

## Reaction Norm of Embryo Growth Rate Dependent on Incubation Temperature in The Olive Ridley Sea Turtle, *Lepidochelys olivacea*, From Pacific Central America

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### Abstract

Olive ridleys (*Lepidochelys olivacea*) and loggerheads (*Caretta caretta*) are two closely phylogenetically related sea turtles that nest in very different thermal habitat. Olive ridleys nest in pan-tropical beaches whereas loggerheads nest in more temperate beaches. In the context of climate change, the temperature in temperate beaches will increase much more than for tropical beach due to buffering effect of air humidity in the later. We have determined the thermal reaction norm for embryonic growth in both species using field records of incubation temperatures and incubation length from loggerheads in Western Mediterranean Sea or olive ridleys from Pacific coast of Guatemala. We show that the optimum temperature for the growth of embryos is lower for loggerheads than for olive ridleys. This makes loggerhead turtles particularly sensitive to increase of beach temperature as it is expected due to effect of global warming in temperature regions. Furthermore, olive ridleys are more resilient to increase of temperatures and should not suffer from sublethal incubation temperatures.

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**Running title:** Reaction norm of embryo growth rate

**Keywords:** Growth, Embryo, temperature, Growth model, Sea turtle, *Lepidochelys olivacea*, *Caretta caretta*

**Received :** Sep 6, 2017

**Accepted :** Nov 27, 2017

**Published :** Dec 19, 2017

**Editor:** Adriana Cortés-Gómez , Laboratoire d'Ecologie, Systématique et Evolution. Université Paris-Sud, Email : adriana.niobe@gmail.com

## Introduction

Global warming presents major challenges to organisms [1,2]. There is a pressing need in conservation biology and climate change research for cross-species, quantitative, objective criteria for assessing the susceptibility of species to climate change-induced extinction [3]. Overall, current estimates of biodiversity loss due to climate change are very variable, depending on the method, taxonomic group, metrics, spatial and seasonal scales considered [4]. Yet, the majority of studies indicate alarming consequences for biodiversity, with the worst-case scenarios leading to extinction rates that would qualify as the sixth mass extinction in the history of the earth [5].

One attempt to address this shortcoming is phenomenological, using climatic descriptions of contemporary ranges coupled with expected temperatures to predict future ranges [6]. These bioclimatic models are best case scenarios because, while they reflect existing interspecific differences in projected ranges under global warming, they also implicitly assume that all species have similar potential to access and exploit predicted climate space. The insidious threat from climate change is that it will weaken or preclude ecological responses by species that

are physiologically constrained; yet, quantitative, objective criteria for assessing relative susceptibility of diverse taxa to warming-induced stress are wanting [3]. Consequently, biologists are endeavouring to develop robust ways to evaluate the differential vulnerability of organisms to climate change [7] and then to evaluate and implement management strategies tailored for species judged most at risk [8].

The extent of adaptive variation in heat-tolerance is likely to have important consequences for the resilience of many ectothermic species in a rapidly warming world [9-13]. This has led to calls for an 'adaptive evolutionary' approach to conservation, which seeks to conserve functional diversity (rather than simply genetic marker diversity) at whatever scale it occurs [14].

*Lepidochelys olivacea*, the olive ridley, and *Caretta caretta*, the loggerhead, are two phylogenetically closely related sea turtles [15] that nest in very different thermal habitats. Olive ridley turtles nest in intertropical zone while loggerheads nest in more temperate beaches [16]. Virtually all biological rates are affected by temperature including development duration [17] and growth rates [18] of ectothermic animals. Egg incubation temperatures affect the duration of

