THE JOURNAL OF EXPERIMENTAL ZOOLOGY 270:432–444 (1994)

Hatchling Sex in the Marine Turtle *Caretta caretta* Is Determined by Proportion of Development at a Temperature, Not Daily Duration of Exposure

ARTHUR GEORGES, COLIN LIMPUS, AND ROS STOUTJESDIJK
Applied Ecology Research Group, University of Canberra, Belconnen, ACT 2616, Australia (A.G., R.S.); and Queensland National Parks and Wildlife Service, North Quay, QLD 4002, Australia (C.L.)

ABSTRACT Mean daily temperature in natural nests of freshwater turtles with temperature dependent sex determination is a poor predictor of hatchling sex ratios when nest temperatures fluctuate. To account for this, a mathematical model has been developed on the assumption that hatchling sex depends on the daily proportion of embryonic development that occurs above the threshold temperature for sex determination rather than the proportion of time spent above the threshold. The model predictions are borne out by experiments using the marine turtle *Caretta caretta*. Average developmental rates, both overall and during the period that sexual differentiation is sensitive to temperature, are unaffected by diel fluctuations about the mean incubation temperature. Sex ratios, on the other hand, were affected by diel fluctuations and ranged from ca. 100% males under regimes 26 ± 0°C and 26 ± 3°C to 100% females for regimes 26 ± 7°C and 26 ± 8°C. These and intermediate sex ratios were in close agreement with model predictions. Demonstration of an impact of temperature on sex, while holding overall developmental rate constant, gives support to hypotheses invoking a direct role for temperature rather than alternative hypotheses invoking overall developmental rate as a more proximal influence on sex. The model explains why mean temperature is a poor predictor of hatchling sex ratios. It urges caution in using "hours above the threshold" for predicting sex ratios, because 1 hr at 1°C above the threshold will not be equivalent to 1 hr at 4°C above the threshold. It provides a general framework for integrating experiments at constant temperatures with those in the field or laboratory using fluctuating regimes. It provides greater scope for exploring how reptiles with temperature dependent sex determination might respond to climatic change or other disturbances to the incubation environment. And it provides an explanation of why secondary factors such a hydric conditions and oxygen potentials might influence hatchling sex, even if temperature acts directly to influence sex ratios rather than through its influence on overall developmental rate. © 1994 Wiley-Liss, Inc.

The influence of temperature on the outcome of sexual differentiation in reptiles is now well established, having been demonstrated for turtles in eight families (though not Chelidae or Trionichidae), for crocodilians and for some lizards (reviewed by Bull, '80, '83; Ewert and Nelson, '91). For most species of turtle, females are produced at high temperatures and males at low temperatures. The reverse is usually true of crocodilians and lizards (Ferguson and Joanen, '82). Typically, a very narrow range of temperatures, referred to as the threshold temperature, produces both males and females and divides the male producing temperatures from the female producing temperatures (Bull '83). A few species have upper and lower thresholds with females produced at both extremes (Yntema, '76; Gutzke and Paukstis, '84; Webb et al., '87). The critical period for sex determination, during which embryonic sex can be irreversibly influenced by temperature, occurs during the middle third to the middle half of incubation (Yntema, '79; Bull and Vogt, '81; Pieau and Dorizzi, '81; Yntema and Mrosovsky, '82; Ferguson and Joanen, '83; Bull, '87; Webb et al., '87).

Most studies of sex determination in turtles have been conducted in the laboratory and less effort has been directed at field studies involving more than a few nests (but see Vogt and Bull, '84; Bull, '85; Schwartzkopf and Brooks, '85). As a result, the influence of temperature on sex ratios in natural nests is poorly understood. In broad terms, studies of sex determination in the field agree with those involving constant temperature experi-

Received November 29, 1993; revision accepted June 29, 1994.

© 1994 WILEY-LISS, INC.

ments; that is, hot exposed nests produce female turtles and cool shaded nests produce males (Bull, '85). However, wide daily fluctuations in nest temperature, thermal gradients within nests, seasonal variation in nest temperatures, and stochastic events such as rainfall which temporarily depress nest temperatures, can all be expected to complicate the influence of environment on sexual differentiation in natural turtle nests (Reed, '80; Georges, '94).

Mean daily temperature in natural nests of freshwater turtles with temperature-dependent sex determination is a poor predictor of hatchling sex ratios when nest temperatures fluctuate. Five nests of the European Pond Turtle, *Emys orbicularis,* that spent more time each day at male-inducing temperatures below the threshold temperature of 28.5°C than at female producing temperatures produced, with one exception, predominantly female hatchlings (Pieau, '82). Hatchling sex ratios in natural nests of *Chrysemys picta* are most closely related to time spent between 20.0°C and 27.5°C, the upper and lower threshold temperatures, and not mean temperature (Schwartzkopf and Brooks, '85). Both the mean and variance in temperature were required to account for sex ratio differences among nests of map turtles in the genus *Graptemys* (Bull, '85). A single mean nest temperature was inadequate as a threshold temperature for natural nests of *Graptemys* because the mean temperature that best discriminated male and female nests decreased as temperatures fluctuated more widely.

By way of explanation, several authors have noted that because embryonic development 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, '81; Pieau, '82; Mrosovsky et al., '84; Bull, '85). 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 has not yet been determined whether the outcome of sexual differentiation depends on the relative time spent at temperatures above and below the threshold temperature or on the relative proportions of development taking place at temperatures above and below the threshold temperature.

In an attempt to restate these observations in the form of a testable hypothesis, Georges ('89) derived model based on the assumption that females will be produced if more than half of embryonic development occurs at temperatures above the threshold temperature and that males will be produced if more than half of daily embryonic development occurs below the threshold temperature. The model predicts that a) overall developmental rate, and therefore incubation period, will be unaffected by diel fluctuations in temperatures, and that b) hatchling sex ratios will be affected by diel fluctuations. A quantitative relationship for predicting hatchling sex ratios under fluctuating regimes is an integral component of the model. In this paper, we test the model using eggs from the marine loggerhead turtle *Caretta caretta.*

MATERIALS AND METHODS

The model

The specific model tested in this paper defines a constant temperature equivalent (CTE) as the iterative solution to equations

$$\mathrm{CTE} = R \cdot \cos[t'] + M,$$

$$t' = \frac{\pi}{2} - \frac{R}{M - T_0} \sin[t'],$$

where M equals mean daily temperature; 2R equals daily range in temperature; T_0 equals the lower limit of temperatures which support development; and t' equals an intermediate value (in radians) eliminated from the equations in the process of solving for the CTE.

Under the model, the outcome of sexual differentiation in a nest with a daily sinusoidal cycle of temperatures about a stationary mean will be equivalent to that in a constant temperature incubator set at the value of the CTE. The derivation of the above equations is given by Georges ('89) who used the term "effective nest temperature" (T') in place of CTE used in this paper.

