# The effect of yolkless eggs on hatching and emerging success of leatherback sea turtles (*Dermochelys coriacea)* in the Tortuguero area, Costa Rica.

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**08/10/2014**

has university of applied sciences

CANADIAN ORGANIZATION FOR TROPICAL EDUCATION AND RAINFOREST CONSERVATION

SEA TURTLE CONSERVANCY

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Tortuguero, Costa Rica

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# ABSTRACT

Leatherback sea turtles lay the highest frequency of yolkless eggs (shelled albumin globs) of all marine turtles. Little is known about possible roles and advantages of these yolkless eggs. This is the first field study in the Tortuguero Area conducted to quantify the relations between yolkless eggs (proportion and number within a clutch) and hatching success, emerging success and predation rates. The data used for statistical analysis were collected by the Sea Turtle Conservancy (STC) on Tortuguero Beach between 2006 and 2013 and by the Canadian Organisation for Tropical Education and Rainforest Conservation (COTERC) on Playa Norte in 2013. In total 175 (160 from Tortuguero Beach and 15 from Playa Norte) leatherback sea turtle nests were suitable for statistical analysis. The mean number of yolkless eggs laid was 68.79 (SD = 18.72). Hatching and emerging success on these beaches respectively 42.65% (SD = 30.76) and 39.21% (SD = 29.67). In this study, no significant relationships have been found between number or proportion of yolkless eggs within a clutch and hatching-, emerging success and predation rate. However, number of normal (yolked) eggs had a significant negative influence on the nest success and a positive influence on number of depredated eggs within a clutch. Clutch size (number of normal + yolkless eggs) had no significant effect on hatching and emerging success. It is recommended to collect data on the state of the yolkless eggs which might give more insight on the influence of nest location and yolkless eggs on the nest success.

# 1 INTRODUCTION

Leatherback turtles (*Dermochelys coriacea)* are internationally listed as vulnerable, facing a high risk of extinction in the immediate future (Wallace *et al*, 2013). Like other sea turtles, leatherbacks are inaccessible during most phases of their life due to their oceanic existence. Population abundance and survival probability estimates are therefore based on capture-mark-recapture studies of females that come ashore to nest (Chaloupka *et al.,* 2001). Although logically the highest priority should be given to the conservation of large juveniles and adult turtles, which represent decades of selective survival, the persistent loss of eggs can have negative consequences for a population (Eckert and Eckert, 1990). Tomillo *et al* (2008) modelled the population response to egg harvesting (poaching) and found that with 90% poaching the population would be extirpated. Thus, protection of all live stages is necessary for the survival of natural populations (Congdon *et al*, 1993).

Dutton *et al.* (2005) discovered that an aggressive program of beach protection and egg relocation most likely leads to an increase in the size of the nesting population. As long as adult survival at sea remains relatively high, beach protection (protecting nests from predators and poachers) provides a simple and effective conservation strategy for Northern Caribbean nesting populations (Eckert and Eckert, 1990).

Adult female leatherbacks migrate to tropical or subtropical nesting beaches every 3 – 5 years (Eckert and Eckert, 1990; Reina *et al*, 2002). They lay three to seven clutches in one season, with an interval of 9 – 10 days between clutches. Each clutch contains an average of 65 – 80 eggs (Steyermark *et al*, 1996; Reina *et al*, 2002; Bell *et al*, 2004; ). In each clutch there are a variable number of shelled albumin globs (SAGs), in literature also referred to as 'yolkless eggs' because they do not contain a yolk. SAGs are packets of excess albumin formed in the oviduct and covered with a thin shell (Dutton and McDonald*,* 1995; Bell *et al*, 2004). These SAGs vary greatly in number (from 10 to 100) as well as shape (10 – 50 mm diameter) within and among clutches of leatherback females and are generally the last of the eggs laid in a clutch.

The average global hatching success of leatherback turtles is ~50%, lower than that of other marine turtle species (Bell *et al*, 2004). Maternal identity, maternal reproductive experience, inter-clutch interval (time between laying clutches), clutch size and possibly the number of SAGs in the clutch can effect hatching success (McGinley, 1989; Dutton and McDonald, 1995; Bell *et al.,* 2004; Caut *et al.,* 2006; Dutton and McDonald, 1995). This study focusses on the effect of number and proportion of yolkless eggs within a clutch on the nesting success of leatherback turtles.

Beneficial functions of SAGs are still unknown. It is possible that the infertile eggs have very low energetic cost to the nesting female, as they do not contain yolk. They may increase hatching and emergence success by increasing interstitial air spaces in the nest, maintaining the moisture in the nest and by buffering the fertile eggs from thermal and predatory disturbances (Frazier and Salas, 1984; Dutton and McDonald*,* 1995). Another hypothesis is that yolkless eggs may act as a barrier, preventing sand from falling between the viable eggs below. This mechanical function is also ultimately related to the need for sufficient oxygen (Dutton and McDonald*.,* 1995). Although the exact function of SAGs is unknown, yolkless eggs seem to have a beneficial effect on the future of the clutch (Caut *et al.*, 2006).

Caut *et al.* (2006) discovered that the nest location has an influence on the state of the yolkless eggs (hydrated or dehydrated) and on the predation rate of the nest. As yolkless eggs dehydrate it is possible they release substances into the surrounding egg chamber, which act as deterrents to mole crickets, a sea turtle egg predator (Dutton and McDonald, 1995). The same study shows that hydrated yolkless eggs are more likely to be predated by mole crickets (Caut *et al.,* 2006). The more hydrated yolkless eggs present at the end of the incubation period, the less likely that the yolked eggs will complete their development. This supports the hypothesis that yolkless eggs might help maintain the physiochemical conditions within the egg chamber (e.g., moisture by dehydrated eggs) (Dutton and McDonald*,* 1995).

