
THE EFFECTS OF SAND TEMPERATURE ON PRE-EMERGENT GREEN SEA TURTLE HATCHLINGS

LUCIANO N. SEGURA¹ AND RODRIGO CAJADE²

¹Laboratorio de Investigaciones en Sistemas Ecológicos y Ambientales (LISEA, CONICET), Dg. 113 469, CP 1900, La Plata, Argentina, e-mail: lsegura79@yahoo.com.ar

²CECOAL, CONICET, Ruta Prov. 5 km 2,5. CP 3400, Corrientes, Argentina.

Abstract.—Sand temperature can play an important role in the survival of pre-emergent sea turtle hatchlings. High sand temperatures may inhibit coordinated muscle action in the ascent process to the sand surface and also may cause a decline in oxygen levels within the nest. These factors can increase mortality of hatchlings prior to emergence. In this study we analyze the effects of sand temperature on pre-emergent Green Sea Turtle (*Chelonia mydas*) hatchlings in Tortuguero, Costa Rica. We also analyze the relationship between sand temperature and egg incubation period, hatching success and hatchling emergence percentage. Nests were selected from June to August 2000, so that hatchlings would emerge during two distinct periods: a period of low temperatures and abundant rainfall mainly during August (wet period, WP) and a period of higher temperatures and lower rainfall mainly during September and early October (dry period, DP). Air and sand temperatures were significantly higher in the DP. Increases in sand temperature reduced incubation period from 58.6 ± 0.6 days in the WP to 56.4 ± 0.5 days in the DP. Mean hatching success and emergence percentage were $86 \pm 3\%$ and $96 \pm 1\%$, respectively, and were not associated with the nest initiation date. However, a negative association was found between the emergence percentage and the mean sand temperature at 40 cm depth in the pre-emergence days. Pre-emergent hatchlings may experience increased mortality when sand temperatures at the egg chamber neck level exceed 33°C . When one takes into account global warming projections, hatchling mortality during pre-emergence days could increase and pose a serious threat to this green sea turtle population.

EFFECTO DE LA TEMPERATURA DE LA ARENA EN LOS DÍAS PREVIOS AL EMERGIMIENTO DE NEONATOS DE TORTUGA VERDE MARINA

Resumen.—La temperatura de la arena puede desempeñar un rol importante en la supervivencia de neonatos de tortugas marinas. Altas temperaturas de la arena podrían inhibir la coordinación muscular en el proceso de ascenso hacia la superficie y también disminuir los niveles de oxígeno dentro del nido. Estos factores podrían incrementar la mortalidad de neonatos en los días previos al emergimiento. En este trabajo analizamos los efectos de la temperatura de la arena sobre el periodo de incubación, el éxito de eclosión y porcentaje de emergimiento de nidos de Tortuga Verde Marina (*Chelonia mydas*) en playas de Tortuguero, Costa Rica. Se seleccionaron nidos desde junio a agosto del año 2000, haciendo coincidir la eclosión con un primer periodo de bajas temperaturas y abundantes precipitaciones (húmedo) y un segundo periodo con mayores temperaturas y escasas precipitaciones (seco). Las temperaturas ambientales y de la arena fueron significativamente mayores en el periodo seco. El aumento en las temperaturas de la arena redujo los días de incubación desde $58,6 \pm 0,6$ días en el período húmedo a $56,4 \pm 0,5$ días en el periodo seco. El porcentaje de eclosión y emergimiento tuvieron promedios de $86 \pm 3\%$ y $96 \pm 1\%$ respectivamente, y no estuvieron asociados con la fecha de inicio del nido. Sin embargo, encontramos una asociación negativa entre el porcentaje de emergimiento y el promedio de temperatura de arena en el cuello del nido en los días previos al emergimiento. Los neonatos podrían morir cuando las temperaturas de la arena superan los 33°C . Considerando el gradual incremento de temperaturas a nivel global, la mortalidad de neonatos en los días previos al emergimiento podría incrementarse y amenazar seriamente a esta población de tortugas verdes marinas.

Key Words.—*Chelonia mydas*; climate change; emergence percentage; hatchling emergence; hatchling success; incubation period; mortality; sand temperature

INTRODUCTION

A new generation of Green Sea Turtles, *Chelonia mydas*, results from the combination of the reproductive qualities of the parents and the diverse environmental conditions of the nesting beach (Miller et al. 2003). These environmental conditions must favor hatchling

development and survival (Georges et al. 1993). Eggs in the chamber and pre-emergence hatchlings are vulnerable to environmental conditions, such as moisture, gas exchange, and temperature (Ackerman 1980, 1991; Mortimer 1990, 1995). Sand grain texture, tidal inundation, sand moisture, and temperature in the nest can all influence the incubation period and hatching

success (Prange and Ackerman 1974; Mortimer 1990; Foley et al. 2006; Yalçin-Özdilek 2007; Madden et al. 2008). In addition to ideal incubation conditions, a coordinated ascent to the surface is essential for hatchling survival (Kurian and Nayak 2003).

Among other physical factors, temperature influences sea turtle nests, where it plays a direct role in the egg incubation phase (Spotila and Standora 1985; Packard and Packard 1988). Several studies have shown that successful incubation in sea turtles occurs within a tight thermal range of 26 to 33° C (Bustard and Greenham 1968; McGehee 1979; Yntema and Mrosovsky 1980; Miller 1982, 1985; Miller et al. 2003). Environmental temperatures also regulate hatchling sex determination during the middle third of incubation (Yntema and Mrosovsky 1982). Warmer temperatures above the pivotal temperature, where a 1:1 sex ratio is produced, yield more females while temperatures below the pivotal temperature shift the ratio towards more males (Yntema and Mrosovsky 1980, 1982). Additionally, environmental temperatures influence the incubation period such that increasing temperatures reduce incubation duration (Miller and Limpus 1981; Limpus et al. 1983; Ackerman 1997), and produce a decrease in the hatchling body size (Booth and Astill 2001; Burgess et al. 2006).

Hatchling mortality before emergence has been attributed to high sand temperatures (> 32° C) in Loggerhead Sea Turtle (*Caretta caretta*) nests in Japan (Matzuzawa et al. 2002). High sand temperatures may inhibit coordinated muscle action in the ascent process to the sand surface (Drake and Spotila 2002) and cause a decline in oxygen levels within the nest (Ackerman 1980). The decline in oxygen levels corresponds with an increase in metabolic heat production, which peaks at the time of hatching (Ackerman 1980). These factors can increase mortality of hatchlings prior to emergence. In Green Sea Turtles, hatchlings usually emerge at night and this is at least partially controlled by a nightly decrease in sand temperatures (Bustard 1967; Mrosovsky 1968). In the days prior to emergence, hatchlings are close to the sand surface, where daily changes in temperature may increase their mortality.

