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Chronology of Sex Determination in the Desert Tortoise (*Gopherus agassizii*)

DAVID C. ROSTAL[1], THANE WIBBELS[2], JANICE S. GRUMBLES[1], VALENTINE A. LANCE[3], AND JAMES R. SPOTILA[4]

[1]*Department of Biology, Georgia Southern University, P.O. Box 8042, Statesboro, Georgia 30460 USA [E-mail: Rostal@gsvms2.cc.gasou.edu; Fax: 912-681-0845];*
[2]*Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170 USA [E-mail: twibbels@uab.edu];*
[3]*Center for Reproduction of Endangered Species, San Diego Zoological Society, San Diego, California 92112 USA;*
[4]*Department of Environmental Science, Engineering and Policy, Drexel University, Philadelphia, Pennsylvania 19104 USA*

ABSTRACT. – **The desert tortoise, *Gopherus agassizii*, possesses a "Pattern Ia" type of temperature-dependent sex determination (TSD). Incubation temperatures of approximately 30.5°C or below produce all males and incubation temperatures of approximately 32.5°C or above produce all females. The results suggest an estimated pivotal temperature (temperature producing a 1:1 sex ratio) of approximately 31.3°C. Sex determination appears sensitive to temperature as early as embryonic stage 15 or before, and as late as stage 21 depending on the specific incubation temperatures utilized. Sexual differentiation of the gonads becomes histologically noticeable between stages 18 and 21, and the gonads show distinct sexual dimorphism by stage 23. The chronology of temperature sensitivity and gonadal differentiation was similar in *G. agassizii* to that reported for several other turtles with TSD. The pivotal temperature was relatively high, and may reflect the relatively warm habitat of *G. agassizii*. The results also provide a basis for predicting hatchling sex ratios in natural populations based on incubation temperatures.**

KEY WORDS. – **Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; sex determination; gonadal differentiation; incubation; histology**

A variety of reptiles possess temperature-dependent sex determination (TSD) (reviewed by Janzen and Paukstis, 1991), including many endangered or threatened species. TSD is of significance in the conservation of endangered reptiles since it can produce highly biased sex ratios (Bull and Charnov, 1989; Wibbels et al., 1991, 1998; Mrosovsky and Provancha, 1992; Mrosovsky, 1994; Hanson et al., 1998) which, in some cases, may not be optimal for the survival of a particular species (Mrosovsky and Yntema, 1980; Morreale et al., 1982). The optimal management of an endangered reptile species with TSD should include the monitoring of hatchling sex ratios produced in a given population (Vogt, 1994; Lovich, 1996). This sort of study is often avoided because it has required sacrificing hatchlings. However, if certain characteristics of TSD are known (e.g., thermosensitive period and pivotal temperature), then hatchling sex ratios can be predicted by monitoring incubation temperatures.

In addition to its conservation significance, the characterization of TSD can also provide insight into its physiology and evolution. For example, comparison of pivotal temperatures among and between species may suggest how environmental temperature selects for specific pivotal temperatures. The period of temperature sensitivity has only been examined in a limited number of reptiles with TSD (reviewed by Wibbels et al., 1991, 1994), yet this information is essential for investigating the physiological basis of TSD.

The purpose of the current study was to generate a general characterization of TSD in the desert tortoise, *Gopherus agassizii*. This tortoise is distributed in the southwestern U.S. and northwestern Mexico and is listed as threatened under the Endangered Species Act (Ernst et al., 1994). As such, the proper management of this species requires an understanding of its reproductive biology, inclusive of TSD. Four species of tortoises have been shown to possess TSD: *Testudo graeca* (Pieau, 1972), *Testudo hermanii boettgeri* (Eendebak, 1995), *Gopherus polyphemus* (Burke et al., 1996), and *G. agassizii* (Spotila et al., 1994; Lewis-Winokur and Winokur, 1995). Spotila et al. (1994) demonstrated that *G. agassizii* possesses TSD but did not address the thermosensitive period or the period of gonadal differentiation. The current study extends the findings of Spotila et al. (1994) by 1) determining the period of temperature sensitivity, 2) examining the chronology of gonadal differentiation, and 3) refining the estimation of the pivotal temperature in *G. agassizii*.