Data collected between 2006 and 2013 by the STC and in 2013 by COTERC will be used to study the effect of clutch size and the number of yolkless eggs on success (hatching and emerging success) of leatherback turtle nests. To answer the main research question the following sub-research questions will be studied: What is the influence of yolkless eggs on Hatching (HS%) and Emerging Success (EM%), number of depredated eggs within a clutch and on the destiny of the nest (predated/not predated). What is the influence of distance to high tide line (HTL) on HS%, EM% and destiny? And what is the influence of number of normal (yolked) eggs and clutch size on HS% and EM%?

# 2 MATERIALS AND METHODS

COTERC used the same turtle monitoring protocol as STC. Any differences will be mentioned in this section.

## 2.1 STUDY SITES

Playa Norte is one of the nesting beaches of leatherbacks, located within the Barra Colorado Wildlife Refuge (BCWR) in the Tortuguero Lowlands (Costa Rica). Since 2006 the Canadian Organisation for Tropical Education and Rainforest Conservation (COTERC) has been conducting the Marine Turtle Monitoring and Conservation Project on Playa Norte annually, under a research permit issued by MINAE (Costa Rican Ministry of Environment and Energy). Researchers, volunteers and interns are based at Caño Palma Biological Station (CPBS). The COTERC study area was a 3.125 mile (approx. 5Km) stretch of Playa Norte, that runned from the Tortuguero River Mouth (10º35’34.4”N - 83º31’28.6”W) to the north end of Laguna Cuatro (10º38’06.9”N - 83º32’31.7”W) (Figure 2, point A-B).

The Sea Turtle Conservancy (STC) is the oldest and most accomplished sea turtle organization in the world which conducts sea turtle research in the same area as COTERC. Researchers and volunteers are based at STC’S John H. Phipps Biological Field Station, located approximately 5 kilometres from CPBS. STC continues to monitor nesting trends, growth rates and reproductive success begun by Dr, Archie Carr in the 1950s. The STC study site was on Tortuguero beach, between the Tortuguero River Mouth (mile -3/8) to five miles south of the Tortuguero River Mouth (mile 5) and between mile 14 and the Jalova Lagoon (mile 18) (Figure 2.1, point C, D, E, F).

As the majority of females emerge at night, night patrols are necessary to collect behavioural and biometric data on these individuals. Each turtle that emerges within the study area is flipper tagged for future identification. If the night patrol team discovers the turtle before oviposition then eggs are counted (yolked and yolkless) and the nest triangulated for future excavation purposes. After the incubation period (~60-65 days), the nest will be excavated two days after seeing the first hatchling tracks or five days after depression. By counting the hatched, un-hatched and dead hatchlings in the excavated nest, calculations can be made to define the nest success of each triangulated nest.

**Figure 2.1:** Study site COTERC, Playa Norte within Barra Colorado Wildlife Refuge, Costa Rica (A = mile one, B = mile 3 1/8) and study site STC (C= mile -2/8, D = mile 5, E= mile 14, F= mile 18) ©Google Earth 2014.

At every 1/8 of a mile, from mile zero to mile 3 1/8 for the COTERC transect and from mile 3/8 to mile five for the STC transect, mile markers were posted to enable spatial analysis. Between mile five and the Parismina Rivermouth (mile 18) for the STC transect mile markers were posted at every 4/8 of a mile. Along Tortuguero Beach there were several permanent structures: Laguna Lodge (at mile 1 4/8) and Mawamba Lodge (at mile 2 3/8). Between mile 2 7/8 and mile 3 3/8 there is a village. Along Playa Norte there were also several permanent structures which consist of two lodges: Turtle Beach Lodge (at mile 2 4/8) and Vista al Mar Lodge (at mile 2/8) and several houses. Parallel to the beach, there was a path that was used by those on foot, bicycle, horseback or car.

Playa Norte and Tortuguero beach are nesting beaches for four different species of marine turtles. The most common nesting species is the Green turtle (*Chelonia mydas*). Table 2.1 provides the common and scientific names of the turtle species nesting in the Tortuguero area as well as their regional nesting season.

**Table 2.1:** Sea Turtle Species in the Tortuguero area. Common and scientific names with abbreviation used in manuscript and typical regional nesting seasons.

| Common and scientific names used in manuscript and typical regional nesting seasons. | | |
|---|---|---|
| **Common Name** | **Scientific Name** | **Nesting Season** |
| Leatherback | *Dermochelys coriacea* | March –June |
| Green Turtle | *Chelonia mydas* | May - October |
| Hawksbill | *Eretmochelys imbricata* | June – October |
| Loggerhead | *Caretta caretta* | May - October |

## 2.2 EXTERNAL MORPHOLOGY

Leatherback turtles are the largest of the seven sea turtles species (Bell *et al.*, 2004). They have a leathery, scuteless black or spotted carapace (carapace exists of small bones covered by leathery skin). The carapace length of an adult female is between 130 and 180 cm and is elongate with seven prominent longitudinal ridges (keels). In adults the head shape is broadly triangular, 25cm in width, with two prominent maxillary cusps and covered with unscaled skin. Adult females weigh between 300 and 500 kg. Leatherbacks are predominately black with variable degrees of white or paler spotting (Figure 2.2). The plastron is relatively small with very little bone (Pritchard and Mortimer, 1999).

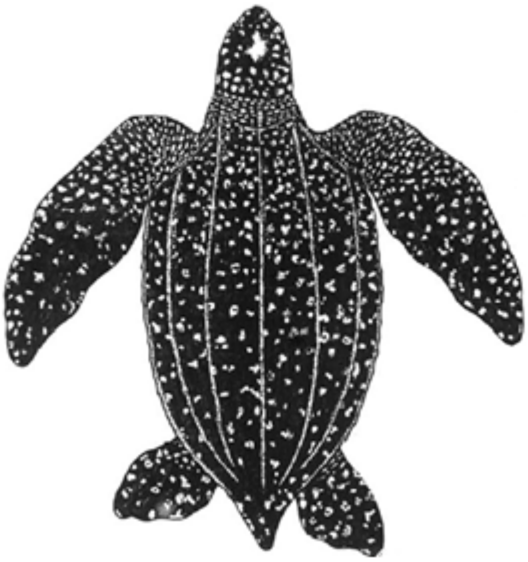

**Figure 2.2:** Top view of Leatherback turtle (Pritchard and Mortimer, 1999).

The entire surface of hatchlings (carapace, plastron and extremities) is covered with small, soft, polygonal scales, seven longitudinal carapace ridges (including edges of shell) boldly outlined in white against a black background. The forelimbs of hatchlings are almost as long as the carapace. The plastron is mottled black and white with an average length of 60 mm (Pritchard and Mortimer, 1999).

