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ARTICULO ORIGINAL

SAND AND INCUBATION TEMPERATURES IN A SEA TURTLE NESTING BEACH AT THE CAYOS DE SAN FELIPE NATIONAL PARK, PINAR DEL RÍO, CUBA, DURING THE 2012-2013 SEASON

Temperatura de la arena y de incubación en una playa de anidación de tortugas marinas en el Parque Nacional Cayos de San Felipe, Pinar del Río, Cuba, durante la temporada 2012-2013

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ABSTRACT

Sand temperature at 50 cm depth was measured in three profiles along El Sijú nesting beach, Cayos de San Felipe National Park, Pinar del Río, Cuba during the 2012-2013 nesting season. Sand and incubation temperatures inside and close to nests of Hawksbill (*Eretmochelys imbricata*), Loggerhead (*Caretta caretta*) and Green turtle (*Chelonia mydas*) were also measured. We compared sand temperatures among locations with different environmental conditions as well as incubation and sand temperatures during the thermo-sensitive period. Using regression analysis, we modelled expected nest temperature from sand temperature for assessing the feasibility of using the latter as a proxy for estimating nesting temperature. Temperature at 50 cm depth of all locations during summer were significantly higher than pivotal while in winter it was significantly lower even in sunny areas. Average sand temperature during the peaks of the nesting seasons of the three species were significantly different ($F_{(2,1214)} = 942.39$, $p < 0.01$), with Green turtle season being the hottest and above pivotal temperature. Mean incubation temperatures in nests of *C. mydas* and *C. caretta* were higher than those of *E. imbricata*. Nests incubated during winter (all of *E. imbricata*) generally became cooler as the winter advanced ($F_{(5,105)} = 47.07$, $p < 0.001$), while those incubated during the period of July to October (summer) had mean temperatures above 30.5°C irrespective of the species. We found significant positive differences in the thermosensitive period between nest and pivotal temperatures in four (the three species) and negative in two (*E. imbricata*) out of eight nests. Furthermore, sand temperature during nesting was significantly higher than 29.2°C in four out of the six nests sampled. These results point out a possible skew in hatchlings sex proportion towards females mainly during summer time, when the peak of *C. mydas*' nesting season occurs. The difference between daily average of nest and sand temperatures increased as the incubation progressed. When modelling expected differences and estimating nest temperatures accordingly, we found strong, significant linear relation (r = 0.91, p <0.05) and no significant differences between observed and estimated temperatures (t = 0.96, p = 0.34). Moreover, we were able to estimate nest temperature with good accuracy (R^2=0.83, average error=± 0.35°C), which indicates that sand temperature could be a good proxy for determining average nest temperature during the middle third of the incubation period, which can provide clues about the occurrence of sex biases.

KEY WORDS: Incubation temperature, sea turtles, climate change

RESUMEN

*Se midió la temperatura de la arena a 50 cm de profundidad (profundidad de anidación promedio) a lo largo de tres perfiles de la playa de anidación de tortugas marinas El Sijú, parque nacional Cayos de San Felipe, Pinar del Río, Cuba durante la temporada de anidación 2012-2013. Además, se midió la temperatura en la arena y de incubación en nidos de carey (*Eretmochelys imbricata*), caguama (*Caretta caretta*) y tortuga verde (*Chelonia mydas*). Se comparó la temperatura de la arena en diferentes posiciones con respecto a las condiciones ambientales así como la diferencia entre la temperatura de incubación y la dela arenas durante el período termo-sensible. Usando un análisis de regresión, se modeló la temperatura esperada del nido estimada a partir de la temperatura de la arena para determinar la factibilidad de usar esta última como proxy para futuras estimaciones de la temperatura en el nido. La temperatura en los perfiles en todos los puntos medidos durante el verano fue superior a la pivote, mientras que en invierno fue significativamente menor, incluso en las áreas soleadas. La temperatura de la arena promedio durante el pico reproductivo de las tres especies fue significativamente diferente (F (2,1214) = 942.39, p <0.01), ya que la temporada de tortuga verde tuvo los valores más altos muy por encima de la temperatura pivote. La temperatura de incubación en nidos de* C. mydas *y* C. caretta *fueron mayores que en los de* E. imbricata. *Los nidos incubados durante el invierno (todos de* E. imbricata*) generalmente redujeron gradualmente la temperatura con el avance del invierno (F(5,105) = 47.07, p < 0.001), los incubaos en el período de julio a octubre, en cambio, tuvieron temperaturas de incubación por encima de los 30.5°C con independencia de la especie. Se encontraron diferencias significativas y positivas entre la temperatura del nido y la pivote durante el período termo-sensible en cuatro nidos (de las tres especies) y negativas en dos nidos (*E. imbricata*) de los ocho analizados. Más aún, la temperatura de la arena durante también fue superior a 29.2°C en cuatro de los seis nidos evaluados. Estos resultados apuntan a un posible sesgo en la proporción sexual en las crías con mayor número de hembras durante el verano, cuando ocurre el pico de* C. mydas. *La diferencia entre la temperatura diaria del nido y de la arena se incrementó con el desarrollo embrionario. Cuando se modeló la diferencia esperadas y se estimó la temperatura tomando esto en consideración, se encontró una relación lineal fuerte y significativa (r = 0.91, p <0.05) y la ausencia de diferencias significativas entre los valores observados y los estimados (t = 0.96, p = 0.34). Más aún, se pudo estimar la temperatura con una buena exactitud (R2=0.83, error medio=± 0.35°C), lo que indica que la temperatura de la arena puede ser un buen proxy para determinar la temperatura del nido durante el segundo tercio del período de incubación, para utilizarla en la estimación de la proporción sexual esperada en estos nidos.*

PALABRAS CLAVE: *Temperatura de incubación, tortugas marinas, cambio climático*

INTRODUCTION

As in most of reptiles, temperature heavily influences different aspects of sea turtle biology. For example, embryonic development proceeds successfully only between 25°C and 35°C and it is known that temperature is the factor determining sex of offspring of all species (Yntema & Mrosovsky 1980, Dalrymple et al. 1985, Shaver et al. 1988, Broderick et al. 2001, Tapilatu et al. 2013).

