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## EFFECTS OF INCUBATION TEMPERATURE ON SEX DETERMINATION IN A COMMUNITY OF NEOTROPICAL FRESHWATER TURTLES IN SOUTHERN MEXICO

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**ABSTRACT:** Incubation temperature has been shown to control sex determination (TSD) in both suborders of living turtles, including freshwater, terrestrial, and sea turtles. This study combines field and laboratory data from four neotropical endemic species (*Dermatemys mawii*, *Staurotypus triporcatus*, *Claudius angustatus*, and *Kinosternon leucostomum*) and two of temperate zone origin (*Chelydra serpentina* and *Trachemys scripta*). The sex of hatchlings incubated in natural nests and controlled laboratory temperatures was studied. Adult sex ratios in natural populations were determined by mark and recapture studies. *Staurotypus* and *Claudius* appear to have genetic sex determination (GSD) while TSD occurred in the other four species. Threshold temperatures of both *Trachemys* and *Chelydra* were lower in these Neotropical populations than in those studied in Temperate Zone climates. Natural populations of species with TSD were found to have variable adult sex ratios, skewed towards one sex or the other. These variations in sex ratio could sometimes be explained by nest site ecology.

**Key words:** Sex determination; Temperature; Freshwater turtles; Cryptodires; Neotropics

INCUBATION temperature has been shown to control sex determination (TSD) in nine families of freshwater, terrestrial, and sea turtles (see Bull, 1980, 1983; Ewert and Nelson, 1991; Miller, 1988; Standora and Spotila, 1985; Vogt and Bull, 1982; Vogt and Flores-Villela, 1986, for reviews). Within this order of reptiles, there are three species with heteromorphic sex chromosomes, other species that have genetic control of sex determination (GSD) but lack heteromorphic sex chromosomes, and those with TSD, which constitute the majority of the species of turtles studied. Most studies have dealt with only one species of turtle in the laboratory or field. Our

study combines both field and laboratory data collected over a span of 8 yr on six species belonging to five different families of turtles that inhabit a neotropical aquatic ecosystem in southern Mexico. In this paper, we address the following issues. (1) Does incubation temperature influence sex determination in *Staurotypus triporcatus*, a species known to have heteromorphic sex chromosomes? Incubation temperature might override a genetic effect in this species, which could be demonstrated by karyotyping. (2) Does incubation temperature influence the sex of neotropical endemic species? Because temperatures in tropical areas are less extreme and offer less diverse temperature gradients both within a habitat and throughout the year than in temperate zones, we predicted that

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we might find more species with GSD. (3) Are threshold temperatures, at which sex determination occurs, modified in populations of wide ranging temperate zone species inhabiting tropical climates? (4) How do adult sex ratios of populations of neotropical turtles with TSD compare to sex ratios in populations of turtles with GSD?

#### STUDY AREAS

Study areas were located in the region of Los Tuxtlas in the southeastern portion of Veracruz, Mexico, 18° N Lat., 85° E Long. (Laguna de Zacatal, Laguna Escondida: see Vogt and Guzman-Guzman, 1988, for a detailed description of these study sites) and the pantano of Lerdo de Tejada, and in southern Chiapas, Mexico, in the Selva Lacandona 16° N Lat., 91° E Long. (Laguna Oaxaca, Rio Lacantun, and Rio Tsendales). Laguna de Zacatal is a temporary lake 150 × 650 m in area and 14 m deep. Laguna Escondida is a 40 m deep permanent lake 1500 × 300 m in size. The pantano of Lerdo de Tejada is part of the Rio Papaloapan system encompassing over 200 ha; the pantano is a marshy area rich in aquatic plants with a water depth of 1–3 m during the wet season and only moist with isolated pools and channels during the dry season. Laguna Oaxaca is an oxbow lake 2500 × 200 m and shallow, only 2–3 m deep, located 150 m from the present channel of the Rio Lacantun. The Rio Lacantun is a 200 m wide, 10–25 m deep fast moving clear river which joins with the Rio Salinas to form the Rio Usamacinta. Rio Tsendales is a clear-water tributary of the Lacantun 50 m wide and 2–10 m deep. Both of these rivers fluctuate 5–10 m in depth during the rainy season and are bordered by lowland evergreen tropical rainforest. Laguna Oaxaca and Laguna Escondida have about half of their shorelines forested and the other half cut for pasture land. Laguna de Zacatal is completely bordered by virgin evergreen tropical rainforest.