## 2.3 IDENTIFICATION BY TRACKS

Adult Leatherback tracks are the widest of the four nesting species on Playa Norte with widths between 150 and 230 cm (Pritchard and Mortimer, 1999). Tracks are symmetrical with diagonal marks made by the forelimbs and usually with a deep groove in the middle formed by dragging the relatively long tail (Figure 2.3). Leatherback turtles generally nest in the open zone, close to the high tide line (Mrosovsky, 1983; Eckert, 1987; Caut *et al,* 2006).

**Figure 2.3:** Leatherback Tracks (photo: Archie Carr National Wildlife Refuge)

## 2.4 DATA COLLECTION

The data are collected during Leatherback season in 2013 by COTERC and from 2006 till 2013 by STC. COTERC data were all collected in the turtle monitoring transect on Playa Norte and STC data were collected on three different beach sections from Tortuguero Beach (Figure 2.1). The same protocols were used by COTERC and STC.

### *2.4.1. NIGHTLY PATROLS*

Each night, Playa Norte was patrolled between mile 0 and mile 3 1/8, for a minimum of six hours. If there was only one team available patrols took place between 20:00 – 02:00. If two teams available then one team would patrol between 20:30 and 01:30 and the other team 23:00 and 04:00. STC patrolled Tortuguero Beach each night with varying frequency and on three different beach sections.

Only red lights were used and as little as possible (lights always faced away from the turtle).

When turtle tracks were found, the Patrol Leader (PL) determined if the turtle was still present or not. If the turtle was not present then the PL determined if the track was a HLF (Half Moon: non-nesting emergence) or NST (Nest) and the team proceeds to collect the following information:

- Date.
- Global Positioning System (GPS) Location and accuracy (accuracy <4m)[1].
- Species.
- Northern Mile Marker (Mile marker to the north of the nest or track).
- Time of encounter.
- Vertical position [2] [3](Figure 2.4):
    - * Open (O): Area of beach that receives 100% of sunlight.
    - * Border (B): Area of beach which is partially shaded by vegetation.
    - * Vegetation (V): Area of beach which is constantly shaded by vegetation.

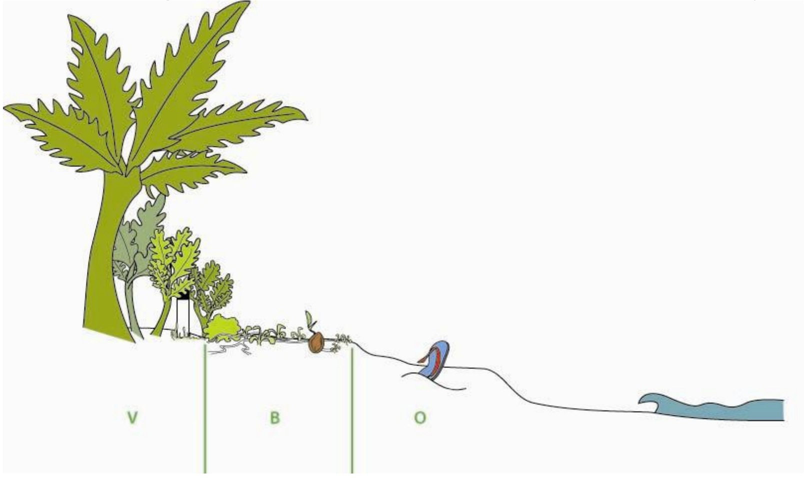

**Figure 2.4:** Vertical position of the nest: V=vegetation, B=Border, O= Open (COTERC, 2012).

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[1] Only COTERC recorded GPS location and accuracy

[2] Relates to the amount of sunlight a nest would receive, not actual vegetation composition.

[3] For HLFs and LIFs vertical position will be the most westward point of the track.
For NST vertical position will be where the eggs are believed to be.

If the turtle was still present then the PL approaches the turtle carefully to determine the nesting stage. Depending on the nesting stage appropriate action can be taken (Table 2.2). The following information was recorded after oviposition.
Type: Half Moon (HLF), Nest (NST), New Record (REC, turtle with no tags, turtle will be tagged), Re-emerger (REM, turtle already has tags), Deceased (DEC, dead turtle) or Hatchling tracks (HAT). To check if REMs have nested in the same season, the data base was checked. Turtles that return multiple times in a season are recorded as Renesters (REN).

**Table 2.2**. Patrol activities as they relate to nesting stage of the encountered female on Playa Norte, Costa Rica

| **Turtle Activities** | **Patrol Response** |
|---|---|
| **Emerging from sea** | Discreetly wait. |
| **Selecting nest site** | Discreetly wait. |
| **Cleaning nesting area**<br>**(Turtle uses all four flippers, carapace still wet and has little sand on it)** | Discreetly wait. |
| **Digging egg chamber**<br>**(Turtle uses rear flippers to dig a deep hole and pat down sand beside the newly dug egg chamber)** | PL and one other team member cautiously approach turtle from behind to prepare for egg counting. |
| **Oviposition** | Observer counts eggs by hand (when possible). Other team members begin triangulation of nest to permanent landmarks. |
| **Covering egg chamber**<br>**(Turtle uses rear flippers to cover egg chamber and pat down surrounding sand)** | Check for tags and/or scarring from lost tags. Apply tags if needed. Obtain biometrics and body check. |
| **Disguising**<br>**(Turtle uses all four flippers to throw sand behind her carapace and refill the body pit with surrounding sand. Carapace will usually be dry and covered by sand)** | Finish data collection and data completion check. |
| **Returning to sea** | Check for tags if possible. Observe. |

### 2.4.2. EGG COUNTING AND TRIANGULATION

When the turtle finished digging her egg chamber an observer would count the eggs physically by holding a latex gloved hand 5 – 10 cm below the cloaca and by feeling the eggs drop onto the hand. Yolked eggs were counted with an egg counter and yolkless eggs were counted separately in their head. When the first couple of eggs (2 – 3 eggs) were laid, the egg counter placed a nest ID (small piece of numbered tape) in the egg chamber which facilitates proper nest identification during excavation.

