

Influence of emergence success on the annual reproductive output of leatherback turtles

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Abstract Reproductive output of leatherback turtles (*Dermochelys coriacea*) is affected by the stochastic nature of emergence success. Average emergence success of nests at Playa Grande, Costa Rica was 0.38 ± 0.27 . Incubation temperature affected development of leatherback turtle eggs and emergence of hatchlings from the nest. We found that high temperatures reduced hatching success and emergence rate and increased embryonic mortality both early and late during incubation at Playa Grande. There was a temporal effect on emergence success that resulted in more hatchlings being produced at the beginning of the season, because of higher emergence success, than toward the end. Likewise, production of hatchlings varied from year to year. The average annual reproductive output was 252 ± 141 hatchlings per female. The 2005–2006 nesting season had the highest emergence success and produced the greatest number of hatchlings per female compared to the

2004–2005 (+120%) and 2006–2007 (+41%) seasons. However, average clutch size (62 ± 10) and clutch frequency (9.45 ± 1.63), were not different among years. Turtles that had nested a high number of years exhibited greater clutch frequency and arrived earlier to nest than turtles that had nested in fewer numbers of years. Nesting when environmental conditions favor high developmental success and emergence rate may constitute an advantageous reproductive strategy.

Introduction

Environmental stochasticity influences reproductive strategies of animal populations (Benton et al. 1995). All sea turtle species are iteroparous, in that individuals reproduce repeatedly during their lives (Cole 1954). Iteroparous reproduction has been explained as an adaptive strategy in response to uncertainty in offspring survival (Murphy 1968). Likewise, low offspring survival requires high investment in reproduction (Stearns 1989). Leatherback turtles (*Dermochelys coriacea*) exhibit the greatest absolute reproductive investment among reptiles (number and mass of eggs and clutches; Wallace et al. 2007). Females lay on average 60–85 eggs per clutch and 5–7 clutches during a nesting season (Van Buskirk and Crowder 1994; Reina et al. 2002). In addition, they reproduce during multiple years with a remigration interval of 2–3 years in Atlantic populations (TEWG 2007) and 3.7 years in Eastern Pacific populations (Santidrián Tomillo et al. 2007). Mortality of eggs and hatchlings is high in sea turtles. Loss of clutches due to tidal inundation, embryonic mortality during development and unsuccessful emergence reduce overall offspring survival and ultimately influence the reproductive output of individual female turtles (Miller 1997). We

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considered the annual reproductive output of female leatherback turtles as the total number of hatchlings that a female produces in a year and that emerge from the nest successfully. Thus, the reproductive output is affected by the number of eggs laid, the number of clutches and the emergence success of the clutches (number of hatchlings emerged related to the number of eggs laid).

Approximately 80% of sea turtle eggs laid in a clutch complete development and hatch (Miller 1997). Additionally, a proportion of the hatchlings die in the nest during emergence (Fowler 1979). Abiotic factors during incubation, such as temperature and humidity affect developmental success in sea turtles (Davenport 1997). High temperature in sea turtle nests increases proportion of female hatchlings (Yntema and Mrosovsky 1979; Morreale et al. 1982) and embryo abnormalities (Billett et al. 1992) and reduces incubation period (Miller 1997), size of hatchlings (Reece et al. 2002), activity of hatchlings due to thermal inhibition (Drake and Spotila 2002) and proportion of hatchlings emerged (Matsuzawa et al. 2002). Extreme levels of humidity (high and low) also reduce hatching success (McGehee 1990) and high humidity increases hatchling size in loggerhead turtles (*Caretta caretta*) (Reece et al. 2002). On the other hand, other studies have found no apparent effect of temperature or humidity on emergence success. For example, Hewavishenthi and Parmenter (2000) found that hatching success in flatback turtles (*Natator depressus*) was not influenced by hydric environment and Matsuzawa et al. (2002) found no relationship between temperature and hatching success in loggerhead turtles. In addition, other factors such as beach location (Caut et al. 2006), clutch size (Hewavishenthi and Parmenter 2002), sand compaction (Peters et al. 1994), tidal inundation (Eckert 1987) and fungal infection (Phillott and Parmenter 2001) influence hatching and emergence successes.

Although overall hatching success of leatherback turtle clutches is low (~50%; Bell et al. 2003), it is also highly variable (Wallace et al. 2007). Several studies have examined the low levels of hatching success in leatherback clutches at Playa Grande, Parque Nacional Marino Las Baulas (PNMB), in Costa Rica. The leatherback nesting population at PNMB has been the subject of a long-term study since the late 1980s (Steyermark et al. 1996, Spotila et al. 2000; Reina et al. 2002; Santidrián Tomillo et al. 2007). Bell et al. (2003) showed that high embryonic mortality and not low fertility rates (~90–95% of eggs were fertilized) caused low hatching success. Wallace et al. (2004) found no relationship between hatching success and temperature or oxygen concentrations in hatchery nests at PNMB. However, Bilinski et al. (2001) found hatching success of eggs incubated in artificial conditions to be related to temperature. Despite the long-term research on nests at PNMB, factors affecting emergence success of in situ leather-

back clutches remain unknown. Most of the studies mentioned above at PNMB were always conducted on clutches relocated to artificial incubation conditions in the hatchery or to incubators. Additionally, these studies focused only on clutches laid during the first 2 months of the season; therefore, information on the last part of the season was lacking. Thus, variability in emergence success in natural in situ conditions throughout the season has yet to be analyzed.