MATERIALS AND MATERIALS

Eggs and Incubation. — Eggs were obtained as part of an ongoing reproductive biology study from a group of desert tortoises maintained at the Desert Tortoise Conservation Center from 1991 to 1993 as described in Rostal et al. (1994b). Female reproductive condition and nesting in the reproductive study group was followed using ultrasonography and palpation (Rostal et al., 1994b). Females were allowed to nest naturally within their outdoor pens and eggs were collected within 48 hrs of laying during May and June 1992. Eggs were incubated in Precision environmental cham-

bers set at 29, 31.3, and 34°C with 0.4% soil moisture as described in Spotila et al. (1994). Incubation temperatures in the chambers were monitored with thermocouples and BAT-12 thermocouple readers. Temperatures inside the egg containers varied ± 0.2°C (Spotila et al., 1994). Temperatures were selected to further refine our estimate of the pivotal temperature as well as to provide a male-producing temperature (29°C) and a female-producing temperature (34°C) based on Spotila et al. (1994). Incubators were accurate to ± 0.5 °C. The course of development was monitored for eggs incubated at 29 and 34°C by dissection of 2 eggs each at regular intervals. Embryos were staged using a dissecting microscope according to the criteria previously described for the snapping turtle, *Chelydra serpentina* (Yntema, 1968). These criteria proved effective for staging desert totoise embryos up to stage 21.

Histology of Gonadal Differentiation. — The gonadal development of embryos incubated at male- and female-producing temperatures were determined for stages 14–23 and at hatching (stage 26). Reproductive tracts were preserved in Bouin's solution (ca. 2–3 embryos per stage per temperature). Fixed tissues were infiltrated with paraffin, sectioned at 6–8 µm, and stained with PAS/hematoxylin (Humason, 1972).

Pivotal Temperature and Thermosensitive Period. — Subsets of eggs were incubated at constant temperatures of 29°C ($n = 7$), 31.3°C ($n = 14$), and 34°C ($n = 13$) throughout incubation, and hatching success and incubation time were determined.

The thermosensitive (temperature-sensitive) period was determined using a shift experiment design similar to that described by Wibbels et al. (1991). Eggs incubated at the male-producing temperature were shifted once to the female-producing temperature (29 to 34°C), or vice versa (34 to 29°C). Eggs were shifted at embryonic stages ranging from 14 to 23, except for control eggs which were maintained at constant 29 or 34°C throughout incubation.

Sex Identification of Hatchlings. — Hatchlings were individually marked and raised on a 12:12 LD cycle between 25 and 28° C for approximately 1.5 yrs prior to sexing. Tortoises were fed processed Zeigler® Iguana Diet (approximately 20% protein) supplemented with shredded carrots. Sex of juvenile tortoises was determined using laparoscopy as described in Rostal et al. (1994a). Mean body mass was 216.1 ± 8.58 g and mean straight carapace length was 102.8 ± 1.66 mm at the time of sexing.

RESULTS

Pivotal Temperature and Thermosensitive Period. — Results of the constant temperature incubations at 29, 31.3, and 34°C resulted in 100% males at 29°C ($n = 7$), 50% males and 50% females at 31.3°C ($n = 14$), and 100% females at 34°C ($n = 12$). These results have been combined with the previous results from Spotila et al (1994) in Fig. 1. The data from the current study suggest an estimated pivotal temperature of 31.3°C.

The results of the single shift experiment are shown in Fig. 2. Embryos shifted from the male-producing temperature (29°C) to the female-producing temperature (34°C) at stage 17 or earlier all became females whereas embryos shifted at stage 18 to 21 resulted in mixed sex ratios (approximately 30% males) with an increasing tendency to become males with increasing embryonic stage. All embryos shifted at stage 23 became males. Conversely, embryos shifted from the female-producing temperature (34°C) to the male-producing (29°C) temperature at stage 15 resulted in approximately 75% males, whereas embryos shifted at stage 16 or 17 resulted in mixed sex ratios with approximately 70% females combined. All embryos shifted at stage 18 or above became females.