The following points are relevant to this paper, but not explicitly covered by Georges ('89). The net amount of development to occur each day (1 day = 2π units) is given by

$$\int_0^{2\pi} \frac{ds}{dt}\, dt = A(M - T_0),$$

where ds/dt is developmental rate. Nett development each day does not depend on R, the magnitude of daily fluctuations, so incubation period will be the same for a fluctuating regime with mean

M as it would for eggs incubated at a constant temperature of M.

It is widely accepted that developmental rate, usually expressed in change of embryo weight per day, is exponentially related to temperature, within limits, according to the Van't Hoff equation: the Q_{10} effect. The departure from linearity that is a feature of this exponential relationship is disregarded in this paper. Instead, the curvelinearity in the relationship between developmental rate and temperature was held to a minimum by choosing rate of change in a linear dimension rather than dry mass an index to developmental rate. The resulting relationship between developmental rate and temperature was linear to close approximation, thus satisfying one of the requirements of the model.

Finally, in a strict sense, the parameter T_0 of the model is the absolute value of the ratio of the intercept and the slope for an approximately linear relationship of developmental rate against incubation temperature *taken over the range of temperatures experienced by the eggs*. The model does not depend implicitly on an ability to extrapolate a relationship which may only be linear to close approximation for the data at hand. When this relationship is approximately linear over the full range of temperatures at which development occurs, the above ratio can be conveniently interpreted biologically as the developmental zero.

Testing the model

Testing the model requires a) calibration by establishing a relationship between developmental rate and incubation temperature and using this relationship to estimate the developmental zero for *Caretta caretta;* b) establishing a relationship between hatchling sex ratios and constant incubation temperature; c) incubating eggs at a male producing mean temperature (say 26°C, Miller and Limpus, '81) but with daily temperature cycles of varying amplitude; d) using the model to calculate constant temperature equivalents (CTEs) for each of these cyclical regimes; e) converting the CTEs to expected sex ratios using the relationship established in (b); and f) comparing the sex ratios expected under the model with those resulting from the cyclical temperature experiments.

Source of eggs

Eggs of *Caretta caretta* were obtained from the rookery at Mon Repos, near Bundaberg in Queensland, during the November to January breeding seasons of 1990 to 1992. Where possible, eggs were collected from nests deposited below the high water mark and destined to be flooded. They were cooled to below 12°C as soon as practicable after collection (Harry and Limpus, '89) and air freighted to Canberra, Australian Capital Territory. In no case did the time between removal of the eggs and setting them in the experimental incubators exceed 72 hr.

Constant temperature experiments

Groups of four eggs were placed in 500 ml circular plastic food containers and covered by moist vermiculite (three parts vermiculite to four parts water, by weight). Water potentials were not measured. Eggs were then systematically allocated across incubators set at 26.0, 27.0, 27.5, 28.0, 29.0, 30.0, 31.0, and 32.0°C so that eggs from each clutch were represented at each temperature. After 5 to 7 days incubation, eggs were checked for a white patch (Thompson, '85), which indicates that development had commenced. Eggs that were not turgid or that lacked the white patch were removed and the remaining eggs were consolidated into the minimum number of containers. Cases of movement induced mortality are well documented (Limpus et al., '79; Parmenter, '80) so extreme care was exercised when inspecting eggs so as not to jolt or rotate them.

The containers were fitted with lids that allowed gas exchange while minimizing water loss. Container weights were monitored during incubation, but replenishment of water was not necessary.

At 26°C, eggs were incubated in a Thermoline Model RI-170 refrigerated incubator. At all other temperatures, eggs were incubated in Labtec water-jacket incubators refitted with Omron E5CS microprocessor controllers and with thermistor probes relocated within the incubation chamber. The incubators were housed in controlled temperature rooms and ambient air temperature in the rooms was maintained at least 5°C below the temperatures maintained in the incubation chambers.

Temperatures were monitored using two AD590 transducer probes placed in close proximity to the eggs of two containers in each incubator. The signals from the AD 590 transducers were amplified by appropriate circuitry, digitized and recorded at 5 min intervals on Unidata Model 7000 data loggers. The apparatus was calibrated against a mercury thermometer certified as accurate to 0.1°C by the National Association of Testing Agencies (NATA) and yielded recorded temperatures to an accuracy of ± 0.2°C. The data were downloaded

automatically to a microcomputer and analysed using SAS (SAS Institute, '88) to produce daily and weekly reports on each incubator.

Water trays were placed in the bottom of each incubator to maintain a high but unmeasured humidity in the incubation chamber.

Cyclic temperature experiments

Groups of ten eggs were placed on beds of moist vermiculite of fixed depth (three parts vermiculite to four parts water, by weight) in 5.5 l circular plastic buckets (inside dia. 21.2 cm, inside depth 18.3 cm). Eggs were evenly spaced to form a ring around the periphery of the inside of the bucket, almost in contact with the walls, and square 4 cm × 4 cm clear plastic partitions were placed between the eggs to reduce cross-infection should one egg come under bacterial of fungal attack. By this arrangement, each egg was identically positioned with respect to the proximal sources and sinks of thermal energy during cyclical experiments. A temperature probe comprising an AD590 transducer was placed between two of the eggs in each bucket, and immediately adjacent to one, at a distance from the wall equivalent to that of the centres of the adjacent eggs. The bucket was then filled with moist vermiculite and a loose fitting lid was set in place. Each bucket was then weighed so that water loss could be monitored and rectified as necessary.

The buckets containing the eggs were placed in programmable refrigerated incubators (Model IM550R with microprocessor control; Clayson Laboratory Equipment, P.O. Box 5401, Brendale, Qld 4500). The incubators were programmed to adjust temperatures to each of six values at a set time each day. The result was that temperature followed the desired sinusoidal curve as a series of discrete steps in the environmental chamber, but as a close approximation for the probe adjacent to the eggs because of the thermal inertia of the eggs, their container and vermiculite (see Fig. 3 of results).

In the first year of the experiment, eggs were incubated under sinusoidal thermal regimes of 26 ± 4°C, 26 ± 6°C, and 26 ± 8°C and in the second year 26 ± 3°C, 26 ± 5°C and 26 ± 7°C. These conditions reflect more the tolerance of *Caretta caretta* embryos to fluctuating temperatures than to conditions experienced by marine turtle eggs in the field. They are, however, well within the range of conditions experienced by shallow-nesting freshwater species (Thompson, '88; Georges, '92). Temperatures adjacent to the eggs were recorded at 5 min intervals, downloaded, and analysed on a daily and weekly basis as for the constant temperature experiments. Humidity in the environmental chambers was maintained at a high but unmeasured level using open water trays.

After 5 to 7 days of incubation, before the critical period for sex determination, the eggs were checked for the white patch and eggs judged not to have commenced development were replaced with eggs from the constant temperature incubator set at 26°C. Again, extreme care was exercised in handling the eggs.