Global warming is considered a major threat for several animals species worldwide (McCarty 2001). Future increases in environmental temperatures will particularly affect animals who maintain a very close relationship with environmental temperatures, such as ectothermal animals (Deutsch et al. 2008; Tewksbury et al. 2008). For sea turtles, climatic warming represents a threat principally due to the important role of environmental temperatures in successful egg incubation. Incubation above the thermal threshold of 33° C will result in greater hatchling mortality and higher numbers of morphological abnormalities (Miller 1985), whereas pivotal temperatures for sex

determination (~ 29° C) will result in a modification in sex proportions of sea turtles populations (Yntema and Mrosovsky 1982; Broderick et al. 2001). Recent models of climate-change predict the feminization of sea turtles populations worldwide (Hawkes et al. 2007; Fuentes et al. 2009). Moreover, rising temperatures could decrease the chances for survival of sea turtles hatchlings due to the smaller size of hatchlings incubated at higher temperatures, thus making them more susceptible to predation (Gyuris 1994).

Green turtle nesting season at Tortuguero beach, Costa Rica, lasts from June through October every year (Carr et al. 1978). The rainy season (wet period, WP) largely dominates these months, and is only interrupted by a brief dry period (DP) in September and early October (Caribbean Conservation Corporation, Report on the 1999 Green Turtle Program at Tortuguero, Costa Rica. 2000. Available from www.cccturtle.org/ccc-costarica.php?page=season-reports [Accessed 26 March 2010]). During the DP, air and sand temperatures increase considerably in comparison with the rest of the nesting season (Caribbean Conservation Corporation. 2000. *op. cit.*). The main goal of this study was to determine whether these high temperatures during the DP affect incubation period, hatching success, and emergence percentage of Green Sea Turtle nests. Because climate change is considered an important threat for most ectothermal animals worldwide (Deutsch et al. 2008; Tewksbury et al. 2008; Hawkes et al. 2009; Fuentes et al. 2010), the present study provides essential information to be considered in predictive models about the potential effects of global warming on nesting success and its implications for the population dynamics of Green Sea Turtles in Tortuguero.

MATERIALS AND METHODS

Study site.—Tortuguero Beach hosts the largest nesting colony of Green Sea Turtles in the Atlantic (Bjorndal et al. 1999; Troëng and Rankin 2005). During the breeding season, females reach these shores from all over the Caribbean Sea to nest (Carr et al. 1978). The Tortuguero nesting beach is located on the Caribbean coast of Costa Rica, between Puerto Limón and the Nicaraguan border (Fig. 1). This unbroken strip of black sand beach extends 29 km from the mouth of Río Tortuguero south to Río Parismina. Along its entire length the nesting beach is closely backed by a natural river system. The study area includes the northern 4 km of Tortuguero beach, close to the CCC Biological Field Station (10°33'N; 83°30'W) and the village of Tortuguero.

Data collection.—From 10 June to 10 August 2000, we randomly selected 32 nests from nesting Green Sea

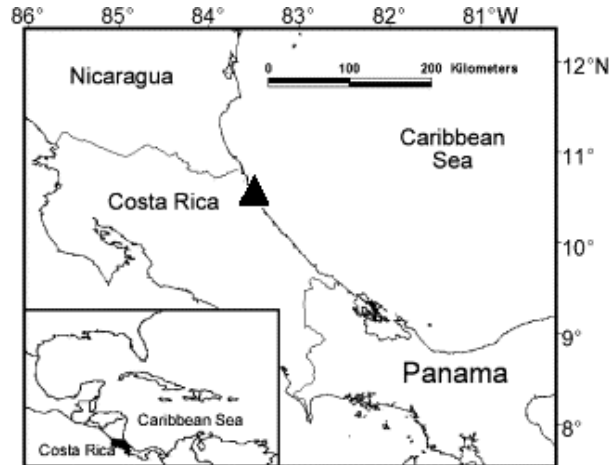


FIGURE 1. Location of Tortuguero Green Sea Turtle nesting beach (black triangle) in the Caribbean Sea, Costa Rica.

Turtles, so that hatchlings from each nests would emerge under two distinct periods: a period of low temperatures and abundant rainfall mainly during August (wet period, WP) and a period of higher temperatures and lower rainfall mainly during September and early October (dry period, DP). All nests were selected in areas with partial sun exposure and located at least 8 m from the high tide line (to avoid flooding) and 2 m from the vegetation line (to avoid excessive shade and roots inside the nest). For each nest, we counted the number of eggs laid.

Daily rainfall, air temperatures, and sand temperatures were recorded for this study. Tortuguero Biological Field Station provided data for local rainfall and air temperature. Sand temperature for the study period was recorded with two thermocouples (Cooper / Constantan Connectors SMP-T-M / F and Teflon Neoflon PFA High Performance Wire TT-T-245, Omega Engineering Inc., Stamford, Connecticut, USA) buried in the sand at 40 and 63 cm depth. We read the thermocouples using a digital thermometer (HH-25TC, Omega Engineering Inc., Stamford, Connecticut, USA). We calibrated thermocouples against mercury thermometers accurate to 0.2–0.3° C prior to sand burial. The chosen depths in the sand for the thermocouples corresponds to the average depth to the top (egg chamber neck level, ECN) and center of the nests (egg chamber level, EC) for Green Sea Turtles on Tortuguero beach (Caribbean Conservation Corporation, 2000. *op. cit.*). We buried the two thermocouples in an area with partial sun exposure, located 15 m above the high tide line (to avoid flooding) and 3 m from the vegetation line (to avoid excessive shade). We recorded sand temperatures daily between 1430 and 1600, corresponding to the time at which Horikoshi (1992) reported the mean daily sand temperature for Tortuguero beach.

Approximately one week before expected hatchling emergence (see Tiwari et al. 2006), we buried two thermocouples 50 cm adjacent to each nest to record the sand temperature in the days prior to hatchling emergence. We placed thermocouples at 40 and 63 cm depth and retrieved them two days after hatchlings emerged (easily recognizable by a small depression in the sand). We monitored nests daily in the morning during the pre-hatching period (approximately 50 days after egg laying). We recorded sand temperatures from each nest daily between 1430 and 1600 (see above). Two days after hatchling emergence, we excavated nests to calculate the hatchling success and emergence percentage. We defined hatchling success as the ratio of eggs hatched to the number of eggs laid, while emergence percentage was defined as the ratio of emerged hatchlings to the number of eggs hatched. Variables we considered for this purpose were: (1) empty egg shells (hatched embryos); (2) unhatched eggs (with or without embryo inside); (3) depredated eggs; and (4) live and dead hatchlings in the ECN or EC.