Gonadal Differentiation. — Genital ridges were evident on the mesonephrons of the embryos by stage 15 at both the male-producing temperature (29°C) and the female-

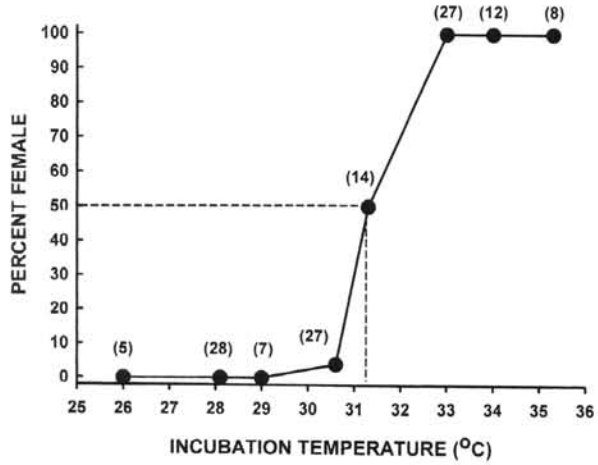

Figure 1. Plot of temperature effect on hatchling sex for desert tortoise (*Gopherus agassizii*) embryos incubated at constant temperatures. Sample sizes are shown in parentheses adjacent to each point. Figure combines results from Spotila et al. (1994) and the present study.

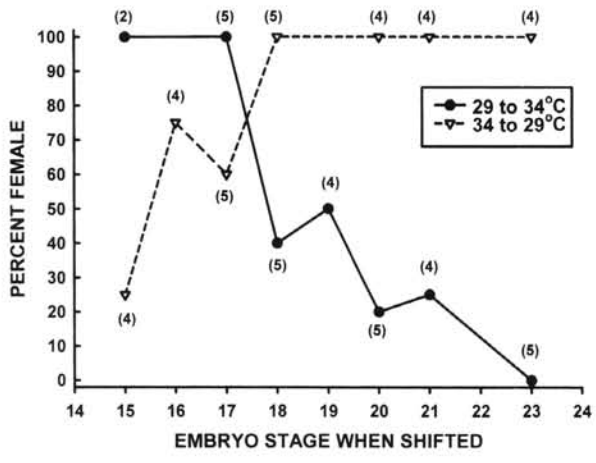

Figure 2. Temperature-shift experiments in which desert tortoise embryos were shifted from a male-producing temperature (29°C) to a female producing temperature (34°C) (or vice versa) once during their incubation. Sample sizes are shown in parentheses adjacent to each point.

