

# Influence of nest temperature on morphology of Leatherback Turtle (*Dermochelys coriacea*) hatchlings incubated in hatcheries in Oaxaca, Mexico

J. García-Grajales, J.F. Meraz-Hernando, J.L. Arcos García, and E. Ramírez-Fuentes

**Abstract:** The influence of nest incubation temperatures on carapace shape and morphological traits of Leatherback Turtle (*Dermochelys coriacea* (Vandelli, 1761)) hatchlings incubated in two hatcheries in Oaxaca, Mexico, was evaluated. This study was carried out from October 2016 through May 2017. On each beach, there are community groups consisting of volunteers not affiliated with universities who protect and relocate the nests to increase hatching success. In each translocated nest, a data logger was placed in the centre of the egg mass. Hatchlings were collected as they emerged from each nest. The carapaces of the hatchlings were photographed and subjected to geometric morphometric analysis; also, hatchlings were weighed and their bodies measured. The mean temperature of 12 nests in each hatchery was recorded, with no significant differences between hatcheries. The principal component analysis revealed an overlapping of the carapace shape under different temperature durations. Temperature had a significant influence on hatchling morphology. Higher mean incubation temperatures produced hatchlings with low mass, smaller appendages, narrower carapace widths, and shorter flipper lengths. Lower mean incubation temperatures produced hatchlings with greater mass, wider appendage widths, wider carapace widths, and longer flipper lengths. Results indicate that the Leatherback hatchlings incubated in hatcheries demonstrate morphology that varies in relation to nest incubation temperature in a similar way to hatchlings produced in natural environments.

**Key words:** carapace, *Dermochelys coriacea*, hatchery, hatchlings, incubation, Leatherback Turtle, morphology.

**Résumé :** L'influence de la température d'incubation dans le nid sur la forme de la carapace et des caractères morphologiques de petits nouvellement éclos de tortues luths (*Dermochelys coriacea* (Vandelli, 1761)) incubés dans deux écloséries de Oaxaca (Mexique) a été évaluée. L'étude a été menée d'octobre 2016 à mai 2017. Sur chaque plage, des groupes communautaires composés de bénévoles sans association à des universités protègent et déplacent les nids pour en accroître le succès d'éclosion. Un enregistreur de données a été placé au centre de la masse d'œufs dans chaque nid. Les petits étaient recueillis à leur émergence du nid. Les carapaces des petits ont été photographiées et ont fait l'objet d'une analyse morphométrique géométrique; aussi, les petits ont été pesés et leurs corps ont été mesurés. La température moyenne de 12 nids dans chaque éclosérie a été enregistrée, aucune différence significative n'étant relevée entre les écloséries. L'analyse en composantes principales révèle un chevauchement de la forme des carapaces pour différentes durées de températures. La température a eu une influence significative sur la morphologie des petits. Des températures d'incubation moyennes plus élevées ont produit des petits de faible masse présentant des appendices plus petits, des carapaces plus étroites et des nageoires plus courtes. De faibles températures d'incubation moyennes ont produit des petits de plus grande masse, présentant des appendices et des carapaces plus larges et de plus longues nageoires. Les résultats indiquent que la morphologie des petits nouvellement éclos de tortues luths incubés en écloséries varie en fonction de la température d'incubation du nid, à l'instar de celle des petits issus de milieux naturels. [Traduit par la Rédaction]

**Mots-clés :** carapace, *Dermochelys coriacea*, éclosérie, petits nouvellement éclos, incubation, tortue luth, morphologie.

## Introduction

Temperature plays an important role in the life cycle of sea turtles (Hulin et al. 2009; Hays et al. 2017). As in most reptiles, the temperature at which sea turtle eggs are incubated determines many of the characteristics of embryos and hatchlings (Glen et al. 2003), such as the rate of embryonic development (Booth et al. 2004); embryo survival, frequency of abnormalities among embryos and hatchlings (Sönmez et al. 2017); pigmentation and scale pattern of

hatchlings (Köhler 2005); body size and mass of the residual yolk at hatching, post-hatching growth, survival, thermoregulation (Köhler 2005; DeGregorio and Southwood 2011); and locomotion performance (Elnitsky and Claussen 2006; Hare et al. 2008). The influence of temperature on developing hatchlings (Booth 2006) can reduce the fitness of neonates (Mickelson and Downie 2010) and impact population dynamics and survival (Fisher et al. 2014).

Nest temperature is determined by a combination of sand temperature and metabolic heating produced by developing embryos

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(Broderick et al. 2001; Booth and Freeman 2006). In most turtles, high egg incubation temperatures result in a female-biased clutch, whereas low incubation temperatures result in a male-biased clutch (Ferreira-Júnior et al. 2011). At a given incubation threshold, called pivotal temperatures (Bull 1980), an equal proportion of males and females are produced. Therefore, different proportions of males and females result from incubation temperatures above and below this threshold (Pieau et al. 1995). The pivotal temperature for Leatherback Turtles (*Dermochelys coriacea* (Vandelli, 1761)) nesting in the Pacific Ocean basin is 29.4 °C (Binckley et al. 1998).

In Mexico, considerable efforts and broader conservation strategies have been devoted toward the protection of sea turtles (García et al. 2003). These actions include the protection of nesting beaches through regular patrols against human poaching, widely implemented nest transference to protected hatcheries, and other general strategies such as a complete ban on the exploitation of turtles and their eggs (García et al. 2003). Particularly, the populations of Leatherbacks in the Pacific Ocean basin have experienced a dramatic decline in nesting numbers in the past few decades (Spotila et al. 2000; Sarti et al. 2007; Benson et al. 2015). Therefore, specific conservation efforts for Leatherback in Mexico have been focused on four index beaches (Mexiquillo, Tierra Colorada, Cahuitán, and Barra de la Cruz), selected because of their intense nesting activity (Sarti et al. 2007; Santidrián-Tomillo et al. 2017). Nevertheless, there are secondary beaches that are considered important nesting sites where Leatherbacks regularly nest (Santidrián-Tomillo et al. 2017). In all of these beaches, nest protection is practiced through egg relocation. The eggs are moved from natural nests to protected hatcheries — a common practice used at rookeries around the World — to increase hatchling recruitment into sea turtle populations (Baskale and Kaska 2005; Maulany et al. 2012; Santidrián-Tomillo et al. 2017; Tapilatu 2017).