Data analysis.—For the analysis, we only considered nests that were undisturbed ($n = 28$), the rest of the nests were depredated ($n = 2$), removed by other nesting turtles ($n = 1$), or could not be re-located ($n = 1$). We defined incubation period as the number of days from egg laying to the emergence of hatchlings. When live hatchlings were found during excavation, we considered as non-emerged those that we found deeper than 40 cm, as we considered these as having a very low probability of reaching the surface by themselves in the following days. In contrast, live hatchlings we found between the sand surface and 40 cm depth we considered successfully emerged, as we considered them to have a high probability of reaching the surface in the following days. To test for an association between the sand temperature in pre-emerging days and emergence percentage, we calculated the mean temperature for the four days before first emergence and the two days after emergence.

Statistical analysis.—We performed a multivariate analysis of variance (MANOVA; $\alpha = 0.05/3 = 0.016$ after Bonferoni correction [see Summers et al. 2004]) to detect fluctuations between the mean, maximum, and minimum air temperatures in the study period (WP and DP). We assessed sand temperatures between periods and depths using a Mann-Whitney U-test. The t ($\alpha = 0.05$) test was used to compare incubation periods and Spearman Rank Correlation Coefficients were used to assess the degree of association between variables. Statistical analyses were performed with the STATISTICA 7.0 package. Reported values are means \pm SE. All data were tested for normality.

TABLE 1. Summary of daily average, minimum, and maximum air temperatures for Tortuguero beach, Costa Rica, during the 2000 Green Sea Turtle (*Chelonia mydas*) breeding season. Values of mean, minimum, and maximum monthly air temperatures are shown for the wet period (August) and dry period (September).

		Mean \pm SE ($^{\circ}$ C)	Minimum ($^{\circ}$ C)	Maximum ($^{\circ}$ C)
Wet period	daily average	26.6 \pm 0.27	24	29.5
	daily minimum	25.3 \pm 0.18	23.3	28
	daily maximum	30.5 \pm 0.38	27	34
Dry period	daily average	27.8 \pm 0.22	25.5	31
	daily minimum	25.3 \pm 0.14	24	27
	daily maximum	33.5 \pm 0.31	30	36

RESULTS

Air temperatures varied during the WP and DP. The average and maximum daily air temperatures (Table 1) were significantly different between the WP and DP (Wilk's $\lambda = 0.55$; $F_{3,37} = 10.17$; $P < 0.001$), with the average ($F_{1,37} = 24.78$; $P < 0.001$) and the maximum ($F_{1,37} = 30.73$; $P < 0.001$) daily air temperatures higher in the DP. No significant differences were found in minimum daily air temperatures (Table 1) between the WP and DP ($F_{1,37} = 0.00$; $P = 0.99$). Accumulated rainfall during August was 591 mm (daily average = 19.0 mm), while in September was 139 mm (daily average = 4.6 mm).

Mean daily sand temperature at 63 cm (EC) was 28.4 \pm 0.17 $^{\circ}$ C (range = 26.6–30.4 $^{\circ}$ C) in the WP and 30.7 \pm 0.19 $^{\circ}$ C (range = 27.8–32.7 $^{\circ}$ C) in the DP, while at 40 cm (ECN) was 28.5 \pm 0.21 $^{\circ}$ C (range = 26.3–31.9 $^{\circ}$ C) for the WP and 31.1 \pm 0.23 $^{\circ}$ C (range = 28.1–34.1 $^{\circ}$ C) for the DP (Fig. 2). The mean daily sand temperature was significantly different between WP and DP both at the

EC level ($U = 78$, $P < 0.001$) or the ECN level ($U = 55.5$, $P < 0.001$). Mean daily sand temperature was not significantly different between EC and ECN levels either in the WP ($U = 473$, $P = 0.89$) or the DP ($U = 368$, $P = 0.22$).

Mean clutch size for the selected nests was 118 \pm 4.4 eggs (range = 72–155 eggs, $n = 28$). Mean distance from the top of the EC to the sand surface was 49 \pm 2.5 cm (range = 30–80 cm) and the mean distance to the bottom of the chamber was 66 \pm 2 cm (range = 48–94 cm; Table 2).

Mean incubation period in this study was 57.6 \pm 0.4 d (range = 54–63 d, $n = 28$; Table 2) and varied with the date of egg laying. We found a significant negative association between the nest initiation date (day 0 = 10 June) and the incubation period ($r = - 0.51$, $P = 0.005$; Fig. 3). For nests whose incubation period was mainly in the WP (July and August), the mean was 58.6 \pm 0.6 d (range = 56–63 d, $n = 16$), whereas for nests whose incubation period was during August and September (partially during the DP), the mean decreased to 56.4 \pm

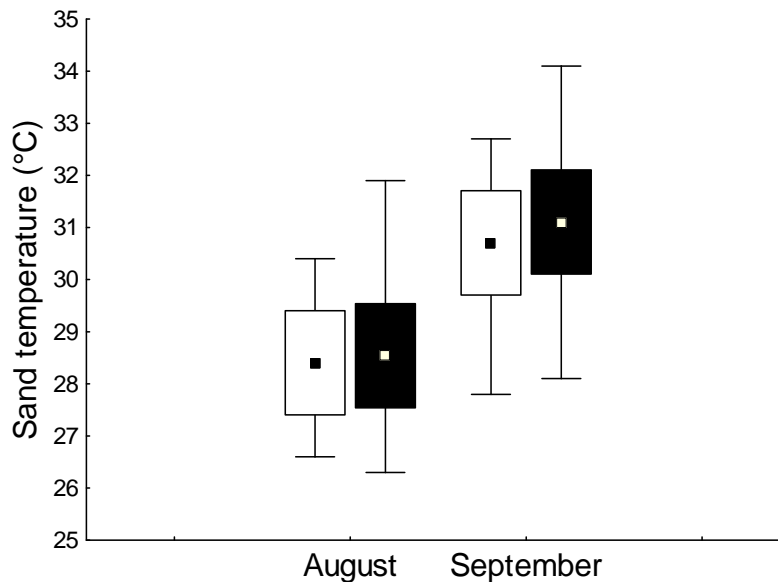


FIGURE 2. Differences in sand temperature during August (wet period) and September (dry period) for Green Sea Turtle (*Chelonia mydas*) at Tortuguero beach, Costa Rica, during the 2000 breeding season. White squares indicate sand temperatures at 63 cm depth (egg chamber level) and black squares at 40 cm depth (egg chamber neck level). Mean values \pm 1 and maximums and minimums are indicated.

Herpetological Conservation and Biology

TABLE 2. Distance from the sand surface to the top (TOP) and bottom (BOTTOM) of the egg chamber, incubation period (INCUB), hatching success (HATCHING) and emergence percentage (EMERG) for the 28 Green Sea Turtle nests monitored in Tortuguero, Costa Rica, during the 2000 breeding season.