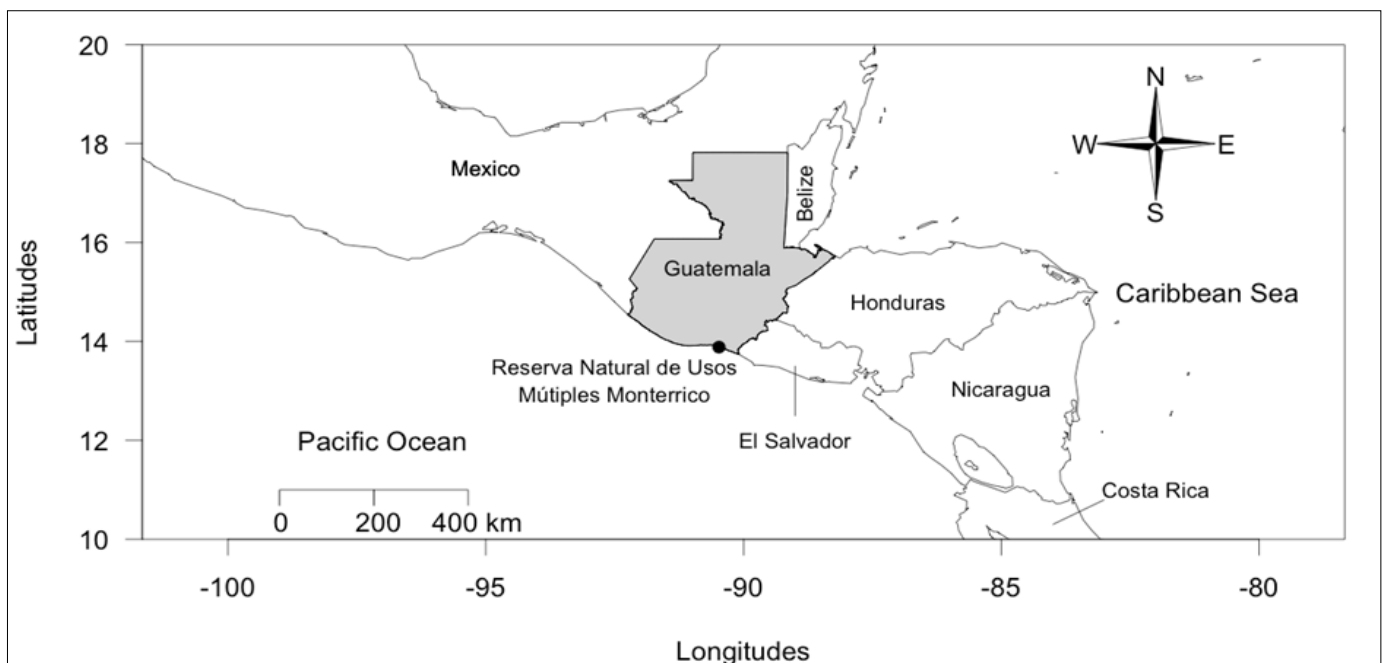


Fig. 1: Map of Central America with greyed Guatemala. Monterrico nesting beach is indicated by a black point within the Monterrico Natural Reserve for Multiple Uses (Reserva Natural de Usos Múltiple Monterrico).