Leatherbacks are globally listed as critically endangered under the International Union for the Conservation of Nature (IUCN) criteria (Wallace et al. 2013), and trends and status of sea turtles in the Pacific Ocean basin have declined precipitously during the last several decades, including a population drop of more than 90% in Mexico (Sarti et al. 1996, 2007). Moreover, the increase in nest incubation temperatures as a result of global warming has the potential to affect the fitness of hatchlings, which may put further pressure on the existing population of this critically endangered species (Mickelson and Downie 2010).

Until now, research indicates that hatcheries provide effective protection of sea turtle nests (Baskale and Kaska 2005); nonetheless, hatcheries do not necessarily provide optimal nest conditions for offspring such as nest incubation temperature, which determines the sex of the hatchlings during the middle third of the incubation period (Binckley et al. 1998). In addition, temperature influences other factors such as those described above. Although there have been previous studies on the effects of nest incubation temperature on Leatherback morphology in the wild (Mickelson and Downie 2010), there are no previous studies in hatcheries of this species that evaluate temperature effects. Therefore, it is important to understand the link between morphological traits of hatchlings and nest incubation temperatures in enclosed protected hatcheries. Whereas different hatchling phenotypes have been reported from natural nests (Glen et al. 2003; Read et al. 2012; Sim et al. 2015), the separate effects of maternal origin and nest environment on hatchling phenotype in natural nests have not been demonstrated because in natural nests the two effects are confounded by each other (Booth 2017). This work was not a split clutch design to experimentally separate nest effects from maternal origin effects in the field nests of Leatherbacks. However, in laboratory experiments that involved the incubation of reptile eggs, both maternal origin of eggs and incubation

environment have been demonstrated to influence hatchling phenotype.

Thus, to know what morphological repercussions occur as a result of mean nest temperatures in the protected hatcheries, our goal in this study was to evaluate the influence of nest incubation temperatures on carapace shape and morphological traits of Leatherback hatchlings incubated in hatcheries in Oaxaca, Mexico.

## Materials and methods

### Study area

The study took place in the San Juan Chacahua and Palmarito secondary nesting beaches of Leatherbacks in the Central Pacific Coast of Oaxaca, Mexico. San Juan Chacahua beach (15°57'48"N, 97°40'52"W to 15°58'54"N, 97°46'54"W) is 12 km in length and is part of the Lagunas de Chacahua National Park, whereas Palmarito beach (15°52'23"N, 97°06'23"W to 15°55'36"N, 97°13'58"W) is about 16 km in length, extending from San José Manialtepec River on the northwest to Punta Colorada on the southeast. The climate is tropical, hot, humid, and characterized by well-defined dry and rainy seasons. The mean annual temperature is 27.5 °C and mean annual rainfall is 800 mm, concentrated between July and October; the dry season can last 8 months, from November to June.

### Fieldwork

This study was carried out from October 2016 through May 2017, during the Leatherback nesting season. On each beach, there are community groups consisting of volunteers without university affiliations who protect and relocate the nests to increase hatching success (García et al. 2003; Vannini et al. 2011). Community groups patrolled both beaches at night from the hours of 2100 to 0600, using an all-terrain vehicle, to record any sea turtle activity. All nests registered were collected, numbered, clutch size recorded, and transported in clean plastic bags to the enclosed hatcheries; these sites were closely monitored daily for threats from natural predators. All relocated nests were manually buried at a depth of 80 cm in the hatchery, which is the mean depth of Leatherback nesting activity reported among Pacific populations (García-Grajales et al. 2019), and this nest depth is required by Mexican law 162 (Diario Oficial de la Federación 2012) for all hatcheries in Mexico.

The enclosed hatchery sites were constructed according to all specifications detailed in Mexican law 162, with a total area of 80 m<sup>2</sup> (10 m × 8 m), which was sufficient to accommodate 100 nests and high enough to avoid being inundated by high tides. The distance between nests was set at 1 m, according to Mexican law 162, to prevent any interaction between them and to allow hatchery personnel to walk among the nests without stepping onto them. Furthermore, because low levels of precipitation and high temperatures have been linked to decreased hatching success and emergence of hatchlings from the nest (Santidrián Tomillo et al. 2012; Hill et al. 2015), to protect the enclosed hatchery from the intensity of the sun, the hatcheries were covered by a sheet of shading net at a height of 1.50 m throughout nesting season. Each year the hatcheries are moved around the area to avoid accumulation of bacteria and other types of contamination that can result in reduced egg survival.

To determine different temperature parameters in the nest environment and their variation during the incubation period, a temperature data logger was placed in the centre of the egg mass in each relocated nest. Only one type of data logger was used (HOBO® UA-002-08; Onset Computer Corporation, Bourne, Massachusetts, USA) in the nests on both beaches, and all were programmed to register the temperature every 30 min. A total of 12 nests (6 nests from San Juan hatchery and 6 nests from Palmarito hatchery) and their temperature data sets were recorded.

Nest contents were excavated 24 h after the emergence of the first hatchling, as suggested by Patiño-Martínez et al. (2010), and the data loggers were retrieved. The total number of eggs (the number of eggs laid into the nest) and the hatching success were evaluated by counting unhatched eggs, dead hatchlings in eggs, and dead hatchlings in nests, and by excluding the shelled albumen globes. The hatching success for each nest was evaluated as the percentage of live hatchlings in the clutch, and the incubation period per nest was determined from the number of days from the date the egg was deposited to the date of emergence of the first hatchling (Yalçın Özdilek et al. 2007).

One hundred and eighty Leatherback hatchlings from both hatcheries were used for a geometric morphometric analysis. For this, we proposed sorting embryo development as slow, moderate, and fast, depending on length intervals of incubation reported (Eckert et al. 2015). Based on Miller et al. (2017), for stages 24–30 of development incubated at 29 °C, embryos complete their development in 55 days; we assumed that embryos took 4 days to develop to the next morphological stage. Therefore, we propose that with the mean nest temperature in the nest chamber, hatchlings that hatched from 58 to 62 days were considered to have slow embryo development (SED); those that hatched between 54 and 57 days after oviposition were considered to have moderate embryo development (MED); and those that hatched within 50 to 53 days were considered to have fast embryo development (FED). We used carapace shape to describe the morphological variation during embryo development because it was easy to take its measurement (Casale et al. 2017).