Rates of development

Incubation period is a crude measure of developmental rate for studies of temperature dependent sex determination because rates may change during incubation and the overall rate may not accurately represent developmental rates during the critical period for sexual differentiation. The terminal point of incubation is often difficult to quantify precisely and embryos with different incubation periods may have different terminal sizes but similar rates of tissue growth (Webb et al., '87; Packard et al., '91). Instead of attempting to use whole incubation measures, we chose to monitor developmental rates directly during the middle half of incubation.

Incubation periods were estimated for each of the temperatures nominally assigned to the constant temperature incubators using the predictive relationship:

$$I = 394350\ T^{-2.612}$$

$$R^2 = 0.96,$$

where I is incubation period (days) and T is incubation temperature (°C) (Limpus et al., '83). Fixed intervals were chosen to correspond to 25%, 40%, 60%, and 75% of incubation, calculated separately for each incubation temperature using the above equation, to span the middle half of embryonic development in each case. The middle half of development includes the period during which sexual differentiation is thought to be sensitive to temperature (Yntema, '79; Bull and Vogt, '81; Pieau and Dorizzi, '81; Yntema and Mrosovsky, '82; Ferguson and Joanen, '83; Bull, '87; Webb et al., '87). On the day corresponding to the end of each of these fixed intervals in each incubator, two eggs were removed and opened. The embryos were separated from the yolk and ex-

tra-embryonic membranes, blotted dry, weighed, and fixed in 10% formalin. Where the two embryos differed markedly in size, a third and sometimes a fourth egg was opened. Remaining eggs were allowed to develop to full term.

Embryos were staged by reference to Yntema's standard developmental series established for *Chelydra serpentina* (Yntema, '68) and their head widths were measured (± 0.1 mm) after fixation using a stereo-microscope with a calibrated, graduated eyepiece. Head width was taken as the maximum, including the optic capsules.

Developmental rates during the temperature sensitive period were obtained by regressing head width against duration of incubation for each incubation temperature. These regressions were linear to close approximation, so change in head width (μm/day) was chosen as the index to developmental rate. A linear regression of developmental rate against incubation temperature was then used to estimate the parameter T_0.

In the cyclical incubators, two to three eggs were opened on each of days 20, 36, 48, and 60 of incubation, so that developmental rates under cyclic temperature regimes could be compared with those at a constant 26°C. Remaining eggs were allowed to develop to full term.

Sex determination

Those eggs that remained unopened in both constant and cyclical temperature experiments were allowed to continue incubation. In the first year of experiments, hatchlings were allowed to emerge in their original containers. In the second year, the eggs were transferred to individual containers one week before anticipated hatching. Hatchlings were killed by intracranial injection of nembutal, injected with 10% formalin, labelled and stored in 10% formalin. The right gonad, kidney, and associated ducts of each hatchling were removed, embedded in wax, sectioned and dyed with haemotoxylin and eosin. The sex of each gonad was assessed by examination under a light microscope according to criteria established by Miller and Limpus ('81). Where an assessment was not possible, the second gonad was examined. In one instance it still was not possible to identify the sex of the hatchling.

RESULTS

Calibration of the model

The constant temperature apparatus maintained very effective control over incubation temperatures. When spikes resulting from inspecting the eggs were removed, temperatures typically wandered no more than ± 0.2°C from their overall mean (in the 28°C incubator, it was not more than 0.3°C). Relationships between maximum head width and duration of incubation were linear to good approximation at all incubation temperatures (R^2 = 0.88–0.99), and rate of change of head width was used as an index to developmental rate (see Table 3).

A relationship between developmental rate and mean incubation temperature (Fig. 1) was adequately modeled by the equation

$$\frac{ds}{dt} = 2.3517\ T - 38.49$$

$$(R^2 = 0.93,\ n = 8),$$

where developmental rate (ds/dt) is in μm/day and mean incubation temperature (T) is in °C. From this equation, the developmental zero for *Caretta caretta* was estimated to be 16.4°C (T_0, corresponding to ds/dt = 0).

Application of the calibrated model to a variety of hypothetical temperature regimes, differing both in mean temperature and in the magnitude of diurnal fluctuations about the mean, yielded the tabulated series of constant temperature equivalents (CTEs) shown in Table 1. Under the model, each of these thermal regimes, embryo survivorship permitting, will influence the out-

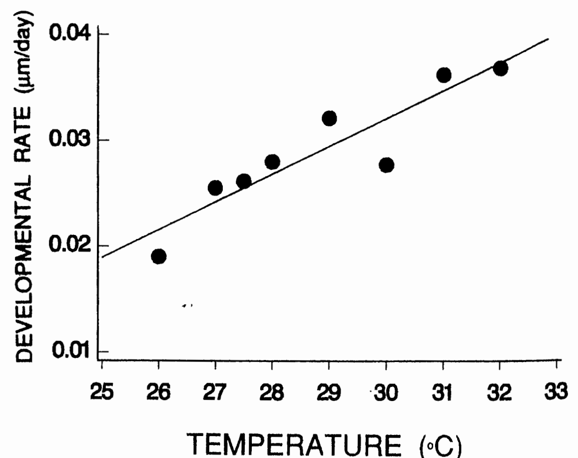

Fig. 1. Relationship between developmental rate and constant incubation temperature for *Caretta caretta* from Mon Repos, Queensland. Developmental rate is rate of change in maximum head width (including the optic capsules) in μm/day.

TABLE 1. Constant temperature equivalents for various combinations of mean nest temperature and daily range in temperature, based on a developmental zero of T_O = 16.4°C [1]

Temperature range (°C)	Mean nest temperature (°C) 25	26	27	28	29	30	31	32
± 0	25.0	**26.0**	27.0	28.0	29.0	30.0	31.0	32.0
± 1	25.1	**26.1**	27.1	28.1	29.1	30.1	31.1	32.1
± 2	25.4	**26.4**	27.4	28.3	29.3	30.3	31.3	32.3
± 3	26.0	**26.9**	27.8	28.7	29.7	30.6	31.6	32.6
± 4	26.6	**27.5**	28.4	29.3	30.2	31.1	32.0	33.0
± 5	27.4	**28.2**	29.1	29.9	30.8	31.7	32.6	33.5
± 6	28.3	**29.0**	29.9	30.7	31.5	32.4	33.2	34.1
± 7	29.2	**30.0**	30.7	31.5	32.3	33.1	33.9	34.8
± 8	30.2	**30.9**	31.6	32.4	33.1	33.9	34.7	35.5

[1]Not all regimes included in the table are conducive to survival of *Caretta caretta* embryos.

come of sexual differentiation as would a constant temperature incubator set at the corresponding value of the CTE. Note that the CTE for a particular thermal regime may differ considerably from the mean temperature and that this difference becomes greater as the magnitude of diurnal fluctuations increases. For a diurnal cycle of fixed magnitude, the discrepancy between the CTE and mean incubation temperature is greatest for small values of mean incubation temperature. The emboldened column of Table 1, corresponding to a mean temperature of 26°C, shows the thermal regimes used to test the model.