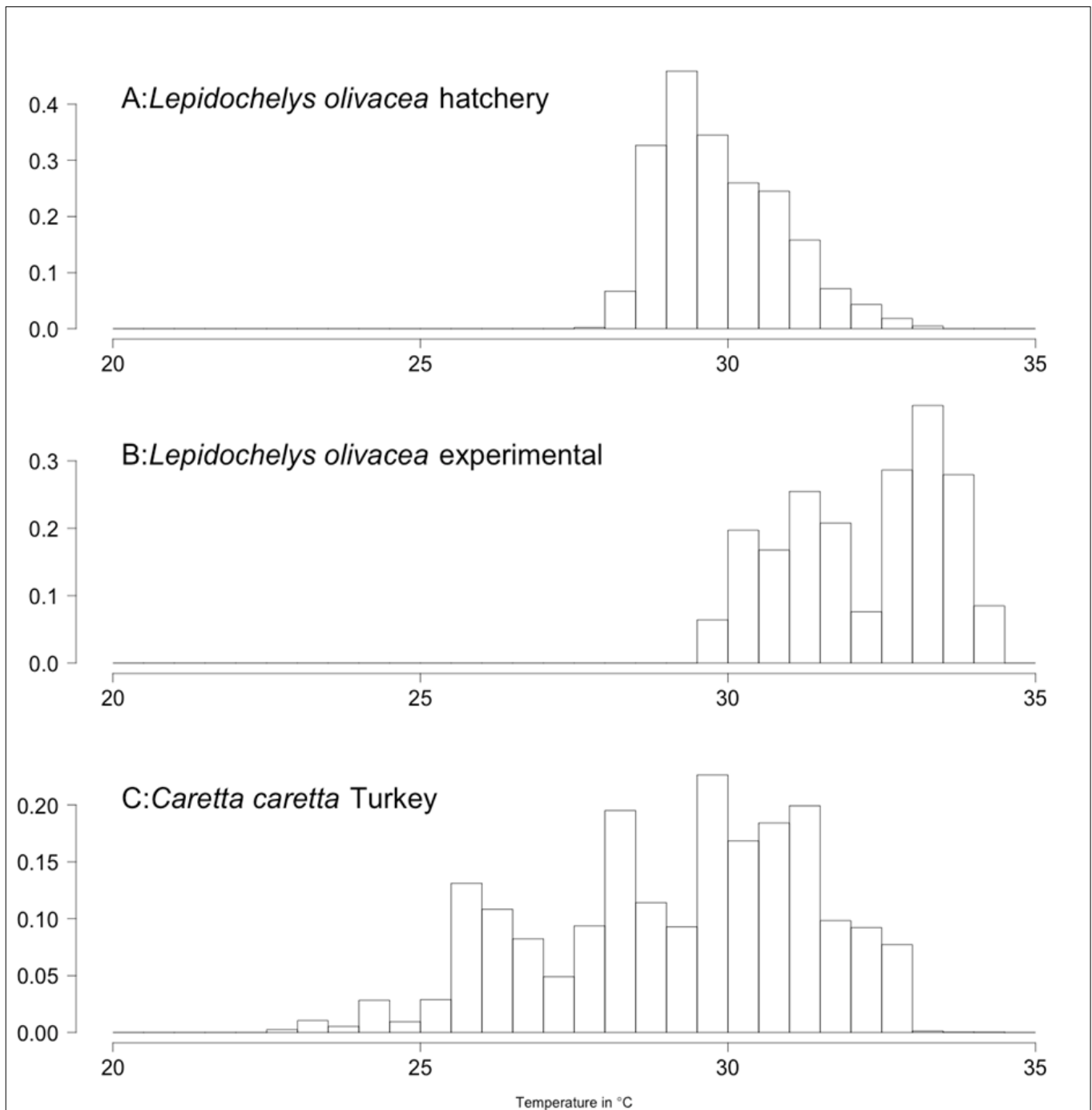


Fig. 2: Temperatures recorded in hatchery (A) and experimental (B) *Lepidochelys olivacea* nests from Monterrico, Guatemala. As a comparison, temperatures recorded in 21 *Caretta caretta* nests from Turkey are shown [42] (C).

embryogenesis [19], the probability of embryo survival [20,21], the sex determination for species with temperature-dependent sex determination such as *Caretta caretta* [22] and the performance, the morphology, and body size at hatching [21,23]. In addition, long-term effects of incubation temperature on the physiology and behaviour of hatchlings has been observed [24,25]. Thus, the fitness of developing embryos is strongly dependent on the temperature within the nest during incubation.

Recently, a general way to model sigmoidal embryo growth with variable incubation temperature during development obtained from *in situ* sea turtle nests has been proposed and tested with data from *Caretta caretta* from Dalyan beach, Turkey [26]. New data of incubation temperatures and incubation durations have been gathered in 2011-2012 for *Lepidochelys olivacea* nesting in Guatemala Pacific coast. These data have permitted to estimate the thermal reaction norm for the embryo growth for this species and to compare this pattern with the loggerhead one.

## Materials and methods

### FIELD DATA

Olive Ridley sea turtles nest along all of the Pacific Coast of Guatemala. Egg collection for human consumption is authorized as long as 20% of the eggs of each collected nest is given to hatcheries located along all the littoral [27]. A total of 1,600 eggs collected on the previous night were bought to various collectors. The dates of incubation beginning were as followed: 16/11/2011 - 140 eggs; 22/11/2011 - 120 eggs; 23/11/2011 - 160 eggs; 24/11/2011 - 1000 eggs; 25/11/2011 - 160 eggs; 26/11/2011 - 20 eggs. We had no control on how the eggs were handled during the previous night but the hatching success was very high. Eggs were grouped in 80 nests (20 randomly selected eggs per nest) in this experiment. Among them, 40 have been incubated in hatchery (hatchery nests) and 40 have been used in 4 different experiments (experimental nests). Half of these 40 nests were buried in sand at 40 cm depth and half at 60 cm depth. Among each group of these 40 experimental nests, half (10) have been incubated in open beach under full shade and half (10) in full sun. Experiment was conducted at the hatchery of the Monterrico Natural Reserve for Multiple Uses.

### ANALYSIS OF INCUBATION DATA

Incubation time, longitudinal temperatures and 10 hatchling straight carapace lengths at the nearest 0.1 mm were registered for all monitored nests. Mean incubation temperatures and incubation durations were analysed using linear model [28] and hatchling sizes were analysed using linear mixed model with nest identity as a random factor [29]. In all cases, fixed factors were depth of nests (40 or 60 cm) and with or without shade and their interaction. Mean incubation temperature and its first order interaction with other factors were added for analysis of mean incubation duration and hatchling size. A backward model selection was used by removing the least non-significant factor one at a time. A single factor was not removed if it was significant when involved in an interaction. F test after ANOVA (ANalysis Of VAriance) was used to detect the influence of factors after linear model [28] and Likelihood Ratio Test (LRT) test after ANOVA was used to detect the influence of factors after linear mixed model using R package glmmADMB [30].

The variability of daily temperatures among nests was measured as the mean of the daily standard deviation of temperatures recorded in nests from each treatment (shading status and depth). Welch modified two-sample t-test with unequal variances were used to test the treatments effect (depth and shading) [31].

### THERMAL GROWTH RATE REACTION NORM

The model of embryo growth integrates in a single framework both the growth rate dependency on temperature and the embryo growth [26]. The parameters for growth rate dependency on temperature that maximized the logarithm of the likelihood ( $\ln L$ ) of the observed hatchling size distribution were search for using the R package embryogrowth [32]. The model is summarized here briefly but a complete description can be found in the original publication [26].