The so called thermo-sensitive phase, which occurs during the middle third of the incubation period (Yntema & Mrosovsky 1980), is the time when offspring's sex determination occurs depending on the relationship between nest and pivotal temperatures (temperature at which a 50:50 hatchling sex ratio is most likely). Eggs incubated at temperatures higher than pivotal result in the production of females; those incubated at cooler temperatures produce males (Esteban et al. 2016). Although variations in conditions among beaches determine variations in sex ratio for the same species (Glen and Mrosovsky 2004), pivotal temperature is relatively constant (Mrosovsky & Pieau 1991). Different authors have found that this parameter is close to 29°C for the hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*) (Mrosovsky 1994; Wibbels 2003) and green turtle (*Chelonia mydas*) (Kaska et al 1998; Godley et al 2002).

There is a growing evidence that the planet's average temperature is gradually rising (McCarthy *et al.*, 2001, Pachauri & Reisinger 2007). Subsequently, taking into account sea turtles' temperature-dependent mechanism for sex determination, it is recognized that global warming may lead to a significant bias towards the production of females in wild populations and might result in the emergence of other malfunctions in the reproductive biology of these species. Climate change will constitute a serious threat to sea turtle survival if they cannot find ways to adapt (Hawkes *et al.* 2009, Hamann *et al.* 2013, Booth 2017).

Several models and scenarios have been developed to project future climate change at global and regional scales (McCarthy *et al.*, 2001, Pachauri & Reisinger 2007). Likewise, there are models for linking atmospheric variables with soil temperature (Hasfurther *et al.* 1972, Dwyer *et al.* 1990 Vose & Swank 1991, Zheng *et al.* 1993 Möllerström 2004). Nevertheless, few models have been specifically developed to link air, sand and nest temperatures for the particular case of sea turtle nesting beaches (Fuentes *et al.* 2009, Godley & Mrosovsky 2004). One obstacle achieving this is the lack of systematic monitoring of sand and incubation temperatures in nesting beaches (Hays et al. 2001, 2017). Therefore, finding and understanding patterns in sand and incubation temperature at nesting beaches is of paramount importance when designing strategies for climate adaptation and long-term conservation, suitable for this group of species (Poloczanska *et al.* 2009, Hamann *et al.* 2013).

Some sea turtle nesting sites in Cuba are among the most important in the insular Caribbean, particularly for *C. caretta* and *C. mydas* (Dow *et al.* 2008). Although systematic sea turtle nesting monitoring has been carried out for over 15 years at various beaches in Cuba, measurements of sand and nest temperatures only began a few years ago (Azanza *et al.* in preparation) and only for *C. mydas*. Cayos de San Felipe National Park is a key area for comparative studies on the incubation temperature among species. This protected area has significant levels of nesting of *C. mydas* and *C. caretta* and it harbors the beach with the highest recorded density of nests of *E. imbricata* in Cuba, which makes the park a very important site for conservation of these species (Moncada *et al.* 2014). In our study, we compare the incubation temperature in nests of these three species of sea turtle and describe the relationship between sand and nest temperature during the thermo-sensitive phase of incubation in San Felipe nesting beaches.

MATERIALS AND METHODS

The study was conducted between March 2012 and April 2013 at El Sijú beach, Cayos de San Felipe National Park, located at 83° 30' W and 21° 56' N. It is a narrow beach, about 3 km long, which is formed by fine grained, white-gray sand. The beach also has visible signs of intense erosion, as according to observations of the authors, coastline advanced 6 meters inland in a profile measured between June and December 2012. Between 17 and 25 nests of *E. imbricata* have been recorded annually during the last five years in this beach, which makes it one of the beaches with the highest nesting density for this species in Cuba (Moncada *et al.* 2014).

Following the recommendations of WWF's guidelines for temperature monitoring on nesting beaches (Baker-Gallegos *et al.*, 2009), three sand temperature profiles were set in sectors with different geomorphological features (Fig. 1). We placed eight sensors in the sand at 50 cm depth in 4 different environments: sunny bare sand (3), sand in sunny areas covered by tall grass (2) and shaded sand (3) (Fig. 2). Sand temperature was continuously and synchronously measured at 2 hours intervals, between March 26th 2012 and April 12th 2013.

In addition, we installed sensors in nine nests: two of green turtles (*C. mydas*), one of loggerhead (*C. caretta*) and six of hawksbill (*E. imbricata*). HOBO® Onset Pendant sensors were used for all measurements. Freshly laid nests were chosen (the night before), which were excavated, a number of eggs equivalent to one third of the expected size of the nest were extracted, and the sensor was placed and secured by a thin cord. Then eggs previously extracted were placed back in the incubation chamber,

