rate function, across a greater range of temperatures than just those constant temperatures that support development. Initial values for the model's parameters are selected, and developmental rate is integrated over each of several temperature traces, generated in the laboratory or taken from field nests, to obtain estimates of the development that should accompany each trace. Each such estimate is then matched to observed development, and the parameters of the model are adjusted iteratively using nonlinear techniques (e.g., PROC NLIN of SAS) to minimize the squared deviations between the two. In this way, we get estimates of the instantaneous developmental rate for temperatures well outside the range of temperatures that support development when held constant.

SAS programs are available in the literature to fit the full Sharpe-DeMichelle model from constant-temperature data (Wagner et al. 1984), or to fit restricted, four-parameter submodels using fluctuating temperature data (Hagstrum and Milliken 1991). The restricted four-parameter models are useful where high-temperature inhibition or low-temperature nonlinearity occur, but not both. Dallwitz and Higgins (1992) provide a FORTRAN program to fit an alternative five-parameter model from uncontrolled temperature traces and accompanying incubation periods. A SAS program for fitting the full six-parameter Sharpe-DeMichelle model using constant and fluctuating temperature traces matched to known developmental increments has not been published, but it is available from the senior author.

Once the Sharpe-DeMichelle function has been determined for the species in question, the CTE can be calculated for each day of incubation as with the degree-hour model. It is the daily median temperature, where tempera-

ture is weighted by its corresponding developmental rate under the Sharpe-DeMichelle or Dallwitz-Higgins models. A program for computing the daily CTE for a given temperature trace based on these underlying models is also available from the senior author.

### Identifying the Thermosensitive Period

Progress of incubation in a natural nest can be estimated by integrating developmental rate along the temperature trace recorded at the same depth as and in the vicinity of the egg. In practice, temperatures taken sequentially using a data logger are interpolated using cubic splines (PROC EXPAND, SAS Institute 1988) to yield temperatures that are evenly spaced at equal but arbitrarily small intervals. The amount of development, $S$, to occur over time increments $t = 1$ to $t = t'$ is then calculated as

$$S = \sum_{t=1}^{t=t'} \frac{ds}{dt} \Delta t \qquad [6]$$

where $ds/dt$ is developmental rate as a function of temperature, as specified by the linear degree-hour, Sharpe-DeMichelle, or Dallwitz-Higgins models described above. It is convenient to express development ($S$) as the percentage of total development and developmental rate as a percentage of total development per day. The TSP will begin when $S = 33.3$ and end when $S = 66.7$, and incubation will be complete when $S = 100$. Note that the TSP will correspond to the middle third of incubation in terms of development (Figure 9.4). It will not correspond to the middle third of incubation in terms of timing or duration.

It is important to note that daily fluctuations in temperature should have no influence on developmental times, over and above that of the daily mean, provided developmental rate and temperature are approximately linearly related over the range of temperatures experienced by the eggs (Georges et al. 1994). Thus, in cases where the degree-hour model is appropriate and temperatures remain above the developmental zero, mean temperature and duration of incubation can be used to estimate the progress of development.

### Predicting Sex

Once the TSP is identified, the CTE statistic provides a major advance over previous approaches to predicting offspring sex ratios from temperature traces because it promises to unambiguously predict sex in cases where the CTE remains below or above the pivotal temperature for all of the TSP. This may well be the case for most nests. Where it will potentially fail is when the CTE crosses the pivotal temperature during the TSP. Mixed-sex nests will not necessarily result when this occurs because switch experiments indicate that irreversible masculinization may occur from a pulse of cool temperatures of only a few days, even if the majority of the TSP is spent in the female-producing domain (Bull and Vogt 1981). How much of the TSP must fall within the female-producing domain to produce females and how much must fall in the male-producing domain to produce males is a question that has not been resolved satisfactorily. Are the embryos presumptive females until a cool pulse of sufficient duration during the TSP switches their developmental trajectory to male? Does the efficacy of a cool pulse depend on the magnitude of the temperature shift as well as its duration? What about two pulses—are their effects cumulative? If so, can degree-hour approaches applied across the TSP provide good predictions of offspring sex? How can the CTE modeling, which applies to individual days, be factored into these calculations?