#### SPECIES STUDIED

Four species are neotropical endemics (*Dermatemys mawii*, *Staurotypus tripor-*

*catus*, *Claudius angustatus*, and *Kinosternon leucostomum*) while two are of temperate zone origin (*Chelydra serpentina* and *Trachemys scripta*). *Dermatemys mawii*, *Staurotypus triporcatus*, and *Claudius angustatus* have a range restricted to southeastern Mexico, Guatemala, and Belize. *Kinosternon leucostomum* is much more widespread ranging from northern Veracruz to Columbia. *Chelydra serpentina* and *Trachemys scripta* are both wide ranging species of temperate zone origin that have invaded the tropics in relatively recent times. The distribution of *Chelydra serpentina* extends from Canada to Columbia and *Trachemys scripta* from Illinois to Brazil.

#### METHODS

We report data collected as part of a long term study on community ecology and reproduction of turtles. Turtles were trapped in large unbaited fyke nets (Vogt, 1980a) and processed in the laboratory or field camp. The study was conducted from March 1981 through December 1988. Individuals of *Claudius angustatus* were hand collected by local professional turtle vendors in the study area. Eggs for incubation experiments were collected by injecting gravid females with 10 units of oxytocin per kilogram of total mass (Ewert and Legler, 1978). Incubation experiments were conducted in an air conditioned laboratory in Precision 800 incubators accurate to  $\pm 0.1$  C at 150 m above sea level. Eggs were placed on moist vermiculite (1:1, vermiculite:water by mass) and enclosed in a clear plastic bag. The entire bag (plastic bowl, vermiculite, and eggs) was weighed at the initiation of the experiments and at weekly intervals thereafter. Water was added to make up for any loss of mass that occurred.

Hatchlings were sacrificed by injection of sodium-pentobarbital within 48 h after hatching, were permanently individually numbered, and were tagged for deposition in the museum collection of UNAM-LT. Hatching is defined as when the turtle crawls out of the egg, not when a longitudinal slit occurs in the egg (Gutzke et al., 1984). Due to water absorption, the

four neotropical species with hard shelled eggs often develop longitudinal slits in the egg shell 1 or 2 mo before hatching. Sex was determined by gross anatomy of fresh gonads under a dissecting microscope, according to the criteria used by Yntema (1976). The female gonad is typically long with a thick cortex and a well-developed Mullerian duct, while males usually have a degenerated Mullerian duct, rudimentary cortex, and a well developed medulla. If the sex of a particular hatchling was not obvious, the gonad was squashed in aceto-orcein stain and viewed under a light microscope. All hatchlings were later preserved in neutral buffered formalin so that histological sections could be made of any questionable material (Bull and Vogt, 1979, 1981). The sex of all hatchlings was later reassessed by two trained observers in double blind tests, to prevent any possibility of observer bias. Hatchlings were collected from wild nests that had been covered with wire mesh or hand collected near the nesting area or taken as eggs in the final stages of development and incubation completed in the laboratory under ambient temperature (uncontrolled natural fluctuating temperatures).

Gonads of a sample of *K. leucostomum* were cleared and sexed using the Vanderheiden technique (Vanderheiden et al., 1985) for comparison of sexing reliability. We sacrificed the hatchlings by injecting 0.1 ml of sodium pentobarbitol into the cranium. The plastron was cut at the bridges and detached from the pelvic musculature so that any residual yolk could be discarded. The entire hatchling was then immersed for preservation in 10% neutral buffered formalin from 1–36 mo prior to clearing with glycerin. It is important to note that the hatchlings were not decapitated and fixed as in other studies that have had a problem using this technique (Mrosovsky and Benabib, 1990). If a hatchling is decapitated, the blood vessels in the cleared testes are not readily distinguishable as they are in specimens that are killed by injection with sodium pentobarbitol; perhaps this is the result of much of the blood being drained from the turtle, leaving the testes translucent.

One clutch of *Staurotypus* was incubated at 25 C and 30 C by M. Ewert in Bloomington, Indiana; karyotypes of these six hatchling *Staurotypus triporcatus* were performed by John Bickham using the usual flame dry procedures (Bickham, 1975).

## RESULTS

Table 1 presents the results of the incubation experiments. Four species were found to have TSD (*K. leucostomum*, *Dermatemys*, *Chelydra*, and *Trachemys*), as evidenced by the number of females versus males being significantly different from 0.5 with  $P < 0.0001$ , 0.001, 0.0001, and 0.0002 respectively using Fisher's Exact Test ( $\alpha = 0.05$ ). As predicted, *Staurotypus* was shown to have GSD and the karyotypic sex of the six hatchlings karyotyped by Bickham coincided with the phenotypic sex of the gonads: two females and four males. *Claudius*, even though lacking heteromorphic sex chromosomes, appears to have GSD, because the sex ratio was not significantly different from 0.5 at the two controlled temperatures tested or in natural nests ( $P = 0.5672$ ).

