

## The Effect of Incubation Environment on Loggerhead Hatchlings and a Potential Advantage of Temperature-Dependent Sex Determination

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I studied the effect of incubation environment on loggerhead hatchlings from 14 clutches by comparing the characteristics of hatchlings from eggs at the periphery of clutches (i.e., those in direct contact with the surrounding soil) to the characteristics of hatchlings from eggs in the center of clutches (i.e., those not in direct contact with the surrounding soil). Eggs from the periphery gained more weight (water) during incubation than eggs from the center. Hatchlings from the center eggs were larger in carapace width than hatchlings from the peripheral eggs. Hatchlings from both the peripheral and center eggs crawled at the same speeds, but hatchlings from the center eggs spent more time powerstroking during the first 24 hours in the water than hatchlings from the peripheral eggs. Hatchlings

from the center eggs also grew faster and were larger after the first two weeks of post-hatchling growth than hatchlings from the peripheral eggs. Temperatures were monitored throughout the incubation period at various locations within three of the clutches. The center of the clutch was the warmest location and the area most likely to produce female hatchlings. As adults, male loggerheads are able to reproduce twice as often as female loggerheads. To help counter the reproductive advantage of males, female loggerheads might get a reproductive headstart by growing faster and maturing at an earlier age than male loggerheads. If this is the case, then temperature-dependent sex determination is advantageous in loggerheads because it associates female hatchlings with the fastest growth rates.

## Statistical Description of Temperature-Dependent Sex Determination in Marine Turtles

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In many reptiles, as well as in some actinopterygians and amphibians, the sexual phenotype is determined at least in part by the incubation temperature of eggs (Pieau *et al.*, 1995). This phenomenon has been called temperature-dependent sex determination (TSD). Its occurrence in reptiles (many turtles, all crocodylians, both sphenodonts, and some lizards) is puzzling from an evolutionary point of view because its adaptive significance remains largely unknown. TSD occurs in all marine turtles and is generally first characterised in a species by the results of incubation at several constant incubation temperatures. Originally, TSD was described simply using the pivotal temperature (also called the critical or threshold temperature), which is the constant incubation temperature producing an equal number of both sexes (Mrosovsky and Pieau, 1991). More recently, a new parameter has been proposed to describe TSD: the transitional range of temperature (TRT) (Mrosovsky and Pieau, 1991). TRT is the range of constant temperatures in which the incubation produces both sexes. TRT is presumed to describe more adequately the consequence of TSD in natural conditions: when the TRT is narrower, more nests should be unisexual.

A new procedure has been recently introduced to calculate conjointly the pivotal temperature, the transitional range of temperature, and their variance (Girondot, 1999). The estimation is performed using maximum likelihood. We present rapidly here the method, its use to characterise TSD in marine turtles, and a test to compare populations.

### Material and Methods

The statistical methodology used is fully described in Girondot (1999) and is summarized here briefly. It is based

on the best fit curve of the sex ratio on the incubation temperatures by the function:

$$sr(t) = 1 / \left( 1 + e^{\left( \frac{1}{S} (P-t) \right)} \right) \quad \text{equation 1}$$

This equation has all the prerequisites for describing TSD: *sr* goes from 0 to 1 for *S* being positive or from 1 to 0 for *S* being negative with a transition more or less sharp according to the *S* parameter and with an *sr* value equal to 0.5 for *t=P*. The range of temperature producing males and females (TRT) is then simply  $|S.k_l|$  with  $k_l$  being a constant dependent on *l*, the sex ratio limit chosen to define TRT (for example  $TRT_{5\%}$  indicates that TRT is defined as the range of temperatures producing between 5% and 95% of males):  $k_l = 2 \ln(l/(1-l))$ .