During oviposition the nest was marked using triangulation. An observer held the measuring tape directly above the centre of the egg chamber while counting the eggs. Palm trees, almond trees or other solid objects on the beach were used for triangulation. The distance from the centre of the egg chamber was measured to three points; central, north and south of the nest. Flagging tape was attached to these three points (knots faced direction of the nest). Central, north and south points had to have at least a 45 degree angle between each other. The distance to the most recent High Tide Line (HTL) was also measured (Figure 2.5).

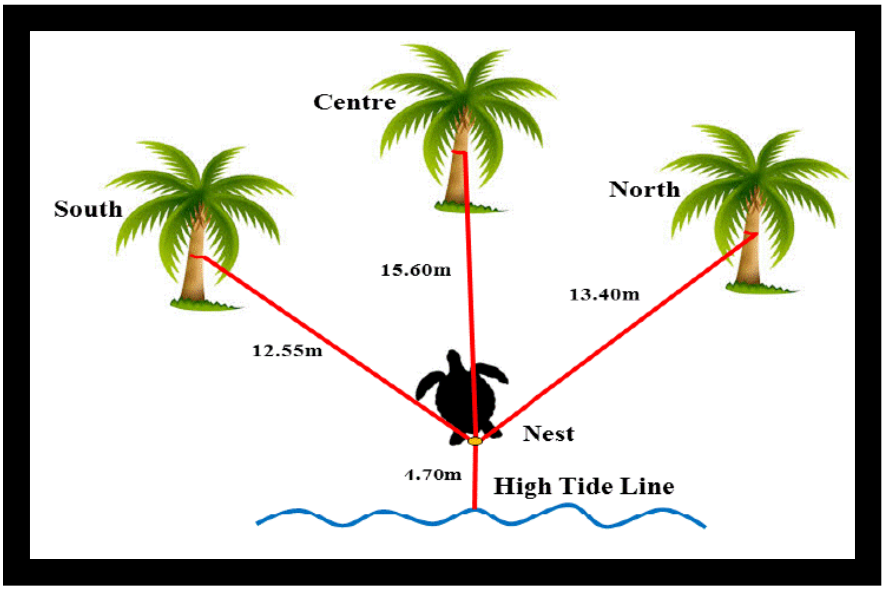

**Figure 2.5:** Triangulation (COTERC, 2013, Leatherback and Green training, unpublished).

### 2.4.3. TAGGING

After oviposition, flippers were checked for tags or tagging scars. Scarring from previous tagging such as Old Tag Notches (OTNs) or Old Tag Holes (OTHs) was recorded (Figure 2.6). If no tags were present then the Leatherback turtle would be tagged twice (one tag in each rear flipper) in the membrane between the rear flipper and the tail using National Band & Tag Co., Newport, USA Monel #49 tags.

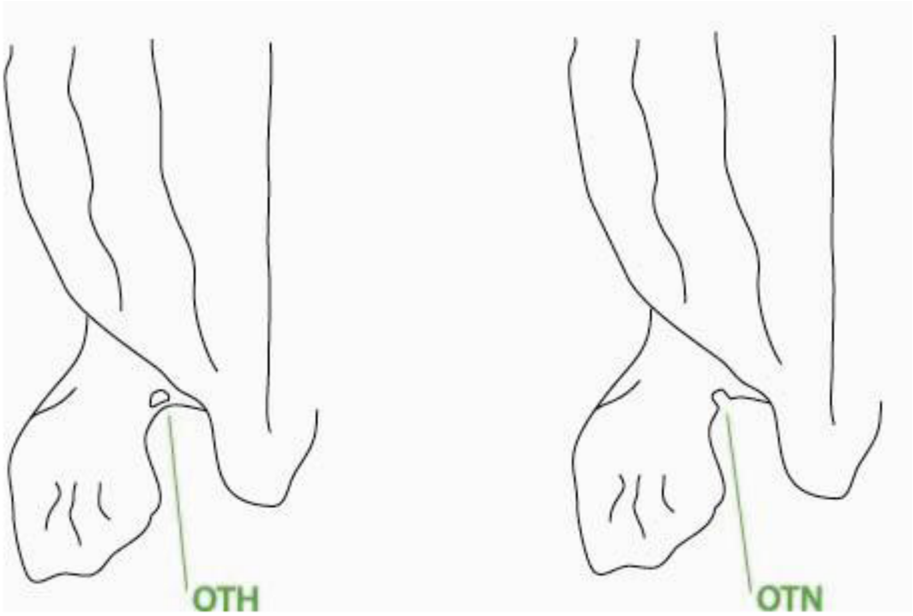

**Figure 2.6:** Old Tag Holes (OTHs) and Old Tag Notches (OTNs) in Leatherback turtles (COTERC, 2012).

### 2.4.4. BIOMETRICS AND BODYCHECK

Biometric data was obtained only for tagged Leatherbacks. Using a flexible measuring tape the minimum Curved Carapace Length (CCLmin) and the maximum Curved Carapace Width (CCWmax) was measured three times each, to the nearest millimetre.

CCLmin was measured from where the skin meats the carapace behind the head to the end of the caudal projection. Measures were made along the right side of the midline ridge. CCLmin was not measured along the crest of the ridge because irregularities in the ridge and the difficulty of keeping the tape on the ridge (Figure 2.7). CCWmax was measured from where the carapace meets the plastron, on the widest part of the carapace. For each turtle, the widest point may vary and therefore the anatomical location on the carapace where CCW will be measured may be not the same (Figure 2.7; Bolten, 1999). After measurements the turtle was checked for Fibropapilloma or other abnormalities (scars, damaged tissue, tumours, etc).