NEST	TOP (cm)	BOTTOM (cm)	INCUB (days)	HATCHING (%)	EMERG (%)
1	37	77	59	81	99
2	49	69	56	93	99
3	50	68	61	76	98
5	36	57	61	88	91
6	31	48	59	75	94
7	62	71	63	94	99
8	49	69	56	94	91
9	49	78	58	95	99
10	42	60	62	85	98
11	36	68	57	98	98
12	52	67	59	96	99
14	40	59	60	96	100
15	49	62	56	93	98
16	51	62	57	97	94
17	38	58	56	88	86
18	68	94	57	94	99
19	52	61	57	87	84
20	38	58	54	92	91
21	44	62	58	98	94
22	46	56	58	97	99
23	54	63	59	97	99
24	31	50	54	44	90
26	30	54	55	40	86
28	68	78	59	96	99
29	57	72	57	91	100
30	70	78	55	73	98
31	80	90	57	84	98
32	58	66	54	72	100

0.5 d (range = 54–59 d, $n = 12$; $t = 2.4$, $df = 26$, $P = 0.02$).

Hatching success varied between 40% and 98% (mean = $86 \pm 3\%$, $n = 28$; Table 2). No significant association was found between the nest initiation date and hatching success ($r = -0.10$, $P = 0.61$; Fig. 4a). The mean emergence percentage was $96 \pm 1\%$ (range = 84–100%, $n = 28$; Table 2) and did not vary with nest initiation date ($r = 0.07$, $P = 0.71$; Fig. 4b). We found a negative association between the emergence percentage and the mean sand temperature at the ECN level in the pre-emergent days ($r = -0.44$, $P = 0.02$; Fig. 5), while this association was expressed instead as a negative tendency at the EC level ($r = -0.35$, $P = 0.07$). Nest depth was not associated with the number of dead hatchlings in the EC ($r = -0.16$, $P = 0.40$) or in the ECN ($r = -0.29$, $P = 0.13$).

DISCUSSION

Air and sand temperature.—Air and sand temperatures on Tortuguero beach were different between the brief DP in September and the WP in August. Sand temperatures at the EC level showed a minimum value of 26.6°C and a maximum of 32.7°C , while at the ECN level minimum and maximum values were 26.3°C and 34.4°C , respectively. These daily fluctuations are not considered extreme and are comparable with those reported from Florida (24.7 – 30.3°C ; McGehee 1979), Australia (25 – 32°C ; Limpus

et al. 1983), and Costa Rica (24.9 – 30.7°C ; Tiwari et al. 2006); but not with those reported from Japan (18 – 33.3°C ; Matsuzawa et al. (2002) where the minimum temperatures are much lower.

Sand temperatures increased with decreasing rainfall, occasionally exceeding the optimum temperatures for incubation of sea turtle eggs (26 – 33°C , Bustard 1972) in September. Booth and Astill (2001) and Matsuzawa et al. (2002) found that temperatures in the center of the nest are slightly higher (approximately 1.7 and 1.0°C , respectively) than temperatures in the surrounding sand, mainly due to increased metabolic activity in pre-emergence hatchlings (Kraemer 1979; Ackerman 1980; Ackerman et al. 1985; Broderick et al. 2001; Matsuzawa et al. 2002). This increase, especially in nests with a large clutch size, could result in EC temperature in excess of 33°C , considered critical for normal embryo development (Bustard and Greenham 1968; McGehee 1979; Yntema and Mrosovsky 1980; Miller 1982, 1985; Miller et al. 2003).

Incubation period and hatching success.—We found a small fluctuation in the incubation period, with a minimum of 54 d and a maximum of 63 d. Similar results in Green Sea Turtles on these beaches can be found in Tiwari et al. (2006), where the ranges were 54 and 68 d, but not in Fowler (1979) or CCC Report 2001 (Caribbean Conservation Corporation, Report on the 2000 Green Turtle Program at Tortuguero, Costa Rica.

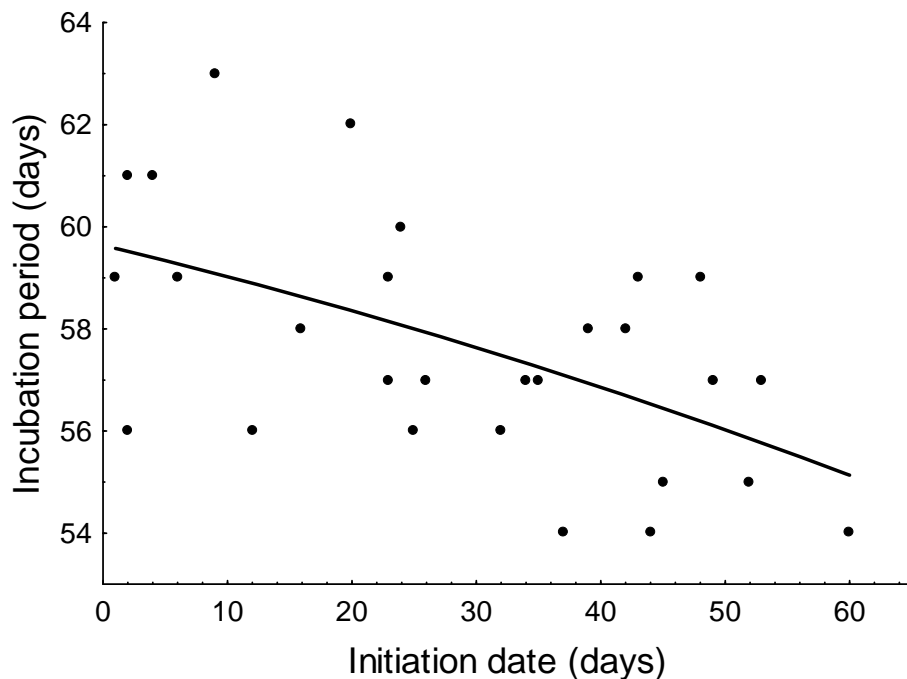


FIGURE 3. Incubation period for each of the 28 nests of Green Sea Turtle nests initiated at different times of the 2000 nesting season in Tortuguero, Costa Rica (day 0 = June 10). Incubation period was defined as the number of days from egg laying to first emergence, including the period from hatching to emergence.