After each nest had been incubating for approximately 45 days, we placed a plastic enclosure around the top of the nest at dusk and we checked these enclosures every half hour between dusk and dawn to ensure that hatchlings were near the surface. Hatchlings were collected as they emerged from each nest with the help of community groups. Only 15 hatchlings selected randomly, excluding those with malformations, from each nest were used. Digital images of the carapace of each hatchling were obtained using a Sony DSC-alfa 350 supported on a tripod and placed perpendicularly 25 cm above the subject. Each hatchling was placed on a self-healing cutting mat. For the geometric morphometric analysis, the techniques developed by Valenzuela et al. (2004), Myers et al. (2006), and Ferreira-Júnior et al. (2011) were adopted. For example, hatchlings were photographed alive because it was necessary to hold them for the photographs to be taken orthogonally to the carapace centre. After photographs were taken, hatchlings were released on the beach. An image file was then obtained with tpsUtil software (Rohlf 2005a). Subsequently, anatomical landmarks were recorded using tpsDig software (Rohlf 2006a). For the carapace shape, a total of twelve type 1 and nine type 2 anatomical landmarks were used (Fig. 1). Type 1 landmarks were formed by the scales that delineate the lateral and marginal scutes, whereas type 2 landmarks included the white scales that adorn the dorsal ridges as white stripes.

The anatomical landmarks of all individuals were superimposed in tpsRelw software (Rohlf 2006b) to generate a consensus. Procrustes distance analysis was then used to evaluate the similarities between shapes and mathematically remove the effects of the digitized position, orientation, and scale. Procrustes distance represents the distances between two points in Kendall's multidimensional space, and it was used to measure the similarity between the shapes (Monteiro and Reis 1999). In this process, the variation in hatchling size and the orientation of the photograph were superimposed, and the variations found were attributed only to the shape of the carapace. From the aligned anatomical landmarks, a mass matrix was calculated as partial warp scores from the thin-plate spline (TPS) (Monteiro and Reis 1999).

We used the TPSs for the analysis of relative warps (visualization of shapes). The generalized Procrustes analysis (GPA) and

TPS functions were created by tpsRelw software. The TPS allowed for visualization of the direction and magnitude of the differences in carapace shape, as well as the identification of areas with higher variation.

To identify the hatchling morphology and size index, each selected hatchling used in the morphometric analysis was weighed immediately prior to the trial using a 100 g Pesola handy spring scale balance. Calipers were used to measure the hatchling carapace length and width, the length and width of the right and left flippers, and head width (Fig. 2) was measured from the cranial base to an accuracy of 0.1 mm.

### Statistical analyses

For the ordination of the variations in carapace shape of the hatchlings in the mass matrix, the principal components analysis (PCA) was used, and each hatchling was coded according to its development sorting (SED, MED, FED) described above.

To evaluate whether carapace shapes vary with incubation duration, a one-factor multivariate analysis of variance (MANOVA) was applied to the weight matrix. XLStatistic (ecology) software was used for this analysis.

The percentage of shape variation determined by incubation duration was calculated by a multivariate regression of shape using tpsRegr software (Rohlf 2005b). The cross-validation test using the R system was used to evaluate the correct classification percentage of specimens and the applicability of this method to other hatchlings (Ferreira-Júnior et al. 2011). Permutation tests were carried out using tpsRegr software.

We performed a PCA on all morphometric measurements to explore and visualize the variation and to reduce the number of shape variables. Principal component (PC) 1 is considered to largely represent size, so we used PC 2 and PC 3 to interpret the shape variation. Prior to the PCA, data were checked for normal distribution with the Anderson–Darling test for normality. Data were log<sub>10</sub>-transformed to meet the above assumptions for parametric statistical tests.

Additionally, separate linear regressions were performed to test the association between mean temperature and each morphological variable (mass, carapace length, carapace width, right flipper length, right flipper width, and head width) for each location (San Juan Chacahua and Palmarito hatcheries). Assumptions of the linear regressions were checked with diagnostic plots. Variables were log<sub>10</sub>-transformed to comply with the assumptions of parametric tests.

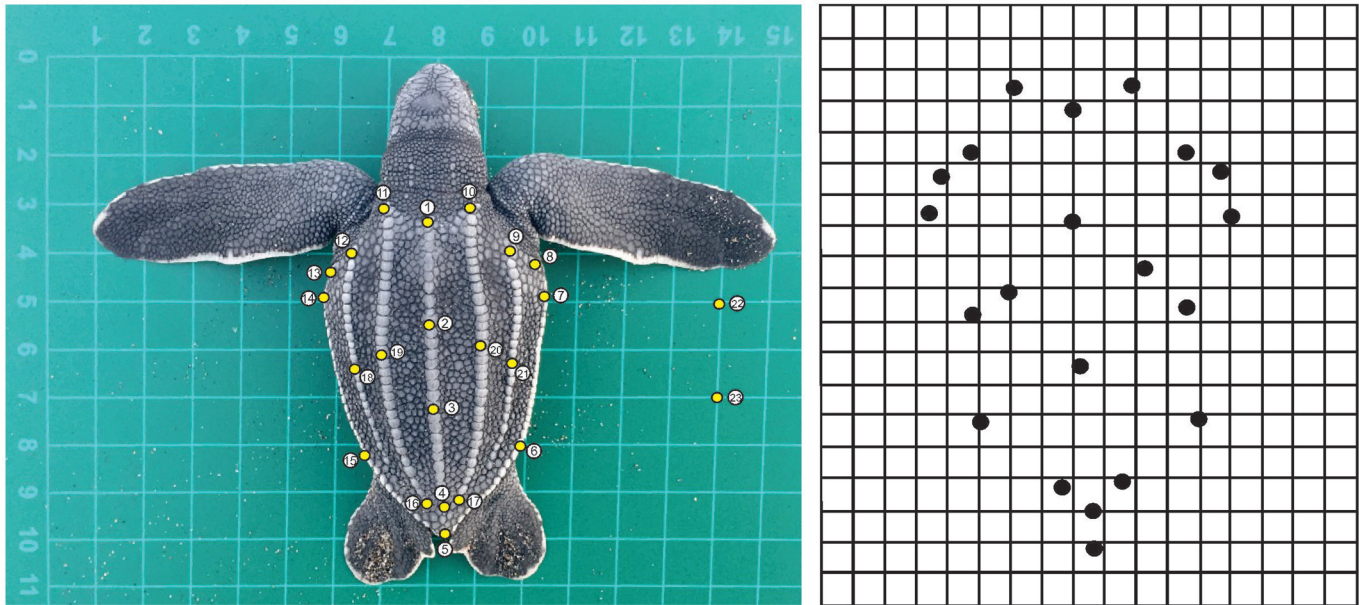
Finally, we used a generalized linear model (GLM) to evaluate the effects of the mean nest temperature on all morphometric measurements (response variables). We identified the contribution of each of the variables and their levels of significance ( $P < 0.05$ ), as well as the confidence interval for each of the variables. All statistical analysis and transformations were carried out using XLStat (ecology) software.