The probability ( $p_i$ ) of the observed distribution of males and females for each *u* incubation temperature is obtained using a binomial distribution with  $m_i$ ,  $f_i$  and  $n_i$  ( $n_i = m_i + f_i$ ) being the observed number of males, females, and total of sexed embryos, respectively, for a particular temperature  $t_i$  and the theoretical sex ratio at this temperature,  $sr(t_i)$  based on equation 1:

$$p_i = C_{n_i}^{m_i} sr(t_i)^{m_i} (1 - sr(t_i))^{f_i} \quad \text{equation 2}$$

The likelihood of the experiment (*L*) is then simply:

$$L = \prod_{i=1}^u p_i \quad \text{equation 3}$$

The adjustment of the *P* and *S* parameters for a particular set of incubation temperatures and sex ratio is performed using maximum-likelihood analysis and the

variance of the parameters are obtained by the values of the second order derivative at the maximum-likelihood  $S$  and  $P$  values (Girondot, 1999). The test for significant difference between  $\lambda$  populations is based on the likelihood ratio test. The null hypothesis ( $H_0$ ) being that no difference exist between population, the likelihood of the combined data of all experiments ( $L_c$ ) is calculated as well as the likelihood of each of the  $\lambda$  experiments ( $L_j$ ). The test is then performed using a  $\chi^2$  with  $2\lambda-2$  DF:

$$\chi^2 = -2 \ln \left( \frac{L_c}{\left( \prod_{j=1}^{\lambda} L_j \right)} \right) \text{ equation 4}$$

Computer program for MS-DOS and MacOS is freely available on the web page: <http://www.biop7.jussieu.fr/perso/girondot.html>

**Results**

The pivotal temperature ( $P$ ) and  $TRT_{5\%}$  are shown in **Table 1** for the available data in literature about marine turtles. Three species need to be discussed in further detail.

*Chelonia mydas*: Only data from Godfrey (1997) are used for the estimations of  $P$  and  $S$ . Data from Mrosovsky *et al.* (1984) does not permit to reach some maximum likelihood. It can be easily explained because only 4 incubation temperatures were studied and the two intermediate temperatures produced inverse sex ratio tendency compared to the two extreme temperatures. This could be the result of difference in cooling due to change of substrat for incubation (Mrosovsky, pers. comm.). Miller and Limpus (1980) estimate sex ratio for Australian population of this species at 3 incubation temperatures but none produced mixed sex ratio whereas at least two are required to estimate  $P$  and  $S$ . Nine intersexes were originally described in this experiment but further studies have shown that their phenotype were in fact identical to the most common sex obtained at this temperature (J.D. Miller and C.J. Limpus, pers. comm.). The probability of the observed number of males and females for each incubation temperature has been established based on the hypothesis

that sex determination is the same than for Suriname Green turtles. The probability of the observed number of males and females is  $>0.99$ ,  $<0.0001$  and  $>0.99$  for incubations at 26, 29 and 33°C respectively. Result at 29°C shows that *C. mydas* from Australia therefore exhibits a significant difference for TSD compared to *C. mydas* from Suriname.

*Dermochelys coriacea*: Binckley *et al.* (1998) reported sex ratio versus incubation temperatures for Leatherbacks nesting at Playa Grande, Costa Rica (Pacific). Based on a visual comparison with data from Atlantic (French Guiana) TSD in Leatherbacks (Rimblot *et al.*, 1985; Rimblot-Baly *et al.*, 1986), they conclude that: “ The two temperature versus % female curves for Atlantic and Pacific leatherback populations are virtually identical in pivotal temperature and overall shape. A reanalysis of these data has concluded that Atlantic and Pacific pivotal temperatures are indeed not significantly different but that TRT is significantly larger for Leatherbacks from Playa Grande (Pacific) compared to French Guiana (Atlantic) (Chevalier *et al.*, 1999).

*Caretta caretta*: TSD has been studied for 6 different geographic origins of animals and permit to use the likelihood ratio test. A significant global difference for TSD is observed (**Fig. 1**). This demonstrates a significant genetic structure of populations for this species as already demonstrated with mtDNA polymorphism (Encalada *et al.*, 1998).

**Conclusions**

The first method that uses all the informations available to describe TSD in reptiles is presented. When applied to available data for marine turtles, this method permits the calculation of pivotal temperature and TRT and also their standard-deviations.

This method can be used also to distinguish populations of *C. caretta*, *C. mydas* and *D. coriacea* based on their sensitivity to temperature. This new statistical tool will permit us to enhance the ecological and evolutionary work on temperature-dependent sex determination.

**Acknowledgments**

This paper has greatly benefited from discussions with Johan Chevalier, Claude Pieau, Michel Laurin, Matthew Godfrey and Nicholas Mrosovsky.

