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on hatching success of the leatherback turtle  
*Dermochelys coriacea*: implications for conservation



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## Abstract

The leatherback turtle, *Dermochelys coriacea*, is Critically Endangered according to UICN, and has a higher embryonic mortality than other sea turtles. A better understanding of patterns and causes of embryonic mortality is therefore necessary for developing effective conservation strategies. I examined the influence of distance to high tide line, mean incubation temperature, section- and zone of the beach on hatching success in Tortuguero, Costa Rica to evaluate the desirability of nest relocation.

Hatching success was positively correlated with distance to the high tide line; nests below the high tide line have significantly lower hatching success. Mean incubation temperature is positively correlated with hatching success due to low survival at low minimum temperatures. Estimates of effects of distance to high tide line and mean temperature from a known fate model in MARK were reversed, probably because washed out nests do not meet data requirements and are excluded. The known fate model also shows that developmental stage significantly affects risk of embryonic mortality.

The strong effect of distance to high tide line indicates that relocation of nests below the high tide line at Tortuguero may improve hatchling recruitment. The inconsistency with the known fate model suggests that there might be conflicting weaker selective forces that favour a more seaward nest placement. The negative effect of low temperature could possibly be coupled with weather conditions; storms and heavy rains accelerate the erosion process and increase risk of wash out or washover. The high embryonic mortality during the first developmental stage could partly be due to infertility, and an effect of badly placed nests dying during the first days of incubation.

**Keywords:** *Dermochelys coriacea*; Tortuguero; Costa Rica; hatching success; spatial distribution; sand temperature.

## Resumen

La Tortuga Baula, *Dermochelys coriacea*, se encuentra en peligro crítico de extinción según el IUCN; y tiene una mortalidad embrional más alta que la de otras tortugas marinas. Es por ello que es necesario entender los patrones y causas de mortalidad embrional de esta especie para el mejoramiento de estrategias de conservación. En este trabajo se investigó la influencia de la distancia a la línea pleamar, temperatura media durante la incubación, sección- y zona de la playa en el éxito de eclosión en Tortuguero, Costa Rica para así evaluar si es factible la reubicación de nidos.

El éxito de eclosión estuvo positivamente correlacionado a la distancia hasta la línea pleamar, y los huevos bajo de la marea alta tuvieron significativamente menor probabilidad de desarrollo. La temperatura media de incubación estuvo positivamente correlacionada al éxito de eclosión, debido a que temperaturas muy bajas conllevan un bajo nivel de supervivencia. A pesar de este hecho, las correlaciones fueron contrarias en el resultado de un modelo Known Fate en el programa MARK y esto probablemente se debe a que los nidos arrasados fueron excluidos. El modelo Known Fate también demuestra que los estados de desarrollo del embrión afectan considerablemente al riesgo de su mortalidad.

El gran efecto de la distancia al pleamar indica que la reubicación de nidos ubicados bajo la línea pleamar en Tortuguero pueda aumentar la natalidad de tortugas baulas. La contradicción del modelo Known Fate indica que podría haber fuerzas opuestas selectivas, aunque menos fuertes, que favorezcan a la anidación más cercana al mar. El efecto negativo de temperaturas bajas podría estar relacionado con el estado del tiempo, ya que las tormentas y lluvias fuertes aceleran el proceso de erosión y aumentan el riesgo de que los huevos sean arrasados. La alta mortalidad embrional durante el primer estado de su desarrollo posiblemente se debe parcialmente a la infertilidad y por otra parte es el resultado de que nidos mal ubicados mueren durante los primeros días de incubación.

**Palabras claves:** *Dermochelys coriacea*; Tortuguero; Costa Rica; éxito de eclosión; distribución espacial; temperatura de la arena.

## Introduction

Sea turtles have provided sustenance to coastal villages for thousands of years. Due to overexploitation, fisheries by-catch and habitat destruction there has been a drastic decline in sea turtle populations during the last century (Troëng and Drews 2004). The leatherback turtle, *Dermochelys coriacea*, is no exception (Spotila *et al.* 1996; Spotila, 2000). The leatherback turtle is present in 64 countries and 80% of those have developing economies (Troëng and Drews, 2004). Benefits of non-consumptive use of sea turtles greatly outweigh that of consumptive use (Troëng and Drews 2004). In order for sea turtles to provide economic and intrinsic benefits to communities, the populations must be restored to healthy levels worldwide.

### *Current status of the leatherback turtle*

Leatherback turtles are Critically Endangered (IUCN 2006). There has been a drastic population decline in the Pacific leatherback rookeries (Sarti *et al.* 1996; Spotila *et al.* 1996; Eckert, 1997; Spotila 2000). The estimated female population size has decreased from 115 000 in 1980 to between 26 200 and 42 900 in 1995, which is less than a third of the 1980 estimate (Spotila *et al.* 1996; Spotila 2000). This raises concerns that Pacific leatherback turtles are going extinct (Spotila 2000). In the face of an increased adult mortality, stable leatherback populations would decrease even if mortality during other life stages is static (Spotila *et al.* 1996). The drastic decline in the Pacific rookeries emphasizes the importance of conservation efforts to maintain a viable population in the Atlantic basin in order to secure the future existence of the species and its unique genetic material.

The primary threats to the survival of the global leatherback turtle population are anthropogenic (IUCN 2006). Egg harvesting on nesting beaches is one of the most serious threats to leatherback sea turtles worldwide (Eckert and Eckert 1990), as for the population nesting on the Caribbean coast of Costa Rica (Troëng 2002). Killing of adult turtles is another main threat to the leatherback turtles nesting on the Caribbean coast of Costa Rica (Troëng 2002). Threats to leatherbacks in the pelagic zones are widely recognized (Spotila *et al.* 2000; Hays *et al.* 2003; Lewison *et al.* 2004). Accidental mortality due to fisheries by-catch is a pressing concern with the pelagic long-line fishery proposed as key threat (Eckert 1997; Spotila *et al.* 2000; Lewison *et al.* 2004), but the risk of entanglement in fixed fishing gear in shelf waters may be greater than previously recognized (James *et al.* 2005). Habitat destruction through sea level rise (Fish *et al.* 2005), contamination, artificial illumination on nesting beaches, ingestion of plastic articles (Troëng 2002) and accumulation of debris that prevents the turtles from ascending the nesting beaches (Chacón 1999) are other threats to leatherback turtles. Global warming could potentially generate higher sand- and nest temperatures, which would result in a mass feminization of hatchlings since sex determination is temperature dependent (Kamel and Mrosovsky 2004).

### *Nesting ecology*

Leatherback turtles reach sexual maturity at the age of 5 to 13 years (Zug and Parham 1996 in Dutton *et al.* 2005). After the first reproductive event, Atlantic leatherback turtles remigrate to nest every 2 to 3 years (Boulon *et al.* 1996). Leatherback nesting season on the Caribbean coast is between March and July, but nesting has been observed from January through August (Chacón 1999). Clutch frequency per reproductive event is estimated to between 3 and 7 by

different authors (Spotila *et al.* 1996), and the internesting period is approximately 9 days (Hilterman and Govere 2004).

Nest site selection in sea turtles consists of beach selection, selection of point of emergence and nest placement (Wood and Bjorndal 2000). Nesting beaches are found through natal homing, but several proximal leatherback nesting populations are genetically indistinguishable (Dutton *et al.*, 1999). This suggests that the natal homing behaviour in leatherbacks could be less precise than that of other marine turtle species, and allow more flexibility to exploit new reproductive habitat and re-colonize beaches where populations have declined (Dutton *et al.* 1999). Since leatherback turtles prefer nesting beaches that are unprotected by offshore reefs and obstructions (Eckert 1987), accessibility is thought to be an important criterion for the selection of point of emergence. Pattern of nest placement has been extensively studied. It has been explained by proximity to the first nest placement (Nordmoe *et al.* 2004), by an erratic and random pattern not clustered around an initial choice (Eckert 1987) and by a significant repeatability of nest site choice relative to the high tide line with a significant difference in distance to high tide line between individual females (Kamel and Mrosovsky 2004). Leatherback turtles strongly prefer to nest in the open sand (Whitmore and Dutton 1985; Nordmoe *et al.* 2005), and thus use vegetation as a cue indicating that the area is not suitable for nesting. Slope is also thought to be a cue used in nest site selection (Wood and Bjorndal 2000; Nordmoe *et al.* 2005).