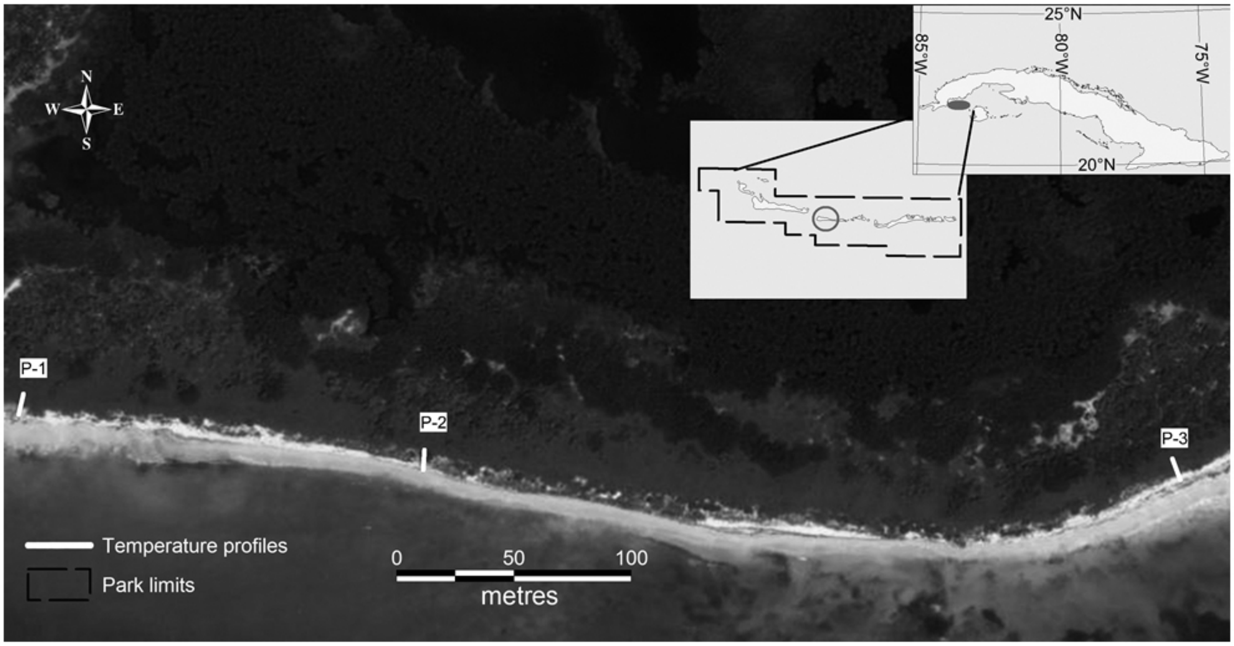

Fig. 1: Location of profiles in El Sijú Beach, Cayos de San Felipe National Park.

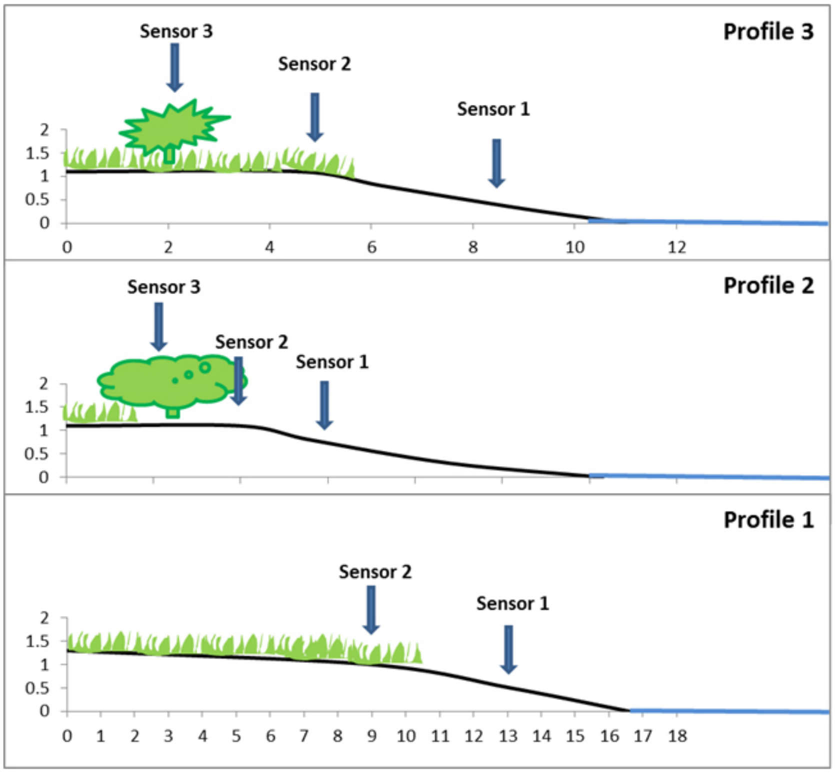

Fig. 2: Temperature profiles.

the nets refilled with the same sand and the surface leveled to be left in conditions similar to those it had before digging the nest. The cord firmly attached to a PVC stake driven deep secured the sensors. The stakes were secured by a rope to a tree or shrub out of reach of storm surges. Additional sensors were also used to measure sand temperature in sites at one meter off the nests, at the same depth and in similar environmental conditions to the corresponding nest. All these sensors were set to measure temperature synchronously at intervals of two hours. We used the HOBOware® Onset® Computer Corp software to download the data collected, while Excel spreadsheets were used to create temperature databases for each sensor.

After the hatching of each nest occurred we gathered the following information: distance to high tide line (meters), nest depth, total number of eggs, number hatched eggs, eggs with no embryo, eggs with embryo and dead hatchlings. With this information we calculated three indicators: incubation rate (number of eggs with embryos hatched or not over the number of eggs multiplied by 100), incubation success (number of hatched eggs over the total of eggs multiplied by 100) and the emergency success (number of hatched eggs minus dead hatchlings over the total of eggs multiplied by 100).