Environmental stochasticity is known to affect recruitment in leatherback turtle populations. Erosion can cause high loss of clutches on some beaches (~45–60%) (Eckert 1987) and tidal inundation prevents development when clutches are consistently washed over by high tides (Leslie et al. 1996). High variability in emergence success due to environmental stochasticity could also affect the overall annual reproductive output of leatherback turtles at PNMB.

The focus of this study was to determine the effects of biotic and abiotic factors on hatchling production through the analysis of emergence success, and specifically, the influence of emergence success to the annual reproductive output of female leatherback turtles both per female and per cohort. There are two processes that affect emergence success (number of emerged hatchlings/number of eggs): incubation of eggs and emergence of hatchlings. To measure success during the period of incubation we looked at the hatching success of clutches (number of hatched eggs/number of eggs laid) and to measure success during emergence we estimated the emergence rate from the nest (number of hatchlings emerged/hatchlings hatched).

First, we studied in situ effects on the success of leatherback turtle clutches by examining the influence of incubation temperature, number of eggs laid in a clutch and physical location on the beach (North to South, distance to vegetation and distance to high tide line) on the emergence success of clutches. Second, we analyzed temporal variability throughout the season and among years in emergence success and its contribution to the annual reproductive output of leatherback turtles at PNMB.

Materials and methods

We conducted the study at Playa Grande (10°20'N, 85°51'W), the main nesting beach at PNMB (length 3.6 km), on the Pacific coast of Costa Rica. We collected data over 3 years (nesting seasons 2004–2005, 2005–2006 and 2006–2007).

We quantified emergence success of clutches (proportion of eggs that result in emerged hatchlings) and also the two processes that determine it (1) hatching success of the clutch (proportion of eggs that develop into hatchlings) and (2) rate of emergence (proportion of hatchlings that emerge

from the nest). We excavated the nest 2 days after the first observance of signs of hatchling emergence, or after 70 days of oviposition, whichever occurred first. Because the average (\pm SD) incubation period at Playa Grande was 59.3 ± 2.5 days, we considered that after 70 days, a nest with no signs of emergence had either 0% emergence success or the emergence event had been missed.

During excavations we counted number of empty shells, number of unhatched eggs and number of dead and live hatchlings. We counted a piece of shell as one when it accounted for at least 50% of the eggshell (Miller 1999). We distinguished four developmental stages in unhatched eggs based on Leslie et al. (1996): (0) there were no signs of development, (1) there were signs of development such as blood vessels or a black spot but the embryo was not visible, (2) embryo was visible, had ocular development but had no pigmentation and (3) embryo had pigmentation. Additionally, we considered an unhatched egg to be of “unknown stage” when we could not determine the developmental stage at which the embryo had died. We combined designated stages 0 and 1 as the early developmental stages and considered stage 3 as the only late developmental stage.

We calculated hatching success by using the formula: $H = S/(S + U)$, where S was number of shells (hatched eggs) and U , number of unhatched eggs. We calculated emergence success by using the formula: $E = (S - (L + D))/(S + U)$, where L was the number of live hatchling left in the nest and D was the number of dead hatchlings found in the nest. Finally, we calculated emergence rate by using the formula: $R = (S - (L + D))/S$.

In situ effects on emergence success

We marked nests while turtles were laying eggs. We measured the distance (± 25 cm) from the nest to the two closest markers on the beach to determine its location. Markers on the beach were located 50 m apart. Additionally, we measured the distance (± 25 cm) from the nest to the vegetation and to the most recent high tide line mark (from the day before or earlier on the same day) to assess the effect of physical location on the beach on emergence success. We counted number of eggs when possible and deposited a thermocouple in the middle of the clutch (~ 70 cm depth, Binckley et al. 1998) to record temperatures during incubation. We used 24 gauge Cu–Cn thermocouples in all marked nests. We recorded temperatures ($\pm 0.1^\circ\text{C}$) using a thermocouple thermometer (BAT-12) by connecting the end of the thermocouple to the thermometer. We took readings in the afternoon every second day during the incubation period, since variation in diurnal temperature of leatherback nests is only 0.3°C at Playa Grande (Binckley et al. 1998). The accuracy of the thermocouple was checked comparing the reading to the actual temperature of water as

measured with a calibrated mercury thermometer. When the reading was not accurate, we opened the head of the thermocouple, fixed connections and checked the end of the thermocouple. We only used thermocouples that read within 0.1°C of the actual temperature. We also monitored sand temperatures at 50 and 75 cm depths throughout the season each year. Binckley et al. (1998) reported that average depth to the middle of the clutch at Playa Grande is ~ 70 cm and found that nest temperatures are intermediate between those of control temperatures at 50 and 75 m depths. We attached thermocouples to two thermoprofiles and placed them (1) at the North end of the beach (outside the beach hatchery) and (2) toward the center of the beach where most turtles nest.