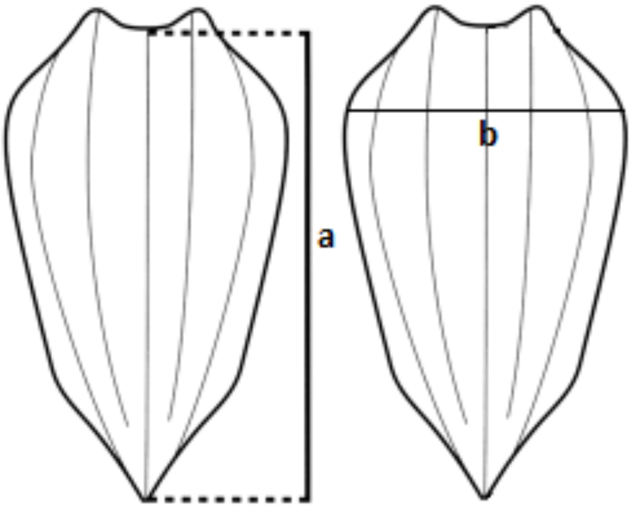

**Figure 2.7:** **a:** Position for measuring minimum curved carapace length. From the anterior edge of the carapace at midline to the end of the caudal projection **b:** Position for measuring maximum curved carapace width (modified from Bolten, 1999).

### 2.4.5. DISGUISING

After data collection the tracks from the female were disguised to prevent double counting and poaching of the nest. Only the tracks were disguised as leatherbacks nest are leatherback eggs are rarely poached on Playa Norte (Fernández, personal comment) and leatherbacks disguise their nest well.

### *2.4.6. MORNING CENSUS*

Every morning a morning census team, consisting of a minimum of two people, walked the transect (from mile 0 to mile 3 1/8) to check for any tracks that were missed by the night patrol team and to check the status of the nests. Morning census took place around sunrise (typically between 05:30 am and 06:00 am).

New encountered tracks were categorized as half moons (HLF), nests (NST) or Hatchling tracks (HAT) (only for marked nests). If a new nest was marked in the night, the morning census team would check if the triangulation was correct by re-triangulating (Figure 2.8). If the triangulation measurements did not coincide, the night patrol team that marked the nest would triangulate the nest again in the afternoon. The nest status and flagging tapes of all marked nests were checked every morning during morning census in the following categories:

- Natural (NAT): Normal condition.
- Poached (POA): Nest has signs of poaching (stick holes, human footprints, normally no egg shells around the nest).
- Predated (PRE): Nest has signs of predation (dog or other animal footprints, egg shells around the nest, nest chamber normally exposed).
- Wet (WET): Nest is wet but not completely inundated.
- Flooded (FLO): Nest has been completely inundated, puddle on top.
- Eroded (ERO).
- Unknown (UNK): If not sure what the condition of the nest was, the nest was classified 'Unknown'. For instance, nest has signs of poaching (footprints and stick holes) but egg shells are found around the nest and the nest chamber is exposed (signs of predation).

Flagging tapes were checked daily if still present or for any degradation by ants or termites and if the knot was still facing the direction of the nest.

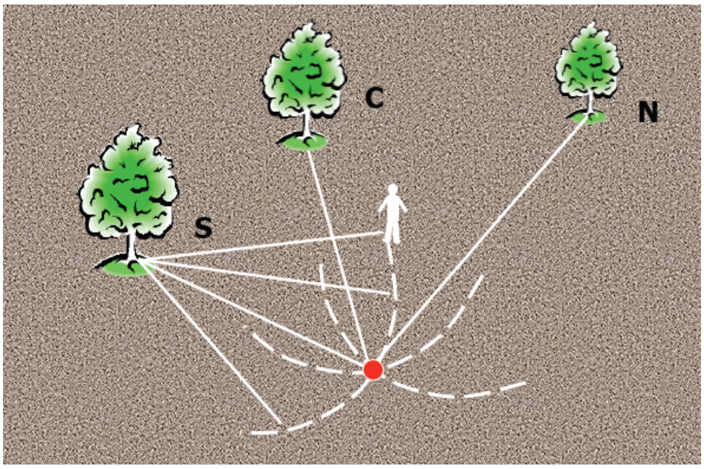

**Figure 2.8:** Triangulation check by morning census team. Distances recorded by night patrol team are measured again, and lines are drawn in the sand . Where the lines cross is the location of the nest (COTERC, 2013, Leatherback and Green training, Unpublished).

After 60 days triangulated nests were monitored for signs of hatching. Signs of hatching include a volcano-shaped depression in the nest area caused by hatchlings digging their way to the surface inside the nest; and hatchling tracks leading away from the nest. The exact location of the nest was re-established by re-triangulation and the nest was marked with three sticks (depression sticks) to easily find the nest the next morning census without triangulating (figure 2.9).

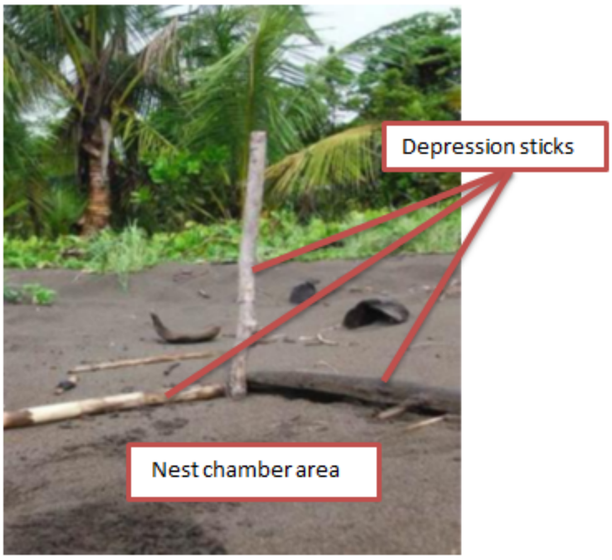

**Figure 2.9:** Depression sticks set up at 60 days of incubation to easily check the nest for depressions (photo by COTERC).

