

# Expansion of the transitional range of temperature for sea turtle *Lepidochelys olivacea* from sex ratio data at controlled incubation temperatures

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## Introduction

Sexual differentiation in sea turtles is determined by incubation temperature (Bull, 1980) during the middle third of the incubation period (Yntema and Mrosovsky, 1980). Warm temperatures produce females and cool temperatures generate males. The pivotal temperature is defined as the temperature of incubation producing 50% of each sex (Mrosovsky et al., 2002). For *Lepidochelys olivacea* incubation experiments at constant temperature show that below 28 °C produces males and above 32 °C produces females. The interval between 28 and 32 °C defines the transitional range of temperatures (TRT) where both sexes can be obtained (McCoy et al., 1983; Mrosovsky and Pieau, 1991). The boundary temperatures producing clutches of one sex are considered as threshold temperatures.

We propose a standard method for determining the threshold temperature, based on the model of Girondot (1999) that describes the functional relationship between the mean temperature during the middle third of the incubation period and the proportion of males produced. Our proposal is important as it consists of a non-lethal method to estimate sex proportions through temperature measurements in the clutches, as opposed to the histological sex determination. The temperature thresholds can be precisely calculated and used to assure only one sex is produced, for example in captive breeding efforts.

## Materials and methods

We estimated the masculinizing and feminizing thresholds temperatures,  $T_m$  and  $T_f$  respectively, of the sea turtle *L. olivacea* by using the model of Girondot (1999) about male proportion  $P_m$  as a function of incubation temperatures  $T$  (eq.1), and we modified the method proposed by Hulin et al. (2009) to obtain equations 2 and 3,

$$P_m(T) = \frac{1}{1 + \exp\left[\frac{P-T}{S}\right]} \quad (1)$$

$$T_m = P - S \ln\left[\frac{1 - 0.99}{0.99}\right] \quad (2)$$

$$T_f = P - S \ln\left[\frac{1 - 0.01}{0.01}\right] \quad (3)$$

Where:

$P$  = Pivotal temperature

$S$  = Shape of the curve between masculinization and feminization temperatures ( $S < 0$ ).

It is not possible to predict 100% of any sex by this method, because the Girondot curve is asymptotic at both sides. We considered that average clutch of *L. olivacea* was around 100 eggs. Then, we proposed that the masculinizing threshold  $T_m$  should be calculated as the temperature corresponding to a proportion of 0.99 of males. In a similar way, the feminizing threshold  $T_f$  is the temperature that produces a 0.01 male proportion. These are the limit values of the male proportion that allows obtaining an integer number of males or females, when it is applied to the total number of eggs of the nest. In order to use this result for conservation practices, it is better to know the boundary temperatures directly from equations 2 and 3 instead of the TRT and the pivotal temperature, as Hulin et al. (2009) proposed, because

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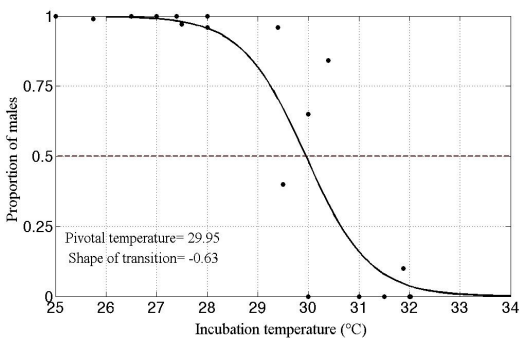
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using the threshold temperatures it is easier to discern when the temperatures in the protected areas produce a bias toward one sex.

Our proposed method is based upon the assumption that the physiological response of *L. olivacea* to incubation temperature for sex determination is independent of the geographical zone where the eggs were deposited. This premise cannot be contradicted because there are several proposed hypotheses to try to explain the physiological responses about temperature sex determination (Salame-Méndez, 1998). From this perspective we used one curve to describe the process. According to our previously global reviews of published data of *L. olivacea* and the reviews from Hays *et al.* (2014), we considered all the articles that included incubations at constant temperatures with several ranges of temperatures (McCoy *et al.*, 1983; Paukstis and Janzen, 1990; Wibbels *et al.*, 1998; López-Correa, 2010; Sandoval, 2012). We used the non-linear regression method of Marquardt (1963) to fit the model, using the Matlab function *nlinfit.m* to estimate the parameters  $p$  and  $s$  of equation 1.

## Results

Nonlinear regression analyses yielded a significant fit of the Girondot curve (Eq. 1) to *L. olivacea* data ( $R^2 = 0.84$ ,  $df = 15$ ,  $P = 1.9 \times 10^{-6}$ ). We found a pivotal temperature of 29.95 °C, and a form factor  $S = -0.63$  (Fig.1). This pivotal temperature is coincident with the formerly known values from experimental data. Our method provides temperature thresholds of 27.1 °C for masculinizing, and 32.9 °C for feminizing (Eqs. 2 and 3), which represent an expansion of the transitional range of temperatures previously known.



**Figure 1.** Nonlinear regression of the Girondot curve ( $R^2 = 0.84$ ,  $P < 0.05$ ).

## Discussion

The estimation of TRT proposed by Hulin *et al.* (2009; Eq. 4) is equivalent to calculate the boundary temperatures or thresholds of masculinization and feminization temperatures on the Girondot fitted curve by using the values of 0.05 and 0.95 respectively, because  $TRT = T_f - T_m$ , but for clutches of more than 20 eggs the method underestimates the TRT. In the case of clutches around or more than 100 eggs, five or more embryos at each side of the curve are considered of one sex, neglecting the estimated male proportion by the model due to the asymptotic nature of the curve. According to our method modification established by equations 2 and 3 to determine new masculinization and feminization temperature limits, this interval is expanded as compared to that proposed by McCoy *et al.* (1983).

For other sea turtle species, our method could be applied regarding the total number of eggs in the nest, after the corresponding curve was fitted, adjusting equations 2 and 3 with the one sex corresponding female and male proportions. In clutches with different number of eggs it is necessary to change the limit values of male proportion in order to obtain the threshold temperatures. For instance, in the case of Flatback turtle the clutches are around 60 eggs, then the feminizing threshold  $T_f$  corresponds to 0.016 of males (1/60), and the masculinizing threshold  $T_m$  produces a 0.984 male proportion. For Hawksbills turtle, with clutches about 160 eggs, the female proportion is 0.00625 and 0.99375 for the male proportion (Table 1).

**Table 1.** Limit values of  $P_m$  for sea turtle species.

Species	Clutch size <sup>1</sup>	Average	$P_m$ limit	
			Females	Males
<i>Natator depressus</i>	50–70	60	0.016	0.984
<i>Dermochelys coriacea</i>	50–90	70	0.015	0.985
<i>Caretta caretta</i>	80–120	100	0.01	0.99
<i>Lepidochelys olivacea</i>	100	100	0.01	0.99
<i>Lepidochelys kempii</i>	100	100	0.01	0.99
<i>Chelonia mydas</i>	100–200	150	0.06667	0.99333
<i>Eretmochelys imbricata</i>	120–200	160	0.00625	0.99375

<sup>1</sup>Bjorndal, 1981.

Our method could also be applied to other sea turtle species, when experimental data on sex ratio at controlled temperature are available, in order to fit the

model for each case. The consequences of the results herein reported could change the considerations about the sex ratio of marine turtles, because our method permits an estimation of sex proportions using only nesting temperature data.

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