A number of authors have used correlative approaches to gain insight to the factors other than mean temperature that operate during the TSP to influence offspring sex ratios. Hatchling sex ratios in natural nests of *Chrysemys picta* were most closely correlated to time spent between 20.0°C and 27.5°C, the upper and lower pivotal temperatures (Schwartzkopf and Brooks 1985). A linear regression involving mean temperature and variance best predicted sex ratios of *Podocnemis unifilis* (Souza and Vogt 1994). However, the most thorough field study of this question was undertaken on *Podocnemis expansa* (Valenzuela et al. 1997; Valenzuela 2001b). Valenzuela (2001b) developed a statistical model to account for the effects of heterogenous daily fluctuations of natural nest temperatures on development and sex ratios using such indices as mean temperature, degree-hours (cumulative temperature units), and time spent below the low constant-temperature survival threshold. Degree-hours and time spent below the survival threshold explained significant variations in sex ratio when laboratory and field data were combined (Valenzuela 2001b). In a previous study in which only partial thermal profiles were available, the number of hours greater than 31°C, during a two-day period only, provided the best prediction of offspring sex ratios in field nests (Valenzuela et al. 1997). However, this statistical result had little biological relevance as the two-day period may not have been representative of a single developmental stage or the entire TSP (Valenzuela et al. 1997; Valenzuela 2001b).

These correlative studies establish the deficiency of

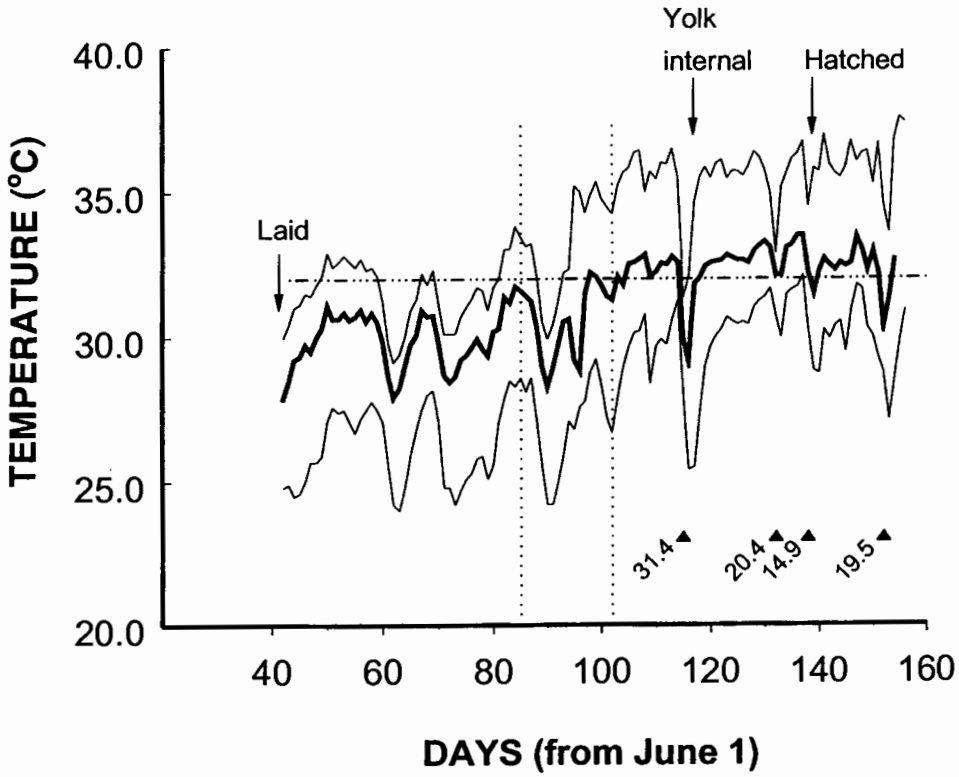


**Figure 9.4.** A temperature trace for the core of a nest of *Carettochelys insculpta* (Ci98–001) showing traces for the maximum and minimum daily temperatures (thin lines) and the constant-temperature equivalent (CTE) (thick line) for the Sharpe-DeMichelle model. Time is in days from June 1, 1998, and temperature is in °C. The known points of laying and hatching are marked. The threshold for sex determination (32°C; Young, unpubl. data) is shown as a horizontal broken line, and the thermosensitive period lies between the two vertical broken lines. Note that the thermosensitive period does not correspond to the middle third of incubation, either in position or duration, owing to the nonstationary trend in temperatures with season. The point of yolk internalization is estimated from the model, and hatching is presumed to be possible after that point. Heavy rainfall or inundation stimulates hatching in this species (Georges 1992; Webb et al. 1986), and four rainfall events of 31.4, 20.4, 14.9 and 19.5 mm are shown. The nest produced male offspring.

mean temperature alone in predicting offspring sex. Those combining a temperature with a duration of exposure, such as the model derived by Valenzuela, provide indirect support for a degree-hour approach, provide a potentially useful approach for predicting offspring sex ratios averaged over a number of nests, and provide hope that accurate prediction of offspring sex from particular nests with a thermal regime that spans the pivotal temperature may one day be possible.