We examined the effect of year and time in the season (date the clutch was laid) on emergence success, hatching success and emergence rate using multivariate analysis of variance (MANOVA). Because there was a significant effect of year on the three variables, we analyzed differences among years with post hoc analysis. We tested the homogeneity of variances using the Levene’s test and subsequently used Tamhane’s test because we could not assume equal variances. We included partial eta squared (η^2) to measure the contribution of each factor to the variation of the variables. Emergence success, hatching success and emergence rate were arcsin transformed before analysis (but not for display in figures). We tested the effect of the overall mean temperature during incubation on emergence success, hatching success and emergence rate across years using multivariate analysis of co-variance (MANCOVA) with mean incubation temperature as a covariate and year as a fixed effect. We excluded year in further analysis because its effect was not significant. We used linear regression to test (1) the effect of temperature during the first and fourth quarter of incubation on the number of dead embryos in the corresponding early and late stages of development; (2) the effect of temperature and number of hatchlings hatched (to account for social facilitation of emergence) on the emergence rate and (3) the effect of physical location on the beach on the emergence success.

Annual reproductive output

Since 1993–1994, we have identified every turtle that nested at PNMB with PIT tags (passive integrated transponder, AVID) as part of the long-term population study. This methodology allowed us to track the fate of clutches laid by individual females within and among years in this study. Additionally, it provided information on how many years each turtle had nested since 1993–1994.

We estimated total number of hatchlings produced per female in each year by multiplying average clutch size (number of eggs) by her estimated clutch frequency (ECF),

which refers to the estimated number of clutches that a female lays between the first and the last time she was seen on the beach (Steyermark et al. 1996) and by the average emergence success of her clutches. We could not mark every single clutch that each female laid on the beach, because sometimes we arrived at the turtle when she had already covered the nest. Consequently, we only included females for which we had at least three nests successfully excavated in order to adequately characterize intra-individual variation across years.

Leatherback turtles often shift between nesting beaches both between and within nesting seasons (Girondot et al. 2007; Georges et al. 2007). Leatherbacks at Playa Grande show a high level of fidelity to beaches within the Park with <1% of the turtles found nesting elsewhere in Costa Rica and occasionally in Mexico. However, there is some level of exchange within the PNMB beaches. Most of the turtles that nest at Las Baulas (~71%) nest exclusively at Playa Grande, a small percentage nest only on nearby Playa Langosta (~10%) and the remaining (~18%) nest at both locations but lay most of their clutches on Playa Grande (Santidrián Tomillo et al. 2007). To account for possible effects of turtles nesting on Playa Langosta on the estimation of reproductive output, we included turtles that were only seen at Playa Grande.

We used MANOVA analysis to assess the effect of year (annual nesting cohorts) and number of seasons nested (age and/or experience) on the average clutch size, ECF, emergence success, annual production of hatchlings and time of arrival (date the first clutch was laid). We further tested for differences among years using post hoc analysis. We used Levene's test to test for homogeneity of variances. We used Tamhane's test when variables had unequal variances and Bonferroni test when variables had equal variances. We included partial eta squared (η^2) to measure the contribution of each factor to the variation of each dependent variable. Finally, we tested the effect of number of seasons nested and time of arrival on the reproductive output of female turtles using an analysis of covariance (ANCOVA). We used number of seasons nested as a fixed effect and time of arrival as the covariate. We measured annual reproductive output per female as the number of hatchlings produced by the female in the year. All statistical tests were carried out using the Statistical Package for the Social Sciences (SPSS, version 15.0, Chicago, USA) (see Table 1 for summary of statistical results).

Results

In situ effects on emergence success

We studied a total of 416 clutches in 2004–2005, 2005–2006 and 2006–2007 (114, 172 and 130, respectively). The

average emergence success (\pm SD) of all clutches included in the study was 0.38 ± 0.27 ($n = 414$), average hatching success was 0.44 ± 0.27 ($n = 416$), and average emergence rate was 0.76 ± 0.25 ($n = 275$) (Table 2).

The MANOVA analysis showed significant differences among years in emergence success ($F_{2,106} = 21.289$, $P < 0.001$, $\eta^2 = 0.287$), hatching success ($F_{2,106} = 20.665$, $P < 0.001$, $\eta^2 = 0.281$) and emergence rate ($F_{2,106} = 6.428$, $P = 0.002$, $\eta^2 = 0.108$). Additionally, time in the season (date laid) had a significant effect on emergence success ($F_{90,106} = 1.731$, $P = 0.03$, $\eta^2 = 0.595$), hatching success ($F_{90,106} = 1.557$, $P = 0.014$, $\eta^2 = 0.569$) and on emergence rate ($F_{90,106} = 1.554$, $P = 0.015$, $\eta^2 = 0.569$). Both number of hatchlings hatched and hatchlings emerged decreased as the nesting season progressed (Table 2). There were no significant interactions between year and time in the season. The post hoc analysis showed higher emergence success, hatching success and emergence rate in 2005–2006 than in 2004–2005 ($P < 0.001$ for all three variables) and 2006–2007 ($P < 0.001$ all three variables), but not differences between seasons 2004–2005 and 2006–2007 ($P = 0.834$, 0.655 and 0.999 , respectively). Clutch size did not affect hatching success ($P > 0.05$).