We compared annual and peak season means of sand temperatures in profiles through ANOVA, and applied one-sample t test of means against reference value to explore differences with pivotal temperature under different environmental conditions both for annual and seasonal means. The average temperature of the sand during the peaks of the nesting seasons of the three species (May-June for *C. caretta*, July-August for *C. mydas* and November-December for *E. imbricata*) was also compared to pivotal temperature. In addition, one-sample t test of means against reference value (pivotal temperature for *E. imbricata*) were performed for each nest and for each sensor in the sand next to the nests.

We used as reference for pivotal temperature the value estimated for Antigua (29.2°C), another locality in the Caribbean (Glen & Mrosovsky 2004). This comparison was also considered appropriate for *C. caretta and C. mydas* nests, since the medians of pivotal temperatures reported for these species are slightly below 29.2°C (Hawkes *et al.*, 2009).

Synchronous temperature records in nests and sand were used to determine an equation that could help estimate nesting temperature during the thermo-sensitive period using sand temperature recorded at average nesting depth. To this aim we used regression analysis to obtain the relationship among the average of the daily differences between nest and sand temperatures, and the number of days elapsed since the beginning of the thermo-sensitive phase (progress of the days of incubation), represented by an exponential curve. We then used this regression model to calculate the expected increase in temperature for each day of the middle third of incubation.

We calculated the expected average of daily temperature by adding the temperature increases estimated for each incubation day to the average of the temperature measured in the sand close to those nests. Using a linear regression, we compared the observed and expected average daily nest temperatures, and evaluated the significance of the relationship by means of T test of difference between means, the coefficient of determination and the standard error.

RESULTS

THERMAL CONDITIONS

Sand and air temperature measured at the three profiles showed similar patterns through time. In general, sand temperature followed a similar daily variation pattern in all measured points, which were much less pronounced than observed thermal fluctuations for air temperature. As is typical for Cuba, the highest daily variations in air temperature were found in winter (Fig. 3).

The three profiles differed significantly in terms of mean annual temperature

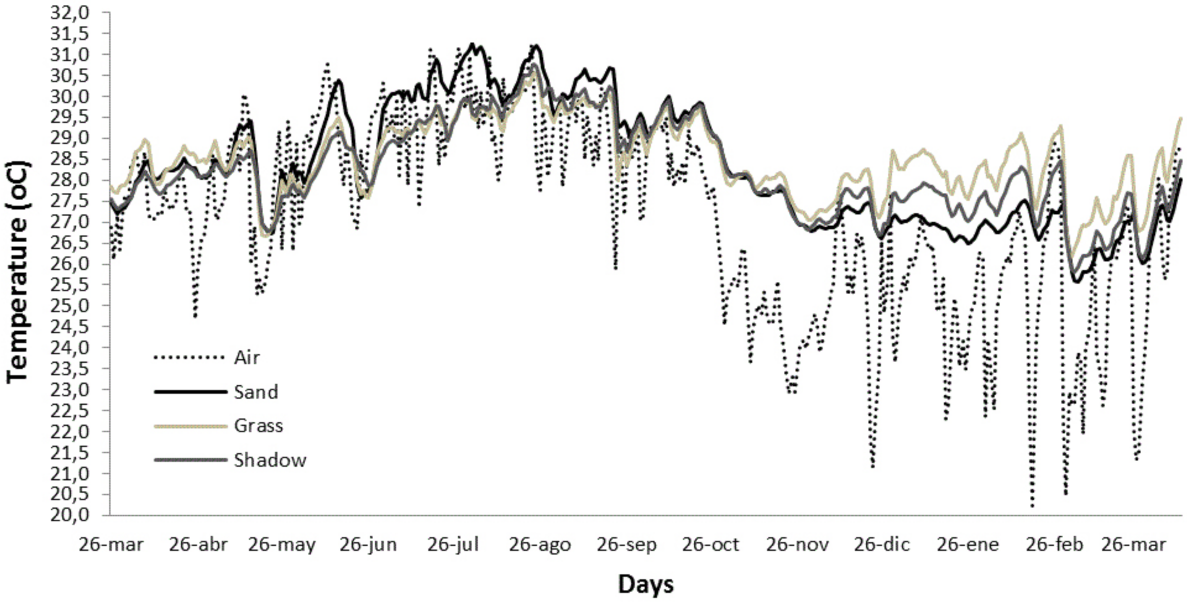

Fig. 3: Daily variation in temperature in four different environments of a nesting beach at San Felipe National Park: air temperature and sand temperature in bare sand, sand covered with grass and under the shadow of shrubs.

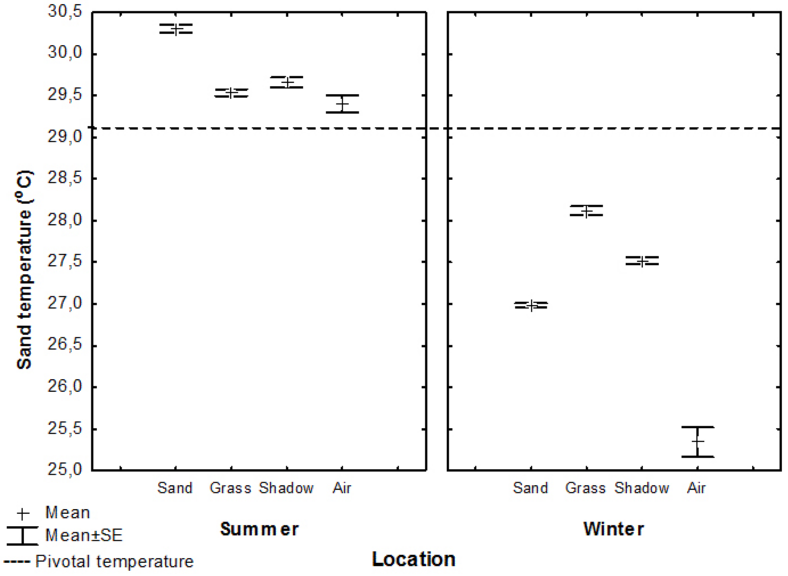

Fig. 4: Mean sand temperature at 50 cm depth in different environments (bare sand, sand covered by tall grass and sand in shady areas), in a) summer and b) winter at El Sijú beach, Cayos de San Felipe National Park, Pinar del Río, Cuba.