Biological temperature-dependent rate models based on Arrhenius' and Eyring's equations have been formulated by Sharpe and DeMichele [33]. The original formulation of Sharpe and DeMichele was modified by Schoolfield *et al.* [34] to remove the very high correlations of parameter estimators. Two kinds of equations using 4 or 6 parameters produced a curve with a maximum at an intermediate temperature and decreased below and above this temperature. The level

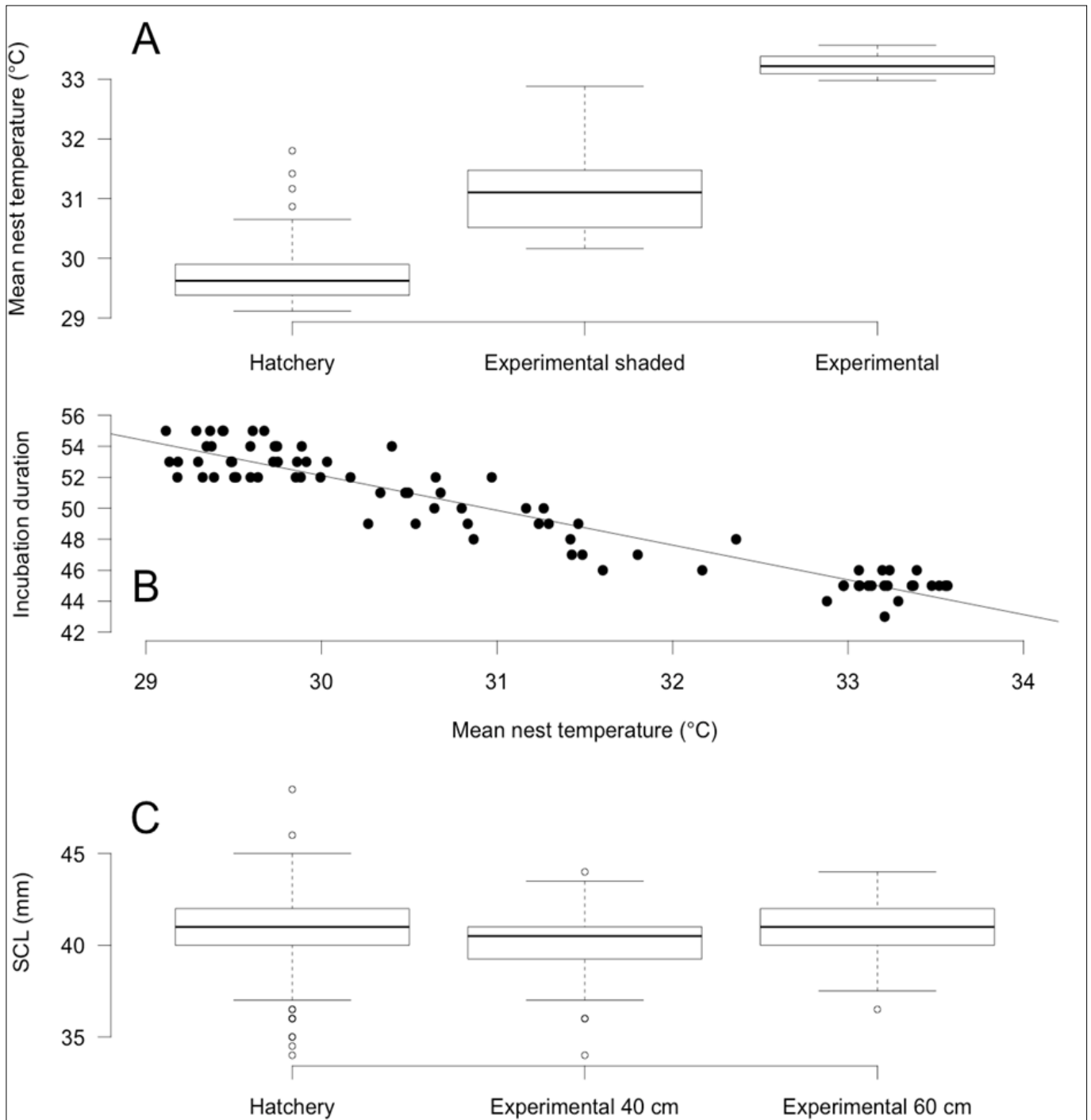


Fig. 3: Descriptive statistics of incubation data for *Lepidochelys olivacea* nests from Monterrico, Guatemala, plotted according to the significant factors explaining the differences between treatments. (A) Mean incubation temperature, (B) incubation duration, and (C) hatchling size.

as well as the position of the maximum can be manipulated using the parameters values.

The early growth of embryos is modelled using a modification of the Gompertz model [35] proposed by Laird [36] (eqn 1):

$$X(t) = X_H \gamma_K \exp \left( \ln \left( \frac{X(0)}{K} \right) \exp (-r(T) t) \right) \text{ eqn 1}$$

Where  $X(0)$  is the size or mass at nesting time (time=0),  $r(T)$  is the growth rate at the beginning of the curve, and  $K$  is the carrying capacity with

$$\lim_{t \rightarrow +\infty} X(t) = K$$

$X_H$  is the hatchling size and  $r_K=2.09$  is a constant used to slowdown growth at the end of incubation [26] to ensure that embryological stages are well positioned during incubation.

The dynamic of  $X(t)$  is governed by the Gompertz differential equation (eqn 2):

$$X'(t) = r(T) \ln \left( \frac{K}{X(t)} \right) X(t) \text{ eqn 2}$$

The gastrula is approximately a disk of 1.7 mm diameter and this size will be used as  $X(0)$  [37].