Effect of temperature on emergence success

The MANCOVA analysis showed a significant effect of mean incubation temperature on emergence success ($F_{1,143} = 46.060$, $P < 0.001$, $\eta^2 = 0.244$), hatching success ($F_{1,143} = 34.915$, $P < 0.001$, $\eta^2 = 0.196$) and emergence rate ($F_{1,143} = 52.611$, $P < 0.001$, $\eta^2 = 0.269$). There were no significant differences among years for any of the variables ($P > 0.05$ for all cases). Likewise, there were no significant interactions between year and mean incubation temperature ($P > 0.05$ all cases). Therefore, we removed year from further analysis. We regressed temperature on both early and late stages of development using quadratic regression. Number of dead embryos in early stages increased with increasing maximum temperature in the first quarter of incubation ($r^2 = 0.088$, $P < 0.001$) and the number of dead embryos in late stages increased as the mean temperature in the last quarter of incubation increased ($r^2 = 0.348$, $P < 0.001$) (Fig. 1). Finally, temperature also affected the process of emergence. Emergence rate decreased with increasing mean temperatures during the last quarter of incubation and with fewer hatchlings involved in the process of emergence ($r^2 = 0.404$, $P < 0.001$).

Physical location on the beach

Emergence success was neither significantly affected by the location on the beach (North to South), nor by the distance to vegetation ($P > 0.05$). However, we found a negative significant relationship between distance to high tide and

Table 1 Summary of statistical analysis (MANOVA, MANCOVA and ANCOVA) conducted on emergence success and reproductive output of leatherback turtles at Playa Grande

Test	Independent variables	Dependent variables	<i>F</i> value	η^2
MANOVA	Year	Emergence success	21.289***	0.287
		Hatching success	20.665***	0.281
		Emergence rate	6.428**	0.108
	Time in the season	Emergence success	1.731**	0.595
		Hatching success	1.557*	0.569
		Emergence rate	1.554*	0.569
	Year \times time in the season	Emergence success	1.137	0.446
		Hatching success	1.142	0.447
		Emergence rate	1.043	0.425
MANCOVA	Year	Emergence success	0.067	0.001
		Hatching success	0.034	0.000
		Emergence rate	1.927	0.027
	Mean incubation temperature (covariate)	Emergence success	46.060***	0.244
		Hatching success	34.915***	0.196
		Emergence rate	52.611***	0.269
	Year \times mean incubation temperature	Emergence success	0.075	0.001
		Hatching success	0.036	0.001
		Emergence rate	1.891	0.026
MANOVA	Year	Emergence success/female	7.622***	0.249
		Clutch size	0.896	0.037
		Estimated clutch frequency	1.612	0.065
		Time of arrival	0.324	0.014
		Production of hatchlings	4.689*	0.169
	Number of years nested	Emergence success/female	0.780	0.064
		Clutch size	2.477	0.177
		Estimated clutch frequency	6.409***	0.358
		Time of arrival	2.740*	0.192
		Production of hatchlings	1.138	0.090
	Year \times number of years nested	Emergence success/female	0.488	0.078
		Clutch size	0.769	0.118
		Estimated clutch frequency	1.354	0.191
		Time of arrival	0.321	0.053
		Production of hatchlings	0.398	0.065
ANCOVA	Number of years nested	Production of hatchlings	1.999	0.105
	Time of arrival (covariate)	Production of hatchlings	7.718**	0.131
	Number of years nested \times time of arrival	Production of hatchlings	1.525	0.107

* $P < 0.05$, ** $P < 0.01$,
*** $P < 0.001$

emergence success ($r^2 = 0.031$, $P < 0.001$). Therefore, clutches that were closer to the high tide line had greater success than those that were further away.

Annual reproductive output

The total number of turtles that nested in 2004–2005, 2005–2006 and 2006–2007 was 54, 124 and 76 turtles, respectively. We calculated production of hatchlings for 19 females (79 clutches) in 2004–2005, 22 (86 clutches) in 2005–2006 and 20 (96 clutches) in 2006–2007. The average annual reproductive output per female was 252 ± 141

hatchlings. Clutch size (average \pm SD) was 62 ± 10 eggs and ECF was 9.45 ± 1.63 clutches. Turtles that nested in 2005–2006 produced the greatest number of hatchlings per female compared to the 2004–2005 (+120%) and 2006–2007 (+41%) (Table 3).

The MANOVA analysis showed significant differences among nesting cohorts in average emergence success per turtle ($F_{2,43} = 7.622$, $P = 0.001$, $\eta^2 = 0.249$) and production of hatchlings ($F_{2,43} = 4.689$, $P = 0.014$, $\eta^2 = 0.169$) but no significant differences in average clutch size ($P > 0.05$), ECF ($P > 0.05$) and time of arrival ($P > 0.05$). Additionally, there was a significant effect of number of seasons nested

Table 2 Average number of hatchlings emerged per nest, emergence success, hatching success and emergence rate of leatherback turtles nests at Playa Grande per month clutches were laid and the average for the year