Moisture and sand grain size have to be adequate for egg chamber construction to be successful. In coarse, dry sand green sea turtle females have difficulty digging egg chambers, and typically make multiple trial nest holes and re-emerge on successive nights before depositing eggs (Mortimer 1990). The mean bottom depth of the egg chamber is approximately 76.0 cm at Tortuguero (De Haro *et al.* 2006). Following a successful construction of an egg chamber about 82 yolked eggs are laid at Tortuguero (De Haro *et al.* 2006). After oviposition the egg chamber is covered and the nest site disguised.

Nest site selection is influenced by both the selective forces that favour nest placement inland and the conflicting selective forces that favour nest placement seaward (Bjorndal and Bolten 1992; Godfrey and Barreto 1994; Kamel and Mrosovsky 2004). Nests too close to the sea are more likely to get washed out or inundated, whereas a nest placement further inland increases the likelihood of desiccation, hatchling disorientation, and predation on nesting females, eggs and hatchlings (Wood and Bjorndal 2000; Kamel and Mrosovsky 2004).

Hatching success is affected by a number of ecological factors such as temperature (Yntema and Mrosovsky 1980; Ackerman 1997), moisture (Bustard and Greenham 1968; Ackerman 1980; Miller 1985; Mortimer 1990), grain size of the sand (Ackerman 1980; Mortimer 1990; Speakman *et al.* 1998), possibly gas exchange (Ackerman 1980; Wallace *et al.*, 2004, Ralph *et al.* 2005), and chloride toxicity (Bustard and Greenham 1968; Whitmore and Dutton 1985).

Temperature affects hatching success and sex determination in sea turtles. The thermal tolerance range for leatherback embryos is approximately 25-27°C to 33-35 °C (Ackerman 1997). Duration of incubation is negatively correlated with incubation temperature (Mrosovsky and Yntema 1980). Leatherback turtles have a temperature dependent sex determination (TDS) system, where high temperatures produce females and low temperatures produce males (Ackerman 1997; Chevalier *et al.* 1999). The pivotal temperature is approximately 29.5°C but varies from region to region (Chevalier *et al.* 1999). The thermo

sensitive period, when sex is determined, occurs during the middle third of incubation (Desvages *et al.* 1993).

Location of oviposition influences both the hatching success and sex determination of the hatchlings (Bjorndal and Bolten 1992), and protection of eggs during incubation and of the hatchlings during their first day of life can have a significant effect on leatherback population stability in the face of an increase in adult mortality (Spotila *et al.* 1996). Intensive beach protection and nest relocation can contribute to obtaining a positive population trend and have been described as “a necessary component of any strategy to recover depleted leatherback populations in the long run” (Dutton *et al.* 2005).

Leatherback clutches have lower hatching success than other sea turtles, which is partly due to a higher embryonic mortality resulting from washover events (Whitmore and Dutton 1985). The higher frequency of washover is a result of the more seaward nest placement (Whitmore and Dutton 1985). The specific causes of the high embryonic mortality of the leatherback turtle in nests that have not been washed over remain unknown.

Understanding the causes of low hatching success would be an important step towards better conservation strategies. Differences in characteristics such as erosion regime, tidal range, sand grain size distribution and temperature between different leatherback nesting beaches influence the effect that different factors have on hatching success. This makes an assessment of how ecological factors affect hatching success in Tortuguero a necessary component when developing a long term conservation strategy for the leatherback turtles nesting on the beach. One of the recommendations of the report from the 2005 leatherback program in Tortuguero is to review the leatherback hatching success at Tortuguero to and evaluate which factors that may be used to predict hatching success (De Haro *et al.* 2006).

The main aim of this study is to evaluate how distance to high tide line, section of the beach and mean temperature during incubation period affect hatching success of leatherback turtles, and determine whether there is a pattern in hatchling survival that motivates nest relocation from any particular zone. I expect hatching success to be positively correlated to distance from the high tide line, and to differ between different sections of the beach. I expect extremely low and high mean temperatures to affect hatching success negatively. A specific objective is to evaluate the usefulness of using the program MARK for modelling hatching success in sea turtles.

## Materials and methods

### *Study area*

Tortuguero is a 35.6 km long beach located on the Caribbean Coast of Costa Rica, between Tortuguero river (N 10°35.80 W083°31.47) and Parismina (N10°19.04 W083°21.39), Figure 1. In 1994 the beach opened up at Jalova lagoon (N10°21.46 W083°23.4) and the southern 6 km are now separated from the main nesting beach (Troëng and Rankin 2005). Along its entire length the nesting beach is closely backed by a natural river system (Fowler 1979). The black sand beach, shown in Figure 2, is constantly subject to alterations resulting from surf erosion and rebuilding. Heavy rains and stormy seas mean the beach is very dynamic. Thus, over a period of only a few days, beach contours can vary considerably and there can also be variation between different years (Fowler, 1979; S. Troëng, personal communication). Stretches of the beach are heavily littered with branches, logs, and hyacinth rafts from the river mouths (Fowler, 1979). The tidal range is less than 1 m and has no apparent effect on the leatherback emerging patterns (Leslie *et al.* 1996 in Wallace *et al.* 2004).

Tortuguero is a part of the leatherback rookery between San Juan River at the Nicaraguan border and Chiriquí beach in Panama. It has between 5 795 and 12 893 nests per year which makes it the worlds' fourth largest, and the population trend is stable to slightly declining (Troëng *et al.* 2004). Between 1995 and 2003 annual leatherback nesting only on the 35.6 km index beach of Tortuguero varied from 574 to 1632 nests per year (Troëng *et al.* 2004).

### *Data collection*

Data on nesting leatherback turtles have been collected from 1998 to 2006 between March and July. The first 5 miles of the beach have been patrolled from 9 pm to 2 am with approximately 2 hours intervals. The beach is divided into 1/8-mile segments with the river mouth of Tortuguero River originally designated as mile zero (Fowler 1979). Accretion cycles have increased beach length, and the river mouth is now 1/8 mile north of mile zero and is designated as -1/8 mile.

All witnessed ovipositions were recorded. At the time of oviposition the location of the nest was determined, using the closest mile marker north of where the nest is located as reference. The zone of the beach in which the nest is located was also determined, using the criteria from Bjørndal and Bolten (1992), where the open zone is defined as the full sun zone, the border zone is the zone between the open and vegetation zones, where there sometimes is shading from the vegetation, and the vegetation zone is the part of the beach where there is complete shade. The location of the nest was marked with three flagging tapes attached to the vegetation behind the nest and the distance from the flagging tapes to the egg chamber was measured. This allowed triangulation to be used when determining the position of the nest later without making it conspicuous to poachers. Distance between the nest and the current high tide line was measured with a measuring tape at the time of oviposition.

Marked nests were inspected daily, at dawn, during the entire incubation period. 75 days after oviposition nests were recorded as belonging to one of the following categories: undisturbed, depredated, invaded by roots, two nests together, unhatched, flooded, washed over, washed out, washed out after hatching, poached, dug up by dogs after hatching, dug up by guide after hatching to show hatchlings to tourists, disturbed after hatching, flagging lost and unknown.

Nests were excavated 72 hours after hatchling emergence. If hatchlings did not emerge within 75 days after oviposition the nest was excavated if possible, and hatching success was determined. Number of empty eggshells representing more than 50% of an egg were counted, as was number of living hatchlings in the nest, number of dead hatchlings in the nest, number of unhatched eggs with fully developed embryo, number of unhatched eggs with obvious, but not fully developed embryos and number of unhatched eggs with no obvious embryo, in consistency with the excavation protocol described by Chacón (1999). Here the number of pipped eggs (eggs containing a hatchling that had succeeded in penetrating the shell but still remained in the egg) was also determined as described by Hilterman and Goverse (2004). Only yolked eggs were used for estimating hatching success. All live hatchlings which were still present in the nest by the time of the excavation were put in a hole and covered with sand to allow them to emerge naturally the same night.

Sand temperature at 70 cm depth, which is the average estimated clutch centre depth (Hilterman and Goverse 2004), was measured every hour from 1998 through 2005 (data for 2006 not yet available) in the open sand zone at the beach of Tortuguero with a temperature logger. Short gaps in temperature data exist due to a replacement lag of a few days after theft of the temperature logger. Missing temperature data was replaced with a linear interpolation done in Matlab 7.0.1.