#### MODEL FITTING

Growth rate  $r(T)$  can be calculated with models (4 or 6 parameters) from Schoolfield et al. [34] model and an incubation temperature  $T$ . With  $X(0)$  and  $K$ , and a time-series of  $r(T)$ , the pattern of change of embryo size for this nest is evaluated using Runge-Kutta method of order 4 for the approximation of solutions of ordinary differential equations.

Estimation of parameters was performed using maximum likelihood with an identity link and a Gaussian distribution of SCL. The standard error of parameters was estimated using the square-root of the inverse of the Hessian matrix which is an asymptotic approximation of the variance-covariance matrix [38]. The models are implemented in the R package *embryogrowth* [32].

#### COMPARISON BETWEEN SETS OF DATA

First, growth rate  $r(T)$  has been fitted for hatchery and experimental *Lepidochelys olivacea* nests separately. Next, all the nests were grouped in a single dataset and growth rate  $r(T)$  has been fitted again. We used AIC and Akaike weight to select between 4 and

6-parameters models. AIC is a measure of the relative quality of fit, which penalized for too many parameters in the model [39] and Akaike weight gives the relative statistical support of several models tested on the same dataset [40]. Likelihood ratio test has been used to test whether a single model for hatchery and experimental nests was sufficient or not to describe observed data.

$$\text{The statistics } LRT = -2 \frac{\ln L_{\text{complex model}}}{\ln L_{\text{simple model}}}$$

(Likelihood Ratio Test, with  $\ln L$  being the logarithm of the likelihood) is distributed as a  $\chi^2$  with the degrees of freedom being the difference of number of parameters between the most complete model and the simplest one [41].

#### Results

##### ANALYSIS OF INCUBATION DATA

Distribution of temperatures recorded in the 80 *Lepidochelys olivacea* nests from the beach of Monterrico, Guatemala are shown in figure 2 as well as the temperatures recorded in *Caretta caretta* nests from Turkey [42]. Average incubation temperatures for the 80 nests ranges from 29.11 °C to 33.56 °C (mean=31.00 °C, sd=1.53 °C). Shaded nests were significantly cooler than those exposed to the sun by 2.11 °C (paired t-tests with Bonferonni correction,  $p < 10^{-9}$ ). Nests from hatchery were also significant cooler than the experimental ones (paired t-tests with Bonferonni correction, 1.32 °C difference between hatchery vs shaded,  $p < 10^{-9}$  and 3.44 °C difference hatchery vs sun,  $p < 10^{-9}$ ) (Fig. 3A). Among the experimental nests, only shading status was significant ( $F_{38}^1 = 155.4, p > 10^{-14}$ ) to explain the difference between nests for average incubation temperatures. Depth and interaction between shading status and depth were not significant ( $F_{38}^1 = 0.33, p > 0.4$  and  $F_{36}^1 = 0.17, p > 0.6$  respectively).

Incubation duration ranged from 43 to 55 days (mean=49.86 days, sd=3.63). Significant effect of mean incubation temperature and shading status for experimental data was observed ( $F_{37}^1 = 47.37, p < 10^{-7}$  and  $F_{38}^1 = 120.71, p < 10^{-12}$  respectively) but not of depth as well as all interactions (all  $p > 0.1$ ). Incubation duration was longer for cooler temperatures and shaded nests (Fig. 3B).