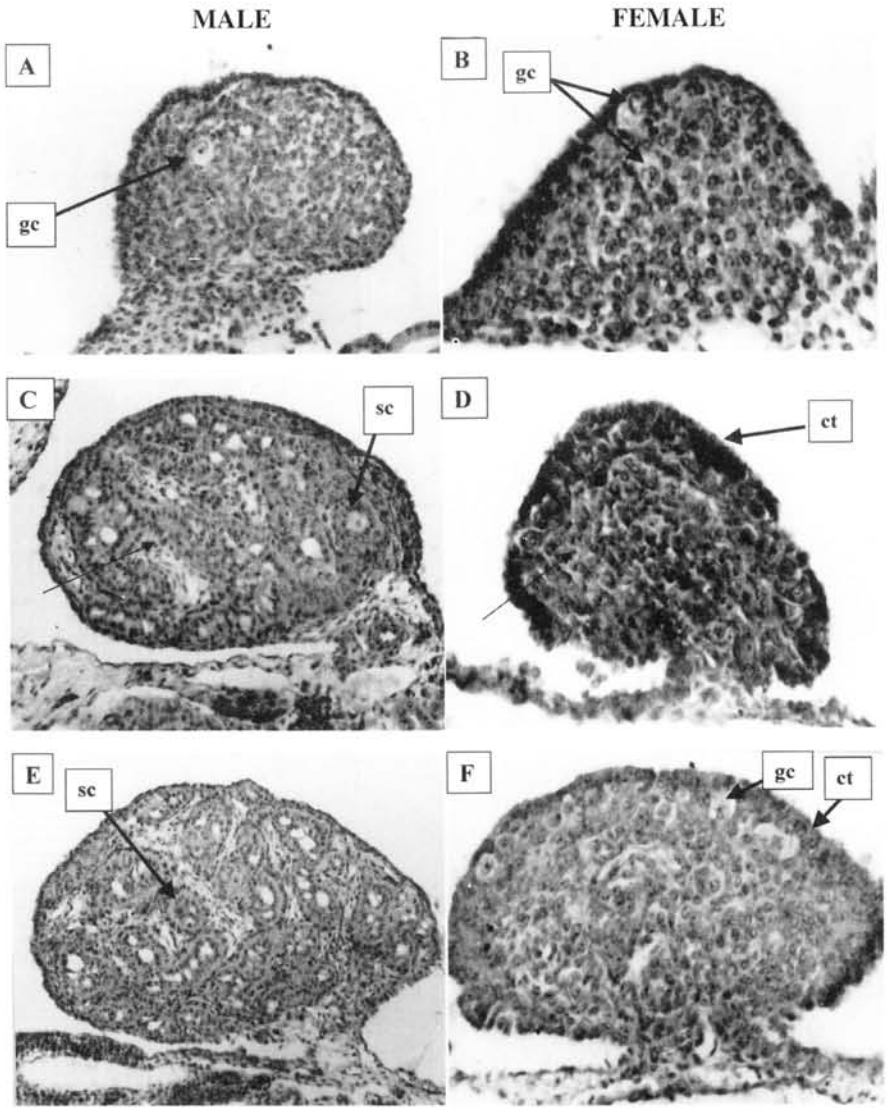

Figure 3. Cross sections of gonads from desert tortoise embryos during various stages of development. Gonads from male-producing temperature (29°C) are shown in **A** (developmental stage 17/18), **C** (stage 21), and **E** (stage 22/23). Gonads from female-producing temperature (34°C) are shown in **B** (stage 17/18), **D** (stage 21), and **F** (stage 22/23). By stage 22/23 sexually dimorphic changes are apparent, with medullary sex chords (sc) becoming prominent at male-producing temperatures. By stage 22/23 at female-producing temperature the cortical region (ct) has begun to thicken and germ cells are often associated with the cortex. Developmental staging based on Yntema, 1968. (gc = germ cell, sc = sex chord, ct = cortex).

producing temperature (34°C). Gonadal development appeared similar at male- and female-producing temperatures up through stage 18 (see Fig. 3). Between stage 18 and 21, sexual differentiation became apparent with the gonadal cortex proliferating at the female-producing temperature whereas medullary cord development occurred at the male producing temperature (Fig. 3). These trends continued through to hatching at which point the testes had distinct medullary cords and lacked a cortex. In contrast, the ovary had a distinct cortex and a regressed medulla.

Incubation Temperatures vs. Days of Incubation and Percent Hatching Success. — Incubation temperature influenced incubation time (days) as well as percent hatching success as expected (Fig. 4). Low (26°C) and high (35°C) incubation temperatures resulted in longer incubation times and significantly lower survival (Table 1). Incubation temperatures ranging from 28 to 34°C resulted in similar incubation times (68 to 89 days) and high hatching success (90 to 100%).

DISCUSSION

Temperature vs. Sex. — The results of the current study provide a general characterization of TSD in *G. agassizii*, including estimates of the effects of specific temperatures on sex determination. Constant temperature experiments in the current study as well as in the study by Spotila et al. (1994) indicate that *G. agassizii* possesses a "Pattern Ia" type of TSD in which relatively cool incubation temperatures produce males and warm temperatures produce females (Ewert et al., 1994). The transitional range of temperatures (at which the sex ratio shifts from producing 100% males to 100% females; Mrosovsky and Pieau, 1991), extends from

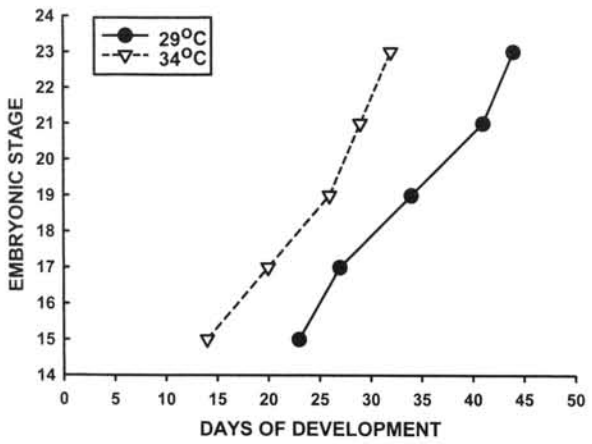

Figure 4. Rate of development (number of days vs. embryonic stage) for desert tortoise embryos incubated at a constant male-producing temperature (29°C) and a constant female-producing temperature (34°C).