The Caribbean Conservation Corporation has kindly allowed me to use data from their leatherback program from the years 1998 – 2006 for the statistical analysis. I participated in the data collection as a Research Assistant during the leatherback program 2006. The Caribbean Conservation Corporation has a research permit from the Ministry of Environment and Energy of Costa Rica to perform all data collection.

### *Statistics*

Using apparent hatching success (number of live hatchlings divided by number of eggs laid) when investigating the effect of abiotic factors results in a loss of important information on time-specific effects. Data from excavations of sea turtle nests provide information on the age of the dead hatchlings, and different developmental stages can be used as re-encounter occasions. The program MARK (White and Burnham 1999) allows increased flexibility in modelling daily egg survival through including the use of individual and time-specific covariates (Dinsmore *et al.* 2002). This approach can give a better understanding of the patterns of embryonic mortality in leatherback turtle. A known-fate model available in the program MARK is used to determine the influence of time and individual covariates on daily egg survival. The model evaluates the influence covariates have on hatching success using model selection by Akaike's Information Criterion, AIC (Akaike 1973 in Dinsmore *et al.* 2002). A set of *a priori* biological hypotheses was developed to form the specific models to explain variation in daily survival of the leatherback eggs. This paradigm for model creation was assessed by Burnham and Anderson (1998) and shown to reduce the possibility of spurious results due to model over-fitting, which could happen if already analyzed models are inspected for fit prior to subsequent analyses.

Distance to the high tide line, mean sand temperature during incubation and section of the beach were evaluated with 17 *a priori* models (Table 1). Distance to high tide line is frequently used as a basis for determining whether nests are to be relocated or not (Eckert and Eckert 1990; Chacón 1999; Dutton *et al.* 2005), incubation temperatures below 25-27°C or above 33-35 °C cause embryonic mortality (Ackerman 1997), and beach width, beach profile

and erosion regime can change with section of the beach. “Erosion prone areas” are mentioned as basis for relocation (Eckert and Eckert, 1990; Dutton *et al.*, 2005). The supposed lower salinity in the proximity of the river mouths could also influence hatching success.

The estimated age of the dead embryo is used to determine whether it is encountered alive or dead at a specific re-encounter occasion. The eggs containing yolk but no visible embryo are set as encountered alive at the first encounter but dead at the second encounter occasion. Eggs containing a visible but not fully developed embryo are set as encountered alive at the first and second encounter occasions, but dead at the third. Eggs with a fully developed embryo are set as alive during the three first encounter occasions but dead on the fourth, pipped eggs are set as alive during the four first encounter occasions and dead on the fifth and dead hatchlings in the nest are set as alive in the fifth stage but dead during the sixth. The number of dead hatchlings is subtracted from the number of hatchlings that have emerged from their eggs, indicated by empty eggshells representing more than 50% of an egg, and the remaining number are set as hatchlings living through the sixth and last encounter occasion and thus succeeded to leave the nest alive. The duration of different developmental stages is determined according to the stages described by Miller (1985) and the estimate of incubation time from Hilterman and Goverse (2005); Table 2.

The Parameter Index Matrices (PIM) are set to five different values, each one corresponding to a specific re-encounter occasion, to allow for time specific effects. The design matrix is used to put further constraints on the data set in order to test specific covariates and for time specific effects as described in White and Burnham (1999). ANODEV-tests are computed for all individual covariates, and for time effects, to evaluate the amount of the total deviation they explain (White and Burnham 1999). A LR-test is computed in MARK to test whether the best model fits data significantly better than the second best (White and Burnham 1999).

The washed out nests can not be included in the known fate model because required information (excavation data) is not available, which could bias the effect of distance to high tide line negatively. Distance to high tide line is frequently used for relocation decisions, and about 20% of the nests at Tortuguero are washed out. To include the washed out nests a generalized linear model (GLM, McCullagh and Nelder, 1989) with binomial distribution (either no surviving hatchlings in nest or >1 surviving hatchling in nest) and a logit link, fitted by stepwise backward removal based on Wald-statistics, is done. Assessed variables are distance to high tide line, section of the beach, zone of the beach and mean incubation temperature. Quick logit regressions are computed for the effect of the distance from the high tide line on the risk for the nest to be washed out, and for the risk of the nest to have no hatching success. A median test is computed to investigate whether apparent hatching success differs between groups of nests grouped by distance to high tide line. Linear regressions with hatching success as dependent variable and minimum and maximum temperatures as independent variables are done. A quadratic regression with hatching success as dependent variable and maximum temperature as independent is also computed. Statistica 6.0 (Statsoft 2004) is used for the statistical analysis. Cubic splines (Schluter 1988) with binomial distributions are used to illustrate the risk of zero survival and of getting washed out as function of distance to high tide line.

## Results

A total of 293 nests were marked and data were collected. Mean hatching success for nests unaffected by anthropogenic interaction was 27%, but was reduced to 24% when nests disturbed by anthropogenic interaction are included. For the statistical analysis, all nests with a destiny influenced by anthropogenic interaction, represented by the categories poached, dug up by dogs after hatching, dug up by guide after hatching and disturbed after hatching were excluded. Not yet excavated nests, all cases where flagging tapes were lost, where the fate remained unknown, where there were missing data and where two nests were together and it was impossible to distinguish their separate destinies were also excluded. This reduced sample size to 198 for the quick logit regressions and median-test, and to 182 for the GLM analysis and regressions since temperature data for 2006 is not yet available. For the analysis in MARK the sample size was further reduced to 140 since washed out nests were not included.

Allowing for time-specific daily survival (time) greatly improved the fit of the known-fate model ( $\Delta AIC_c=16186.07$ ), due to a variation in embryonic mortality between different developmental stages (Figure 3). The individual covariates distance to high tide line, section of the beach, temperature and an interaction between section and distance to high tide line also improved the fit of the model, and were thus all included in the best model (Table 3). The best four models all included distance to high tide line and mean incubation temperature, while section and distance to high tide line\*section only appeared in two of the four each. Distance to high tide line was negatively correlated with hatching success (shown as +/- SE) ( $\beta=-0.082 \pm 0.013$ ), as was mean incubation temperature ( $\beta=-0.048 \pm 0.012$ ), while section of the beach ( $\beta=0.043 \pm 0.013$ ), and an interaction between section and distance to high tide line ( $\beta=0.030 \pm 0.013$ ) were positively correlated with hatching success.

The best model, S(time + distHTL + section + mean temp + distHTL\*section) fitted data significantly better than the second best model (LR-test;  $\chi^2=4.97$ , df=1, p=0.026). The 13 models with the highest  $AIC_c$  values, indicating a higher error and thus an inferior fitted model, all had weights ( $w_m$ ) < 0.002 and  $\Delta AIC_c$  between 12.43 and 16254.56, and are not displayed in Table 3. Estimated hatching success for all nests modelled (not including washed out nests) was 37%, but varied between individual years with the lowest estimate being 26% (2005) and the highest 67% (2000).

An ANODEV-test of the individual covariates used in the known fate model revealed that the developmental stage (time) strongly affected the daily mortality (p<0.0001), but none of the individual covariates, distance to high tide line, section or mean temperature, explained a significant part of the deviation (all p>0.84).

Both distance to high tide line (GLM; Wald Z=8.6, p=0.0031, df=1; Table 4) and mean temperature during the incubation period (GLM; Wald Z=7.3, p=0.0066, df=1; Table 4) significantly affected hatching success, but section (GLM; Wald Z=0.0098, p=0.92, df=1; Table 4) and zone of the beach (GLM; Wald Z=0.23, p=0.63, df=1; Table 4) did not significantly affect hatching success and were therefore not included in the best-fit generalized linear model. Distance to high tide line ( $\beta=0.094 \pm 0.032$ ) and mean temperature ( $\beta=0.29 \pm 0.11$ ) were both positively correlated with hatching success, ( $\beta \pm SE$ ).

Nests at greater distance from the high tide line had a lower risk of not hatching (logistic regression;  $y=\exp(-0.054+(0.10)*x)/(1+\exp(-0.054+(0.10)*x))$ ,  $\chi^2=14.2$ , df=1, p=0.00016).