Threshold (T) temperatures (defined in Bull et al., 1982a, as the temperature that produces 50% male and 50% female) of both *Chelydra* and *Trachemys* were lower by 2 and 1 C, respectively, from conspecific populations in temperate zones. Threshold temperatures of all four species with TSD were remarkably similar.

Sex ratio (proportion males) of the adult populations of turtles studied varied as follows: *Kinosternon* in Laguna de Zacatal ( $n = 665$ ) was 0.5 while in Laguna Escondida ( $n = 760$ ) it was 0.69. *Staurotypus* in Laguna Oaxaca ( $n = 386$ ) was 0.44. *Claudius* ( $n = 286$ ), for hand captures of adults from Lerdo de Tejada, was 0.75 while that of 26 wild caught hatchlings was 0.5. *Dermatemys* was 0.25 in Laguna Oaxaca ( $n = 41$ ) and 0.20 in Rio Tsendales ( $n = 106$ ). In contrast, *Trachemys* was skewed in the opposite direction at these sites: 0.65 in Laguna Oaxaca ( $n = 198$ ) and 0.76 in Rio Tsendales ( $n = 85$ ). We have not caught a sufficient number of *Chelydra* at any site in Mexico to be able to make any statements about the adult sex ratio.

TABLE 1.—Sex ratio as a function of incubation temperature: data presented as percent male; sample size in parentheses.

Species	Temperature (C)					Ambient
	25	27	28	29	30	
<i>Kinosternon</i>	70 (35)	—	T 48 (62)	18 (65)	25 (36)	59 (34)
<i>Dermatemys</i>	100 (14)	—	T 44 (9)	0 (9)	—	—
<i>Staurotypus</i>	63 (27)	—	—	—	60 (25)	40 (21)
<i>Claudius</i>	52 (61)	—	—	56 (7)	—	41 (28)
<i>Chelydra</i>	85 (13)	T 2 (10)	12 (33)	0 (3)	0 (2)	—
<i>Chelydra*</i>	100	—	100	T	0	—
<i>Trachemys</i>	100 (16)	53 (34)	T 26 (53)	0 (44)	0 (47)	78 (9)
<i>Trachemys**</i>	—	—	100 (21)	T 37 (16)	0 (17)	—

T = threshold temperature.

\* Data from Wisconsin turtles (Yntema, 1979).

\*\* Data from Alabama turtles (Bull et al., 1982a).

### DISCUSSION

Table 1 suggests that *Kinosternon leucostomum* could be in the process of evolving GSD at these sites, because no temperature produced 100% of either sex (Bull et al., 1982b). This may be the case, but in all four species of neotropical endemics, other factors may be intervening. All have embryonic diapause and embryonic aestivation, which leads to a wide range of incubation times even at the same temperature (90–265 days); thus factors of humidity and/or embryonic metabolism may be confounding the analysis of the temperature effect (Ewert and Vogt, unpublished data). The data in Table 1 differ from those presented by Ewert and Nelson (1991) for this species, where the 12 hatchlings at 27–30 C were all females but both males and females hatched from 12 eggs incubated at 22.5–25 C. The geographic origin of their material is not known, originating from different regions within the wide range (Panama to Mexico) inhabited by this species.

It is intriguing that *Claudius* appears to have GSD without any apparent chromosomal changes, while *Staurotypus*, a closely related species in the same family, has recently evolved heteromorphic sex chromosomes (Sites et al., 1979). This suggests that TSD may be the primitive state and that GSD can occur without the evolution of heteromorphic sex chromosomes.

The variability of threshold temperatures within the same species on the same continent emphasizes the importance of experiments from the population of turtles

at the specific sites of interest; clearly data cannot be extrapolated from other parts of the range of the species. As was found in temperate zone species (Bull et al., 1982a), threshold temperatures within the same species were reduced at lower latitudes. In southern Mexico, *T. scripta* nest in February–April when temperatures are not extremely high (Vogt, 1990). Also nesting areas for both *Chelydra serpentina* and *Trachemys scripta* are often in or beneath vegetation rather than on hot exposed sandy beaches. This is also the situation with *Kinosternon leucostomum*, which often nests within the closed canopy forest. *Dermatemys mawii* nests in exposed river banks of either sand or laterite soil within 1–3 m of the river edge. These differences in nesting ecology may explain part of the variance in the sex ratios of the adult populations. Nesting habitat differences between the populations of *Kinosternon leucostomum* from the two lagunas reflect not the availability of open nesting habitat but rather the predation of nests and nesting females by the indigenous people and their dogs. Predation of both nests and nesting females is higher on the deforested and populated side of the lake; thus, the bias towards males in this population may be the result of differential predation on females and the female-producing nests of the hotter sites.