approximately 30.5 to 32.5°C. The pivotal temperature (producing an approximate 1:1 sex ratio) estimated in the current study (31.3°C) is slightly lower (by 0.5°C) than that predicted by Spotila et al. (1994). However, the estimate by Spotila et al. (1994) was projected from temperatures which produced mostly all males or females, whereas the current study included a temperature which actually produced a 1:1 sex ratio. Therefore, although pivotal temperature estimates can vary due to factors such as interclutch variation (Mrosovsky, 1988; Etchberger et al., 1991; Ewert et al., 1994; Lang and Andrews, 1994), the estimate from the current study appears more reliable than that of Spotila et al. (1994). This is one of the highest pivotal temperatures reported for any chelonian (Alho et al., 1985; Ewert et al., 1994; Mrosovsky, 1994; Wibbels et al., 1998). The relatively high pivotal temperature estimated for *G. agassizii* may relate to its warm xeric environment. *Gopherus polyphemus* in the southeast U.S. displays a pivotal temperature (ca. 29°C) similar to other temperate turtle species (Burke et al., 1996; Demuth, 2001). As such, the higher pivotal temperature displayed by *G. agassizii* could represent a prime example of how environmental temperature may influence a particular pivotal temperature.

Table 1. Incubation time and hatching success of desert tortoise (*Gopherus agassizii*) eggs incubated under controlled conditions at the Desert Tortoise Conservation Center. Data from Spotila et al. (1994)* and present study; *n* = number of eggs set.

Temperature (°C)	*n*	Incubation Time (days)	% Hatching Success
26.0*	10	125	50
28.1*	29	89	96
29.0	7	87	100
30.6*	29	72	93
31.3	14	78	100
32.8*	29	68	93
33.0*	10	73	90
34.0	13	76	93
35.3*	28	85	29

Thermosensitive Period of Sex Determination. — The temperature-shift experiment provided an estimate of the thermosensitive period of sex determination in *G. agassizii*. In the case of shifts from female-producing temperature (34°C) to male-producing temperature (29°C), one embryo was committed to female sex determination by stage 15, and all other became committed by stage 18. In contrast, in shifts from male-producing to female-producing temperature, the sex of embryos remained labile until at least stage 17 and in some embryos as late as stage 21. These findings suggest that temperature sensitivity can begin before stage 15, and depending on the specific temperature-shift regimen, some embryos remain sensitive to a temperature-shift until at least stage 21. In general, these findings are similar to the thermosensitive periods estimated for several other species of turtles (Yntema, 1979; Bull and Vogt, 1981; Pieau and Dorizzi, 1981; Yntema and Mrosovsky, 1982; Wibbels et al., 1991). However, precise comparisons between studies are confounded by the fact that the specific temperatures and shift protocols used in each study can significantly affect the results (Wibbels et al., 1991). Therefore, the results should be considered a general estimate of the thermosensitive period.

The temperature-shift experiment also revealed that the sexual fate of embryos incubated at the female-producing temperature (34°C) becomes irreversible earlier than embryos incubated at the male-producing temperature (29°C). This finding has been previously reported for several reptiles with TSD (reviewed by Wibbels et al., 1991). However, the opposite case has also been reported for species of turtles in which the sexual fate of embryos at male-producing temperature becomes irreversible earlier than that of embryos at female-producing temperatures (Bull and Vogt, 1981; Pieau and Dorizzi, 1981). Such variation could relate to interspecific differences in TSD, or to differences in the temperatures and protocols used in the various studies.

In regards to the actual days of incubation corresponding to the thermosensitive period, the embryos reached stage 15 by approximately day 15 to 23 of incubation, and stage 21 by approximately 28 to 42 days of incubation, depending on incubation temperature (34 or 29°C, respectively, in both cases). Considering that the total length of incubation at these two temperatures is approximately 87 and 76 days, respectively, the thermosensitive period occurs during the approximate second quarter of incubation.

Gonadal Differentiation. — Gonadal differentiation in *G. agassizii* was similar in both chronology and morphology to that reported for other turtles with TSD (Pieau and Dorizzi, 1981; Wibbels et al., 1991), with gonads beginning to show sexual differentiation between stages 18 to 21, and showing distinct sexual dimorphism by stage 23. Comparing the chronology of gonadal differentiation to the thermosensitive period indicates that the onset of temperature sensitivity precedes any obvious sexual differentiation of the gonads at the temperatures examined (29 and 34°C). Further, at the female-producing temperature (34°C), the sexual fate of the embryo became irreversible before any

obvious sexual differentiation of the gonads. At the male-producing temperature (29°C), the sexual fate of the embryos became irreversible at a time when the gonads were already beginning to undergo sexual differentiation. In reference to the actual days of incubation, the gonads exhibited distinct sexually differentiation (i.e., stage 23) by approximately days 32 or 42 of incubation, depending on incubation temperature (i.e., 34 or 29°C, respectively).