Sex determination

Hatchling sex ratios varied with constant incubation temperature in agreement with previous studies (Limpus et al., '83; '85). *Caretta caretta* does not have a threshold represented by the typical narrow range of temperatures. Mixed sexes were produced at constant incubation temperatures ranging from 28°C to 30°C, with the proportion of females increasing with increasing

TABLE 2. Developmental rates (change in head width) and hatchling sex ratios for embryos of Caretta caretta *incubated at a range of constant temperatures* [1]

Nominal temperature (°C)	Actual mean temperature ± SD	Developmental rate µm/day (R^2,n)	Unsexed mortalities	Males	Females	Sex ratio (% females)
26	25.92 ± 0.05	22.0 (0.95,18)	4	25	1	3.8
27	27.10 ± 0.04	25.5 (0.88,12)	0	23	1	4.2
27.5	27.54 ± 0.05	26.2 (0.98,11)	—	—	—	—
28	28.07 ± 0.09	28.0 (0.95,14)	3	11	9	45.0
29	29.10 ± 0.02	32.1 (0.94,12)	1	14	12	46.2
30	30.04 ± 0.03	27.7 (0.96,12)	2	9	17	65.4
31	31.14 ± 0.03	36.2 (0.97,12)	4	0	20	100.0
32	31.89 ± 0.03	36.9 (0.99,10)	4	0	24	100.0

[1]Actual mean temperatures were calculated from temperature traces after removal of spikes resulting from inspection of the eggs. Developmental rates are calculated for the middle half of incubation only. All regression co-efficients were significant at the 0.0001 level.

incubation temperature (Table 2). The relationship between hatchling sex ratio and incubation temperature was modelled using a ramp function (Fig. 2) with the predictive equations

% Females = 0.0	T < 26.6°C
% Females = 20.79 T - 552.34 (R^2 = 0.93)	26.6 < T < 31.4°C
% Females = 100.00	T > 31.4°C

Cyclic experiments

The cyclical temperature apparatus yielded temperature traces that agreed well with the desired sinusoidal cycles of given means and amplitudes (Fig. 3). These cycles were highly reproducible with the minimum varying by only 0.3 to 0.5°C from day to day and the daily maximum varying by only 0.5 to 0.9°C (Table 3). While precise, the statistics of the actual sinusoidal cycles deviated a little from the nominated values (Table 3), but this was of little consequence to the experiment because the actual statistics for the sinusoidal cycles were used in all calculations.

Developmental rates, and ultimately incubation period, were little affected by the magnitude of the diurnal fluctuations about the common mean incubation temperature of ca. 26°C, for embryos that successfully completed incubation (Table 4). The data are suggestive of a decline in developmental rate with increasing magnitude of daily fluctuations, especially for 26 ± 7°C and 26 ± 8°C, but the trend was not significant (F = 1.46; df = 6,49; *P* = 0.21). Incubation periods for embryos incubated at 26 ± 4°C, 26 ± 6°C and 26 ± 8°C were not significantly different from those obtained from a constant 26°C (Table 4; F = 0.27; df = 3,53; *P* = 0.85). Nor was there any indication of differences in Yntema's stage achieved by embryos of the same age incubated under any of the thermal regimes (Table 4). From these observations, it can be concluded that average developmental rates, during the period that sexual differentiation is sensitive to temperature, are unaffected by diurnal fluctuations about the mean incubation temperature, in this case ca. 26°C. This is consistent with the predictions of the model (see Materials and Methods).

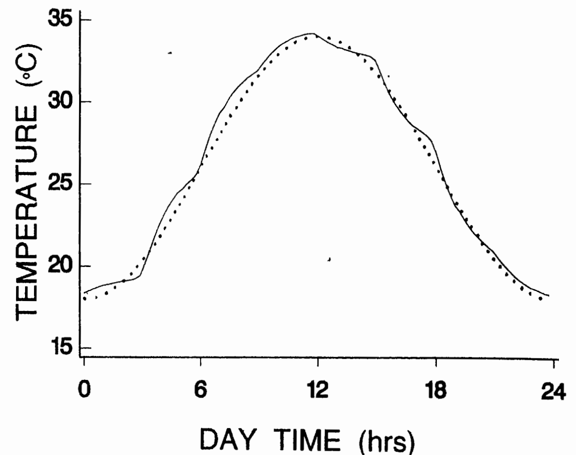

Fig. 3. A graph showing the close agreement between the desired sinusoidal cycle in daily temperatures (dotted line) and the temperatures observed in the Clayson programmable incubators (solid line). This example is for 26 ± 5°C.

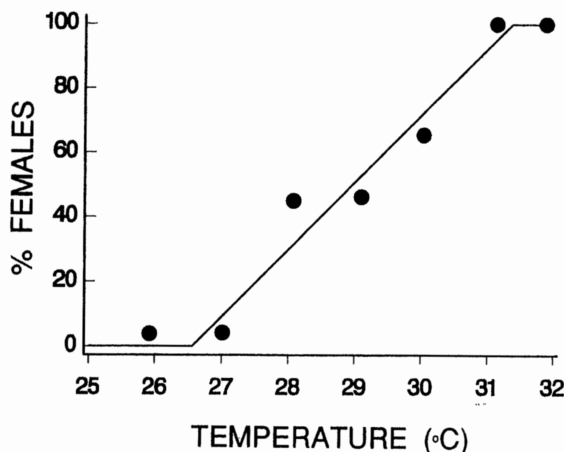

Fig. 2. Relationship between hatchling sex ratio and constant incubation temperature for *Caretta caretta* from Mon Repos, Queensland. A ramp function was used to provide a predictive equation for this relationship (see text).

Testing the model

Applying the model to the actual means and ranges in temperature for the cyclical experimental data yielded CTEs for each incubator (Table 5). If, as under the model, the outcome of sexual differentiation in the cyclical incubators is identical to that in constant temperature incubators set at the corresponding CTE, it is possible to use these CTE values and the relationship between constant temperature and sex ratio (Fig. 2) to predict the hatchling sex ratio expected to emerge from the cyclical regimes. There was no significant difference between the observed sex ratios and those predicted from the model CTEs (Weighted regression, H_0: β = 1, t = 0.85; df = 5; *P* = 0.43).