The sex ratio differences between the populations of *Trachemys scripta* do reflect a difference in available nesting habitat. Rio Tsendales is bordered by forest with no open areas along the shoreline.

The main nesting area is a forested island in the center of the river, with closed canopy forest on both sides of the river. Even though more male *Trachemys* were captured than females due to greater male movement (determined by mark and recapture), the differences in male abundance between these two areas cannot be explained by differential capture rates of the sexes, because the same methodology was used in both areas during the same times of the year. These results may be a reflection of lower temperature nesting habitat, as was found for *Graptemys* in Wisconsin (Vogt, 1980b; Vogt and Bull, 1984).

Even though *Staurotypus* produces a 0.5 sex ratio, the adult population appears to be biased towards females, but recapture data show that females move more than males and are recaptured more frequently. The opposite is suggested for *Claudius*, because wild caught hatchlings and incubation experiments show that there is a primary sex ratio of 0.5. Differential mortality of nesting females or differences in the movement patterns between males and females may have produced these results.

We emphasized that before one is able to speculate on the effect that TSD has on the adult sex ratio, all factors must be studied. This is especially apparent from the data for *Claudius* and *Staurotypus*. *Staurotypus triporcatus*, a species with GSD, had a balanced sex ratio (0.5) in all populations studied; while the sex ratio of hatchling *Claudius* was 0.5, the manner in which the adult population was surveyed produced a male bias. It appears that the evolution of GSD has enhanced the capability of maintaining a balanced sex ratio where sympatric populations of other species with TSD have sex ratios radically different from 0.5.

It is mandatory for conservation biologists who are beginning to manage freshwater turtle populations to note that threshold temperatures are variable, and that experiments must be undertaken on the population to be managed rather than relying on data from other areas, or they will risk producing biased sex ratios, or worse, all males as was produced by the conservation effort with *Chelonia mydas*

in Costa Rica for many years. It is important that freshwater turtle biologists learn from the mistakes made in sea turtle biology management and not waste effort, expense, and turtles in a misguided notion that head starting is a beneficial practice. Iverson (1989) derided the idea of farming turtles as a solution to turtle conservation; he later stated, "There is no reasons (sic) why the 'head start' programs applied to marine turtles cannot be applied more aggressively to terrestrial and freshwater turtles" (Iverson, 1991). This should not be confused with the so often used term "head starting" referring to how sea turtles are often grown in captivity for several years in artificial living conditions and fed unnatural diets before being dumped into the sea as fattened morsels for larger predators. What Iverson meant (personal communication) was that through the manipulation of nests to produce the desired sex ratio, the protection of nests from predators, floods, and other environmental stresses, and the manipulation of habitat to produce more nesting beaches and feeding grounds, a population of turtles can be given a much needed jump-start to incrementing its abundance. He did not intend to imply that hatchling turtles should be raised in captivity for later release. Conservation efforts must strive to protect the standing crop of adult females in the population; they have proven their fitness and are relatively predation free. To concentrate one's efforts on the early life history stages, as Iverson (1991) suggested, at expense of the adults is not a sound practice, because natural predation is always going to be high on juvenile turtles free living in nature.

#### RESUMEN

En los dos subordenes de tortugas existentes que incluyen tortugas terrestres, de agua dulce, y marinas, se ha demostrado que la determinación del sexo es controlada por la temperatura de incubación de los huevos (TSD). En este estudio se combinan los datos de campo y de laboratorio con cuatro especies de tortugas endémicas del Neotrópico: *Dermatemys mawii*, *Staurotypus triporcatus*, *Claudius angustatus*, y *Kinosternon leucostomum*; y dos

especies de zonas templadas: *Chelydra serpentina* y *Trachemys scripta*. Se realizaron experimentos de incubación en el laboratorio y se obtuvieron datos de proporciones sexuales en nidos naturales. Por medio de estudios de marcaje y recaptura se estimó el sesgo de los sexos de los adultos en poblaciones naturales. *Staurotypus* y *Claudius* poseen determinación genética del sexo (GSD), y las otras cuatro especies presentan TSD. La temperatura umbral fue más baja en las poblaciones neotropicales de *Chelydra* y *Trachemys* comparada con la encontrada en estudios previos con poblaciones de zonas templadas. Las poblaciones naturales de las especies que poseen TSD presentaron razones sexuales variables, con sesgos hacia uno o otro sexo. Estas variaciones en el sesgo de los sexos se puede explicar en parte por la ecología de los nidos.

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