2001. Available from www.cccturtle.org/ccc-costarica.php?page=season-reports [Accessed 26 March 2010], where, although the means were similar, (62 and 57 d, respectively), ranges were larger than from our study (53–81 and 47–76 d, respectively). The small fluctuation found in this study is probably due to the short period of time monitoring the nests (June–September), while the monitoring conducted by Fowler (1979) and CCC Report 2001 (Caribbean Conservation Corporation. 2001. *op. cit.*) was more extensive (June–November). However, this small fluctuation was significantly different between the WP and DP. Sand temperatures in the DP were higher than in the WP, and this led to acceleration in embryo development during incubation. Other studies have also shown that incubation period is negatively related to nest temperature (Miller and Limpus 1981; Miller 1985; Ackerman 1997). An increase in temperature speeds up physiological processes, including growth (Schmidt-Nielsen 1997), so a decrease in incubation period with an increase in nest temperature is expected. Embryo development is faster with higher sand temperatures in the nest (Miller 1985). For example, Loggerhead Turtle (*Caretta caretta*) eggs developed at a minimum sand temperature of 26–27° C showed an incubation period of approximately 13 weeks, while when the sand temperatures exceeded 32° C the incubation period was approximately 7 weeks (see Miller et al. 2003).

However, when temperatures in the EC exceed 33° C for a relatively long period, eggs rarely hatch (Bustard and Greenham 1968; McGehee 1979; Yntema and Mrosovsky 1980; Miller 1982) and there is also an increased possibility of developmental abnormalities (Miller 1985).

Hatching success was not associated with nest initiation date, probably due to the relatively stable temperatures found in the EC. However, two nests in particular showed much lower hatching success (nests 24 and 26, see Table 2). In these nests, distance between the top of the EC and the sand surface was lower than in the rest of the nests (31 and 30 cm, respectively). Due to proximity to the sand surface, these nests were possibly exposed to greater daily fluctuations in sand temperature during incubation, often exceeding the optimum thermal range for incubation (26–33° C; Bustard and Greenham 1968; McGehee 1979; Yntema and Mrosovsky 1980; Miller 1982, 1985; Miller et al. 2003). Greater daily fluctuations in sand temperatures could cause unstable environmental conditions for embryo development, and may increase the number of unhatched eggs when temperatures exceed the optimal thermal range of incubation.

Emergence percentage.—Although the emergence percentage was not associated with the nest initiation date, our results indicate that pre-emergent hatching

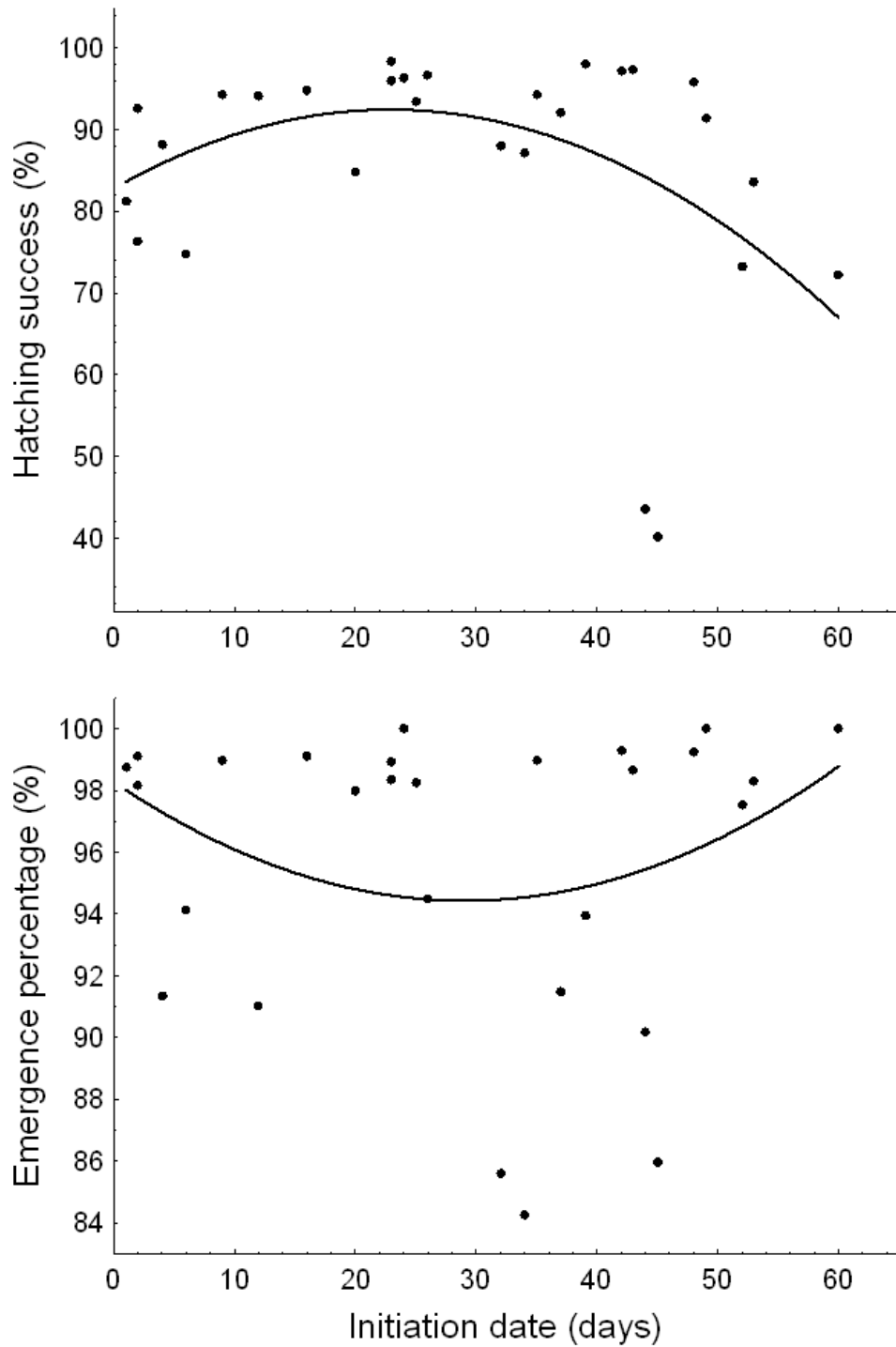


FIGURE 4. Hatching success (A) and emergence percentage (B) for each of the 28 Green Sea Turtle nests initiated at different times of the 2000 nesting season in Tortuguero, Costa Rica (day 0 = June 10). Hatching success was defined as the ratio of eggs that hatched to the number of eggs laid. Emergence percentage was defined as the ratio of hatchlings that emerged to eggs that hatched.