Incubation Temperature, Mortality, and Embryonic Development. — Hatching success was high at incubation temperatures ranging from 28 to 34°C (Table 1). In contrast, high mortality was encountered at a relatively low incubation temperature (26°C) and at a relatively high incubation temperature (35.2°C). These findings are consistent with those reported previously for *G. agassizii* (Spotila et al., 1994). Survival at 34°C was 95% while survival at 35°C was 30%. At temperatures which produced high hatch rates, the total time of incubation ranged from 68 (32.8°C) to 89 (28.1°C) days. Monitoring of development at 34°C (76 days) and 29°C (87 days) indicated that embryos developed from stage 1 through 23 over a period of approximately 32 or 44 days, respectively, and then required from 44 to 43 days, respectively, to develop from stage 23 through 26 (i.e., hatchling). Thus, the last few stages of embryonic development encompass a relatively long time period in *G. agassizii*.

Predicting Hatchling Sex Ratios. — The results of the current study provide information which could be used to predict hatchling sex ratios produced in *G. agassizii* populations. For example, the results indicated the incubation temperature affects sex determination during the second quarter of the total incubation period, depending on the specific incubation temperature. Further, the results, together with those of Spotila et al. (1994), indicated that temperatures of approximately 32.5°C or higher produce all females, whereas those of approximately 30.5°C or lower produce all males, and temperatures between those ranges produce mixed sex ratios. Such information could potentially be used to predict sex ratios if incubation temperatures within nests are monitored and if those temperatures fall within the ranges mentioned above. It should be noted, however, that the data in the current study as well as those by Spotila et al. (1994) are based on constant temperatures. Temperatures within natural nests fluctuate, and if temperatures fluctuate between the male and female ranges, they may be difficult to interpret. Thus further studies are needed to address that particular case, however, in other situations temperatures within nests may clearly indicate the production of either males or females (Hanson et al., 1998).

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LITERATURE CITED

ALHO, C.J.R., DANNI, T.M.S., AND PÁDUA, L.F.M. 1985. Temperature-dependent sex determination in *Podocnemis expansa* (Testudinata: Pelomedusidae). Biotropica 17(1):75-78.

BULL, J.J. AND CHARNOV, E.L. 1989. Enigmatic reptilian sex ratios. Evolution 43:1561-1566.

BULL, J.J. AND VOGT, R.C. 1981. Temperature-sensitive periods of sex determination in emydid turtles. J. Exp. Zool. 218:435-440.

BURKE, R.L., EWERT, M.A., MCLEMORE, J.B., AND JACKSON, D.R. 1996. Temperature-dependent sex determination and hatching success in the gopher tortoise (*Gopherus polyphemus*). Chelonian Conservation and Biology 2(1):86-88.

DEMUTH, J.P. 2001. The effects of constant and fluctuating incubation temperatures on sex determination, growth, and performance in the tortoise *Gopherus polyphemus*. Can. J. Zool. 79:1609-1620.

EENDEBAK, B.T. 1995. Incubation period and sex ratio of Hermann's tortoise, *Testudo hermanni boettgeri*. Chelonian Conservation and Biology 1(3):227-231.

ERNST, C.H., LOVICH, J.E., AND BARBOUR, R.W. 1994. Turtles of the United States and Canada. Washington: Smithsonian Institution Press, 578 pp.

ETCHBERGER, C.R., PHILLIPS, J.B., EWERT, M.A., NELSON, C.E., AND PRANGE, H.D. 1991. Effects of oxygen concentration and clutch on sex determination and physiology in red-eared slider turtles (*Trachemys scripta*). J. Exper. Zool. 258:394-403.

EWERT, M.A., JACKSON, D.R., AND NELSON, C.E. 1994. Patterns of temperature-dependent sex determination in turtles. J. Exper. Zool. 270:3-15.

HANSON, J., WIBBELS, T., AND MARTIN, R.E. 1998. Predicted female bias in hatchling sex ratios of loggerhead sea turtles from a Florida nesting beach. Can. J. Zool. 76:1850-1851.