	Number of nests			Number of hatchlings emerged per nest			Emergence success			Hatching success			Emergence rate		
	2004–2005	2005–2006	2006–2007	2004–2005	2005–2006	2006–2007	2004–2005	2005–2006	2006–2007	2004–2005	2005–2006	2006–2007	2004–2005	2005–2006	2006–2007
October	6	13	4	29 ± 12	34 ± 20	25 ± 13	0.44 ± 0.15	0.57 ± 0.22	0.39 ± 0.23	0.47 ± 0.15	0.59 ± 0.21	0.41 ± 0.23	0.85 ± 0.17	0.93 ± 0.05	0.95 ± 0.03
November	35	53	35	23 ± 17	34 ± 17	29 ± 16	0.38 ± 0.26	0.61 ± 0.22	0.49 ± 0.23	0.43 ± 0.26	0.65 ± 0.23	0.53 ± 0.23	0.78 ± 0.19	0.89 ± 0.14	0.90 ± 0.08
December	45	69	51	17 ± 14	28 ± 16	16 ± 12	0.24 ± 0.22	0.53 ± 0.26	0.28 ± 0.22	0.31 ± 0.23	0.57 ± 0.26	0.36 ± 0.22	0.72 ± 0.26	0.86 ± 0.17	0.65 ± 0.26
January	28	37	40	9 ± 11	17 ± 13	10 ± 8	0.12 ± 0.17	0.32 ± 0.22	0.20 ± 0.19	0.18 ± 0.18	0.38 ± 0.24	0.28 ± 0.20	0.57 ± 0.27	0.75 ± 0.25	0.54 ± 0.30
Year	114	172	130	18 ± 15	28 ± 17	18 ± 15	0.27 ± 0.25	0.51 ± 0.27	0.31 ± 0.24	0.32 ± 0.25	0.56 ± 0.26	0.38 ± 0.24	0.71 ± 0.25	0.84 ± 0.19	0.70 ± 0.28

Emergence success, hatching success and emergence rate were quantified in seasons 2004–2005, 2005–2006 and 2006–2007

on ECF ($F_{4,45} = 6.409$, $P < 0.001$, $\eta^2 = 0.358$) and time of arrival ($F_{4,45} = 2.740$, $P = 0.040$, $\eta^2 = 0.192$) but not on the other dependent variables ($P > 0.05$ all cases). Therefore, turtles that nested in more years laid more clutches and arrived earlier to nest in a given year than turtles that had nested in fewer numbers of years. There were no significant interactions between year and number of years nested ($P > 0.05$ all cases). The post hoc analyses showed higher emergence success per female in 2005–2006 than in 2004–2005 ($P < 0.001$) and 2006–2007 ($P = 0.005$), but no significant differences between 2004–2005 and 2006–2007 ($P = 0.371$). Likewise, annual production of hatchlings was higher in 2005–2006 than in 2004–2005 ($P < 0.001$) but not significantly different between 2004–2005 and 2006–2007 ($P = 0.201$) and between 2005–2006 and 2006–2007 ($P = 0.088$).

Finally, there was a significant effect of time within the year on hatchling production. The ANCOVA analysis showed a significant effect of time of arrival on the annual reproductive output of leatherback turtles ($F_{1,51} = 7.718$, $P = 0.008$, $\eta^2 = 0.131$) with no significant interactions between number of years nested and time of arrival. Consequently, turtles that arrived early to nest produced more hatchlings than those that arrived later in the nesting season (Fig. 2).

Discussion

The annual reproductive output of leatherback turtles is affected by the emergence success of nests. The temporal effects on emergence success result in more hatchlings produced at the beginning of the season than at the end. The stochastic nature of emergence success can have implications on the reproductive strategies exhibited by leatherback turtles.

In situ effects on emergence success

Emergence success decreased as the nesting season progressed, resulting in more hatchlings being produced at the beginning of the season than at the end. Hatching success and emergence success were lower than previously estimated. Prior studies at PNMB and at other leatherback nesting beaches estimated an average hatching success of 0.50 (Bell et al. 2003) and average emergence success of 0.41 (Wallace et al. 2007), but we found an overall hatching success of 0.44 ± 0.27 and emergence success of 0.38 ± 0.27 for the 3 years. Because past studies only included clutches laid at the beginning of the season, excluding the months of lowest success, these were overestimated. Likewise, because of the different success of clutches in different years, it is necessary to include multi-

Fig. 1 Effect of temperature during the incubation period on emergence success, number of early stage mortalities and number of late stage mortalities in leatherback turtle clutches at Playa Grande, Costa Rica in seasons 2004–2005, 2005–2006 and 2006–2007. **a–c** Emergence success decreases as the mean temperature during incubation increases. **d–f** Number of dead embryos in early stages increases with the maximum temperature during the first quarter of the incubation period. **g–i** Number of dead embryos in late stages increases with average temperature during the last quarter of the incubation period