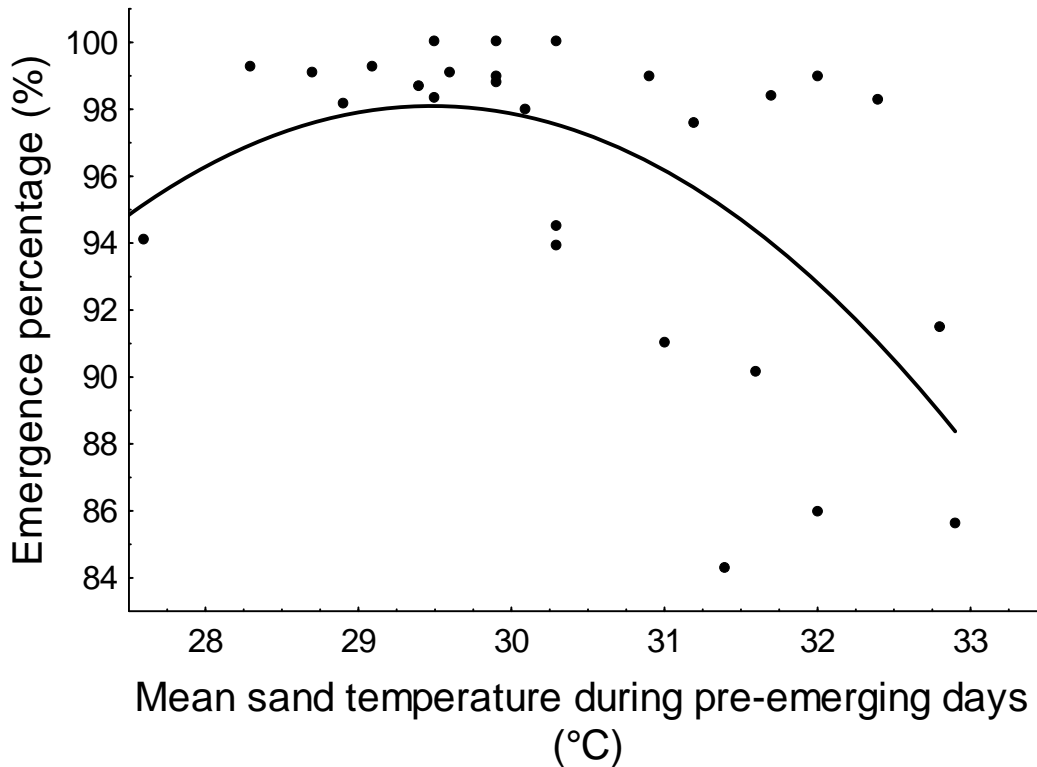


FIGURE 5. Relationship between mean sand temperature in pre-emerging days (four days before first emergence and two days after emergence) and emergence percentage for each of the 28 Green Sea Turtle nests of the 2000 nesting season in Tortuguero, Costa Rica.

mortality is negatively influenced by sand temperature and that sand temperature fluctuations in the ECN were more pronounced than in the EC, often exceeding 33° C. These results are consistent with those reported by Matsuzawa et al. (2002), where high sand temperatures increased hatchling mortality in artificial Loggerhead nests in Japan. Our increase in mortality could be explained in several ways. First, warming could directly kill sea turtle hatchlings when temperatures are very high (for example, exceeding 33° C). Emergence percentage decreased when sand temperature in pre-emergence days exceeds 31° C and is considerably lower when it exceeded 33° C (Fig. 5). In addition, it has been demonstrated that nest temperature could be slightly higher than the surrounding sand. For example, Broderick et al. (2001) found a marked rise in nest sand temperature attributed to hatchling metabolic heating on Green Sea Turtle nests on Ascension Island. Matsuzawa et al. (2002) reported similar results for Loggerhead Turtle nests in Japan. Second, hatchlings could get caught in the ECN due to this overheating. Hatchling ascendant movements decrease with increasing temperature, probably due to thermal inhibition of coordinated muscle movement (see Mrosovsky 1968; O'Hara 1980; Moran et al. 1999). Finally, poor gas exchange in the nest due to high temperatures and

increased oxygen consumption by hatchling metabolism (Ackerman 1980) could increase hatchling mortality.

Other studies also indicate that hatchling mortality is high in the pre-emergence period, mainly due to causes other than sand temperature. Mortimer (1990) suggests that the lack of moisture in the sand and cave-ins can reduce the emergence percentage of Green Sea Turtle on Ascension Island. Peters et al. (1994) suggest that compactness of beach sand can obstruct emergence of Loggerhead Sea Turtle hatchlings in Turkey. Although we did not measure the sand moisture potential, it was moist enough not to collapse at the time of nest excavation. We also observed that sand compaction did not appear to inhibit the emergence of hatchlings from nests. In addition, unhatched eggs were still turgid upon excavation.

As with many oviparous reptiles, sea turtles have a life history, physiology, and behavioral traits that are strongly influenced by environmental temperature (Hawkes et al. 2009; Fuente et al. 2010). Therefore, projected temperature changes due to global warming could seriously affect sea turtle populations in both their terrestrial reproductive habitat and in their ocean habitat (see Hawkes et al. 2009). Some recent models suggest that the impacts of climate warming may be particularly severe in the tropics, the region predicted to experience

the highest increase in temperatures (Deutsch et al. 2008; Tewksbury et al. 2008). However, although much effort has been expended over the last two decades to understand and mitigate the threats to marine turtles (Lutcavage et al. 1997; Watson et al. 2005), the threat of climate change on this taxon has, until recently, been given little attention (see Hawkes et al. 2009). As demonstrated in this study, pre-emergent Green Sea Turtle hatchlings experience increased mortality when sand temperatures in pre-emergence days exceed 33° C. These results provide important information for management and conservation of sea turtles, not just on Caribbean beaches but worldwide. If temperatures in the tropics increase in the future, many nesting colonies in the tropics will need additional management actions to protect sea turtle nests, for example by moving the nests to more shaded areas, or by cooling nests that are about to hatch by partial shading or sprinkling with water.

Although Tortuguero Beach supports the largest Green Sea Turtle rookery in the Atlantic system and the number of Green Sea Turtles nesting annually has increased over the past 30 years (Bjorndal et al. 1999, 2005; Troëng and Rankin 2005; but see Campbell 2003), in many beaches in the world there are decreasing numbers of breeding female Green Sea Turtles (see Seminoff 2004). All of these populations could experience excessive nest failure as a result of global warming. At Tortuguero Beach the population of Green Sea Turtles could be exposed to greater risk of nest failure during the brief dry period.