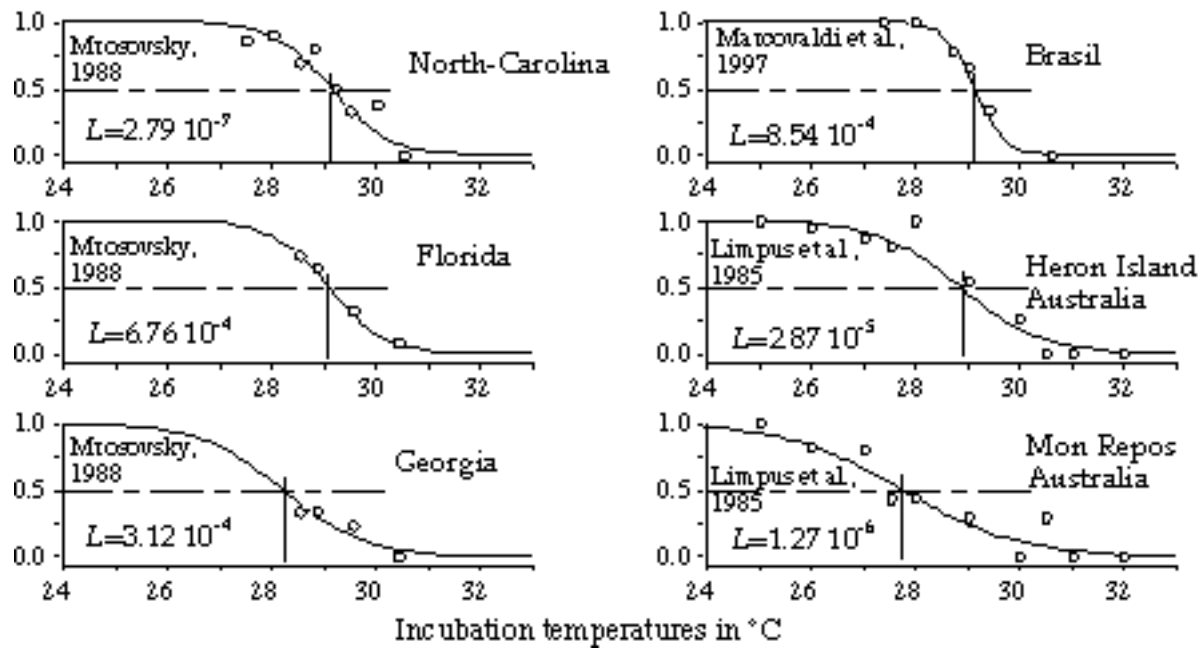
**Table 1:** Pivotal temperature ( $P$ ) and  $TRT_{5\%}$  for marine turtles (SD is standard deviation).

Species	Locality	P (SD)	$TRT_{5\%}$ (SD)	Ref.
<i>C. mydas</i>	Suriname	29.14 (SD 0.18)	3.23 (SD 0.464)	1
<i>L. olivacea</i>	World	30.06 (SD 0.24)	3.95 (SD 0.047)	2, 3
<i>C. caretta</i>	North-Carolina, USA	29.15 (SD 0.11)	3.24 (SD 0.353)	4
<i>C. caretta</i>	Georgia, USA	28.19 (SD 0.17)	4.65 (SD 0.706)	4
<i>C. caretta</i>	Florida, USA	29.09 (SD 0.11)	3.18 (SD 0.647)	4
<i>C. caretta</i>	Mon Repos, Australia	27.85 (SD 0.22)	5.89 (SD 0.765)	5
<i>C. caretta</i>	Heron Island, Australia	28.87 (SD 0.22)	4.59 (SD 0.294)	5
<i>C. caretta</i>	Brazil	29.15 (SD 0.06)	1.75 (SD 0.070)	6
<i>E. imbricata</i>	Antigua	29.27 (SD 0.04)	0.97 (SD 0.007)	7
<i>D. coriacea</i>	Costa Rica, Pacific	29.43 (SD 0.01)	1.17 (SD 0.040)	8

1: Godfrey, 1997; 2: McCoy *et al.*, 1983; 3: Standora and Spotila, 1985; 4: Mrosovsky, 1988; 5: Limpus *et al.*, 1985; 6: Marcovaldi *et al.*, 1997; 7: Mrosovsky *et al.*, 1992; 8: Binckley *et al.*, 1998.

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**Figure 1:** Significant TSD difference between *Caretta caretta* “populations” ( $\chi^2=107.12$ , 10 DF,  $p<0.01$ ). The sex ratios (Y axes) are shown in “male frequency”.

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