($F_{(2, 2505)}$ = 50.85, p <0.001): Profile 1 was the hottest, while profile 3 was the coolest. Among different zones of the beach, the average annual temperature were significantly below pivotal temperature with an average of 28.31°C in bare sand (t= -11.85, p <0.01), 28.50 in sand covered by tall grass (t= -15.46, p <0.01), 28.25 in shadow (t= -16.94, p <0.01) and 27.11 the air temperature (t= -17.79, p <0.01).

The average temperature of all locations during summer (Fig. 4a) were significantly higher than pivotal temperature (sand: $\bar{X}$ =30.30; t= 25.91, p <0.01, grass: $\bar{X}$ =29.53; t= 8.19, p <0.01 and shadow: $\bar{X}$ =29.65; t= 7.63, p <0.01). Average daily temperature in winter (Fig. 4b) was significantly lower than pivotal even in sunny areas (sand: $\bar{X}$ =26.98, t= -84.04, p <0.01; grass: $\bar{X}$ =28.12, t= -20.30, p <0.01 and shadow: $\bar{X}$ =27.52, t= -44.98, p <0.01).

The average sand temperature during the peaks of the nesting seasons of the three species (May-June for *C. caretta*, July-August for *C. mydas* and November-December for *E. imbricata*) showed significant differences ($F_{(2,1214)}$ = 942.39, p <0.01) (Fig. 5). During the nesting peak of *C. mydas* sand temperature reached the highest levels (over 30 °C) over pivotal temperature. Temperatures were below

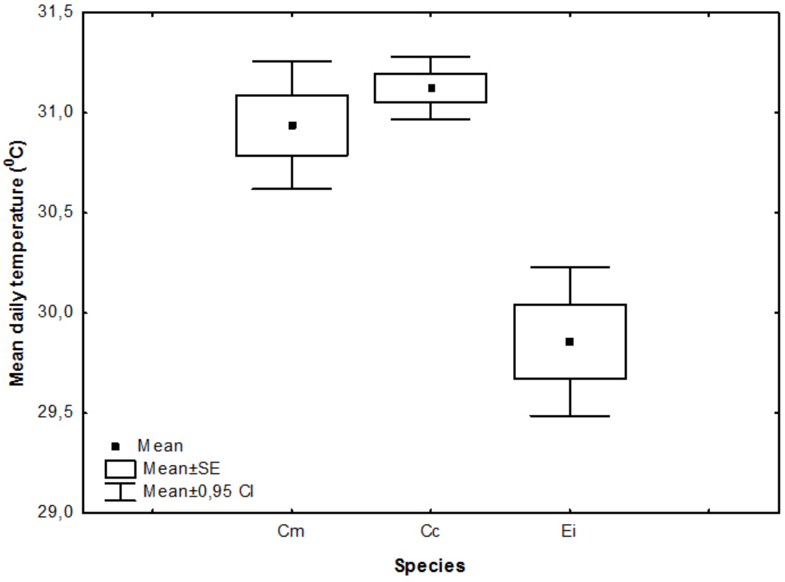

Fig. 5: Mean sand temperature at 50 cm depth during the months when the reproductive peaks of loggerhead (*Caretta caretta*), green turtle (*Chelonia mydas)* and hawksbill (*Eretmochelys imbricata*) occur at El Sijú beach, Cayos de San Felipe National Park, Pinar del Río, Cuba.

Table 1: Nest characteristics. Sp species (Cm *Chelonia mydas*, Cc *Caretta caretta*, Ei *Eretmochelys imbricata*), DHT distance to high tide line (meters), NE total number of eggs, NH number hatched eggs, ENE eggs with no embryo, EE eggs with embryo, DH dead hatchlings, IR incubation rate, IS incubation success, ES emergency success, ND nest depth

# Nest	Sp	DHT (m)	NE	NH	Not hatched eggs		DH	IR (%)	IS (%)	ES (%)	ND (cm)
					ENE	EE					
81	Cm	5,00	154	106	30	18	9	80,52	68,83	62,99	56
87	Cc	2,10	126	101	14	11	0	88,89	80,16	80,16	47
128	Ei	14,20	120	72	41	7	0	65,83	60,00	60,00	45
131	Ei	16,10	72	1	60	11	0	16,67	1,39	1,39	39
132	Ei	6,30	224	23	200	1	0	10,71	10,27	10,27	¿?
133	Ei	5,80	131	45	23	63	0	82,44	34,35	34,35	¿?
142	Ei	13,00	159	52	77	30	7	51,57	32,70	28,30	41
143	Ei	13,00	181	114	50	17	1	72,38	62,98	62,43	39

pivotal temperature in May and June when the nesting peak of *C. caretta* occurs, as well as in winter, during the nesting peak of *E. imbricata*.