### Results

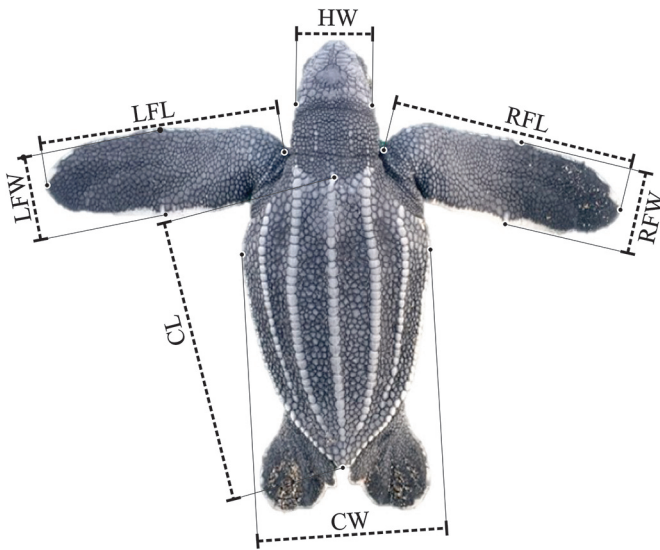
The temperature was recorded between 51 and 62 days, with a mean ( $\pm$ SE) of  $57.04 \pm 2.7$  days. The mean ( $\pm$ SE) temperature was  $30.43 \pm 0.78$  °C for the San Juan Chacahua hatchery and was  $30.53 \pm 0.41$  °C for the Palmarito hatchery, without significant differences between hatcheries (ANOVA,  $F_{[1,18]} = 1.83$ ,  $P = 0.085$ ; Figs. 3a and 3b).

Examining variation in carapace shape of Leatherback hatchlings using MANOVA, significant effects related to incubation duration were found (Wilks'  $\lambda = 0.794$ ,  $F_{[23,678]} = 80.53$ ,  $P = 0.001$ ). Permutation tests also indicated significant differences between carapace shapes ( $P < 0.001$ ). Using cross-validation analysis, we found that 86.3% of hatchlings were correctly classified. The PCA revealed an overlapping of the carapace shape when SED, MED, and FED were compared (Fig. 4). In those hatchlings incubated with SED, the cranial region of scutes was broader and longer,

**Fig. 1.** Anatomical landmarks used in geometric morphometric analysis of carapace shape of Leatherback Turtle (*Dermochelys coriacea*) hatchlings (left), and the consensus generated by the superposition of carapaces of all hatchlings (right). Colour version online.



**Fig. 2.** Leatherback Turtle (*Dermochelys coriacea*) hatchling standard measurements used for morphological data. HW, head width; CL, carapace length; CW, carapace width; RFW, right flipper width; LFW, left flipper width; RFL, right flipper length; LFL, left flipper length. Colour version online.



and the shape was more rounded, compared with those of hatchlings incubated with FED whose caudal and cranial regions developed faster and were more compressed, shorter and close to the medium scutes. The hatchlings that have MED showed more characteristics related to faster development.

#### Hatchling morphology and size index

Eight biometric measurements were obtained from 180 hatchlings among 12 nests (Table 1). Mean nest incubation temperatures monitored during this study were above the critical threshold temperature for Leatherback hatchling sex determination (29.4 °C) during the majority of the incubation period (Figs. 3a and 3b).

Therefore, it was estimated that 100% of the hatchlings measured were female (García-Grajales et al. 2019), and hatchling sex was not a factor in this study.

Seven PC scoring systems were calculated for both hatcheries, and the results indicated that variations in hatchling morphology and size were concentrated in PC 1, PC 2, and PC 3. These three components comprised 76% of the morphology and size variation between individuals (Table 2).