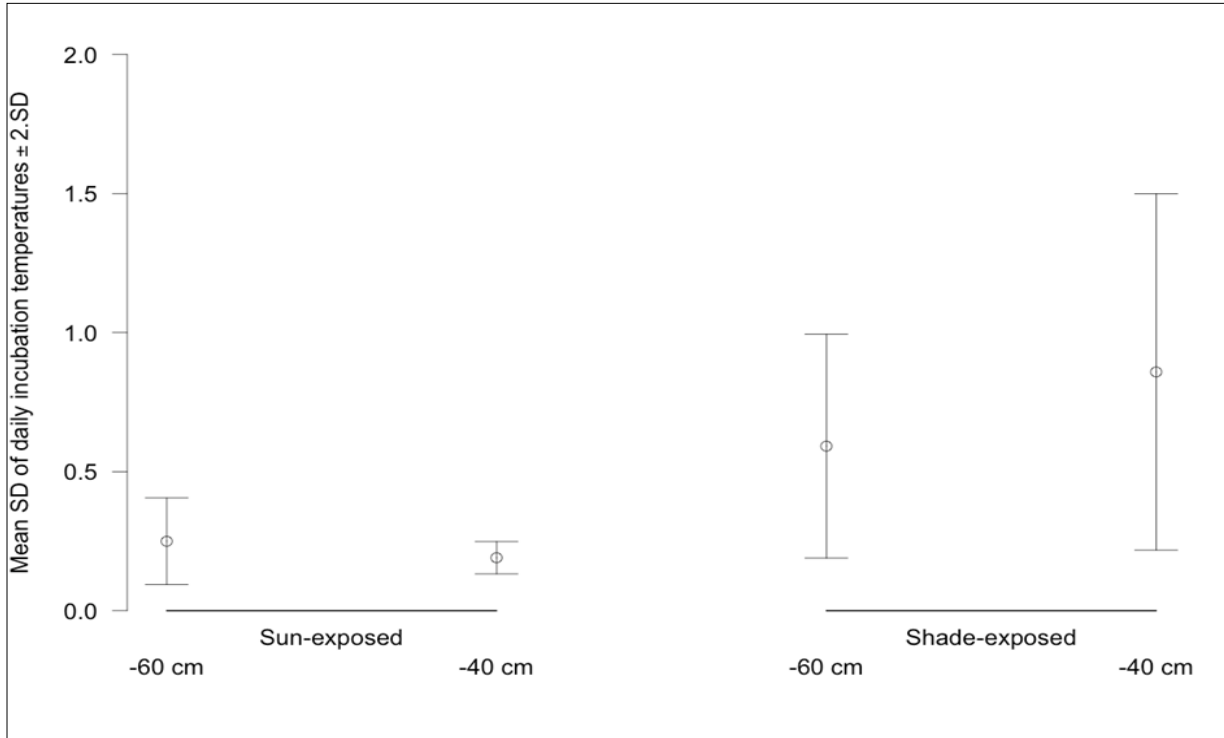


Fig. 4: Average of the daily standard deviation of temperatures recorded in nests from each treatment (shading status and depth), for *Lepidochelys olivacea* nests from Monterrico, Guatemala.

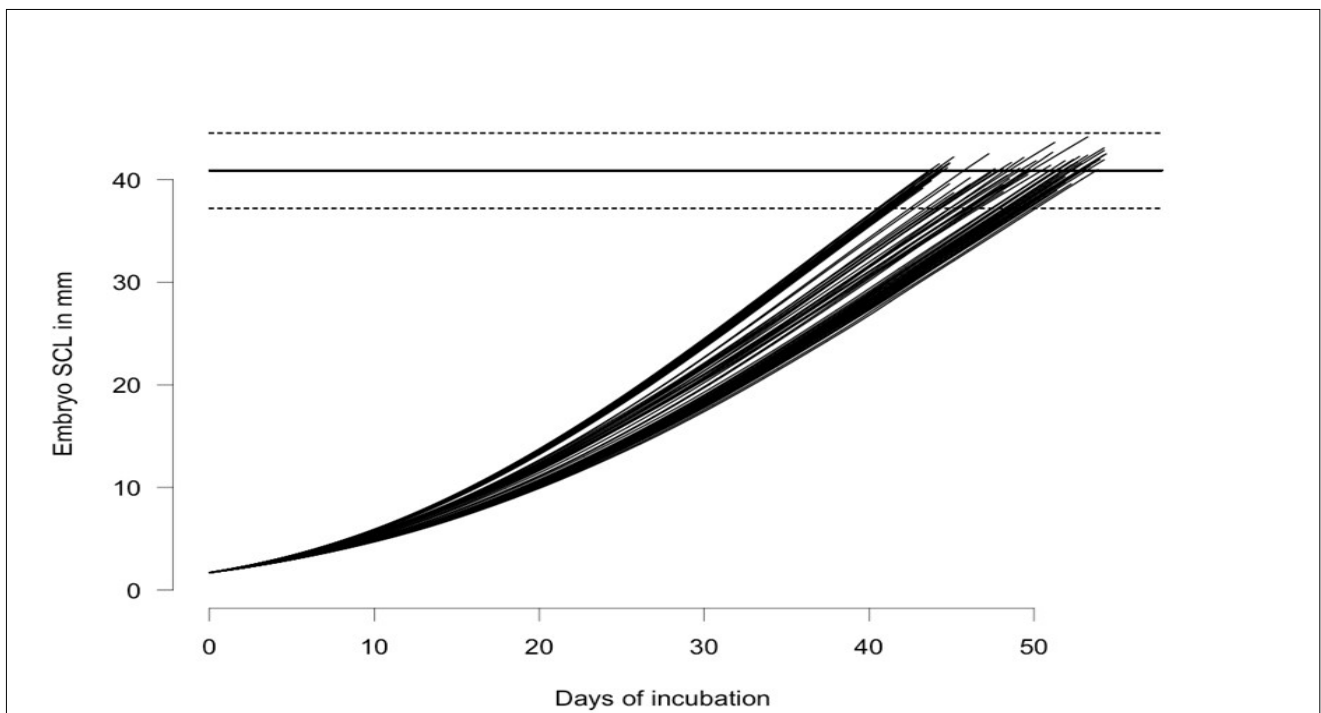


Fig. 5: Fitted straight carapace length (SCL) depending on incubation time for the 80 monitored nests of *Lepidochelys olivacea*, from Monterrico, Guatemala. The horizontal lines are the observed hatching size and twice the standard deviation.



Straight carapace length of hatchlings (mean 40.86 mm,  $sd=1.82$ ) was significantly different for experimental data according to depth factor (deviance= 5.492,  $df=1$ ,  $p<0.02$ ) but not for any other factors (all  $p>0.05$ ). Embryos incubated at 40 cm were smaller (40.22 mm,  $sd=1.54$  mm) than those incubated at 60 cm (41.07 mm,  $sd=1.36$  mm) (Fig. 3C).