Acknowledgments.—We especially thank Manjula Tiwari for her support in acquiring the field data and the Research Assistants of the "Green Turtle Program 2000" for their help in data collection: Melinda Stockmann, Andrés Ortega, Catalina Reyes, Zunilda Hudgson, Alvaro Opazo, Luis Fernández, Ricardo Hernández, Damien Hussy, Manuel Ramírez, Sylvia de la Parra, Mery Martínez, and Reem Hajjar. We thank the staff of the Caribbean Conservation Corporation (CCC) in the Biological Field Station at Tortuguero, to its Scientific Director Sebastian Troëng and its Field Coordinator Jeff Mangel for their support and use of instrumentation. Luciano N. Segura and Rodrigo Cajade were supported by fellowships from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

LITERATURE CITED

- Ackerman, R.A. 1980. Physiological and ecological aspects of gas exchange by sea turtle eggs. *American Zoologist* 20:575–583.
- Ackerman, R.A. 1991. Physical factors affecting the water exchange of buried eggs. Pp. 193–211 *In* *Physical Influences on Embryonic Development in Birds and Reptiles*. Ferguson, M.J.W., and D.C. Deeming (Eds.). Cambridge University Press, New York, New York, USA.
- Ackerman, R.A. 1997. The nest environment and the embryonic development of sea turtles. Pp. 83–106 *In* *The Biology of Sea Turtles*. Lutz, P.L., and J.A. Musick (Eds.). CRC Press, Boca Raton, Florida, USA.
- Ackerman, R.A., R.C. Seagrave, R. Dmi'el, and A. Ar. 1985. Water and heat exchange between parchment-sheller reptile eggs and their surroundings. *Copeia* 1985:703–711.
- Bjorndal, K.A., J.A. Wetherall, A.B. Bolten, and J.A. Mortimer. 1999. Twenty-six years of Green Turtle nesting at Tortuguero, Costa Rica: An encouraging trend. *Conservation Biology* 13:126–134.
- Bjorndal, K.A., A.B. Bolten, and M.Y. Chaloupka. 2005. Evaluating trends in abundance of immature Green Turtles, *Chelonia mydas*, in the Greater Caribbean. *Ecological Applications* 15:304–314.
- Booth, D.T., and K. Astill. 2001. Incubation temperature, energy expenditure and hatchling size in the Green Turtle (*Chelonia mydas*), a species with temperature-sensitive sex determination. *Australian Journal of Zoology* 49:389–396.
- Broderick, A.C., B.J. Godley, and G.C. Hays. 2001. Metabolic heating and the prediction of sex ratios for Green Turtles (*Chelonia mydas*). *Physiological and Biochemical Zoology* 74:161–170.
- Burgess, E.A., D.T. Booth, and J.M. Lanyon. 2006. Swimming performance of hatchling Green Turtles is affected by incubation temperature. *Coral Reefs* 25:341–349.
- Bustard, H.R. 1967. Mechanism of nocturnal emergence from the nest in Green Turtle hatchlings. *Nature* 214:317.
- Bustard, R.H. 1972. *Sea Turtles: Their Natural History and Conservation*. Taplinger Publishers, New York, New York, USA.
- Bustard, R.H., and P. Greenham. 1968. Physical and chemical factors affecting hatching in the Green Sea Turtle, *Chelonia mydas*. *Ecology* 49:269–276.
- Campbell, C.L. 2003. Population assessment and management needs of a Green Turtle, *Chelonia mydas*, population in the western Caribbean. Ph.D. dissertation, University of Florida, Gainesville, Florida, USA. 124 p.
- Carr, A.F., M.H. Carr, and A.B. Meylan. 1978. The ecology and migrations of sea turtles, 7. The West Caribbean Green Turtle colony. *Bulletin of the American Museum of Natural History* 162:1–46.
- Deutsch, C.A., J.J. Tewksbury, R.B. Huey, K.S. Sheldon, C.K. Ghalambor, C. David, D.C. Haak, and P.R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* 105:6668–6672.

- Drake, D.L., and J.R. Spotila. 2002. Thermal tolerances and the timing of sea turtle hatchling emergence. *Journal of Thermal Biology* 27:71–81.
- Foley, A.M., S.A. Peck, and G.R. Harman. 2006. Effects of sand characteristics and inundation on the hatching success of Loggerhead Sea Turtle (*Caretta caretta*) clutches on low-relief Mangrove Islands in Southwest Florida. *Chelonian Conservation and Biology* 5:32–41.
- Fowler, L.E. 1979. Hatching success and nest predation in the Green Sea Turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. *Ecology* 60:946–955.
- Fuentes, M.M.P.B., J.A. Maynard, M. Guinea, I.P. Bell, P.J. Werdell, and M. Hamann. 2009. Proxy indicators of sand temperature help project impacts of global warming on sea turtles in northern Australia. *Endangered Species Research* 9:33–40.
- Fuentes, M.M.P.B., M. Hamann, and C.J. Limpus. 2010. Past, current and future thermal profiles of Green Turtle nesting grounds: Implications from climate change. *Journal of Experimental Marine Biology and Ecology* 383:56–64.
- Georges, A., C.J. Limpus, and C.J. Parmenter. 1993. Natural history of the *Chelonia*. Pp. 120–128 *In* Fauna of Australia: Amphibia and Reptilia. Glasby, C.J., G.J.B. Ross, and P.L. Beesley (Eds.). Australian Government Publishing Service, Canberra, Australia.
- Gyuris, E. 1994. The rate of predation by fishes on hatchlings of the Green Turtle. *Coral Reefs* 13:137–144.
- Hawkes, L.A., A.C. Broderick, M.H. Godfrey, and B.J. Godley. 2007. Investigating the potential impacts of climate change on a marine turtle population. *Global Change Biology* 13:923–932.
- Hawkes, L.A., A.C. Broderick, M.H. Godfrey, and B.J. Godley. 2009. Climate change and marine turtles. *Endangered Species Research* 7:137–154.
- Horikoshi, K. 1992. Eggs, survivorship, and sex ratios of Green Turtle hatchlings in Tortuguero, Costa Rica. Ph.D. Dissertation, University of Florida, Gainesville, Florida, USA. 167 p.
- Kraemer, J.E. 1979. Variation in incubation period of Loggerhead Sea Turtle, *Caretta caretta*, clutches on the Georgia coast. M.S. thesis, University of Georgia, Athens, Georgia, USA. 57 p.
- Kurian, A., and V.N. Nayak. 2003. Influence of environmental factors on the hatching success of Olive Ridley Turtles: a preliminary study. *Kachhapa* 8:8–11.
- Limpus, C.J., P. Reed, and J.D. Miller. 1983. Islands and turtles: the influence of choice of nesting beach on sex ratio. Pp. 397–402 *In* Proceedings of Inaugural Great Barrier Reef Conference. Baker, J.T., R.M. Cater, P.W. Sammarco, and K.P. Stark (Eds.). James Cook University Press, Townsville, Queensland, Australia.
- Lutcavage, M.E., P. Plotkin, B.E. Witherington, and P.L. Lutz. 1997. Human impacts on sea turtle survival. Pp. 387–409 *In* The Biology of Sea Turtles. Lutz, P.L., and J.A. Musick (Eds.). CRC Press, Boca Raton, Florida, USA.
- McCarty, J. 2001. Ecological consequences of recent climate change. *Conservation Biology* 15:320–331.
- Madden, D., J. Ballesterro, C. Calvo, R. Carlson, E. Christians, and E. Madden. 2008. Sea turtle nesting as a process influencing a sandy beach ecosystem. *Biotropica* 40:758–765.
- Matsuzawa, Y., K. Sato, W. Sakamoto, and K.A. Bjorndal. 2002. Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of Loggerhead Sea Turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. *Marine Biology* 140:639–646.
- McGehee, M.A. 1979. Factors affecting the hatching success of Loggerhead Sea Turtle eggs (*Caretta caretta caretta*). M.S. thesis, University of Central Florida, Orlando, Florida, USA. 73 p.
- Miller, J.D. 1982. Development of marine turtles. Ph.D. Dissertation, University of New England, Armidale, New South Wales, Australia. 123 p.
- Miller, J.D. 1985. Embryology of marine turtles. Pp. 269–328 *In* Biology of the Reptilia. Gans, C., F. Billett, and P.F.A. Maderson (Eds.). Wiley-Interscience, New York, New York, USA.
- Miller, J.D., and C.J. Limpus. 1981. Incubation period and sexual differentiation in the Green Turtle *Chelonia mydas*. Pp. 66–73 *In* Proceedings of the Melbourne Herpetological Symposium. Banks, C.B., and A. Martin (Eds.). The Royal Melbourne Zoological Gardens, Melbourne, Australia.
- Miller, J.D., C.L. Limpus, and M.H. Godfrey. 2003. Nest site selection, oviposition, eggs, development, hatching and emergence of Loggerhead Turtles. Pp. 125–143 *In* Ecology and Conservation of Loggerhead Sea Turtle. Bolten, A.B., and B.E. Witherington (Eds.). University Press of Florida, Gainesville, Florida, USA.
- Moran, K.L., K.A. Bjorndal, and A.B. Bolten. 1999. Effects of the thermal environment on the temporal pattern of emergence of hatchling Loggerhead Turtles *Caretta caretta*. *Marine Ecology Progress Series* 189:251–261.
- Mortimer, J.A. 1990. The influence of beach sand characteristics on the nesting behavior and clutch survival of Green Turtles (*Chelonia mydas*). *Copeia* 1990:802–817.
- Mortimer, J.A. 1995. Factors influencing beach selection by nesting sea turtles. Pp. 45–51 *In* Biology and Conservation of Sea Turtles. Bjorndal, K.A. (Ed.). Smithsonian Institution Press, Washington, D.C., USA.
- Mrosovsky, N. 1968. Nocturnal emergence of hatchling sea turtles: control by thermal inhibition of activity. *Nature* 220:1338–1339.