The probability of the nest to produce at least one surviving hatchling at different distances from high tide line is illustrated by a cubic spline with a binomial distribution, Figure 4. Greater distance to high tide line also implied a lower risk of being washed out (logistic regression;  $y = \exp(-0.20+(0.13)*x)/(1+\exp(-0.20+(0.13)*x))$ ;  $\chi^2=18.6$ ,  $df=1$ ,  $p<0.0001$ ). The probability of the nest not to be washed out at different distances from high tide line is illustrated by a cubic spline with a binomial distribution, Figure 5. The nests below the high tide line had significantly lower hatching success (median test;  $\chi^2= 10.3$ ,  $df = 4$ ,  $p = 0.036$ ; Figure 6).

Hatching success was positively correlated to minimum temperature during incubation period (linear regression;  $y = -80.12+3.97*x$ ;  $R^2=0.061$ ,  $F=12.09$ ,  $df1=1$ ;  $df2=187$ ,  $p=0.00063$ ; Figure 7), but maximum temperature was quadratically correlated to hatching success; both too low- and too high maximum temperatures influenced hatching success negatively (quadratic regression;  $y= -2389+160.35*x -2.65*x^2$ ;  $R^2=0.059$ ,  $F=5.82$ ,  $df1=2$ ,  $df2=195$ ,  $p=0.004$ ; Figure 8). When washed out nests were excluded from the analysis, hatching success was negatively correlated with maximum temperature during incubation period, though (linear regression;  $y= 192 -4.82*x$ ;  $R^2=0.038$ ,  $F=5.12$ ,  $df1=1$ ,  $df2=131$ ,  $p=0.0253$ ; Figure 9). All  $\alpha=0.05$ .

## Discussion

### *Distance to high tide line*

My results are consistent with my hypothesis that distance to the high tide line strongly influences hatching success for leatherback turtle nests at Tortuguero. Distance to the high tide line affects hatching success through an elevated risk of getting washed out close to the high tide line. Washover, which can result in embryonic mortality even if the nest is not washed out (Whitmore and Dutton 1985), is probably contributing to the elevated embryonic mortality close to the high tide line. The importance of distance to high tide line for hatching success is consistent with results from other leatherback turtle nesting beaches (Eckert and Eckert 1990; Dutton *et al.* 2005).

The significant repeatability of nest site choice relative to the spring high tide line in individual leatherback turtles found by Kamel and Mrosovsky (2004) is consistent with the importance of the distance to the high tide line found in my study. The difference in distance from the nest to the high tide line between individual females reported by Kamel and Mrosovsky (2004) suggests that this could be a basis for selection (but results are inconsistent; see Eckert 1987; Nordmoe *et al.* 2004). Do the results of the study imply that the nest site selection of the leatherback population nesting at Tortuguero is maladaptive, and should be under directional selection?

Several proximal leatherback nesting populations are genetically indistinguishable (Dutton *et al.* 1999). This suggests that natal homing behaviour in leatherback turtles could be less precise than that of other marine turtle species. Turtles tagged at nesting beaches from San Juan River at the Nicaraguan border to Chiriquí beach in Panama frequently nest at Tortuguero (Troëng *et al.* 2004). One of the consequences of this could be that nest site selection in leatherback turtles is adapted to a region rather than one specific nesting beach as suggested by Kamel and Mrosovsky (2004), and leatherback turtles thus select sub-optimal nest site locations in some parts of their nesting range, which is manifested through a too seaward nest placement at Tortuguero.

Surprisingly, the  $\beta$ -estimate for distance to high tide line in MARK indicated a negative correlation with hatching success, probably due to washed out nests not being included in the analysis. Even if distance to high tide line did not explain a significant part of the deviation for the modelled nests, the inclusion of a distance to high tide line with a negative correlation in the best known fate model indicates that there could possibly be some weak selection for a more seaward nest placement. Desiccation is known to generate lower hatching success at larger distance from high tide line (Wood and Bjørndal 2000), and could possibly explain this trend.

A higher hatching success at a larger distance from the sea can also be counteracted by increased likelihood of hatchling disorientation and predation on hatchlings as a result of further inland nest placement (Wood and Bjørndal 2000; Kamel and Mrosovsky 2004). An alternative explanation is that an adequate amount of washover events could have some advantage. Washover events could cool the nest, which can be important for sex ratio. If the sex ratio of the hatchlings for Tortuguero is female biased, male hatchlings might be more valuable, and thus worth the elevated risk of embryonic mortality the more seaward nest placement implies.

## *Temperature*

Mean temperature during the incubation period had a significant effect on hatching success, but it was positively correlated rather than having an intermediate optimum as I expected. The positive correlation is mainly due to a higher embryonic mortality at low minimum temperatures; maximum temperature is quadratically correlated with hatching success and affect hatching success negatively both at too low and too high temperatures; the degree of explanation is low for both correlations though. Minimum temperatures at Tortuguero during extreme occasions were below the temperatures known to cause embryonic mortality (Ackerman 1997). High variance in embryonic mortality rate at a constant minimum temperature (0-70% at  $< 22^{\circ}\text{C}$ ) suggests that the lower hatching success at low minimum temperatures might not be due only to temperature induced mortality. A plausible explanation would be that low temperatures are associated with extreme weather conditions, which are likely to result in an elevated risk of wash out or washover due to more violent wave regimes, or flooding due to augmented precipitation. This would be consistent with the change in beach contours over only a few days which heavy rains and storms bring about as described by Fowler (1979).

Differences in survival between nests at the same temperature could also possibly be due to differences in nest depth and the developmental stage of the clutch. Since the temperature logger is buried at the average clutch depth, extremely deep or shallow nests could have temperatures different from the one measured by the logger. Metabolic heating increases incubation temperature during the later part of incubation (Wallace *et al.* 2004) which could possibly cause the effect of low temperature to differ between developmental stages.

When the washed out nests are excluded, mean temperature was negatively correlated with hatching success, indicated by the negative  $\beta$  in the known fate model. Maximum temperature is also negatively correlated with hatching success when washed out nests are removed. If lethal temperatures frequently occur at Tortuguero, a more seaward nest placement may have the advantage of washover cooling the sand. This effect was much weaker than the increased mortality due to wash out a more seaward nest placement generate.

## *Development stage*

The high  $\Delta\text{AIC}_c$  and ANODEV results for adding time specific effects reveal that the developmental stage of an egg greatly affect the daily survival. The first stage, the first 5 days of incubation before the embryo is visible, had the highest rate of embryonic mortality but this might be biased due to infertile eggs. Whitmore and Dutton (1985) estimate 6,1% of the leatherback eggs on Babusanti beach, Suriname, to be infertile, but no such estimate has been done for Tortuguero. The high mortality at the first stage may also reflect that eggs in badly placed nests are prone to die during the first days of incubation due to for example washover. The high embryonic mortality prior to the embryo being visible is consistent with the results from oxygen sampled nests in a (not washed over) hatchery, where 75% of embryonic mortality occurs at this stage (Ralph *et al.* 2005). This could indicate that some intrinsic effect or elevated sensitivity to disturbance might occur at this stage, but conclusions remain speculative. Daily embryonic mortality was low during the second stage, from visible embryo to full embryo. During the stages when the hatchling pips the egg and hatches, mortality was higher again, suggesting that emergence is a critical step in a hatchling's life. The daily

mortality during the last 5 days of incubation, when the hatched hatchling absorbs the yolk sac before emerging from the nest, was low.

### *Section of the beach*

The best fitted known-fate model included both section and a distance to high tide line\*section interaction, but section was not included in the best fitted GLM-model. This could possibly be due to the linear nature of the GLM. Another possibility is that section affects the distance to high tide line (some parts of the beach are so narrow that nests are placed closer to the sea), so distance to high tide line explains some of the variation in hatching success between different sections of the beach; section was easily interacting with distance to high tide line and mean incubation temperature when many covariates were included in a GLM. This is supported by the improved fit when including interaction distance to high tide line\*section in the known-fate model. The positive  $\beta$  indicates that hatching success could possibly be lower in the proximity of the Tortuguero river mouth. This trend could be an effect of the area being more erosion prone, or possibly that the supposed lower salinity could affect nests negatively. Since washed out nests are not included that result has to be very conservatively interpreted, though.