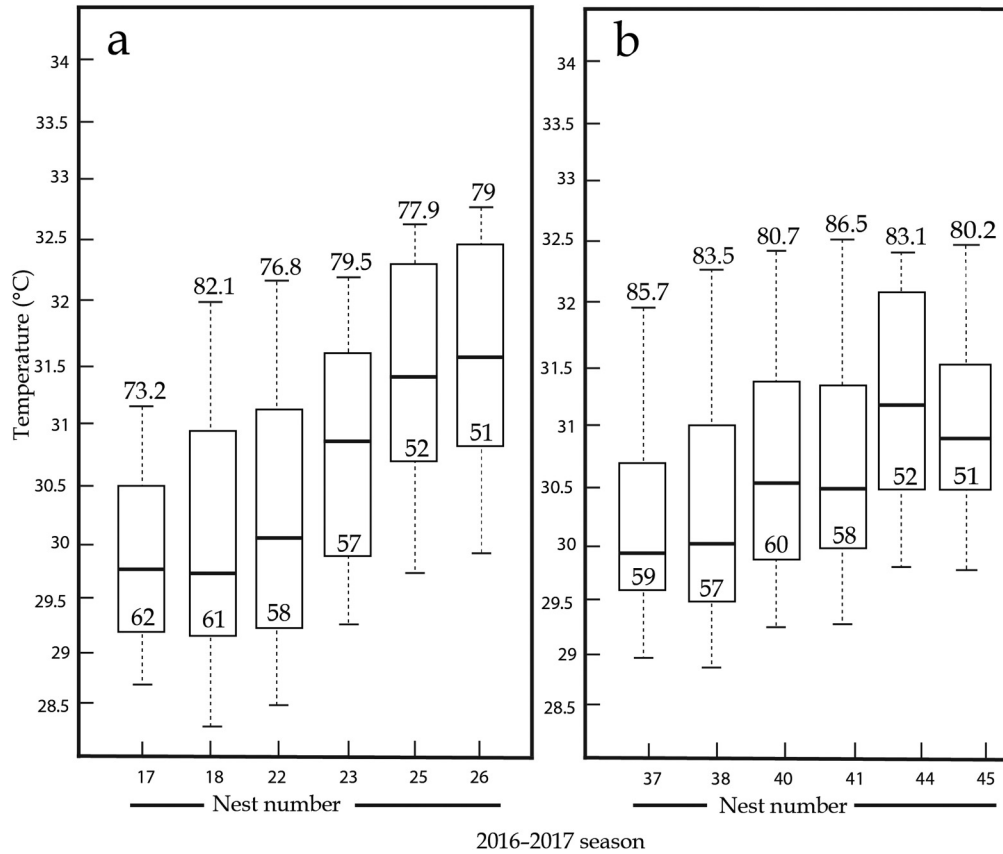
Mean nest incubation temperature had a significant influence on hatchling morphology (size and biometric measurements) based on PCA scores (Figs. 5a and 5b). Higher mean incubation temperatures produced hatchlings with low mass, smaller appendages, narrower carapace widths and shorter flipper lengths. Lower mean incubation temperatures produced hatchlings that had greater mass, wider appendage widths, wider carapace widths, and longer flipper lengths, whereas intermediate nest incubation temperatures produced hatchlings with morphological characteristics similar to those with higher incubation temperatures.

With respect to the influence of mean nest temperature on morphology, linear regressions for both locations (Figs. 6a–6f) showed incubation temperature to be negatively associated with all biometric measurements (Table 3). The GLM showed differences in some morphological traits between the two localities (group 1 is the San Juan Chacahua hatchery and group 2 is the Palmarito hatchery) (Table 4). Mass of hatchlings in the San Juan Chacahua hatchery were less heavy than mass of hatchlings in the Palmarito hatchery.

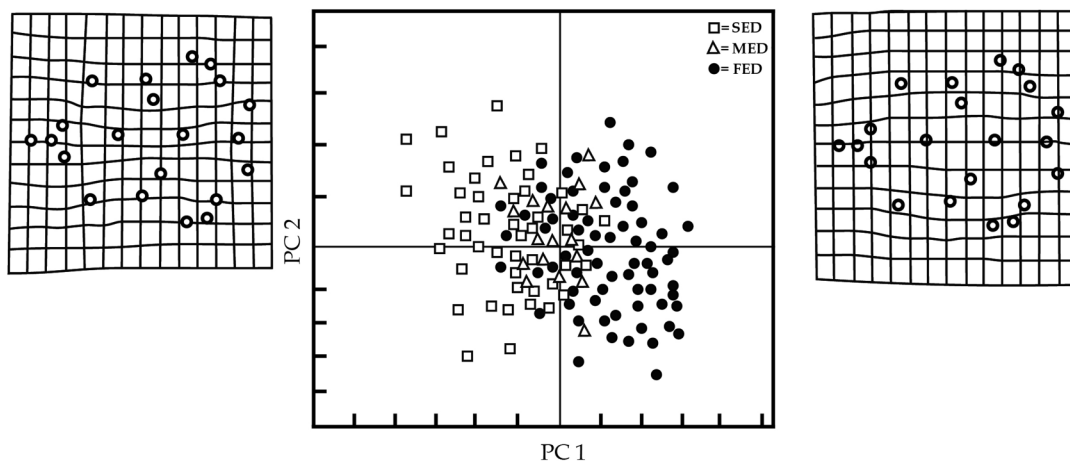
#### Discussion

Incubation temperatures have several consequences to developing reptile embryos. For example, nest incubation temperature exerts strong influence on the development rates of turtle embryos, and thus, variation in nest temperatures consequently leads variation in incubation duration (Ferreira-Júnior et al. 2011). Our results in terms of incubation temperature profiles are similar to those presented by Steckenreuter et al. (2010) in Papua New Guinea and by Mickelson and Downie (2010) in Tobago, West Indies, where incubation temperatures of Leatherback nests ranged between 26.3 and 36 °C, with the only difference being that those studies were conducted in natural environments.

**Fig. 3.** Temperature profiles obtained from 12 Leatherback Turtle (*Dermochelys coriacea*) nests produced in hatcheries (San Juan Chacahua (a) and Palmarito (b)) of Oaxaca, Mexico. Thick horizontal lines within the boxes represent median temperatures. Whiskers represent the maximum and minimum values recorded. Numbers inside the boxes represent the lengths of the incubation period (days), whereas the numbers above the upper whiskers represent nesting successes (%).



**Fig. 4.** Representation of the first two axes of the principal components analysis of carapace shape of Leatherback Turtle (*Dermochelys coriacea*) hatchlings. PC, principal component; SED, slow embryology development; MED, moderate embryology development; FED, fast embryology development.