## Discussion

There are a number of practical implications stemming from knowledge that it is proportion of development at a temperature that is important for sex determination and not simply duration of exposure to a temperature, implications that become clearer from the modeling presented above. While the pivotal temperature for sex determination established in the laboratory is a fairly well-defined concept, its definition in a field context is less well defined. The boundary between male-producing and female-producing conditions depends on both mean nest temperature and the magnitude of daily fluctuations in temperature (Bull 1985; Georges et al. 1994). The CTE provides a means of evaluating a nest against the pivotal temperature established under constant conditions in the laboratory, whether it is calculated under the degree-hour model (Georges et al. 1994) or the more complex nonlinear scenarios (as yet untested). The CTE, as a single value extracted from the complex daily thermal regime of a natural nest, promises to be of

considerable practical utility, especially if coupled with the degree-hour approaches applied to the aperiodic variation in temperature across the TSP (Valenzuela 2001b).

Regimes for monitoring temperatures in reptile nests for study of sex determination will need to consider daily variation in temperature if the nests are shallow. Spot temperatures each day are of little value. Temperatures monitored only in the core of the nest fail to recognize the contribution of temperature fluctuations on offspring sex, and that there are likely to be strong gradients in the magnitude of those fluctuations with depths above 30 cm (Georges 1992; Demuth 2001). Ideally, each nest should be fitted with three probes, one immediately above the top egg, one in the core of the nest, and one at the bottom of the nest chamber.

The foundation for using incubation period as a surrogate for temperature in predicting offspring sex is weak, especially for species that have shallow nests. Under the linear model describing the relationship between developmental rate and incubation temperature, and probably under all but extreme cases of nonlinearity, incubation period will be closely linked to mean nest temperature. Sex, on the other hand, will be influenced by both mean temperature and variability in temperature. In this sense, the average developmental rate reflected by incubation period and the thermal influences important for sex are uncoupled, and incubation period will not necessarily be a good basis for predicting sex ratio, especially in the case of individual nests.

Finally, the approaches above are of value not only in reconciling the results of laboratory and field studies, but also in reconciling disparate results of laboratory studies where cyclic temperature regimes are applied (Paukstis et al. 1984; Packard et al. 1991; Georges et al. 1994).

Clearly, the relationship between incubation temperature and development, and understanding how the form of this relationship influences the interplay between the thermal environment of a nest and the outcome of sexual differentiation is critical for reconciling laboratory and field data. From the insights derived from the degree-hour approaches, in general, and the formulation of the CTE statistic, in particular, we now understand why mean nest temperature is a poor indicator of hatchling sex ratios. We can understand why indicators such as hours spent above the threshold, hours above 30°C, degree-hours above 31°C, and other related indices perform better than the mean in predicting sex in empirical approaches such as multiple regression, but have a theoretical foundation for preferring the CTE if the assessment is to be made on a day-by-day basis.

The degree-hour approaches and their nonlinear counterparts, coupled with the CTE statistic, provide us with a general framework for integrating experiments using constant temperatures with those in the field or laboratory using fluctuating regimes, but there is still work to be done. These approaches adequately integrate the effects of average temperature and periodic daily variation in temperature, but we have yet to find a satisfactory model for integrating the effects aperiodic variation in temperature across the TSP with those of average temperature. The nonlinear approaches outlined in this paper have yet to be tested in studies of reptile sex determination, either in the laboratory or in the field. Nor have we applied these ideas to species with dual thresholds: Are degree-hours above the high pivotal temperature in turtles with dual thresholds equivalent in their feminizing effect to degree-hours below the low pivotal temperature?

If we look beyond the practical application of these models to ecological implications, the models discussed in this paper yield important insights. They explain why mixed sex ratios occur in more nests than would be expected from the very narrow pivotal temperature range of many species, even in the absence of gradients in mean temperature with depth (Georges 1992; Demuth 2001). The models provide us with more scope for exploring how reptiles with TSD might respond to climatic change, latitudinal variation in climate, or other disturbances to the incubation environment, because they identify a range of additional parameters that shallow-nesting species can manipulate in order to compensate for climatic change or variation with latitude. We have focused on the outcome of sexual differentiation as the phenotypic trait of interest, but the approaches used to model sex may apply equally well to other phenotypic traits, especially those without a posthatching metabolic component, such as coloration or morphometrics. This latter aspect would be a fruitful area for further investigation in both reptiles (Shine and Harlow 1996; Doody 1999) and insects.