NESTING TEMPERATURE

The data recorded in of some of the nests was not useful for analysis, which limited the scope of our study. Data from nests # 86 (*C. mydas*) and # 131 (*E. imbricata*) were lost due to malfunctioning sensors; nest # 132 (*E. imbricata*) had almost no embryonic development, so nest and sand temperatures were practically the same, which limited its use in comparisons. In the case of nest # 143 (*E. imbricata*) there was no sensor available at the time of incubation to record the temperature of the sand, so no comparison was possible in this case. A summary of the characteristics of nests, as well as its hatching and emergence success is presented in Table 1.

Mean incubation temperatures in *C. mydas* and *C. caretta* nests were higher than those of *E. imbricata* (Fig. 6). However, nests incubated during winter (all of *E. imbricata*) became cooler as the winter advanced ($F_{(5,105)} = 47.07$, $p < 0.001$), while those incubated during the period of June to October (summer) had mean temperatures above 30.5°C irrespective of the species.

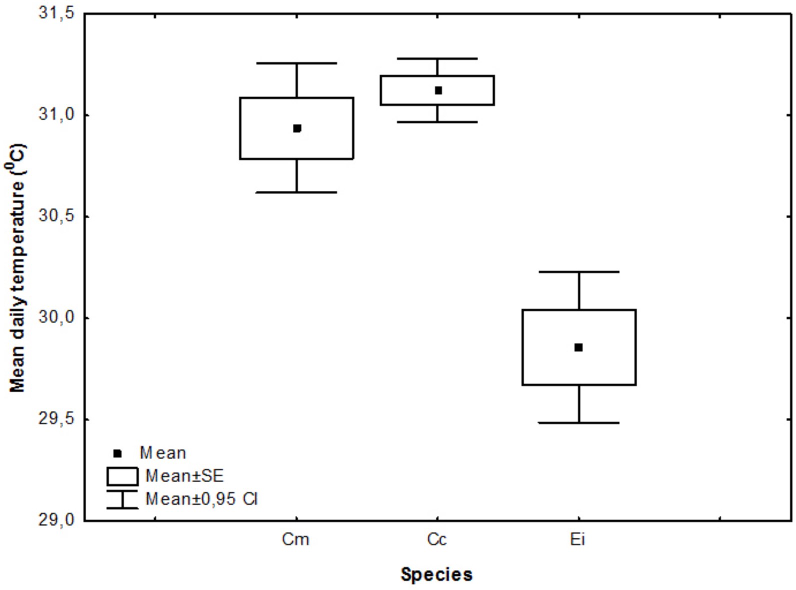

Fig. 6: Mean incubation temperature in nests of *Chelonia mydas* (Cm), *Caretta caretta* (Cc) and *Eretmochelys imbricata* (Ei) at El Sijú beach, Cayos de San Felipe National Park, Pinar del Río, Cuba.

Table 2: Nests characteristics and mean incubation temperature for sampled nests of *Eretmochelys imbricata* (Ei), *Caretta caretta* (Cc) and *Chelonia mydas* (Cm).

Nest	Species	Date of middle third of incubation	Mean nest temperature during 2nd third of incubation (°C)	Sensor depth (cm)	Number of eggs incubated
81	Cm	Jul 30 to Aug 15	30.83	50	124
87	Cc	Aug 1 to Aug 15	31.08	40	112
128	Ei	Sep 25 to Oct 11	30.99	37	79
131	Ei	Sep 17 to Nov 5	29.9	34	12
132	Ei	Oct 18 to Nov 3	29.11	30	24
133	Ei	Oct 18 to Nov 3	31.58	38	108
142	Ei	Nov 27 to Dec 18	29.53	30	82
143	Ei	Nov 28 to Dec 20	28.11	35	131

We observed a significant increase in nest temperature with respect to sand temperature as incubation progressed, although in general the nest temperature pattern was similar to that of the sand at the same depth. We found that nest temperature during the thermo-sensitive period was significantly higher than the sand temperature at the same depth ($F_{(1,174)} = 72.54$, $p < 0.01$). The nests were on average 1.4°C warmer than the sand.

Table 2 shows mean incubation temperatures in nests and two characteristics that influence this variable (nest depth and number of eggs). Temperature in the thermo-sensitive period showed no significant differences with pivotal ($t = -0.36$, $p = 0.72$; $t = 1.32$, $p = 0.20$) in only two (nests # 132 and # 142) out of six nests in which the middle third of incubation occurred before November 10th. One (# 143) of the two nests incubated in winter had an average temperature significantly lower than pivotal ($t = -4.94$, $p < 0.05$).

Similarly, we found that sand temperature during nesting was significantly higher than 29.2 °C in four out of the six nests sampled, which coincided with those where the temperatures were the highest and significantly higher than pivotal. We did not find differences with pivotal temperature in one of the nests, and the sand close to one of the nest we monitored in winter had mean temperatures significantly lower than pivotal (see Table 3).

Table 3: Mean sand temperature measured at one meter of distance from six marine turtles' nests and significance of difference with pivotal temperature (29.2°C).