Considering the course of natural selection, shape is one of the most important expressions of the phenotype (Roff 1992). As far as turtles are concerned, influence of shape in the survival of hatchlings offer an important contribution for the understanding of population biology (Myers et al. 2007). Normally, studies aimed at assessing survival and behaviour of hatchlings use body size

and mass of individuals as variables, and indicate a strong correlation between hatchling body and survival (Janzen et al. 2007; Tucker et al. 2008). Therefore, carapace shapes are correlated with the ability to swim, and consequently, to avoid predation (Myers et al. 2007). As carapace shape is highly heritable, it may constitute a key role in the early life stages of turtles (Myers et al. 2006). The use

**Table 1.** Descriptive statistics for Leatherback Turtle (*Dermochelys coriacea*) hatchlings ( $n = 180$ ).

Measurement	San Juan Chacahua hatchery			Palmarito hatchery		
	Mean $\pm$ SD	Minimum	Maximum	Mean $\pm$ SD	Minimum	Maximum
Mass (g)	38.63 $\pm$ 2.81	31.2	45.3	42.35 $\pm$ 3.01	34.5	47.8
Carapace						
Length (mm)	57.35 $\pm$ 3.16	50.4	66.3	60.13 $\pm$ 2.16	53.75	67.4
Width (mm)	36.82 $\pm$ 2.25	31.2	42.7	38.42 $\pm$ 2.25	33.28	44.21
Right flipper						
Length (mm)	50.12 $\pm$ 2.63	49.4	64.9	58.13 $\pm$ 3.12	50.31	65.27
Width (mm)	16.32 $\pm$ 1.03	15.1	19.8	17.99 $\pm$ 1.17	16.52	20.43
Head width (mm)	16.84 $\pm$ 0.78	14.8	19.7	17.84 $\pm$ 0.93	16.31	20.35

**Table 2.** Principal components analysis (eigenvalues and coefficient loading scores of the seven principal components (PC)) applied to morphological measurements of Leatherback Turtle (*Dermochelys coriacea*) hatchlings.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
<b>San Juan Chacahua hatchery</b>							
Eigenvalue	2.579	1.368	0.689	0.624	0.234	0.124	0.068
Proportion of variance	0.482	0.135	0.114	0.104	0.074	0.068	0.023
Cumulative proportion of variance	0.482	0.617	0.731	0.835	0.909	0.977	1.000
Variable							
Mass	<b>0.511</b>	0.27	0.116	0.006	0.315	-0.743	0.135
Carapace							
Length	<b>0.480</b>	0.312	0.115	0.317	0.431	0.123	0.144
Width	<b>0.402</b>	0.130	-0.798	-0.299	-0.307	0.235	-0.028
Right flipper							
Length	<b>0.461</b>	0.161	<b>0.471</b>	0.018	0.025	0.046	0.144
Width	0.199	<b>-0.686</b>	-0.021	0.685	0.465	0.347	0.234
Head width	0.308	<b>-0.561</b>	0.338	-0.484	0.316	0.248	0.149
<b>Palmarito hatchery</b>							
Eigenvalue	2.463	1.247	0.724	0.635	0.183	0.115	0.057
Proportion of variance	0.413	0.128	0.105	0.100	0.162	0.072	0.020
Cumulative proportion of variance	0.413	0.541	0.646	0.746	0.798	0.87	1.000
Variable							
Mass	<b>0.53</b>	0.29	0.123	0.007	0.346	-0.758	0.146
Carapace							
Length	<b>0.493</b>	0.318	0.119	0.321	0.439	0.129	0.149
Width	<b>0.418</b>	0.145	-0.893	-0.299	-0.314	0.243	-0.029
Right flipper							
Length	<b>0.472</b>	0.174	<b>0.486</b>	0.021	0.028	0.051	0.135
Width	0.203	<b>-0.697</b>	-0.028	0.694	0.473	0.349	0.243
Head width	0.314	<b>-0.573</b>	0.341	-0.497	0.322	0.252	0.151

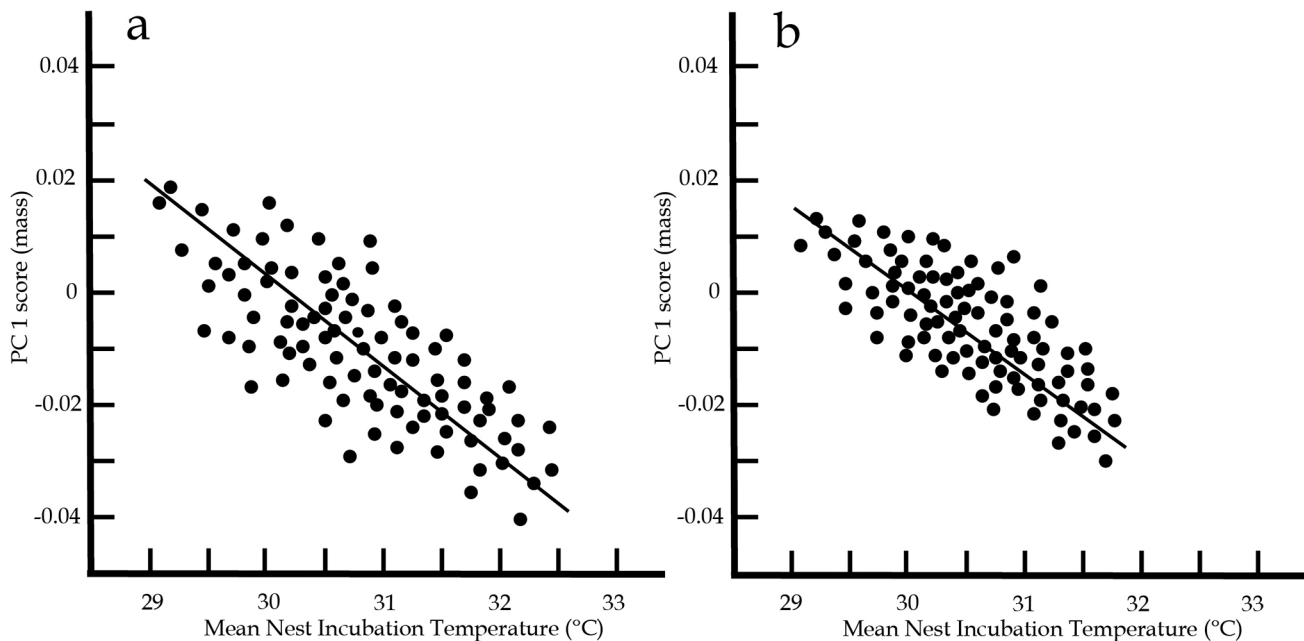
Note: Significant values based on  $P < 0.05$  are set in boldface type.

of geometric morphometric analysis in our study reveals significant differences in the carapace shapes of Leatherback hatchlings associated with length of incubation. To date, no similar studies have been carried out on Leatherbacks; however, similar results have been found for other species such as the Giant South American River Turtle (*Podocnemis expansa* (Schweigger, 1812)), Painted Turtle (*Chrysemys picta* (Schneider, 1783)) (Valenzuela et al. 2004), and Loggerhead Sea Turtle (*Caretta caretta* (Linnaeus, 1758)) (Ferreira-Júnior et al. 2011). With respect to the overlapping carapace shapes observed, it is probable that other factors interact to shape the final shells of hatchlings (Ferreira-Júnior et al. 2011), such as multiple paternity of nests (Crim et al. 2002), more than one laying during a nesting season (Eckert et al. 2015), maternal characteristics (Myers et al. 2006, 2007), temperature oscillations during incubation, embryo development rate (Georges et al. 2004), and sex (Reece et al. 2002).