TABLE 3. Thermal characteristics of the cyclic temperature experiments[1]

Nominal temperature regime (°C)	Actual mean temperature (°C)	Mean daily minimum (°C)	Mean daily maximum (°C)	Mean daily range (°C)	Constant temperature equivalent (°C)
26 ± 0	25.9	—	—	—	25.9
26 ± 3	26.1	23.0 (22.8–23.1)	29.1 (28.8–29.3)	3.1 (2.9–3.2)	27.0
26 ± 4	26.8	23.0 (22.8–23.2)	30.6 (30.3–30.8)	3.8 (3.7–3.8)	28.1
26 ± 5	25.8	20.5 (20.4–20.7)	31.0 (30.7–31.3)	5.2 (5.1–5.4)	28.2
26 ± 6	26.4	20.5 (20.3–20.7)	32.4 (32.2–32.7)	6.0 (5.9–6.1)	29.4
26 ± 7	26.0	18.7 (18.4–18.9)	33.1 (32.9–33.4)	7.2 (7.1–7.3)	30.1
26 ± 8	26.3	18.3 (18.1–18.6)	34.2 (33.8–34.7)	7.9 (7.6–8.2)	31.0

[1]Ranges are given in parentheses. The constant temperature equivalent was calculated using the model equations and the observed mean daily temperatures and ranges. T_o = 16.4°C.

The success of the model in predicting hatchling sex ratios from the fluctuating temperature regimes is strikingly demonstrated in Figure 4. Had mean incubation temperature been the prime determinant of sex, then no relationship at all would have been evident.

DISCUSSION

The results give strong support to the proposition that it is daily proportion of development occurring at a temperature rather than time spent at that temperature that is important for sexual

TABLE 4. A comparison of developmental progress under cyclic conditions with a mean of 26°C[1]

Nominal temperature regime (°C)	Actual mean temperature (°C)	Yntema's stage Mean head width (± SE) 25% (day 20)	45% (day 36)	60% (day 48)	75% (day 60)	Developmental rate (μm/day)	Incubation period (days)
26 ± 0	25.9	13 0.38 ± 0.02 mm n = 4	16–18 0.91 ± 0.01 mm n = 6	19/20–21/23 1.05 ± 0.03 mm n = 4	23–25 1.32 ± 0.05 mm n = 5	22.0	80.6 (76–86) n = 21
26 ± 3	26.1	13–14 0.40 ± 0.02 mm n = 3	16–17 0.90 ± 0.01 mm n = 3	19/20–21/23 1.03 ± 0.01 mm n = 2	24 1.34 ± 0.02 mm n = 2	23.9	—
26 ± 4	26.8	13 0.46 mm n = 1	18 0.90 ± 0.00 mm n = 2	21–23 1.12 mm n = 1	25 1.47 ± 0.03 mm n = 3	22.3	80.2 (79–82) n = 17
26 ± 5	25.8	14 0.45 ± 0.01 mm n = 3	16–17 0.92 ± 0.00 mm n = 3	21–23 1.04 ± 0.02 mm n = 2	24 1.36 ± 0.01 mm n = 2	23.5	—
26 ± 6	26.4	15 0.59 ± 0.01 mm n = 2	18 0.89 ± 0.12 mm n = 2	21–23 1.09 ± 0.08 mm n = 2	25 1.40 mm n = 1	19.4	80.2 (79–83) n = 17
26 ± 7	26.0	13 0.35 ± 0.03 mm n = 3	15–16 0.81 ± 0.01 mm n = 2	19–20 0.95 mm n = 1	19/20–21/23 1.04 ± 0.03 mm n = 2	18.1	—
26 ± 8	26.3	13 0.41 mm n = 1	14–16 0.62 ± 0.11 mm n = 2	—	—	13.1	80.5 (80–81) n = 2

[1]Incubation period was estimated from the relationship developed by Limpus et al. ('83) to be 79 days at 26°C. Mean head widths and wet weights (±SE) are shown below each stage.

TABLE 5. Hatchling sex ratios for embryos of Caretta caretta *incubated with a mean temperature of ca. 26°C and daily fluctuations in temperature of varying magnitude*[1]

Nominal temperature regime (°C)	Actual temperature regime (°C)	Constant temperature equivalent (°C)	Unsexed mortalities (non-starters)	Males	Females	Observed sex ratio (% Females)	Expected sex ratio (% Females)
26 ± 0	25.9 ± 0.2	25.9	4 (0)	25	1	3.8	0.0
26 ± 3	26.1 ± 3.1	27.0	6 (1)	23	1	4.2	9.6
26 ± 4	26.8 ± 3.8	28.1	7 (2)	10	3	23.1	31.4
26 ± 5	25.8 ± 5.2	28.2	12 (0)	11	7	38.9	34.8
26 ± 6	26.4 ± 6.0	29.4	4 (0)	7	8	53.3	58.6
26 ± 7	26.0 ± 7.2	30.1	21 (5)	0	9	100.0	74.4
26 ± 8	26.3 ± 7.9	31.0	29 (0)	0	1	100.0	92.5

[1]The observed sex ratios are compared with those expected on the basis of constant temperature equivalents computed from the model. Constant temperature equivalents are converted to expected sex ratios using the relationship shown in Figure 2.

differentiation. This in itself, is not an entirely novel suggestion (Bull and Vogt, '81; Bull, '85; Pieau, '82; Mrosovsky et al., '84), but the model of Georges ('89) restates the hypothesis quantitatively, allowing rigorous testing and unambiguous exploration of its implications. These implications are important for our understanding of temperature dependent sex determination in field nests with temperatures that fluctuate widely each day. The consequences of the model are therefore greater for shallow nesting freshwater species whose nest temperatures fluctuate widely (up to 18°C in *Emys orbicularis;* Pieau, '82) than for marine species with deep nests where diel fluctuations in temperature are modest (0.5–1.0°C in *Chelonia mydas*; Morreale et al., '82). Sinusoidal cycles are a good approximation of the daily variation in nest temperatures for shallow-nesting species, though temperatures tend to rise more rapidly than they cool each day (Thompson, '88; Georges, '92).

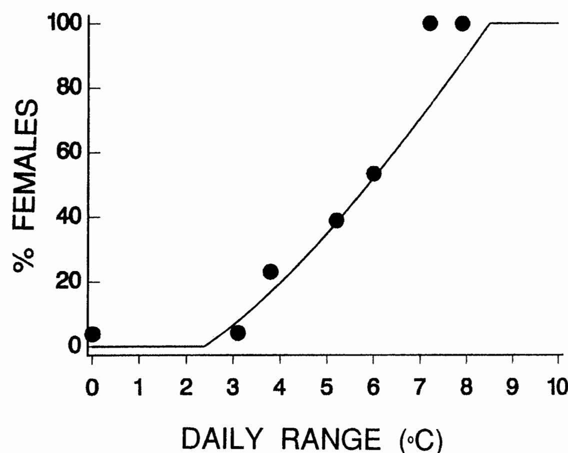

Fig. 4. Relationship between hatchling sex ratio and the magnitude of daily fluctuations in temperature (±R) about a fixed mean of ca. 26°C. The dots represent actual sex ratios to emerge from the cyclical experiments. The line shows the sex ratio predicted by the model.