Herpetological Conservation and Biology

- O'Hara, J. 1980. Thermal influences on the swimming speed of Loggerhead Turtle hatchlings. *Copeia* 1980:773–780.
- Packard, G.C., and M.J. Packard. 1988. The physiological ecology of reptilian eggs and embryos. Pp. 523–605 *In* *Biology of the Reptilia*, Vol 16, Ecology B. Gans, C., and R. Huey (Eds.). Alan R. Liss Press, New York, New York, USA.
- Peters, A., K.J.F. Verhoeven, and H. Srijbosch. 1994. Hatching and emergence in the Turkish Mediterranean Loggerhead Turtle, *Caretta caretta*: natural cause for egg and hatchling failure. *Herpetologica* 50:369–373.
- Prange, H.D., and R.A. Ackerman. 1974. Oxygen consumption and mechanism of gas exchange of Green Turtle (*Chelonia mydas*) eggs and hatchlings. *Copeia* 1974:758–763.
- Seminoff, J. 2004. Sea turtles, Red Listing and the need for regional assessments. *Marine Turtle Newsletter* 106:4–6.
- Schmidt-Nielsen, K. 1997. *Animal Physiology. Adaptation and Environment*. 5th Edition. Cambridge University Press, New York, New York, USA.
- Spotila, J.R., and E.A. Standora. 1985. Environmental constraints on the thermal energetics of sea turtles. *Copeia* 1985:694–702.
- Summers, K., T.W. Cronin, T. Kennedy. Cross-breeding of distinct color morphs of the strawberry poison frog (*Dendrobates pumilio*) from the Bocas del Toro Archipelago, Panama. *Journal of Herpetology* 38(1):1–8.
- Tewksbury, J.J., R.B. Huey, and C.A. Deutsch. 2008. Putting the heat on tropical animals. *Science* 320:1296–1297.
- Tiwari, M., K. Bjorndal, A. Bolten, and B. Bolker. 2006. Evaluation of density-dependent processes and Green Turtle *Chelonia mydas* hatchling production at Tortuguero, Costa Rica. *Marine Ecology Progress Series* 326:283–293.
- Troëng, S., and E. Rankin. 2005. Long-term conservation of the Green Turtle *Chelonia mydas* nesting population at Tortuguero, Costa Rica. *Biological Conservation* 121:111–116.
- Yalçin-Özdilek, Ş., H.G. Özdilek, and F.S. Ozaner. 2007. Possible influence of beach sand characteristics on Green Turtle nesting activity on Samandağ Beach, Turkey. *Journal of Coastal Research* 23:1379–1390.
- Yntema, C.L., and N. Mrosovsky. 1980. Sexual differentiation in hatchling Loggerheads (*Caretta caretta*) incubated at different controlled temperatures. *Herpetologica* 36:33–36.
- Yntema, C.L., and N. Mrosovsky. 1982. Critical periods and pivotal temperatures for sexual differentiation in Loggerhead Sea Turtles. *Canadian Journal of Zoology* 60:1012–1016.
- Watson, J.W., S.P. Epperly, A.K. Shah, and D.G. Foster. 2005. Fishing methods to reduce sea turtle mortality associated with pelagic longlines. *Canadian Journal of Fisheries and Aquatic Sciences* 62:965–981.



LUCIANO N. SEGURA is a biologist graduated at the Universidad Nacional de La Plata, Argentina. He was a Research Assistant in the “2000 Green Turtle Program” in Tortuguero, Costa Rica. He began researching in Argentina follow the Comisión de Investigación Científica de la provincia de Buenos Aires (CIC) in 2005, and since 2009 follow the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). He is currently completing the PhD “Biología reproductiva del Cardenal Común (*Paroaria coronata*) en talares del NO de la provincia de Buenos Aires, Argentina” at the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. (Photographed by Luciano N. Segura)



RODRIGO CAJADE is a Wildlife Biologist interested in the study of natural history of amphibians and reptiles. In 2006, he obtained a BS in biology from the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (UNLP), Buenos Aires, Argentina. In 2007 he obtained a Phd grant from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina, CONICET. Currently, he is working on his PhD project in the UNLP. His more recent participation in a scientific research is collaboration in the amazing discovering of the underwater acoustic communication in the anuran larvae of *Ceratophrys ornata*, directed by Dr. Guillermo S. Natale and in collaboration with other Argentinean colleagues. (Photographed by Rodrigo Cajade)