Several authors suggest that geometric morphometric analysis is probably more effective and easier to perform than linear measurements to identify morphological differences in carapace shapes (Hildebrand et al. 1997; Michel-Morfin et al. 2001; Glen

et al. 2003; Ferreira-Júnior et al. 2011). Traditional morphometric analysis was done by measuring linear distances (such as length, width, and height), and multivariate statistical tools were used to describe patterns of shape variation within and among groups. This approach also sometimes used counts, ratios, areas, and angles. Although the advantage of this method was its simplicity, its biggest problem was that linear distance measurements are usually highly correlated with size, which makes shape analysis difficult (Valenzuela et al. 2004). Another problem was the measurements taken from two different shapes could produce equal results because the data did not include the location of where the measurements were taken relative to each other, and it was also not possible to reconstruct a graphical representation of the shape from taken measurements (Congdon et al. 1999; Ruber and Adams 2001; Adams et al. 2004). To overcome these problems with traditional morphometric analysis, the geometric morphometric analysis was created. In this study, however, we used both methods in a complementary way because of the difficulty in analyzing the shapes of the flippers.

**Fig. 5.** Relationship between Leatherback Turtle (*Dermochelys coriacea*) hatchling morphology (principal component 1 (PC 1) = mass) and mean nest incubation temperature of San Juan Chacahua hatchery (a) and Palmarito hatchery (b).



Until now, the influence of incubation temperatures on hatchling morphology have been focused principally on tropical snakes (Webb et al. 2001), soft-shelled turtles (Du and Ji 2003), lizards (Braña and Ji 2000), and in some species of sea turtles such as the Green Sea Turtle (*Chelonia mydas* (Linnaeus, 1758)) (Glen et al. 2003; Booth et al. 2004; Burgess et al. 2006) and the Loggerhead Sea Turtle (Fisher et al. 2014). Additionally, While et al. (2018) demonstrates taxonomic bias in studies of thermal developmental plasticity of reptiles. As Mickelson and Downie (2010) point out, studies investigating the influence of incubation temperature on sea turtles have been limited, but our results are similar to those few existing studies. For example, our study has demonstrated that nest incubation temperature has a significant influence on Leatherback hatchling morphology, namely that higher incubation temperatures produce hatchlings with smaller measurements or sizes. In this sense, Mickelson and Downie (2010) found a negative correlation between incubation temperature and Leatherback hatchling size. Elevated incubation temperatures have resulted in significantly reduced carapace lengths in such species as Green Sea Turtles (Glen et al. 2003; Booth et al. 2004; Burgess et al. 2006) and Loggerhead Sea Turtles (Read et al. 2012; Sim et al. 2015).

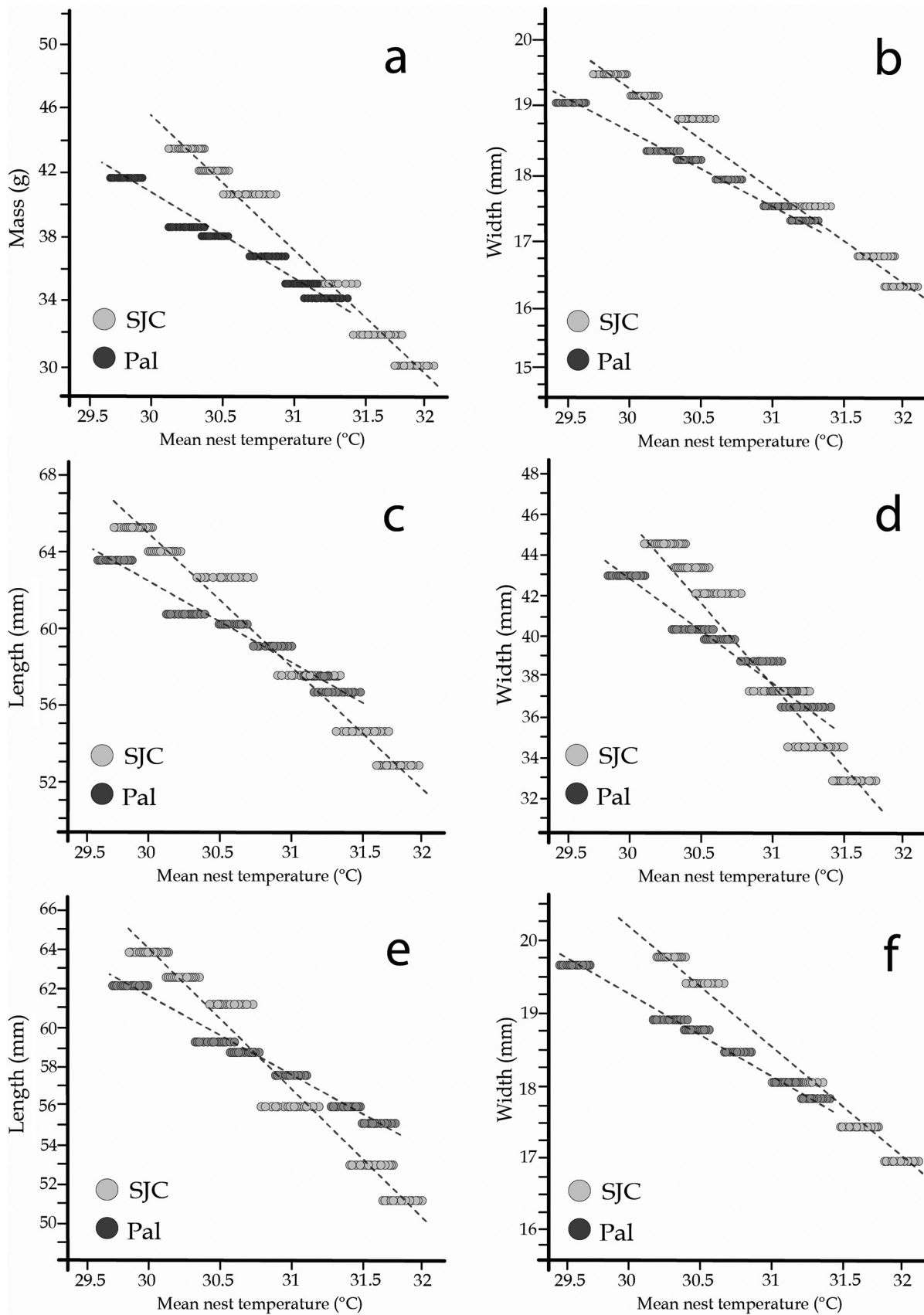
Downes and Shine (1999) explain that morphological traits influenced by incubation temperature vary in magnitude and direction among reptiles and are linked to the ability of hatchlings to evade beach and nearshore predators and to survive in the open ocean. This ability to survive is directly related to the locomotor performance of hatchlings, and the probability of being predated upon is directly proportional to the time spent in coastal waters (Gyuris 1994). Therefore, the ability of a hatchling to quickly upright itself is important. On other hand, the potential effects of incubation temperature on hatchling survival might be advantageous in avoiding predation because larger body sizes produced by cooler nests might allow hatchlings to avoid gape-limited predators (Salmon and Scholl 2014; Salmon et al. 2015); the hatchlings also have larger yolk reserves and are more likely able to survive longer without eating (Booth 2006). However, a decrease in hatchling recruitment to the population might occur if the smaller hatchlings experience a higher rate of