Consider first the practical implications of the model. While threshold temperature established in the laboratory is a fairly well defined concept, there is no equivalent concept in a field context when nest temperatures fluctuate daily. The boundary between male producing and female producing conditions will depend both on mean nest temperature and the magnitude of daily fluctuations in temperature (see the data of Bull, '85 and the reinterpretation by Georges, '89). Field studies will need to monitor both of these parameters. Provided temperatures vary each day approximately according to a sinusoidal cycle (see Thompson, '88; Georges, '92), the thermal regime of a nest can be converted to a constant temperature equivalent using the model equations. This CTE can then be used to estimate a predicted sex ratio for the nest, by comparing the CTE to a threshold temperature established in the laboratory under constant conditions. If more complex diel cycles in nest temperature are evident, perhaps because of a particular daily pattern of exposure to sun and shade, then the approach outlined in the Appendix to this paper is recommended. When

studying the effects of seasonal trends in nest temperature on sex determination, it will be necessary to consider trends in both daily mean and daily variance in temperature, and from these to calculate seasonal trends in the CTE, if patterns of hatchling sex ratios are to be interpreted in the context of laboratory thresholds. It is the ability of the model to provide a single value from a fluctuating temperature regime, a value that can be used to predict hatchling sex ratios, that promises to be of considerable practical utility.

In an ecological context, shallow nesting species may have greater scope than previously suspected for responding to climatic change, environmental disturbance or latitudinal shifts in distribution. Not only can they respond, through natural selection, by shifting their threshold temperature and where they nest in respect to exposure to solar radiation, but adjustment of the relationship between developmental rate and incubation temperature will affect the impact of thermal fluctuations on sex determination, and increasing or decreasing nest depth will profoundly modify the magnitude of the fluctuations themselves. There may even be scope for modifying the daily proportion of development above the threshold temperature required to produce females. We have assumed it to be 50%, and for *Graptemys* sp. (Georges, '89) and *Caretta caretta,* the fit of the model supports this assumption. However, there is scope for this parameter to vary among species.

Eggs of marine species with deep nests experience very modest diurnal fluctuations in temperatures (Morreale et al., '82). These species will have less flexibility in parameters of the model that can be adjusted as an effective response to latitudinal shift or climatic change. This may explain why intraspecific latitudinal variation in the threshold and interspecific variation in the threshold consistent with differences in reproductive pattern, are evident in marine species (*Caretta caretta:* Limpus et al, '85; *Chelonia mydas:* Limpus, unpublished data; *Chelonia mydas* vs. *Dermochelys coriacea*: Mrosovsky et al., '84) but have not yet been demonstrated clearly for smaller freshwater species (Bull et al., '82b). For marine species, adjusting the threshold may be their only option.

The temperature range that produces both sexes is very narrow for most species studied in the laboratory, and it has been argued that, as a result, the effective heritability of threshold temperature is low in natural populations (Bull et al., '82a). If egg temperatures fall outside the narrow range that produces both sexes, genotypic variation in the threshold temperature will have little influence on sex determination. Thermal gradients within nests (Standora et al., '82; Webb and Smith, '84; Georges, '92), often spanning the threshold temperature, will enhance opportunities for genotypic variation in the threshold to be expressed. Furthermore, the model predicts that thermal gradients within nests will be effectively magnified when nest temperatures fluctuate, because the uppermost eggs will experience greater fluctuations each day than the lowest eggs (Wilhoft et al., '83; Thompson, '88; Georges, '92).

Eggs at the top of a nest of *Carettochelys insculpta* varied up to 9.2°C each day compared to 5.2°C for the deepest eggs (Georges, '92). In one such nest, mean temperatures over a day differed by only 0.6°C between top and bottom eggs [mean and range 31.1 ± 2.3°C (SD) and 31.7 ± 4.6°C, respectively]. If we assume for sake of illustration, that T_0 is 23.6°C for this tropical species (crudely estimated from the developmental rates of Webb et al., '86), then the corresponding CTEs for top and bottom eggs are 33.9°C and 31.8°C, respectively with an effective thermal gradient of 2.1°C. This is more than treble the gradient in mean temperature and is of a magnitude sufficient to span the threshold in many species. While field studies involving large number of nests have shown that most nests produce one sex or the other, thermal gradients within nests, inflated by the effect of diel fluctuations, may in part explain the high proportions of nests that produce mixed sexes in some studies (e.g., 20% of nests in *Graptemys ouachitensis,* 30% for *G. pseudogeographica*; Vogt and Bull, '84). These proportions are greater than would be expected on consideration of the range of temperatures that produce mixed sexes in the laboratory. This may in turn provide greater scope for variation in threshold temperature to evolve under natural selection.

One hypothesis providing a general view of the influence of environment on sexual differentiation has the consequences of differing overall developmental rates rather than temperature per se as the determinant of sex (Webb and Smith, '84; Webb et al., '87). Embryos developing slowly differentiate as one sex; those developing rapidly differentiate as the other sex. Under this hypothesis, temperature exerts its influence by altering metabolic and development rates, which presumably modifies the timing of differentiation of the gonad in relation to the hormonal environment within the embryo, and any factor affecting the rate of embryonic development is expected also to

affect sexual differentiation. However, recent studies that have manipulated developmental rate independently of temperature by varying substratum water potentials (Packard et al., '91), oxygen potentials (Etchberger et al., '91), and even genome size (Lockwood et al., '91) have failed to demonstrate an effect of developmental rate independent of temperature. Water potential was reported to affect sex ratios of *Chrysemys picta* embryos incubated under constant temperatures (Gutzke and Paukstis, '83) but attempts to reproduce this result for *C. picta* have failed (Packard et al., '89) and sex determination in *Chelydra serpentina* is unaffected by hydric conditions (Packard et al., '84, '87).

In this paper, we were able to demonstrate an influence on sex ratios, with outcomes varying from ca. 100% males to 100% females, while holding mid-term and overall developmental rates constant. It appears that alternative hypotheses invoking the direct effect of temperature on cellular and hormonal processes (Crews et al., '89; Deeming and Ferguson, '89) are currently the most promising.

Studies of the influence of factors other than temperature on sexual differentiation are nevertheless worthy of further examination. The model makes no predictions on the effect of factors other than temperature when incubation temperature is held constant. However, when temperatures fluctuate appreciably, any factor that modifies the relationship between developmental rate and temperature (and hence T_0) has the potential to influence hatchling sex ratios. Paukstis et al. ('84) found that substratum water potential modifies the influence of temperature on developmental rates in *Chrysemys picta* (see incubation periods listed in their Table 1), and further demonstrated an influence of water potential on sex ratios when temperatures fluctuated between 18°C and 31°C. Under this regime, the CTE would have been about 28.4°C. This is within the range 28.3°C to 29.5°C established as the upper threshold for *C. picta* (Bull et al., '82b) and the experiment of Paukstis et al. ('84) therefore is appropriate for detecting subtle shifts in sex ratio resulting from differences in substrate water potential. While consistent with model predictions, attempts to reproduce the fluctuating temperature experiments of Paukstis et al. have not been successful (Packard et al., '91).