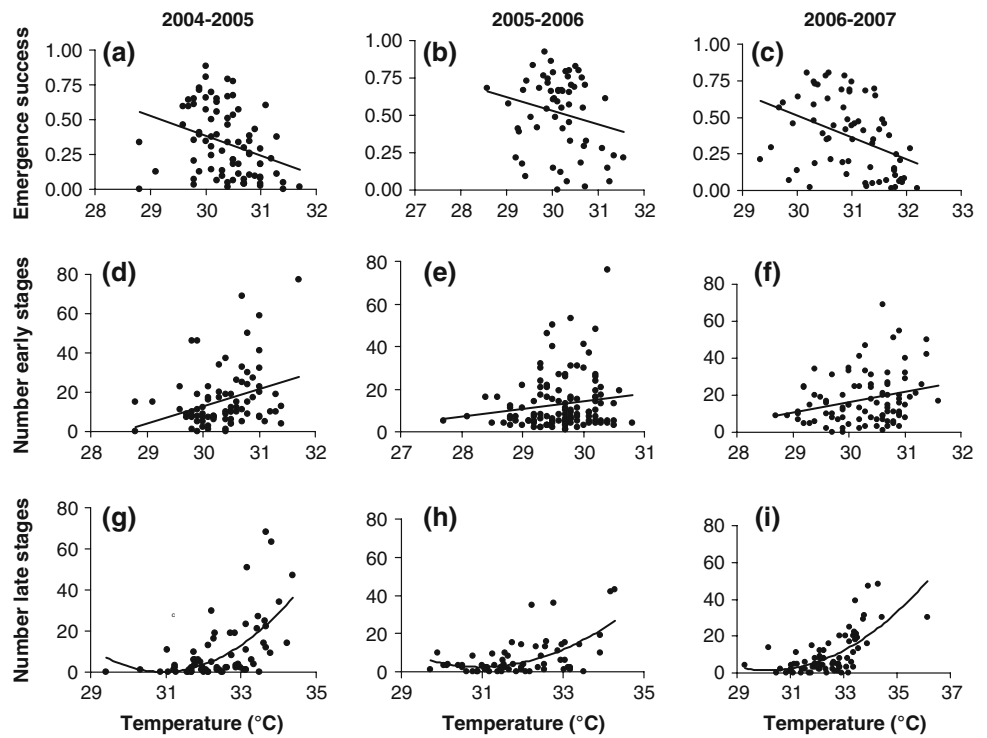


Table 3 Average clutch size, estimated clutch frequency (ECF), emergence success and annual reproductive output (total number of hatchlings produced in the year and that emerged from the nest) of

female leatherback turtles at Playa Grande in seasons 2004–2005, 2005–2006 and 2006–2007

Season	Number of females that nested	Number of females included in the analysis	Clutch size	ECF	Average arrival time	Emergence success per female	Annual reproductive output
2004–2005	54	19	61 ± 14	9.21 ± 1.51	8 Nov	0.24 ± 0.18	135 ± 108
2005–2006	124	22	63 ± 16	9.01 ± 1.78	2 Nov	0.53 ± 0.18	297 ± 129
2006–2007	76	20	65 ± 15	9.93 ± 1.54	4 Nov	0.33 ± 0.19	210 ± 120

ple years for the estimations to accurately characterize interannual variability in clutch success.

High temperatures during incubation had a negative effect on emergence success, reducing production of hatchlings. Temperature during incubation is known to affect survival of eggs in different reptile species. For instance, high temperatures reduce hatching success in snakes (Lin et al. 2005), lizards (Ji et al. 2002), freshwater turtles (Packard et al. 1987) and desert tortoises (Spotila et al. 1994). Likewise, we found a thermal effect on development of leatherback turtle eggs at PNMB with high temperatures resulting in lower emergence success. Additionally, eggs and hatchlings were sensitive to high temperatures at all stages during the incubation period and emergence. Number of dead embryos in early stages was related to temperature during the first part of incubation, number of dead embryos in late stages was affected by temperature in the last quarter and temperature also affected hatchlings during emergence.

Hatchling sea turtles generally emerge synchronously from the nest (Bustard 1967), which facilitates emergence by cooperation among hatchlings and reduces predation once hatchlings are on the beach (Carr and Hirth 1961; Bustard 1967). Proportion of hatchlings that successfully emerged at PNMB increased with number of hatchlings involved in the process of emergence, suggesting social facilitation during emergence, which increased survival. Additionally, high temperature also played an important role, decreasing the proportion of hatchlings that made it to the surface. Tolerable temperatures for development range between 25–27 and 33–35°C in sea turtle eggs (Ackerman 1997). Hatchling leatherback turtles start exhibiting uncoordinated movements at 33.6°C and have critical thermal maxima (CTM) of 40.2°C (Drake and Spotila 2002). Although temperatures above the CTM are uncommon in the nest, temperatures higher than 33.6°C are frequent at the end of the incubation period. Locomotion of hatchlings in the nest might be limited by temperature (Drake and

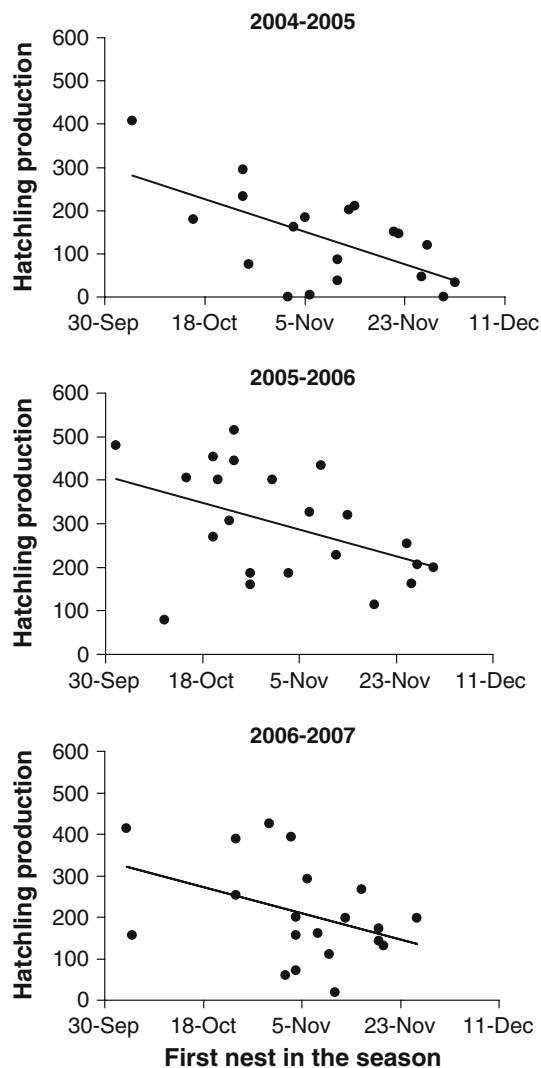


Fig. 2 Temporal effect on the annual reproductive output of female leatherback turtles at Playa Grande, Costa Rica in 2004–2005, 2005–2006 and 2006–2007. Annual reproductive output is the total production of hatchlings per female in the year

Spotila 2002), which in turn increases their mortality before emergence is complete.