### *Using MARK for modelling hatching success in sea turtles*

The results from the known-fate model in MARK were not consistent with the results from the GLM-model. The known-fate model provided information on the great importance of the stage of development for daily survival, which is an interesting area for future research. Nevertheless, the data requirements of both the known-fate model and the nest survival model available in MARK do not allow washed out nests to be incorporated in the models. Even if the time of wash out can be monitored, it is impossible to know if some of the eggs have died prior to being washed out. Thus, the known-fate model underestimates the importance of the distance to high tide line, which my study has shown is an important factor for hatching success at Tortuguero. This underestimation was clearly manifested through the contradicting results on both distance to high tide line and mean sand temperature during incubation. Using MARK to investigate the influence of different factors on sea turtle hatching success is thus very problematic if a high percentage of the nests are washed out.

Notwithstanding, a known fate model in MARK can be a useful complement for investigating time specific effects and effects of individual covariates for nests not being washed out. The surprising results from the known fate model that do not include washed out nests, where hatching success had a tendency to be negatively rather than positively correlated with distance to high tide line and mean incubation temperature, show that the approach can provide valuable information on effects that are normally overridden by the risk of getting washed out. Knowledge of those more subtle effects is also important when relocating eggs.

### *Implications for management and conservation*

If leatherback turtle hatching success is significantly lower in certain areas of the beach, this constitutes a basis for relocation to areas with higher hatching success. My study indicated that there is a basis for nest relocation at Tortuguero. Nests closer to the high tide line had a higher embryonic mortality, and there was a significant difference in hatching success

between the nests below the high tide line and those above it. Relocation of nests below the high tide line can potentially increase the number of hatchlings produced at Tortuguero.

Nest relocation together with beach protection has been reported to contribute to a positive population trend at US Virgin Island St Croix (Dutton *et al.* 2005). Relocation, however, is a controversial topic. There are costs of relocation, including reduced hatching success due to handling (Limpus 1979; Chan *et al.* 1985; Eckert and Eckert 1990), an increased risk of embryonic deformity (Eckert and Eckert 1990), and the potential of biasing sex ratio (Eckert and Eckert, 1990; Mrosovsky and Yntema 1980). There are also evolutionary concerns about the practice of nest relocation. If nest site selection is an individual, heritable trait and there is variation in this trait in the leatherback population, nest relocation could end up selecting for turtles that make poor nest site selections. There are different opinions on whether individual females nest at a consistent distance from the high tide line, and whether there is a significant difference between individual females (Kamel and Mrosovsky 2004; Nordmoe *et al.* 2004). It is not known if nest site selection is a heritable trait.

Using conservative estimates when deciding on below which point to relocate is important for the Critically Endangered leatherback turtle. Nests below high tide line had practically zero survival at Tortuguero, and could be relocated without the risk of decreasing hatching success. Relocation directly after oviposition is strongly recommended (Limpus 1979; Chan *et al.* 1985). Evaluation of hatching success and incubation period of the relocated nest would be useful for assessing the consequences of the relocation practice. A shortened incubation period indicates a higher nest temperature and thus a higher proportion of female hatchlings (Mrosovsky and Yntema 1980), and would give valuable information on the risk of biasing sex ratio.

The low hatching success of the nests that are neither washed out nor exposed to too low temperatures is still not understood, and is an interesting field for future investigation. Continued monitoring is important for providing more data that can further improve the understanding of the nesting biology of leatherback turtles, and thus help increase hatchling production and maintain a viable leatherback population.

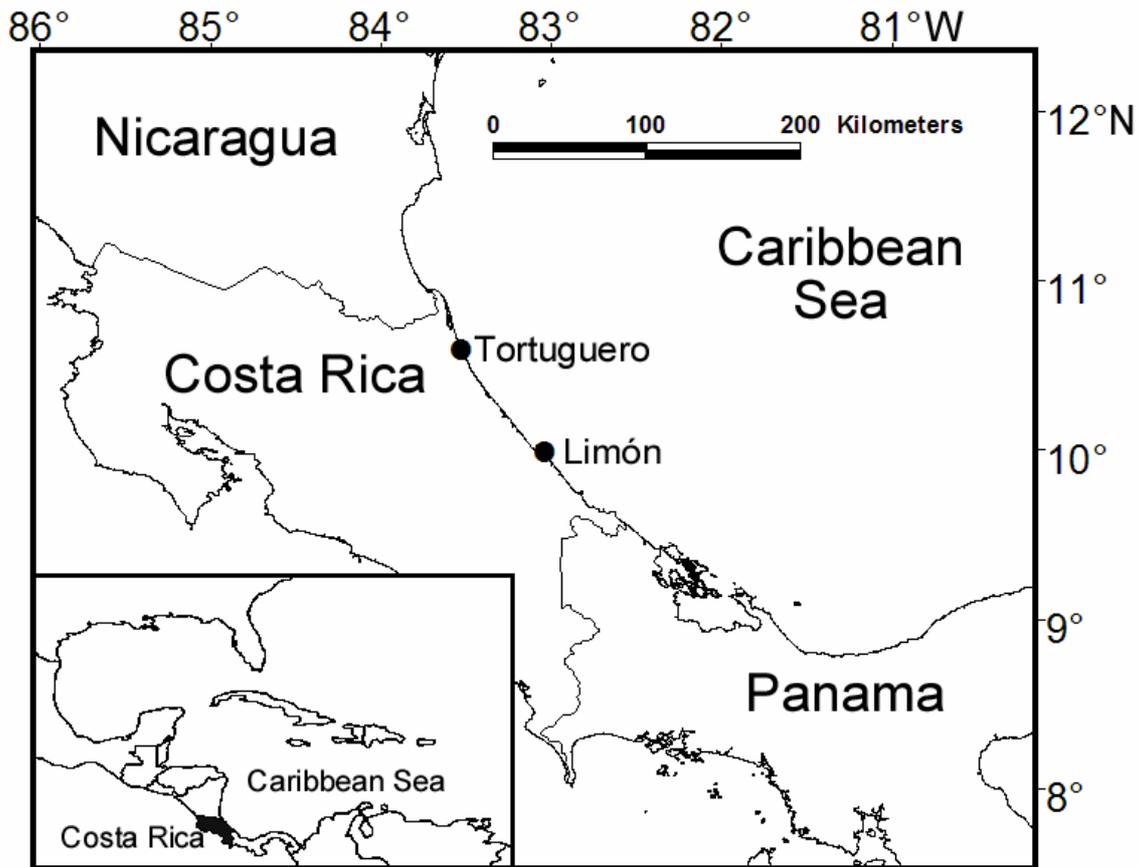


Figure 1. Location of Tortuguero.



Figure 2. The nesting beach of Tortuguero.