We believe that further experiments are required to test this aspect of the model's predictions. Packard et al. acknowledge differences between their experiments and those of Paukstis et al. In particular, cyclic temperatures were held at a daily maximum for four hours in one study but only for one hour in the other. This would lead to substantial differences in the CTE for the two experiments and may, in addition to the other factors identified by Packard et al. ('91), explain failure of the two studies to agree.

Clearly, the model tested in this paper has important implications for understanding temperature dependent sex determination in natural nests. It explains why mean temperature is a poor predictor of hatchling sex ratios. It recommends caution in using "hours above the threshold" or "hours above 30°C" for predicting sex ratios, because 1 hr at 1°C above the threshold will not be equivalent to one hour at 4°C above the threshold. It provides a general framework for integrating experiments using constant temperatures with those in the field or laboratory using fluctuating regimes. It provides us with greater scope for exploring how reptiles with temperature dependent sex determination might respond to climatic change or other disturbances to the incubation environment. And it provides us with an explanation of why secondary factors such as hydric conditions and oxygen potentials might influence hatchling sex, even if temperature directly affects sex determination rather than through its influence on overall developmental rate.

ACKNOWLEDGMENTS

We would like to thank the members of the Applied Ecology Research Group for the discussions which assisted in formulation of the ideas presented in this paper. Kevin Bearton and the staff of Chave Enterprises, Bundaberg, provided the cold room used in preparing the eggs for transportation to Canberra from Bundaberg. Staff and volunteer assistants of the Queensland Turtle Research Project at Mon Repos assisted in locating nesting turtles to supply eggs for this study. Mike Thompson, Rod Kennett, and Peter Whitehead provided valuable critical comments on a draft of this paper. Mike Palmer-Allen and Mark Carson provided technical assistance. Alex McNee proofread the final draft. This research was funded by the Australian Research Council with initial support from the Conservation Commission of the Northern Territory.

APPENDIX

The model as it stands will not cater for species with dual threshold temperatures, for situations where daily cycles in temperature are not

sinusoidal or for situations where temperatures drop each day below T_0. Nor is the model particularly useful when temperatures cycle about a mean that regresses with season during the period critical for sex determination. A generalization of the model may assist with these more complex scenarios. The model equations given above for CTE are a solution to the following equality (Georges, '89):

$$\int_0^{t'} \frac{ds}{dt}\, dt = \int_{t'}^{\pi} \frac{ds}{dt}\, dt,$$

with

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

where ds/dt equals developmental rate, T equals temperature in °C, T_0 equals the temperature at which development ceases when temperature drops, t' equals time when temperature reaches the point above and below which half of development occurs, and A equals the slope of the developmental rate vs. temperature relationship.

If we take the zero point for temperature as T_0, that is, the temperature at which development ceases rather than arbitrary value of 0°C, then the CTE will occur when

$$\int_0^{t'} T\, dt = \int_{t'}^{\pi} T\, dt,$$

but $\int_{t_1}^{t_2} T\, dt$ is simply a measure of the degree.hours spent between time t_1 and t_2. The CTE can therefore be interpreted as the temperature above and below which half of the degree.hours occur, where temperature T is measured relative to the developmental zero T_0.

In practice, this means we should consider the proportion of degree.hours spent at female and male producing temperatures rather than the proportion of time spent there. If more than 50% of degree·hours are spent each day at female producing temperatures then we should expect female hatchlings. Otherwise we should expect males.

It is a simple matter to calculate the degree.hours (relative to T_0) spent above the threshold temperature regardless of the shape of the daily cycle in egg temperatures. It is also straightforward to calculate degree.hours spent at female producing temperatures for species with dual thresholds, and instead of relating seasonal trends in nest temperatures to hatchling sex ratios, greater insight may be gained from examining trends in the daily proportion of degree·hours spent at female producing temperatures.

LITERATURE CITED

Bull, J.J. (1980) Sex determination in reptiles. Quart. Rev. Biol., *55:*3–21.

Bull, J.J. (1983) Evolution of sex determining mechanisms. Benjamin/Cummings Publ. Co., London.

Bull, J.J. (1985) Sex ratio and temperature in turtles: Comparing field and laboratory data. Ecology, *66:*1115–1122.

Bull, J.J. (1987) Temperature-sensitive periods of sex determination in a lizard: Similarities with turtles and crocodilians. J. Exp. Zool., *241:*143–148.

Bull, J.J., and R.C. Vogt (1981) Temperature sensitive periods of sex determination in Emydid turtles. J. Exp. Zool., *218:*435–440.

Bull, J.J., R.C. Vogt, and M.G. Bulmer (1982a) Heritability of sex ratio in turtles with environmental sex determination. Evolution, *36:*333–341.

Bull, J.J., R.C. Vogt, and C.J. McCoy (1982b) Sex determining temperatures in turtle: A geographic comparison. Evolution, *36:*326–332.

Crews, D., T. Wibbels, and W.H.N. Gutzke (1989) Action of sex steroid hormones on temperature induced sex determination in the snapping turtle (*Chelydra serpentina*). Gen. Comp. Endocrinol., *75:*159–166.

Deeming, D.C., and M.W.J. Ferguson (1989) The mechanism of temperature dependent sex determination in crocodilians: A hypothesis. Am. Zoologist, *29:*973–985.

Etchberger, C.R., J.B. Phillips, M.A. Ewert, C.E. Nelson, and H.D. Prange (1991) Effects of oxygen concentration and clutch on sex determination and physiology in red-eared slider turtles (*Trachemys scripta*). J. Exp. Zool., *258:*394–403.

Ewert, M.A., and C.E. Nelson (1991) Sex determinations in turtles: Diverse patterns and some possible adaptive values. Copeia, *1991:*50–69.

Ferguson, M.W.J., and T. Joanen (1982) Temperature of egg incubation determines sex in *Alligator mississippiensis.* Nature, *296:*850–853.

Ferguson, M.J.W., and T. Joanen (1983) Temperature-dependent sex determination in *Alligator mississippiensis.* J. Zool., London *200:*143–177.

Georges, A. (1989) Female turtles from hot nests: Is it duration of incubation or proportion of development that matters? Oecologia, *81:*323–328.

Georges, A. (1992) Thermal characteristics and sex determination in field nests of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. Aust. J. Zool., *40:*511–521.