predation after emergence because of poor locomotor performance abilities.

The majority of studies that have investigated the influence of incubation temperature on morphology of sea turtle hatchlings have focused on natural nests of Green Sea Turtles (Glen et al. 2003; Booth 2006; Burgess et al. 2006), Loggerhead Sea Turtles (Ferreira-Júnior et al. 2011), and only one study on Leatherbacks (Mickelson and Downie 2010). Our results fill the gap in research on the effects of mean incubation temperature on the morphology of Leatherback hatchlings produced in enclosed hatcheries.

The main advantage of Leatherback hatchery management is that this type of incubation provides a number of eggs incubated in guarded areas, free from disturbance, natural predators, beach erosion, and flooding (García et al. 2003); therefore, the rate of hatching success can be increased (Baskale and Kaska 2005; Maulany et al. 2012; Pazira et al. 2016). Additionally, the strategy of releasing offspring to reduce their vulnerability to predators — by eliminating their initial migration from nest to sea — would increase survival by decreasing the length of time a hatchling is exposed to predators (Sieg et al. 2011; Pazira et al. 2016). However, hatchery management has some disadvantages; for example, the relocation of nests could cause potential negative effects, such as an alteration in sex ratio, because temperatures in hatchery clutches were cooler due to decreases in the number of metabolizing embryos (Godfrey and Mrosovsky 1999). Furthermore, hatchery management should be discussed on the basis of genetics and evolution (Sari and Kaska 2017). The increase in nest incubation temperatures because of global warming has the potential to affect hatchling fitness, which may put further pressure on the existing population of this critically endangered species. In the present study, mean nest incubation temperature affected the morphology of Leatherback hatchlings in a similar way as those produced in a natural environment, the difference being that the nests evaluated in hatcheries showed an increase in their rate of hatching success. However, keep in mind that we carried out our study within only one season, and similar studies should be conducted over several seasons to be able to obtain more accurate morphological hatchling data based on mean nest incubation temperature in hatcheries.

**Fig. 6.** Influence of nest temperature on morphology of Leatherback Turtle (*Dermochelys coriacea*) hatchlings based on linear regressions for both locations (SJC, San Juan Chacahua hatchery; Pal, Palmarito hatchery).



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**Table 3.** Results of linear regressions for the influences of nest temperature on morphology of Leatherback Turtle (*Dermochelys coriacea*) hatchlings in both locations (SJC, San Juan Chacahua hatchery; Pal, Palmarito hatchery).

Variable	Locality	R <sup>2</sup>	P
Mass	SJC	0.34	0.001
	Pal	0.49	0.001
Carapace length	SJC	0.29	0.037
	Pal	0.37	0.042
Carapace width	SJC	0.28	0.039
	Pal	0.19	0.025
Right flipper length	SJC	0.363	0.04
	Pal	0.42	0.049
Right flipper width	SJC	0.17	0.021
	Pal	0.19	0.027
Head width	SJC	0.09	0.017
	Pal	0.11	0.022

Our results suggest that the morphology of Leatherback hatchlings that were incubated in hatcheries varies in relation to mean nest incubation temperature in a similar manner as those produced in natural environments. Both techniques, morphometric geometric analysis and linear measurements, can be used in a complementary way to study morphological traits of Leatherbacks in both natural environments and enclosed hatcheries. Finally, our results should encourage more thorough experimental studies relating to body shape and size, as well as locomotor performance and behaviour, to understand the importance of morphology in the survival of Leatherback hatchlings in their early life stage.

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**Table 4.** Comparisons of means and standard errors (SE) of the morphological traits of Leatherback Turtle (*Dermochelys coriacea*) hatchlings between hatcheries (group 1 is the San Juan Chacahua hatchery and group 2 is the Palmarito hatchery).

Trait	Group	Mean	SE	F	df	P
Mass	1	38.63	2.81	0.363	1, 58	<b>0.03</b>
	2	42.35	3.01			
Carapace length	1	57.35	3.16	4.27	1, 65	<b>0.04</b>
	2	60.13	2.16			
Carapace width	1	36.82	2.25	2.43	1, 19	0.127
	2	38.42	2.25			
Right flipper length	1	50.12	2.63	3.52	1, 53	<b>0.038</b>
	2	58.13	3.12			
Right flipper width	1	16.84	0.78	3.81	1, 62	0.127
	2	17.84	0.93			
Head width	1	16.84	0.78	0.363	1, 49	0.551
	2	17.84	0.93			

Note: Significant differences ( $P < 0.05$ ) are set in boldface type.

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