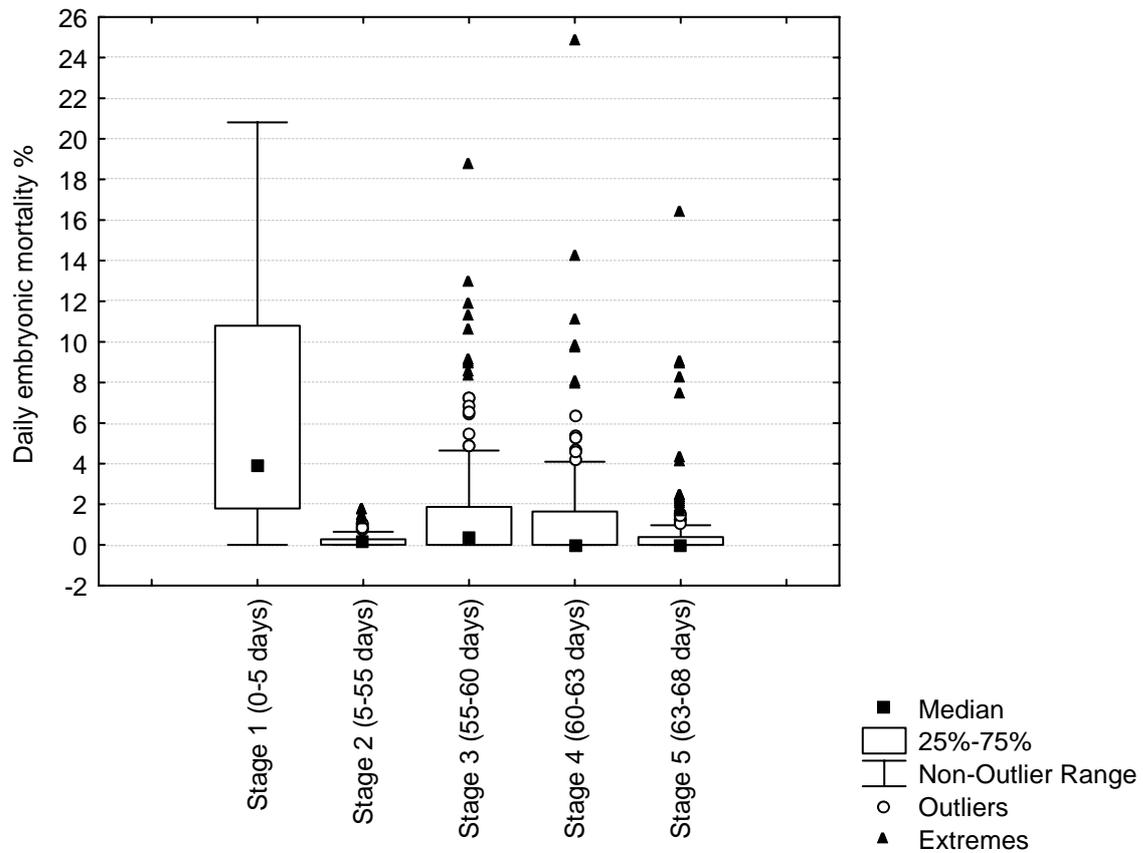


Figure 3. Daily embryonic mortality for leatherback turtle embryos at Tortuguero (%) during different developmental stages. During stage 1 no obvious embryo can be seen, during stage 2 the embryo is visible, during stage 3 the embryo is fully developed, during stage 4 the hatchling pips the egg and emerges, and during stage 5 the hatched hatchling absorbs the yolk sac.

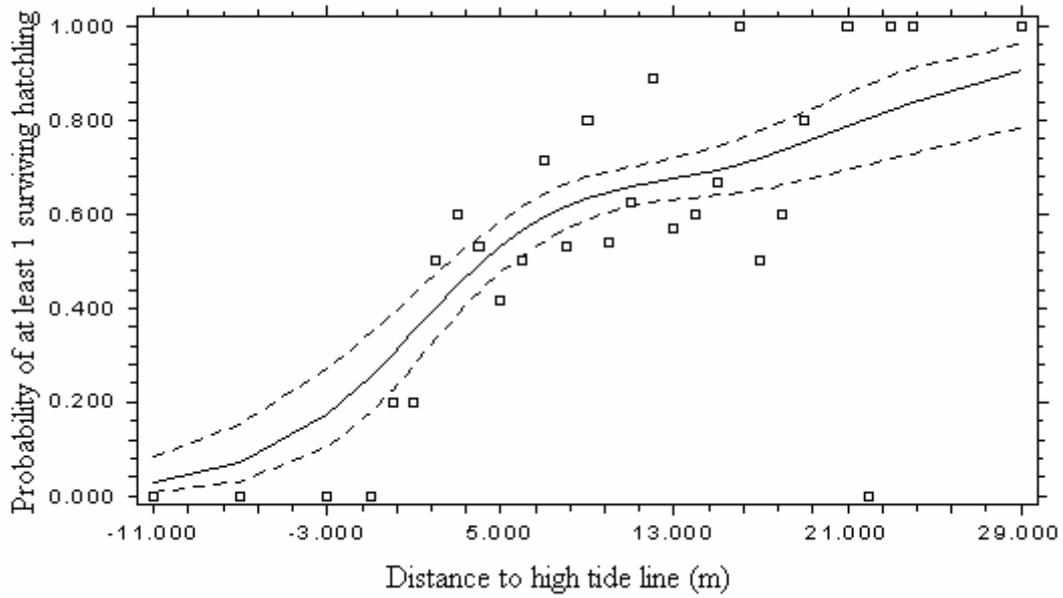


Figure 4. Probability of at least one surviving hatchling in leatherback turtle nest at Tortuguero as a function of distance to high tide line (m), illustrated by a cubic spline with a binomial distribution. Dashed curves indicate  $\pm$ SE of predicted values  $f^{\wedge}(z)$  from 50 bootstrap replicates of the fitness function,  $n=198$ .

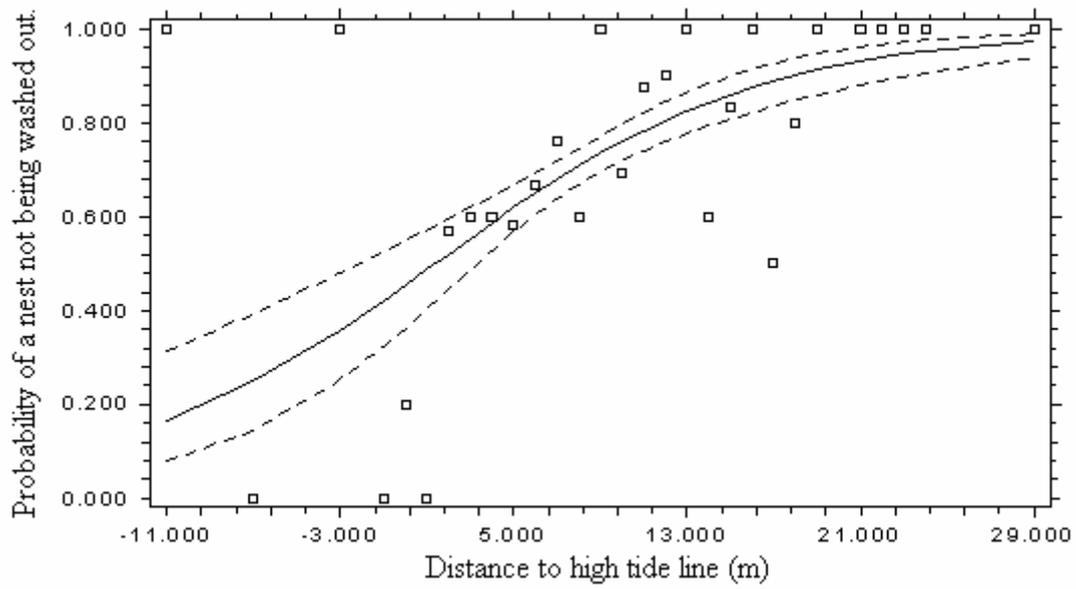


Figure 5. Probability of a leatherback turtle nest at Tortuguero not to get washed out as a function of distance to high tide line (m), illustrated by a cubic spline with a binomial distribution. Dashed curves indicate  $\pm$ SE of predicted values  $f^{\wedge}(z)$  from 50 bootstrap replicates of the fitness function,  $n=198$ .