There were large differences in emergence success among years. Weather differences among years could explain this difference. Temperatures were lowest in 2005–2006 which resulted in an overall higher emergence success that year. Additionally, levels of precipitation affect sand temperatures. Houghton et al. (2007) reported a cooling effect of rain on leatherback turtle nests in Grenada, West Indies. Likewise, Matsuzawa et al. (2002) reported increased temperatures after the rainy season ended on a loggerhead turtle nesting beach in Japan. Levels of precipitation in Northwest Costa Rica are high at the beginning of the nesting season (rainiest months are September and October) and low or inexistent at the end of the nesting season (December through March). Intra and inter-annual variability in precipitation levels affect

temperatures on the beach, which in turn can affect emergence success as reported on other sites (Houghton et al. 2007; Matsuzawa et al. 2002).

Emergence success decreased as the nesting season progressed at Playa Grande. Ambient and sand temperatures in this area increased from the beginning to the end of the nesting season (Fig. 3). Thus, decreased success of clutches toward the end of the season could be explained by increased temperatures given the effect on hatching success and emergence rate. Bell et al. (2003) suggested that while fertility of leatherback eggs was usually high (>90%), it might be reduced in turtles that arrive later in the nesting season. The effect of temperature that we found on hatching success suggests that the increase in temperature through the season explains the temporal effect on hatchling production because embryos died during development. However, a combination of both, high temperature and lower fertility at the end of the season could result in the low success of the late clutches.

Sex ratios are female-biased at Playa Grande and vary intra and inter-annually (Binckley et al. 1998). Proportion of female hatchlings increases throughout the season

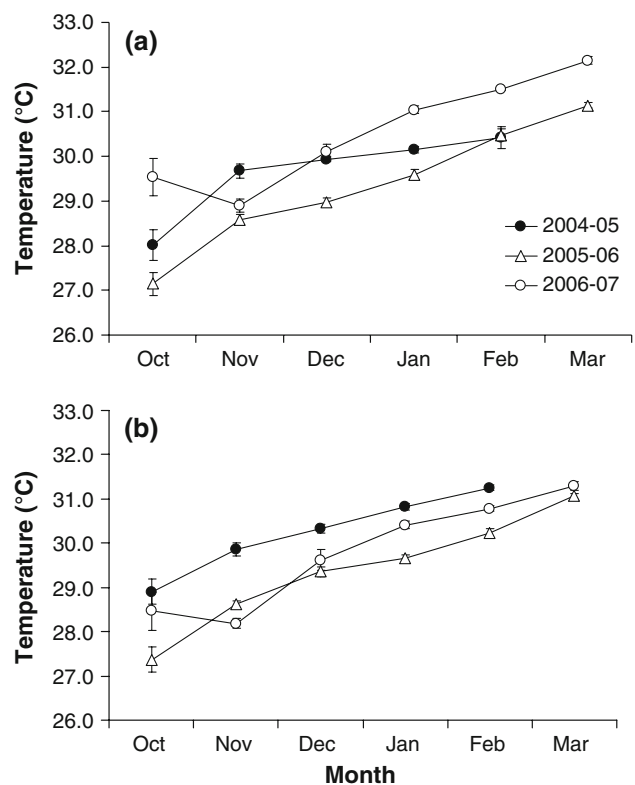


Fig. 3 Changes in mean (\pm SE) sand temperature (50 and 75 depths) throughout the season at Playa Grande, Costa Rica in 2004–2005, 2005–2006 and 2006–2007. **a** Thermoprobe was located at the North end of the beach (outside the beach hatchery). **b** Thermoprobe was located toward the center of the beach in 2004–2005, 2005–2006 and 2006–2007

because of increasing temperature and vary among years. Binckley et al. (1998) reported female proportions as 100, 93.5 and 74.3% in 3 years of study. Because temperature varies intra and inter-annually, time of arrival and year of nesting affect sex ratios as well as offspring survival. Therefore, both variables should be included in future studies on population dynamics.

Despite the significant effect of temperature on emergence success, the low coefficients of these relationships suggest that factors other than temperature, not included in the present analyses, also affect the success of clutches. For instance, humidity, fungal infection during incubation and sand characteristics have been related to developmental success of sea turtle eggs (Mortimer 1990; Phillott and Parmenter 2001; López-Castro et al. 2004) and should be considered in future studies at PNMB. Temperature as well as precipitation may affect sand properties and gas exchange (Kraemer and Bell 1980). In addition to characteristics of the incubation environment, biotic factors related to differences among mothers might also play a role in differences in emergence success and thus deserve study. Additionally, errors in the methodology can have lowered the effect of temperature that we found in the analysis. For example, the thermocouple could have been misplaced (not touching eggs or far from the center of the clutch) as it happens sometimes when the turtle is covering the nest. Likewise, there are differences in temperature within the clutch (top, center, and bottom) that were not measured in this study (Binckley et al. 1998).