Nest	Period measured	Sensor depth (cm)	Mean sand temperature at nest depth (°C)	Significantly different from pivotal
81	Jul 30 to Aug 15	50	30.10	Yes
87	Aug 1 to Aug 15	40	30.21	Yes
128	Sep 25 to Oct 11	37	29.45	No
132	Oct 18 to Nov 3	30	29.58	Yes
133	Oct 18 to Nov 3	38	30.35	Yes
142	Nov 27 to Dec 18	30	27.60	Yes

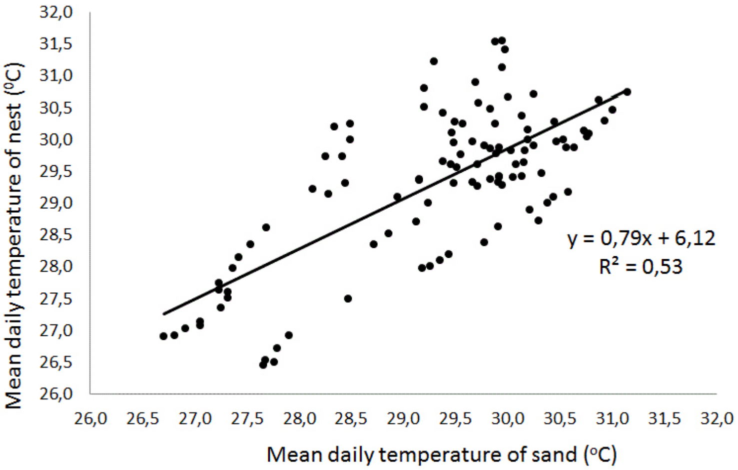

Fig. 7: Relation between mean daily temperature of nests and mean daily sand temperature during the middle third of the incubation period, showing significant but not strong linearity.

We found a weak but significant linear relationship ($p < 0.05$, N = 105; Fig. 7) when relating daily average nest temperature during the middle third of the incubation period with sand temperature at the same depth. In turn, nest temperature increased not linearly with respect to the sand temperature as the incubation progresses (Fig. 8), and it was suitably represented by an exponential curve.

The exponential regression model allowed calculating the expected increases in temperature for each incubation day, which was used as a correction factor for estimating expected incubation temperatures.

We found a significant lineal relation between the expected and the observed temperatures. Incubation temperatures were estimated with a coefficient of determination $R^2 = 0.83$ and average error of ± 0.35°C. Furthermore, no significant differences between the two variables ($t = 0.96$, $p = 0.34$) were found. These results suggest that the fitted line (Fig. 9) accounts for most of the variability observed in the data, result that supports the viability of this approach to obtain a proxy for average incubation temperature from sand temperature.

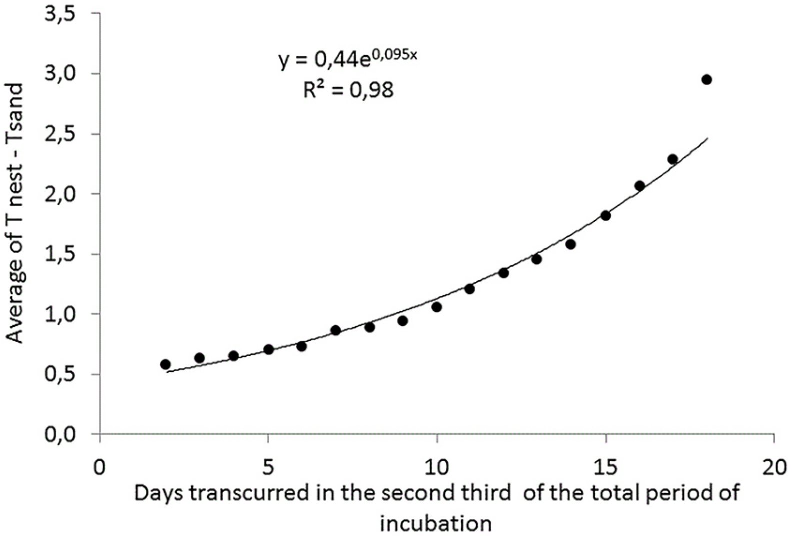

Fig. 8: Nest temperature increases exponentially during the middle third of the incubation period in relation to sand temperature at the same depth. Expected increments for each day are calculated using the regression model in order to estimate nest temperature from sand temperature.

DISCUSSION

Sand temperature at beaches is a function of several factors like its geographical position, sea surface temperature, physical and geomorphological characteristics, vegetation coverage, season and time of the day (Godfrey *et al.*, 1996, Naro-Maciel *et al.* 1999, Hays *et al.* 2001, Matsuzawa *et al.* 2002 Reece *et al.* 2002, Houghton *et al.* 2007, Fuentes *et al.* 2009). In our case, the

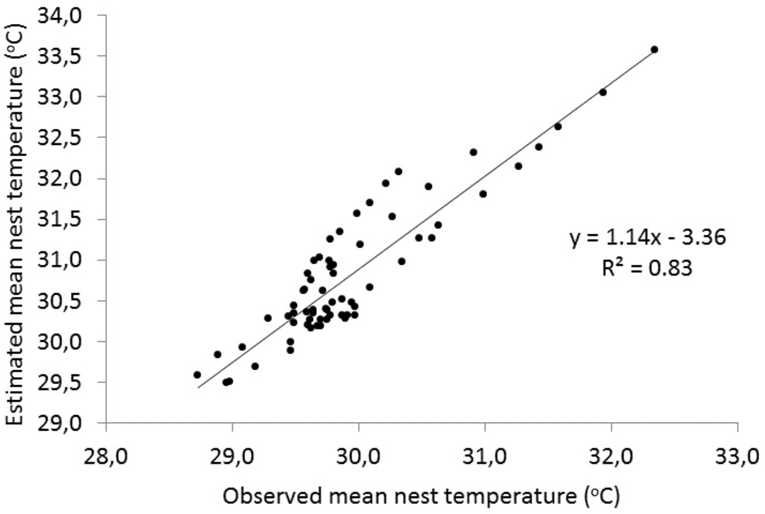

Fig. 9: Linear relation between estimated and observed nest temperatures.

homogeneous geographical and geomorphological characteristics of the sector we studied at El Sijú beach in the Cayos de San Felipe National Park, explain the similarity in the variation of temperatures in the 3 profiles throughout the year. Therefore, the differences found in sand temperature at average nesting depth were more conditioned by the vegetation cover and season of the year, than by differences in geomorphology or physical properties of the sand.