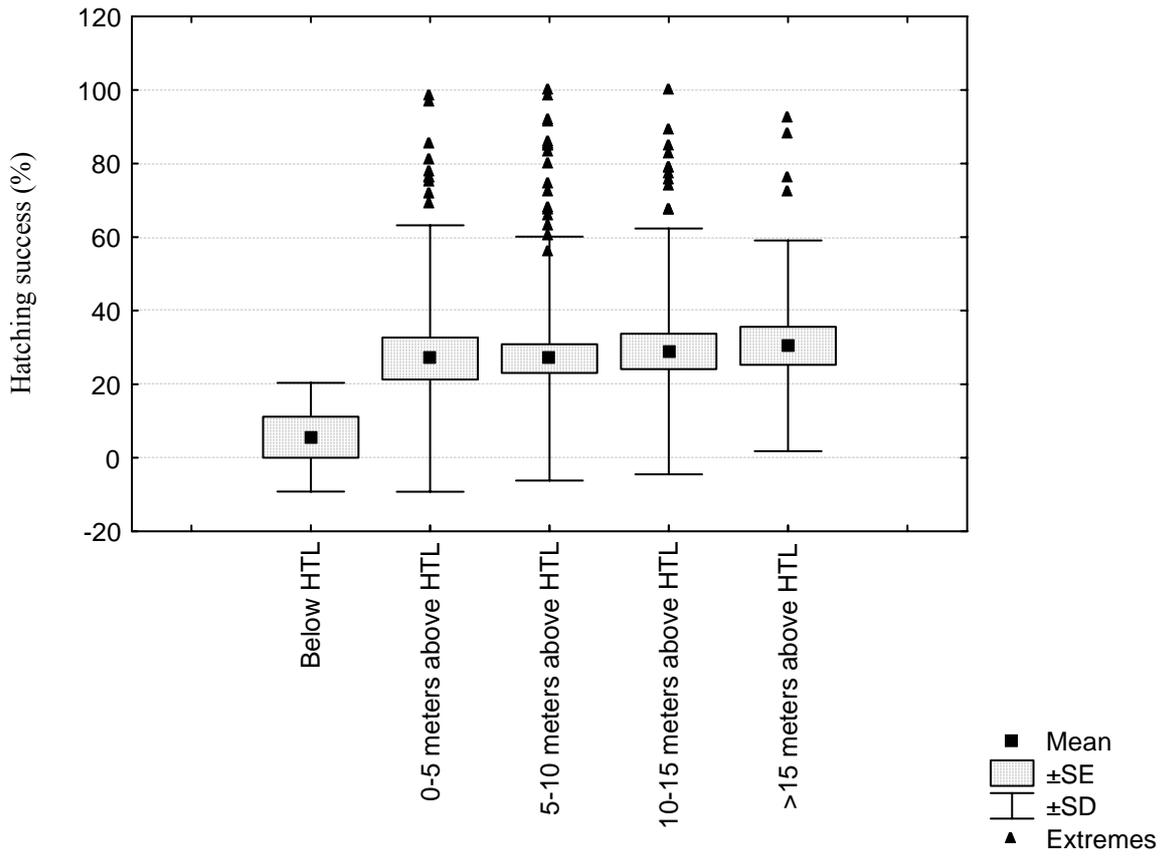


Figure 6. Hatching success (%) of leatherback turtle nests the beach at Tortuguero grouped by distance to high tide line; hatching success is significantly lower below high tide line;  $\chi^2 = 10.31$ ,  $df = 4$ ,  $p = 0.036$ .

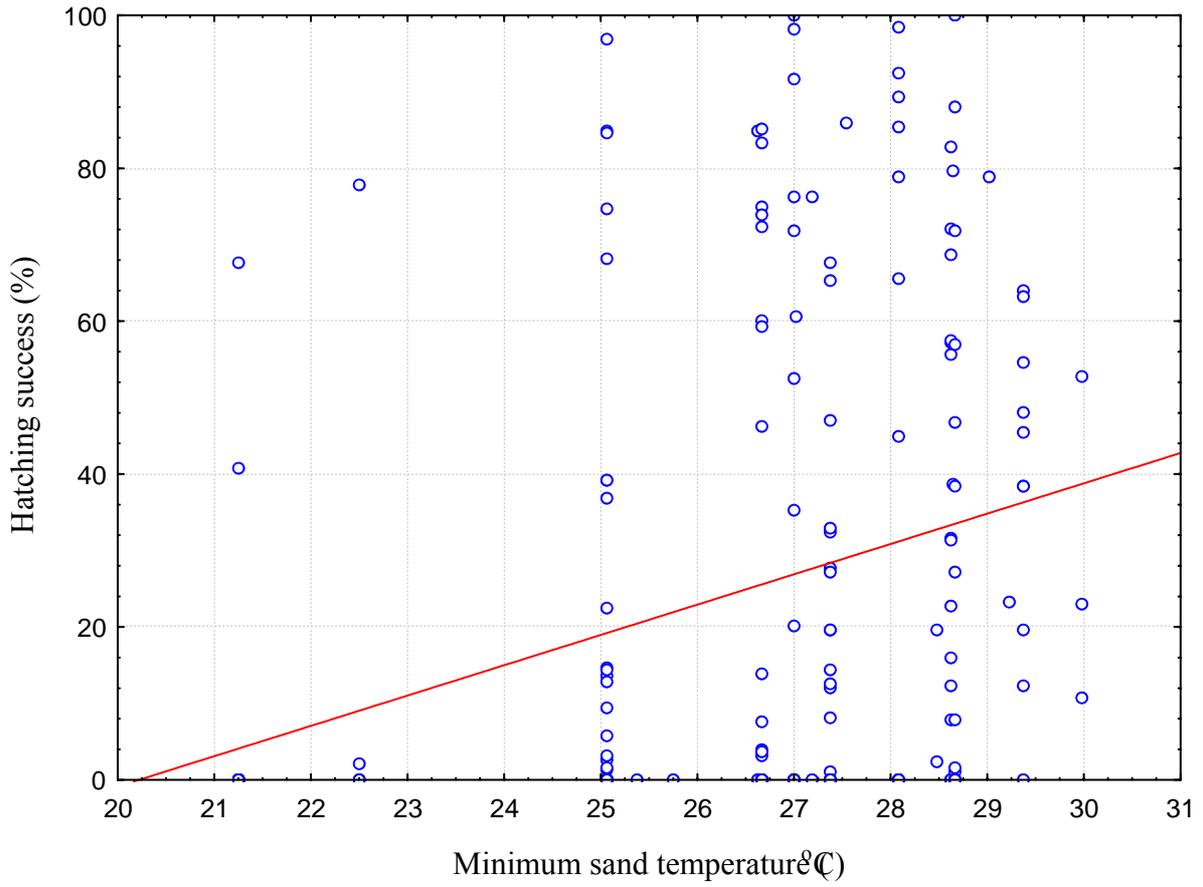


Figure 7. Hatching success (%) of leatherback nests as a function of minimum temperature during incubation period.  $y = -80.18 + 3.96 * x$ ;  $R^2 = 0.061$ ;  $F = 12.09$ ;  $df1 = 1$ ;  $df2 = 187$ ;  $p = 0.00063$ .

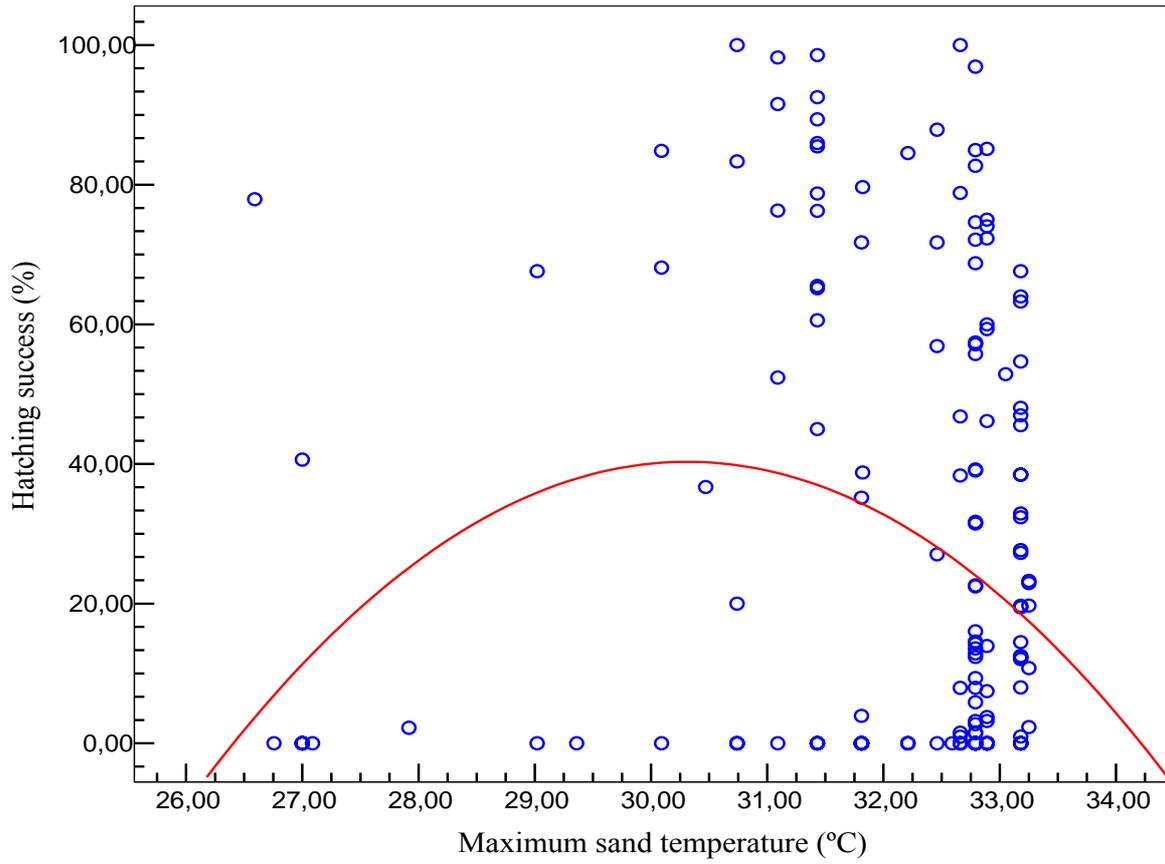


Figure 8. Hatching success of leatherback turtle at Tortuguero as a function of maximum sand temperature during the incubation period (°C).  $y = -2389,79 + 160,35 \cdot x - 2,65 \cdot x^2$ ;  $R^2 = 0,059$ ;  $F = 5,82$ ;  $df1 = 2$ ;  $df2 = 195$ ;  $p = 0,004$ .