Finally, we found that emergence success of leatherbacks at PNMB was not related to clutch size and was not affected by location on the beach (North to South). Hewavishanthi and Parmenter (2002) found that hatching success of flatback turtles was not affected by position on the beach but was correlated to clutch size. Playa Grande is a high energy beach and, therefore, beach characteristics vary from year to year. Consequently, the environmental conditions around the nest may also vary temporally and spatially along the beach, affecting emergence success. Distance to vegetation did not affect emergence success at Playa Grande, but distance to high tide had a significant effect on emergence success. Clutches that were close to the high tide line had a greater success than those that were further away. The very low r^2 value found in the analysis suggests a weak effect. However, more specific analysis considering that the distance to the high tide line is variable throughout the season may show a greater effect of tides that was not obvious with a single measurement.

Annual reproductive output

Environmental variability in emergence success influences the annual reproductive output of leatherback turtles at

PNMB. Investment in reproduction was similar among years, but emergence success and number of offspring produced varied. Turtles that had previously nested a high number of years (up to five, maximum recorded) laid more clutches and arrived earlier to nest than turtles that had nested fewer years.

A combination of age and experience appears to result in the higher reproductive investment and the early arrival of turtles that had nested multiple years. Even if we cannot determine the age of turtles, females that have nested 5 years are probably older than those that have nested only 1 year, considering that the average remigration interval between years is 3.7 years (Santidrián Tomillo et al. 2007). However, there is some uncertainty in knowing the life history traits of individual females since individual females differ in level and variability of reproductive output (Wallace et al. 2007). This variability may drive weak relationships as suggested by the low coefficients of the analysis. The average ECF of the turtles included in the study (9.45 ± 1.63) was higher than previously estimated (~ 7 , Reina et al. 2002). Because the turtles included in the study had only nested at Playa Grande, we selected turtles with high site fidelity and therefore, the ECF was higher than the average for the total nesting population.

Each turtle at Las Baulas nests on average seven times in a year, every 9–10 days (Reina et al. 2002). Therefore, the total nesting period of each turtle is about 2 months. Early turtles nest in October–November and their production of hatchlings is greater than that of late turtles that nest in January–February when production is the lowest. Early breeding has been associated with higher reproductive success in bird species (Price et al. 1988). For example, older male American redstarts (*Setophaga ruticilla*) arrive earlier to breeding grounds than younger ones and their reproductive success is both related to arrival time and age (Lozano et al. 1996). In American redstarts the quality of wintering habitat influences arrival time and reproductive success (Norris et al. 2004). Likewise, female leatherback turtles that arrive early in the season produce more hatchlings and therefore, have higher annual reproductive output (Fig. 2).

Cost-benefits associated with early arrival have been hypothesized to drive arrival time in bird species (Møller 1994). For instance, in barn swallows (*Hirundo rustica*) the benefit obtained from early arrival was a higher reproductive output and the cost to pay was the variable weather conditions at the breeding grounds and at grounds before migration (Møller 1994). Similarly, benefits of early arrival of leatherbacks at PNMB are increased hatchling production and costs of early arrival could be related to variable and/or extreme weather conditions. The nesting season at PNMB extends from early October to early March and the rainy season in this area extends from May to November. The rainiest months are September and October. Bad

weather during the rainy season causes higher tides and greater erosion. Additionally, excessive rainfall could result in the suffocation of the eggs as shown by Kraemer and Bell (1980) in loggerhead turtles. As a result, some clutches need to be relocated to safer areas on the beach, above the high tide line, where the eggs can develop. Relocation of eggs occurs more often at the beginning of the season when tides are the highest than later on in the season. Therefore, clutches that are laid at the beginning of the nesting season have a greater probability of being washed away than those laid later on when the weather conditions are more stable. Because sea turtles lay several clutches in a season, if early clutches are washed away, females can still produce offspring in a year because later nests have a greater probability of surviving. If early clutches develop successfully, females will benefit from higher hatchling production. Finally, time of arrival of females of some bird species (Cristol 1995) and salamanders (Spotila and Beumer 1970) occur after the arrival of the males. Time of arrival of female leatherback turtles related to the arrival of males may also be of importance, since appropriate arrival date could increase mating probability, quality of offspring and overall reproductive output.

In conclusion, annual reproductive output of female turtles is affected by the variable nature of emergence success which ultimately affects population dynamics. We hypothesize that natural selection will favor turtles that nest in years of high production as well as those that, within a year, arrive to nest when conditions for development and emergence are optimal. Turtles that nested in 2005–2006 produced 120 and 41% more hatchlings than turtles that nested in 2004–2005 and 2006–2007, respectively. Therefore, turtles that nest in optimal years have a greater annual reproductive output than turtles that nest in other years, despite exhibiting similar reproductive investments. Additionally, more turtles nested at PNMB in 2005–2006 (124) than in 2004–2005 (54) and 2006–2007 (76). Number of nesting turtles per year fluctuates at PNMB and the probability of turtles remigrating to nest is related to oceanographic conditions in the Pacific Ocean (Saba et al. 2007). The relationship between years in which individual turtles nest and their overall hatchling production still needs to be explored.

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