The fact that during the summer (June, July and August) the temperature of the sand at all points measured in sunny areas was significantly higher than pivotal, indicates the risk of nests of being biased toward the production of females, at least during the studied period. If we consider that the studied year was not hotter than historical mean (INSMET 2014, Resumen Mensual, Año 2012); sand temperatures hotter than pivotal may be frequent at the beach, especially for *C. mydas* and *C. caretta*, since their peak season occurs precisely during the hottest period of the year in Cuba. High female production has been predicted for loggerhead nesting in the westernmost area of the Cuban archipelago (Azanza et al. 2017) and verified for both species (Calderón 2015).

On the other hand, sand temperatures were on average significantly cooler than pivotal in winter (November, December and January), even in sunny areas, which is consistent with the decline in solar radiation, air and sea temperatures during this season. The highest temperatures were recorded in grassy areas while the coolest in shady areas, although the difference between the extreme values was limited to 1.22°C. During this period *E. imbricata* is the only nesting species in the area. Together with the fact that they usually lay the eggs in shady areas, the species could balance the production of hatchlings of both sex.

However, although the average temperature during the middle third of the incubation period can be a guide to estimate sex ratios, it cannot be considered an accurate indicator (Glen & Mrosovsky 2004). Moreover, although the abiotic factors exert a major influence on the incubation temperature of the eggs, the metabolic heat can produce temperature increases in the range of one to several degrees (Bustard & Greenham 1968, Mrosovsky & Yntema 1980, Godfrey *et al.* 1997 Hays *et al.*, 2001) during the middle third of the incubation period, when the sex determination occurs. Metabolic heat produced a significant increase in temperature of the nests monitored in our study, a result consistent with the findings of other authors (Bustard & Greenham 1968, Mrosovsky & Yntema 1980, Godfrey *et al.* 1997, Hays *et al.* 2001). This increase reached almost 2°C on average (1.91 ± 0.58) during the middle third of the incubation period, a value somewhat higher than those found in a study on the island of Antigua (Glen & Mrosovsky 2004).

The sex ratio of sea turtles resulting from a specific nest or from an entire nesting beach depends on other factors besides those already discussed here. For instance, the incubation temperature shows significant variations (one or more degrees) between the bottom and top of the nest (Kaska *et al.* 1998 2001 Hays & Houghton, Ricardo *et al. 2013*), depending on the size of the fertile clutch and the position of the eggs in the nest. These means that measurements in a single position inside the nest those not represent the universe of conditions inside of it, hence, real sex determination for each embryo.

Despite no assessment was carried out about temperature variations in different parts of the nests, the fact that sand temperature in summer was hotter than pivotal, together with the increases due to metabolic heat, makes likely the risk of bias towards the production of females in the case of *C. mydas* and *C. caretta*, at El Sijú beach.

Davenport (1997) acknowledged that the incubation temperature varies not only between different beaches, but also on the same beach throughout the year, across the profile, and at different nest depth. However, in our case the relatively limited variations in nest depths (ranging from 39 cm to 56 cm), along with the comparatively homogeneous geomorphological conditions along the beach could explain that we found significant differences in the incubation temperature only between nests laid on different seasons (summer and winter) but not amongst those incubated in either of the seasons. The only exception was nest # 132, an extreme case that having only 24 eggs incubated, remained much colder than the others laid in the same season.

For all the above we can conclude that on this beach, except in the case of extreme differences in the number of eggs hatched, the nest temperature during the middle third of the incubation period seems to depend more on the depth of the nest, shadow received, time of year and local weather than on species. This could also explains potential differences in incubation temperature between species, which should be mainly due to seasonal differences in sand temperature, because nesting peaks for *Chelonia mydas/ Caretta caretta* and *E. imbricata* occur in summer and winter respectively, and therefore do not necessarily correspond to biological differences between species.

The weak but significant linear relationship between sand and nest temperatures is explained by the fact that the latter increments gradually and not linearly as the incubation progresses due to increases in metabolic heat. By applying a correction to the average difference found for each day of incubation, the linear relation is greatly improved, which should be taken into account in developing models for estimating nesting temperature from sand temperature. As a result, an exponential model, rather than a linear one, accurately predicts expected differences between sand and nest temperatures as the second third of the incubation progresses. Using the expected temperature increases as a correction factor allows the estimation of the mean incubation temperatures using data obtained from monitoring of sand temperature at nesting depth. The strong, significant relation and high coefficient of determination found between observed and estimated daily mean temperature of nets supports the previous assertion.

Since the differences between nest and sand temperatures vary with nest depth, time of the year and the size of the fertile clutch, a greater and more comprehensive replication of the type of experiment we carried out is needed. Collecting data for different depths, seasons, clutch sizes and species, will allow the production of sets of curves to predict more accurately the expected differences in temperature, and to gain in accuracy when estimating the average temperature of incubation. To this end, attention should also be paid to the physical and climatic differences of the beaches, so similar studies need to be replicated in beaches with significantly different physical-geographical conditions.

Estimation of nest temperature from sand temperature is especially useful when monitoring is to be carried out post-nesting, cases in which a significant perturbation of incubation may occur if the nests are dug and some eggs taken out of the nest in order to place the sensors.

Taking into account the limitations noted by Glen & Mrosovsky (2004) on the use of nest temperature to estimate the sex ratio, we consider that it is necessary to continue and expand these investigations to use monitoring of sand temperature for early warning on potential impacts of climate change on the conservation of this group of species, while actual sex ratio determination is done by other means.

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