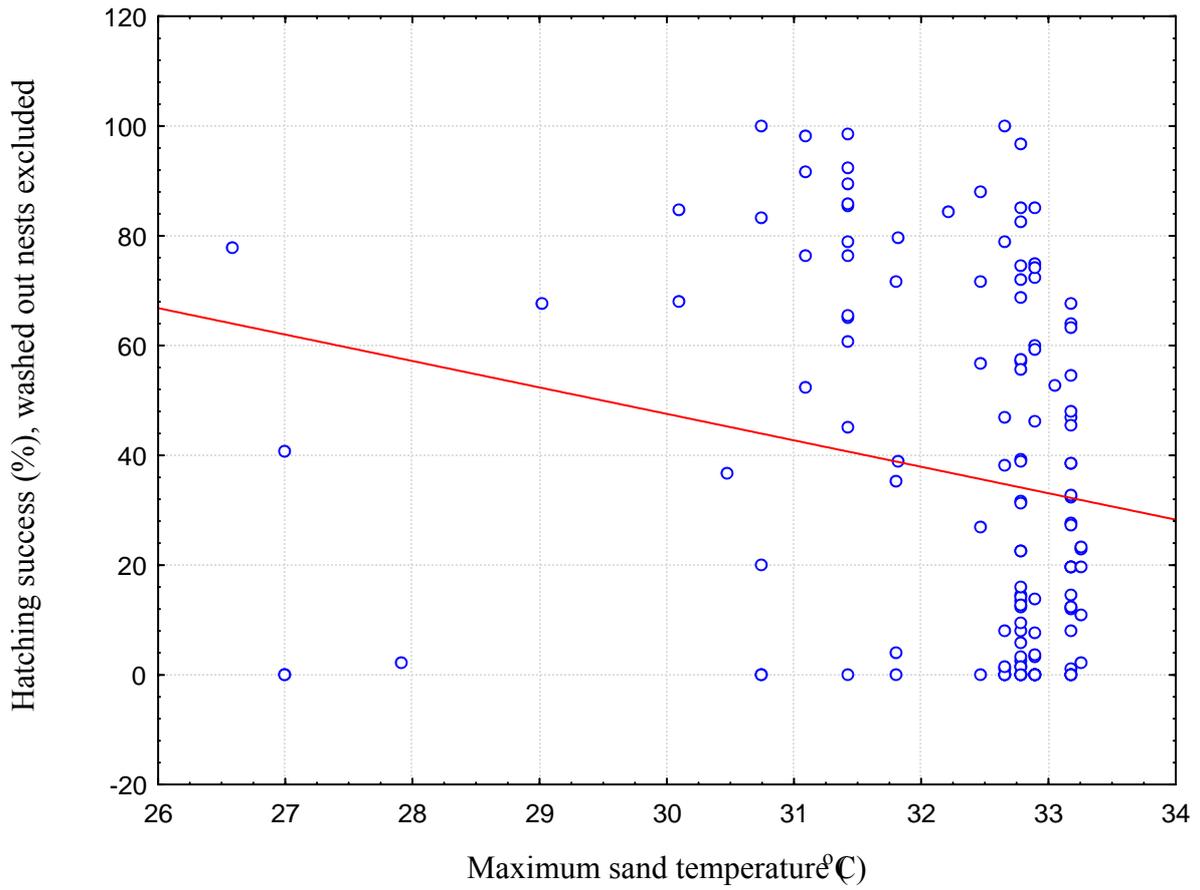


Figure 9. Hatching success as a function of maximum temperature when washed out nests are excluded.  $y=192.08-4.82*x$ ;  $R^2=0.0376$ ;  $F=5.121$ ;  $df1=1$ ;  $df2=131$ ;  $p=0.0253$ .

Table 1. A priori defined candidate model for evaluating which factors influence hatching success with a known fate model in MARK. HTL= high tide line.

Model no.	Model description	Model structure
1	Simple.	S.
2	Time effect.	S(time)
3	Time and distance to HTL effect.	S(time+distHTL)
4	Time and section effect.	S(time+section)
5	Time and temperature effect.	S(time+mean temp.)
6	Time and distanceHTL*section effect.	S(time+distHTL*section)
7	Time, distance to HTL and section effect.	S(time+distHTL+section)
8	Time, distance to HTL and temperature effect.	S(time+distHTL+mean temp.)
9	Time, distance to HTL and distanceHTL*section effect.	S(time+distHTL+distHTL*section)
10	Time, section and temperature effect.	S(time+section+mean temp.)
11	Time, section and distanceHTL*section effect.	S(time+section+distHTL*section)
12	Time, temperature and distanceHTL*section effect.	S(time+temp+distHTL*section)
13	Time, distance to HTL, section and temperature effect.	S(time+distHTL+section+mean temp.)
14	Time, distance to HTL, section and distanceHTL*section effect.	S(time+distHTL+section+distHTL*section.)
15	Time, distance to HTL, temperature and dist.HTL*section effect.	S(time+distHTL+meantemp+distHTL*section)
16	Time, section, temperature and distanceHTL*section effect.	S(time+section+meantemp+distHTL*section.)
17	Time, distance to HTL, section, temp. and dist.HTL*section effect.	S(time+distHTL+section+meantemp+distHTL*section.)

Table 2. Developmental stages of leatherback turtle embryos classified as embryo, full embryo, pipped hatchling, dead hatchling, and living through the entire period. Morphological descriptions and estimations of % of developmental time from Miller (1985) and the incubation period of 63.2 days (Hilterman and Goverse, 2004) were used.

Developmental stage	% of development time	Days of incubation	Description
1.	7,6 +/- 0.5%	4.80	Embryo about 0.5 cm long.
2.	86,0 +/- 2%	54.35	Ratio embryo volume to yolk ranges between 1.5 and 4:1. Basic hatchling morphology and pigmentation present.
3.	94% +/- 2%	59.40	Pipped. Egg shell is penetrated but hatchling is still in the egg.
4.	100%	63.20	The turtle is outside the egg, yolk mass is mostly withdrawn into the abdomen and is covered by pigmented tissue. Extra embryonic membranes remain attached
5.	-	68.20	Hatchlings are ready to emerge from the nest. Yolk mass is completely withdrawn into the abdomen and the extra embryonic membranes are absent.

Table 3. Akaike's Information Criterion ( $AIC_c$ ) ranking of the four best of 17 a priori models used to estimate hatching success of the leatherback turtles in Tortuguero 1998-2005, where K is number of parameters,  $\Delta AIC_c$  is the difference between the model and the best model and  $w_m$  is the weight of the model.

Model no.	Model structure	K	$AIC_c$	$\Delta AIC_c$	$w_m$
17	S(time+distHTL+section+mean temp+distHTL*section.)	9	29355.90	0.00	0.80071
16	S(time+distHTL+section+mean temp.)	8	29358.87	2.97	0.18129
15	S(time+distHTL+mean temp+distHTL*section.)	8	29364.25	8.35	0.01232
8	S(time+distHTL+mean temp.)	7	29366.74	10.83	0.00355

Table 4. GLM for the effect of individual covariates on hatching success of leatherback turtles in Tortuguero. The best fitted GLM-model only includes intercept, distance to high tide line and mean temperature (all  $p < 0.05$ ).

Individual covariate	Degrees of freedom	Wald Z	Wald p	$\beta$	Standard error	Comment
Intercept	1	7.19	0.0073	7.192	3.349	
Distance to HTL	1	8.72	0.0031	0.094	0.032	
Mean temperature	1	7.38	0.0066	0.293	0.110	
Section of the beach	1	0.0098	0.9212	-0.013	0.129	Removed
Zone of the beach	1	0.23	0.6332	-0.215	0.442	Removed

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