Possible depressions were first visually detected and then with the help of a pencil that was gently pushed into the depression area. If the sand underneath gave way very easily it was considered a depression.

### 2.4.7. INCUBATION DURATION AND NEST SUCCESS

Incubation duration was determined by counting the days since the eggs were laid to the date the first hatchling tracks were observed. Two days after the first sight of hatchling tracks the nest was excavated. In case of triangulated nests where no evidence of emergence was observed, the nest was excavated after 75 days of incubation. The following data were collected from each excavated nest:

- Egg Depth (cm): Distance between sand surface and first egg (shell) encountered.
- Nest Depth (cm): Distance between sand surface and bottom of the egg chamber.
- Number of yolkless eggs.
- Number of hatched eggs (shells (S) ≥50% of original shell are counted).
- Number of hatchlings in-nest (Alive (L) and dead (D)).
- Number of un-hatched eggs (UH):
    - * Without embryo: No signs of embryo (infertile egg).
    - * With embryo (Figure 2.10):
        - Stage 1: Embryo occupies less than 25% of the egg.
        - Stage 2: Embryo occupies between 25% and 50% of the egg.
        - Stage 3: Embryo occupies between 50% and 75% of the egg.
        - Stage 4: Embryo occupies between 75% and 100% of the egg.
    - * Unknown: Embryo has been predated/destroyed (P) and determination in what state development stopped is impossible.
    - * Number of pipped eggs: Hatchling broke through the shell, but failed to emerge from the shell (UHT).

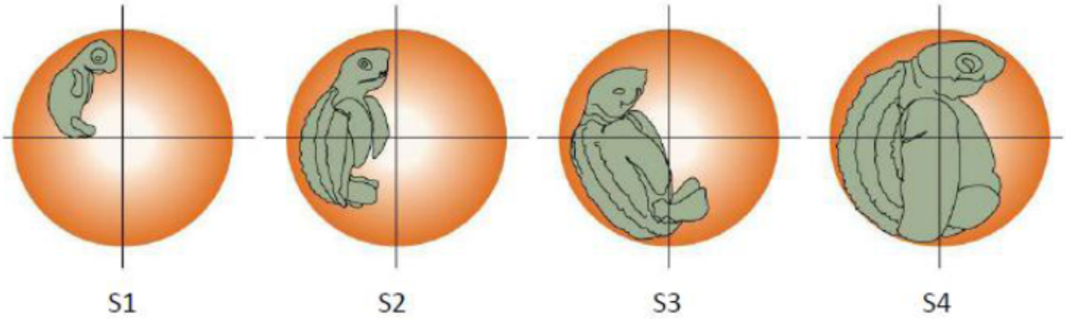

**Figure 2.10:** Embryonic stages used as criteria during nest excavations (Chacón *et al,* 2007).

## 2.5 DATA ANALYSIS

Hatching success refers to the number of hatchlings that emerge out of their shell. This number should be equal to the number of empty shells in the nest. Emergence success refers to the number of hatchlings that reach the beach surface. This number should be equal to the number of empty egg shells in the nest minus the number of dead and alive hatchlings remaining in the nest chamber (Table 2.3, Miller, 1999). Mean success rates will be calculated by averaging the success rate of each nest.

**Table 2.3:** Classifications, descriptions and formulas used for determine hatching and emerging success rates described by Miller (1999).

| **Classification** | **Description** |
|---|---|
| Shells **(S)** | Number of empty shells counted (≥50% complete) |
| Live in nest **(L)** | Live hatchlings left in nest |
| Dead in nest **(D)** | Dead hatchlings that have left their shells |
| Undeveloped **(UD)** | Unhatched eggs with no obvious embryo |
| Unhatched **(UH)** | Unhatched eggs with obvious embryo |
| Unhatched term **(UHT)** | Unhatched apparently full term embryo in egg shell or pipped |
| Predated **(P)** | Nearly complete shells containing egg residue, includes shells predated by animals, bacteria, fungi and vegetation |
| **Hatching success (HS%)** | **#Shells/(#Shells+#UD+#UH+#UHT+#P) x 100** |
| **Emerging success (EM%)** | **#Shells-(#L+#D)/(#Shells+#UD+#UH+#UHT+#P) x 100** |

All relocated nests were left out of the analysis as these were not natural circumstances. Nests with an inaccurate or missing egg-count (based on field notes) as well as nests where the Nest ID was not found during excavation were left out of the analysis as there could not be determined if it was the marked nest or unmarked nest.

A two way ANOVA was used to check if Playa Norte and Tortuguero beach had comparable levels of leatherback nest success (HS% and EM%). As there was no significant difference between the HS% and EM% of Playa Norte and Tortuguero beach (p=0.54; p=0.77) data of COTERC and STC were combined for the analysis.

The effect of yolkless eggs and clutch size on HS% and EM% was tested using a linear regression analysis with HS% and EM% as dependent variables and number- and percentage of yolkless eggs within a clutch and clutch size as independent variables.

The effect of yolkless eggs on the percentage of depredated normal (yolked) eggs within one clutch was tested using a linear regression analysis with the percentage of depredated eggs as dependent variable and number and percentage of yolkless eggs as independent variable. A logistic regression analysis was applied to test the influence of number of yolkless eggs and distance to HTL on the nest-state at the end of incubation. Nest-state was categorized in two groups: Predated (partially predated and predated) or not-predated (poached, partially poached, washed, eroded nests and natural nests).

Variables used for regression analysis were tested for normality with the Shapiro-Wilk test. The skewness (measurement of the deviation of the distribution from symmetry) of a normal distribution is close to zero, as normal distributions are symmetrical. The kurtosis (measurement of ‘peakedness’ of the distribution) of a normal distribution is zero. If the skewness and kurtosis are clearly different from zero, then the variable is not normally distributed. All statistical analysis were applied using IBM SPSS Statistics 20©.