An effect of shading at 40 cm ( $t = -6.7727$ ,  $df = 9.15$ ,  $p < 0.0001$ ) and 60 cm ( $t = -5.0015$ ,  $df = 11.441$ ,  $p < 0.001$ ) and depth for shade ( $t = -2.3516$ ,  $df = 15.382$ ,  $p$ -value  $< 0.04$ ) and sun-exposed nests ( $t = 2.1895$ ,  $df = 11.557$ ,  $p < 0.05$ ) were noticed on the daily standard deviation temperatures.

#### TEMPERATURE DEPENDENT EMBRYONIC GROWTH RATE

Parameters maximizing likelihood of observed hatchling size for each nest have been fitted first using the total set of 80 nests using the 4 and the 6-parameters equation describing instantaneous growth rate dependency to temperature. AIC for 4-parameters model was 380.97 whereas it was 390.05 for the 6-parameters model. Akaike weight gives a very strong support to retain the 4-parameters model ( $p=0.99$ ). In a second step, parameters have been fitted separately for hatchery nests ( $\ln L=-97.83$ ), for experimental nests ( $\ln L=-79.79$ ) and for all nests together ( $\ln L=-186.55$ ) with LRT being 1.90 ( $df=4$ ,  $p=0.75$ ). Thus, a single model for the two categories of nests was sufficient.

The fitted pattern of embryo growth for the 80 nests is shown in figure 5. It should be noted that all fitted embryo sizes at the end of the incubation are comprised within the 95% confidence interval of observed hatchling sizes.

The fitted instantaneous growth rate according to temperature is shown in figure 6 for *Lepidochelys olivacea* from Guatemala. The curve fitted for Mediterranean *Caretta caretta* [42,43] is also shown for comparison.

#### Discussion

Temperature during incubation of ectothermic animals can have profound consequences on the fitness of individuals and then selection should act to adapt response of embryos to temperature.

The effect of shading on incubation temperature was anticipated based on several previous reports for

turtles [44-47] but also lizards [48]. Indeed, we found that the shaded experimental nests have a lower mean incubation temperature than the experimental nests exposed to the sun. Hatchery nests are also shaded and have still a lower temperature (Fig 3A). However, we did not detect a significant effect of depth probably because the differential between both depths (40 and 60 cm) was not sufficient to produce enough change in temperature. As a consequence, shaded nests take longer to emerge when compared to the nests directly exposed to sun and incubation temperature could modulate this effect (Fig 3B).

Hatchlings from eggs incubated at 40 cm were significantly smaller than those from eggs incubated at 60 cm (Fig. 3C) but a direct effect of temperature or shading was not observed. We do not have definitive explanation for this effect. Mass of hatchling has been shown to be dependent on incubation temperature in many reptiles [49-52] but also on dryness of substrate [53]. On the other hand, effect on size is not consistent among studies nor within the same study. For example, no effect has been observed in the turtle *Pelodiscus sinensis* [23] but a slight decrease of carapace length has been measured in the sea turtle *Caretta caretta* as the moisture increased [54]. But, in the same experiment and for the same turtles, carapace width and plastron length did not show any consistent pattern of increasing with higher moisture [54].

Here we do not detect an effect of temperature on size but an effect of depth of the nest. This effect could be mediated by moisture difference between two depths: it is possible that eggs incubated at 40 cm lost more water than those located at 60 cm, the latter being closer to the water table (sea water that infiltrates by porosity in sand) [55]. Eggs incubated in dryer substrate accumulate less water during incubation [56]. The mechanism linking substrate water content and size of embryos has been well studied in the freshwater *Chrysemys picta* [57]. Water within the eggs plays a key role in the mobilization of yolk reserves by reptile embryos and consequently influences hatchling mass [57].

*Caretta caretta* nests mainly out of the intertropical region whereas *Lepidochelys olivacea* has a distribution more centred on the equator [16]. The northernmost nest for a sea turtle has been deposited by a *Caretta caretta* in South of France at the latitude