HUMASON, G.L. 1972. Animal Tissue Techniques. W.H. Freeman, San Francisco.

JANZEN, F.J. AND PAUKSTIS, G.L. 1991. Environmental sex determination in reptiles: ecology, evolution, and experimental design. Quart. Rev. Biol. 66:149-179.

LANG, J.W. AND ANDREWS, H.V. 1994. Temperature-dependent sex determination in crocodilians. J. Exp. Zool. 270:28-44.

LEWIS-WINOKUR, V. AND WINOKUR, R.M. 1995. Incubation temperature affects sexual differentiation, incubation time, and posthatching survival in desert tortoises (*Gopherus agassizii*). Can. J. Zool. 73:2091-2097.

LOVICH, J.E. 1996. Possible demographic and ecologic consequences of sex ratio manipulation in turtles. Chelonian Conservation and Biology 2(1):114-117.

MORREALE, S.J., RUIZ, G.J., SPOTILA, J.R., AND STANDORA, E.A. 1982. Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science 216:1245-1247.

MROSOVSKY, N. 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. Can. J. Zool. 66(3):661-669.

MROSOVSKY, N. 1994. Sex ratios of sea turtles. J. Exp. Zool. 270:16-27.

MROSOVSKY, N. AND PIEAU, C. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex

determination in reptiles. Amphibia-Reptilia 12(2):169-179.

MROSOVSKY, N. AND PROVANCHA, J. 1992. Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year study. Can. J. Zool. 70:530-538.

MROSOVSKY, N. AND YNTEMA, C.L. 1980. Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. Biol. Conserv. 18:271-280.

PIEAU, C. 1972. Effets de la temperature sur le developpement des glandes genitales chez les embryons de deux cheloniens, *Emys orbicularis* et *Testudo graeca*. C.R. Acad. Sci. Paris 274(D):719-722.

PIEAU, C. AND DORIZZI, M. 1981. Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis*. Journal Morph. 170(3):373-382.

ROSTAL, D.C., GRUMBLES, J.S., LANCE, V.A., AND SPOTILA, J.R. 1994a. Non-lethal sexing techniques for hatchling and immature desert tortoises (*Gopherus agassizii*). Herpetological Monographs 8:83-87.

ROSTAL, D.C., LANCE, V.A., GRUMBLES, J.S., AND ALBERTS, A.C. 1994b. Seasonal reproductive cycle of the desert tortoise (*Gopherus agassizii*) in the eastern Mojave desert. Herpetological Monographs 8:72-82.

SPOTILA, J.R., ZIMMERMAN, L.C., BINCKLEY, C.A., GRUMBLES, J.S., ROSTAL, D.C., LIST, A.JR., BEYER, E.C., PHILLIPS, K.M., AND KEMP, S.J. 1994. Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. Herpetological Monographs 8:103-116.

VOGT, R.C. 1994. Temperature controlled sex determination as a tool for turtle conservation. Chelonian Conservation and Biology 1(2):159-162.

WIBBELS, T., BULL, J.J., AND CREWS, D. 1991. Chronology and morphology of temperature-dependent sex determination. J. Exp. Zool. 260:371-381.

WIBBELS, T., MARTIN, R.E., OWENS, D.W., AND AMOSS, M.S., JR. 1991. Female-biased sex ratio of immature loggerhead sea turtles inhabiting the Atlantic coastal waters of Florida. Can. J. Zool. 69:2973-2977.

WIBBELS, T., BULL, J.J., AND CREWS, D. 1994. Temperature-dependent sex determination: a mechanistic approach. J. Exp. Zool. 270:71-78.

WIBBELS, T., ROSTAL, D., AND BYLES, R. 1998. High pivotal temperature in the sex determination of the olive ridley sea turtle, *Lepidochelys olivacea*, from Playa Nancite, Costa Rica. Copeia 1998(4):1086-1088.

YNTEMA, C.L. 1968. A series of stages in the embryonic development of *Chelydra serpentina*. J. Morph. 125:219-252.

YNTEMA, C.L. 1979. Temperature levels and periods of sex determination during incubation of eggs of *Chelydra serpentina*. J. Morph. 159:17-28.

YNTEMA, C.L. AND MROSOVSKY, N. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. Can. J. Zool. 60:1012-1016.

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