# 3 RESULTS

The results from all regression analysis where the depended variable was not normally distributed are not completely reliable. Regression analysis should only be applied if the dependent variable is normally distributed. The scatterplots give a good impression if there is a relationship between both variables or not.

## 3.1 HATCHING AND EMERGING SUCCESS

In total 160 nests recorded between 2006 and 2013 by STC and 15 nests recorded in 2013 by COTERC were used for statistical analysis with HS% and/or EM% as dependent variables.

The average number of normal yolked eggs laid within one clutch was 68.79 (SD = 18.72) and the average number of yolkless eggs laid within one clutch was 33.21 (SD=15.94). Average HS% and EM% were respectively 42,65% (SD=30.76) and 39.21% (SD= 29.67). Yolkless and yolked eggs were normally distributed (respective- skewness = 0,108; -0,21, kurtosis = -0,516; 0,181). HS% and EM% were not normally distributed (respective- skewness = -0,077; 0,092, kurtosis = -1,241; -1,162).

The number of yolked eggs within a clutch has a very highly significant effect on HS% (p = 0.001) and EM% (p = 0.000; figure 3.1). There is a small decline in HS% and EM% if the number of yolked eggs increase (respectively B= -0,147 and B=-0,162). Clutch size (number of yolked + yolkless eggs) has no significant relationship with HS% or EM% (respectively p = 0,122; p = 0,065).

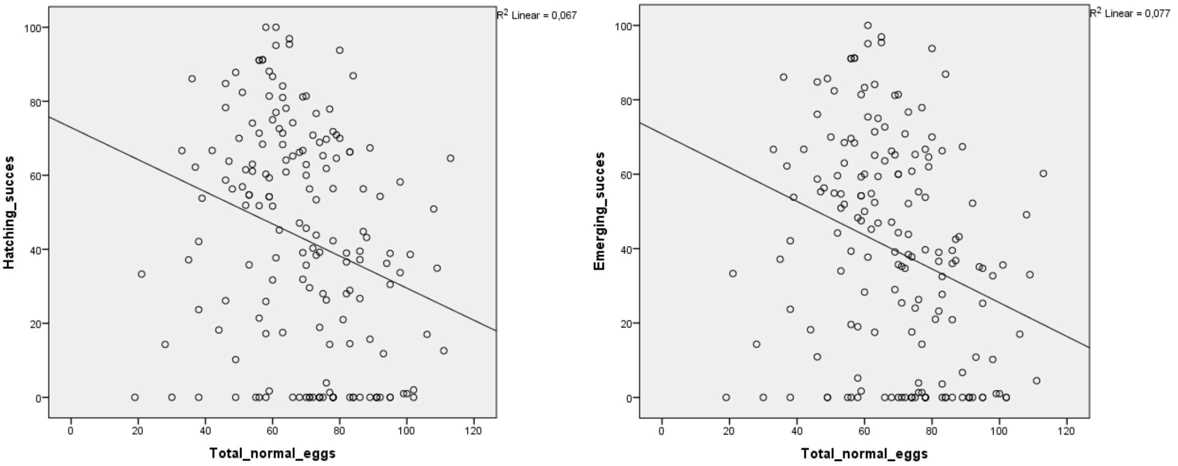

**Figure 3.1:** Scatterplots including regression lines with hatching success (left) and emerging success on y-axis and total number of yolked (normal) eggs on x-axis.

The number of yolkless eggs within a clutch has no significant effect on HS% and EM% (p= 0,34; p = 0,28; Figure 3.2).

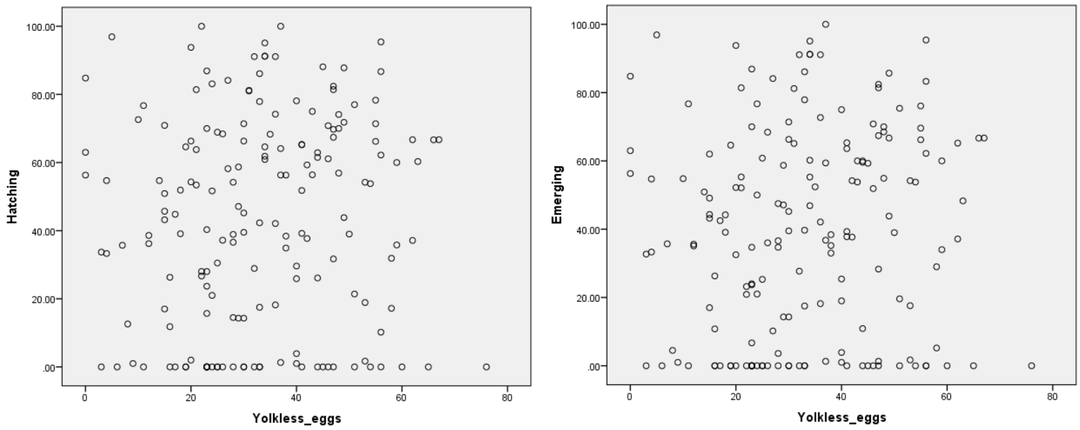

**Figure 3.2:** Scatterplots with Hatching success (left) and Emerging Success (right) on y-axis and number of yolkless eggs within one clutch on the x-axis.

## 3.2 NEST LOCATION

93% of all nests laid between 2006 and 2013 were located in the open zone. The remaining 7% were located in the border zone. No nests were laid in the vegetation zone (figure 3.3).