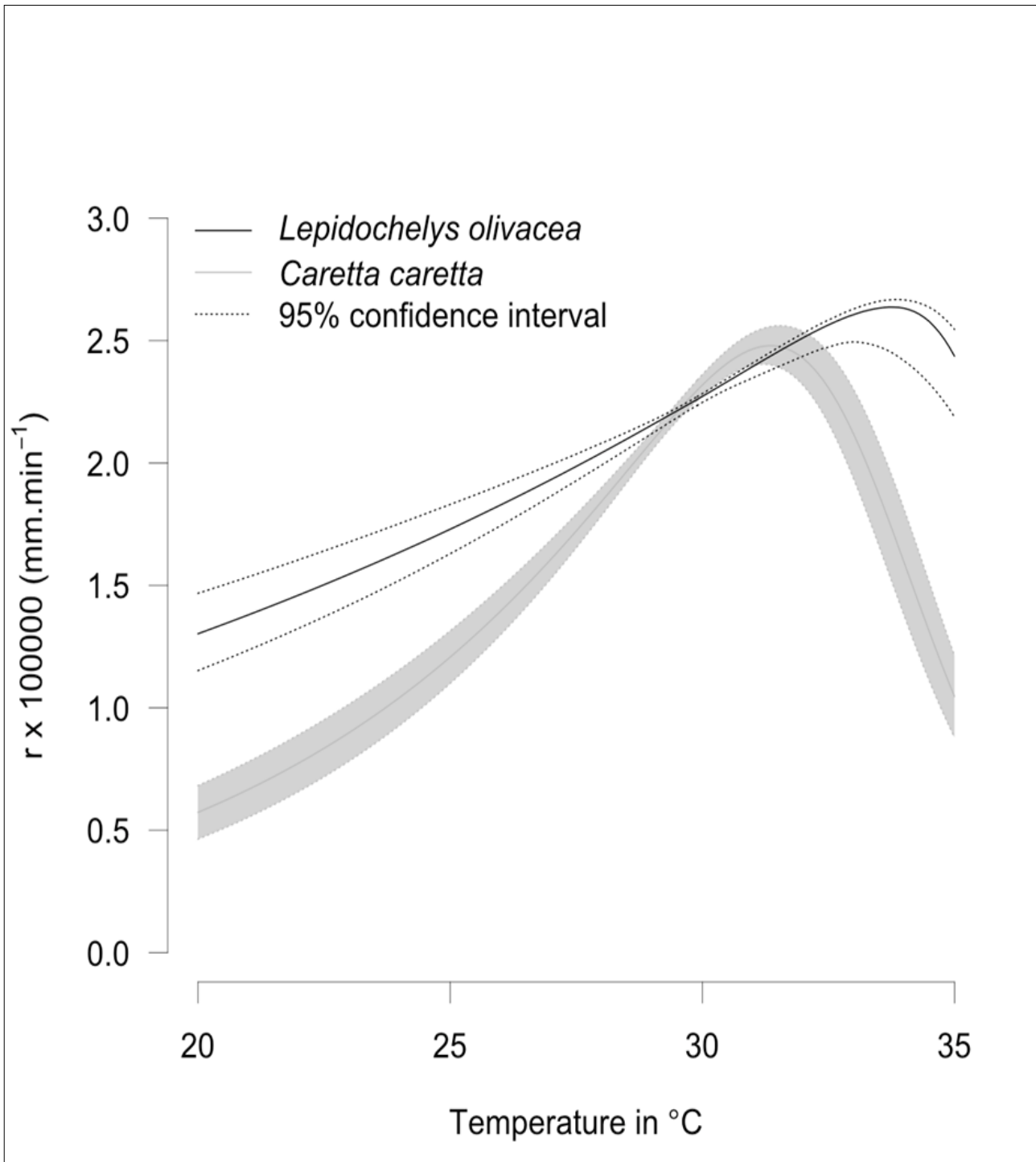


Fig. 6: Fitted growth rate  $r(T)$  of straight carapace length (SCL) depending on incubation temperature  $T$  for *Lepidochelys olivacea* (this study) and *Caretta caretta* [43]. The envelope in dashed lines is the 2.5% and 97.5% quantiles.

43°16.05'N [58]. As a consequence, the nest temperatures experienced by *Lepidochelys olivacea* (Fig. 2A and B) are generally much higher than temperatures experienced by *Caretta caretta* (Fig. 2C). The growth rate depending on temperature in olive ridleys showed a pattern of increase from 20 °C to 35 °C. However, it should be noted that the part of the curve from 20 °C to 27 °C is extrapolated based on Schoolfield model of thermal reaction norm [34] because no lower temperature than 27 °C was recorded in *Lepidochelys olivacea* nests from Guatemala (Fig. 2). On the other hand, temperature as low as 22 °C was recorded in *Caretta caretta* nests in Turkey [42] and the pattern of growth rate for these two species at low temperatures was very similar (Fig. 6). However, the pattern differs completely for incubation at high temperatures. The growth rate continued to increase up to 35 °C for *Lepidochelys olivacea* whereas it went down for *Caretta caretta* around 32 °C. This is consistent with the observation that incubation duration increased at 32 °C as compared to 31 °C [43] and that no *Caretta caretta* hatchlings survived when incubated at 32 °C and above [21].

The variability of temperatures among different nests incubated in same conditions was measured. We detect an effect of depth but much more an effect of shading (Fig. 4). Such a measure is particularly important because it permits to explain why, in the same beach and at the same time, heterogeneity of incubation conditions was observed. The thermal microhabitats have been overlooked for sea turtle and generally for reptiles. This is particularly important in the context of prediction of the impact of climate change. The climate models available give prediction at the regional scale [59] very far away from the beach scale and even intra-nesting beach level as shown here. This downscaling is challenging to be able to produce realistic models of incubation of reptile eggs.

### Acknowledgments

We thank the hatchery of the Monterrico Natural Reserve for Multiple Uses and the authorities of Guatemala for authorization to use the eggs collected for this experiment. We thank two anonymous referees for their very valuable suggestions that have enhanced the clarity of this manuscript and we thank also the editor Adriana Cortés-Gómez for her help with this manuscript.

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