Georges, A. (1994) The influence of fluctuating temperatures on hatchling sex ratios: A model and proposed test using *Caretta caretta.* Proceedings of the Australian Marine Turtle Conservation Workshop. Sea World Nara Resort, Gold Coast, 14–17 November, 1990. Australian Nature Conservation Agency, Canberra.

Gutzke, W.H.N., and G.L. Paukstis (1983) Influence of the hydric environment on sexual differentiation of turtles. J. Exp. Zool., *226:*467–469.

Gutzke, W.H.N., and G.L. Paukstis (1984) A low threshold temperature for sexual differentiation in the painted turtle, *Chrysemys picta.* Copeia, *1984:*546–547.

Harry, J.L., and C.J. Limpus (1989) Low temperature protec-

tion of marine turtle eggs during long distance relocation. Aust. Wildl. Res., *16:*317–320.

Limpus, C.J., V. Baker, and J.D. Miller (1979) Movement induced mortality of loggerhead eggs. Herpetologica, *35:*335–338.

Limpus, C.J., P. Reed, and J.D. Miller (1983) Islands and turtles: The influence of choice of nesting beach on sex ratio. In: Proceedings: Inaugural Great Barrier Reef Conference, Townsville, August 28–September 2, 1983. J.T. Baker, R.M. Carter, P.W. Sammarco and K.P. Stark, eds. JCU Press, Townsville, pp. 397–402.

Limpus, C.J., Reed, P., and Miller, J.D. (1985). Temperature dependent sex determination in Queensland sea turtles: Intraspecific variation in *Caretta caretta*. In: Biology of Australian Frogs and Reptiles. G. Grigg, R. Shine, and H. Ehmann, eds. Surrey Beatty and Sons, Sydney, pp. 343–351.

Lockwood, S.F., B.S. Holland, J.W. Bickham, B.G. Hanks, and J.J. Bull (1991) Intraspecific genome size variation in a turtle (*Trachemys scripta*) exhibiting temperature-dependent sex determination. Can. J. Zool., *69:*2306–2310.

Miller, J.D., and C.J. Limpus (1981) Incubation period and sexual differentiation in the Green Turtle *Chelonia mydas* L. In: Proceedings of the Melbourne Herpetological Symposium. C.B. Banks and A.A. Martin, eds., pp. 66–73.

Morreale, S.J., G.J. Ruiz, and E.A. Standora (1982) Temperature-dependent sex determination: Current practices threaten conservation of sea turtles. Science, *216:* 1245–1247.

Mrosovsky, N., P.H. Dutton, and C.P. Whitmore (1984) Sex ratios of two species of sea turtle nesting in Suriname. Can. J. Zool., *62:*2227–2239.

Packard, G.C., M.J. Packard, and G.F. Birchard (1989) Sexual differentiation and hatching success by painted turtles incubating in different thermal and hydric environments. Herpetologica, *45:*385–392.

Packard, G.C., M.J. Packard, and T.J. Boardman (1984) Effects of the hydric environment on metabolism of embryonic snapping turtles do not result from altered patterns of sexual differentiation. Copeia, *1984:*547–550.

Packard, G.C., M.J. Packard, K. Miller, and T.J. Boardman (1987) Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. Ecology, *68:* 983–993.

Packard, G.C., M.J. Packard, and L. Benigan (1991) Sexual differentiation, growth, and hatching success by embryonic painted turtles incubated in wet and dry environments at fluctuating temperatures. Herpetologica, *47:*125–132.

Parmenter, C.J. (1980) Incubation of green sea turtle (*Chelonia mydas*) eggs in Torres Strait, Australia: Effect on hatchability. Aust. Wildl. Res., *7:*487–491.

Paukstis, G.L., W.H.N. Gutzke, and G.C. Packard (1984) Effects of substrate water potential and fluctuating temperatures on sex ratios of hatchling painted turtles (*Chrysemys picta*). Can. J. Zool., *62:*1491–1494.

Pieau, C. (1982) Modalities of the action of temperature on sexual differentiation in field-developing embryos of the European pond turtle *Emys orbicularis* (Emydidae). J. Exp. Zool., *220:*353–360.

Pieau, C., and M. Dorizzi (1981) Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis*. J. Morphol., *170:*373–382.

Reed, P.C. (1980) The sex ratios of hatchling loggerhead turtles: The progeny of two nesting females. Honours thesis, James Cook University, Queensland, Australia.

SAS Institute (1988) SAS/STAT Users Guide. Release 6.03 Edition. SAS Institute Inc., Cary, N.C., 1028 pp.

Schwartzkopf, L., and R.J. Brooks (1985) Sex determination in northern painted turtles: Effect of incubation at constant and fluctuating temperatures. Can. J. Zool., *63:*2543–2547.

Standora, E.A., S.J. Morreale, G.J. Ruiz, and J.R. Spotila (1982) Sex determination in green turtle (*Chelonia mydas*) hatchlings can be influenced by egg position within the nest. [Abstr.] Bull. Ecol. Soc. Am., *63:*83–84.

Thompson, M.B. (1985) Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In: Biology of Australian Frogs and Reptiles. G. Grigg, R. Shine and H. Ehmann, eds. Surrey Beatty and Sons, Sydney, pp. 387–395.

Thompson, M.B. (1988) Nest temperatures in the pleurodiran turtle., *Emydura macquarii*. Copeia, *1988:*998–1002.

Vogt, R.C., and J.J. Bull (1984) Ecology of hatchling sex ratio in map turtles. Ecology, *65:*582–587.

Webb, G.J.W., and A.M.A. Smith (1984) Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni*. Symp. Zool. Soc. London, *52:*319–355.

Webb, G.J.W., D. Choquenot, and P.J. Whitehead (1986) Nests, eggs and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelidae) from northern Australia. J. Zool., London, *1B:*521–550.

Webb, G.J.W., A.M. Beal, S.C. Manolis, and K.E. Demsey (1987) The effects of incubation temperature on sex determination and embryonic development rate in *Crocodylus johnstoni* and *C. porosus*. In: Wildlife Management of Crocodiles and Alligators. G.J.W. Webb, S.C. Manolis, and P.J. Whitehead, eds. Surey Beatty and Sons, Sydney, pp. 507–531.

Wilhoft, D.C., E. Hotaling, and P. Franks (1983) Effects of temperature on sex determination in embryos of the snapping turtle *Chelydra serpentina*. J. Herpetol., *17:*38–42.

Yntema, C.L. (1968) A series of stages in the embryonic development of *Chelydra serpentina*. J. Morphol., *125:* 219–252.

Yntema, C.L. (1976) Effects of incubation temperatures on sexual differentiation in the turtle. *Chelydra serpentina*. J. Morphol., *150:*453–462.

Yntema, C.L. (1979) Temperature levels and period of sex determination during incubation of eggs of *Chelydra serpentina*. J. Morphol., *159:*17–28.

Yntema, C.L., and N. Mrosovsky (1982) Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. Can. J. Zool., *60:*1012–1016.