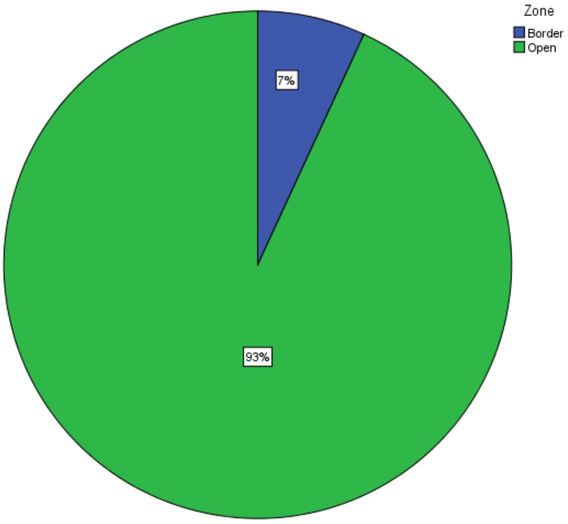

**Figure 3.3:** Percentage of nests laid within border and open zone between 2006 and 2013.

Distance to HTL has no significant effect on HS% (p = 0.907) and EM% (p = 0.826). Distance to HTL was normally distributed (skewness = 0,624; kurtosis = 0,212

## 3.3 PREDATION

7.3% (3.4 + 3.9) of all nests that were included in the statistical analysis (n = 178) were predated or partially predated (Table 3.1) The number of yolkless eggs had no significant effect on the percentage of depredated normal eggs within one clutch (p = 0.62). Number of normal eggs within a clutch did have a positive significant effect on the number of depredated eggs within a clutch (p < 0.01; B = 0.127; figure 3.4).
The number of depredated eggs were not normally distributed (skewness = 2,126; kurtosis = 7,085).

**Table 3.1:** Frequency's and percentages of hatched, predated, partially predated and unhatched nests laid on Tortuguero Beach between 2006 and 2013 and on Playa Norte in 2013.

| Destiny of nest | | | | | |
|---|---|---|---|---|---|
| | | Frequency | Percent | Valid Percent | Cumulative Percent |
| Valid | Hatched | 131 | 73,6 | 73,6 | 73,6 |
| | **Partially predated** | **6** | **3,4** | 3,4 | 77,0 |
| | **Predated** | **7** | **3,9** | 3,9 | 80,9 |
| | Unhatched | 34 | 19,1 | 19,1 | 100,0 |
| | Total | 178 | 100,0 | 100,0 | |

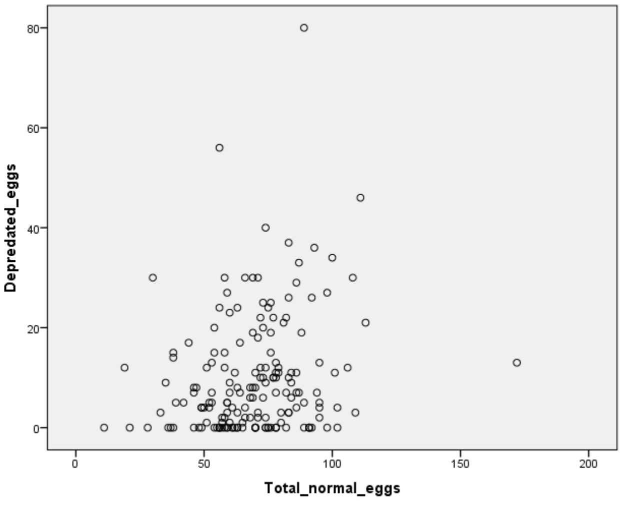

**Figure 3.4:** Scatterplot of the number of depredated eggs within a clutch on the y-axis and number of normal eggs within a clutch on x-axis.

# 4 DISCUSSION AND CONCLUSION

Leatherback hatching and emerging success has been studied on a number of different nesting beaches around the world and show a variety of different numbers ranging between 20%-70% (Caut *et al*, 2006). Bell *et al* (2004) found that HS% differs between females, with some mothers having success rates between 71-81% and some between 23-32% (Las Baulas, Costa Rica). Success rates on Playa Norte and Tortuguero Beach differ between 0 and 100% with an average of 42.88 HS% and 39.43 EM%. HS% and EM% in this study were not normally distributed which can be expected by the high SD found (SD = 30.82 and 30.02). This could be explained by the high diversity in success rates between mothers (Bell *et al*, 2004). The average number of yolked eggs within a clutch laid was 69.38 and the average number of yolkless eggs laid was 33.15. Maros *et al* (2003) and Bell *et al* (2004) found the same averages for number of eggs (58-114 yolked; 21-56 yolkless).

No significant relation has been found between number or proportion of yolkless eggs and HS% and EM%. However, no data of the state of the yolkless eggs (hydrated, dehydrated or predated) was available for statistical analysis. Caut *et al* (2006) only found a significant relation between dehydrated yolkless eggs and HS%. Not only dehydrated yolkless eggs, but the interaction between distance to HTL and distance to vegetation line increase HS% (Caut *et al*, 2006). No relations have been found between distance to HTL and HS% and EM% in this study and no data of distance to vegetation has been collected.

Dutton and McDonald (1992) also studied the effect of yolkless eggs on hatching success. They reburied some clutches with all the yolkless eggs and some without any on the same areas of the beach (Sandy Point, St. Croix in the US, Virgin Islands) and the same season. No significant difference was found in mean hatching success between the clutches with and without yolkless eggs. Yolkless eggs are generally the last eggs laid in a clutch (Caut *et* al, 2006). One of the hypotheses in Dutton and McDonald's study (1992) was that yolkless eggs may satisfy or partially satisfy predators before they encounter the viable eggs below. They recommend repeating this study on a beach were predation is more of a threat to leatherback nests than on Sandy Point. Only 7.3% of all the nests laid on Playa Norte and Tortuguero Beach together were classified as predated of partially predated.

Number of normal eggs within a clutch had a negative influence on HS% and EM% and a positive influence on the number of depredated eggs within a clutch. Even though the influences of normal eggs on the nest success are minimal, they are significant. Stancyk *et al* (1980) found no difference in hatching success between whole and partial clutch-transplants and control loggerhead nests on the beach. Also no relation has been found by Wyneken *et al* (1988) between clutch size and proportion of eggs depredated by bacteria. Notably, only the number of normal eggs within a clutch had an influence on nest success and clutch size (number of normal- + yolkless eggs) had no influence on nest success.

To gain more insight in the effects of yolkless eggs on nest success it is highly recommended to collect more data on yolkless eggs. Collecting data on the state of yolkless eggs at the end of incubation might give more insight in possible interactions between nest location, yolkless eggs and nest success (Caut *et al*, 2006).

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