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Vulnerability of loggerhead turtle eggs to the presence of clay and silt on nesting beaches

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ABSTRACT

Sea turtle nests usually suffer a high mortality on important nesting grounds. Understanding the main factors that influence hatching success and productivity on important rookeries of endangered populations is essential to properly manage and protect them. The amount of clay can be high on some nesting beaches and could affect egg incubation. In the main loggerhead rookery in the Eastern Atlantic (Boa Vista, Cape Verde), clays and silts are very common on near 10% of the main nesting beaches, and turtles do not avoid clay substrates when they lay their eggs. Nests incubated on sandy substrates rich on clay and silt had a very high mortality. The same occurs on nests with the eggs stained with clay and incubated in sand free of clay. The eggs experimentally incubated with a covering layer of clay (30%, 50% and 80% of the eggshell) suffered an important loss of water, which in the extreme cases was irreversible, causing the death of the embryos. 75% of the eggs with 80% of their eggshells covered with clay died, while the mortality rate of the control eggs was only 25%. The salt content of the clay/silt seems not to be responsible for the egg dehydration and death. The hatchlings born from eggs with only 30% of the eggshell covered by clay were slower that those born from control eggs. Substrates with a significant presence of clay/silt can seriously disrupt embryonic development, reducing the emergence success of nests and should be avoided on nest relocation programs.

1. Introduction

The environmental features of the incubation site of the nest (Packard and Packard, 1988; Ackerman, 1991) directly affect emergence success of sea turtle nests. Physical, chemical and biological properties of the egg incubation chamber decisively determine parameters such as temperature, moisture of the substrate and exchange of gases, which establish the conditions for egg incubation (Read et al., 2013; Fisher et al., 2014; Patino-Martinez et al., 2014; Cheng et al., 2015). These variables are highly influenced by the specific characteristics of the nesting site, such as the grain size of the substrate, the distance or height from the sea, the slope of the beach, the vegetation, the salinity, the compaction of the substrate, the organic matter content, etc. (Mortimer, 1995; Garmestani et al., 2000). The effect of substrate grain size on embryonic development and its relationship to the movement of water and gases within the nest have been widely studied in sandy substrates (Foley et al., 2006; Mann, 1977; Speakman et al., 1998), but they have been much less studied in substrates with clay and/or silt (Ackerman, 1991).

Beaches are composed mainly of sand, but they are in fact mixtures of different types of substrates: sand, silt (0.0625–0.0039 mm) and clay (<0.0039 mm), which provide diverse properties to the incubation environment of sea turtle nests. The soil deposits in beaches can be a mixture of materials of diverse origins: marine (marine deposits), continental from soil erosion (alluvial deposits), and/or aeolian, transported by the wind (aeolian deposits and sand sheets). The clay content is usually higher in dune sand compared to beach sand (Shepard and Young, 1961).

On beaches near river deltas or coastal wetlands, shallow substrate layers with a significant amount of clay and silt can be found (Trenhaile, 2009; Collins and Sitar, 2011). Moreover, silt and clay can be deposited on beaches close to estuarine rivers and in areas where beaches are contiguous to steep clay slopes. The amount of small size clay/silt particles present can be quite high on beaches in areas affected by strong erosion processes; some human activities such as deforestation, intense underground freshwater exploitation or excessive cattle load grazing, can favor the erosion and accumulation of clay and silt on beaches. The percentage of clay/silt in these substrates can have a significant effect on embryonic development, on nest mortality (personal observations; Sarmiento-Ramírez et al., 2014) and, possibly, on the biological characteristics of the hatchlings. Due to characteristics such as small grain sizes (<2 μm), low permeability, low drainage,

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high cationic exchange and high compaction properties, substrates with clay and silt are poorly ventilated soils with high water retention, and, high salt retention in the small interstitial spaces, which can dehydrate the eggs compared to other sandy substrates (Hillel, 1980). However, the effect of substrates with clay and silt on the incubation of sea turtle nests has not been properly studied (Garrett et al., 2010; Sarmiento-Ramírez et al., 2014).

The nesting behavior of the seven sea turtle species is similar (Miller, 1995). They exhibit strong philopatry in their nesting (Lee et al., 2007; Carr and Stancyk, 1975) and, in some species and instances show strong nesting-site fidelity (Lee et al., 2007; Hatase et al., 2002; Schroth et al., 1996; Carr and Stancyk, 1975). The knowledge of the environmental and behavioral factors that control nest-site selection is still very limited (Miller et al., 2003). In some cases, a high number of nests are laid in areas that are unsuitable for incubation, which causes high mortality in natural nests (personal observation). For example, in the Cape Verde islands, an important and threatened loggerhead rookery in the Atlantic (Marco et al., 2012), turtles searching for a nesting site often encounter muddy areas. In some instances when a turtle enters the mud, it finds it harder to crawl, gets exhausted and dies in the heat of the day. On many other occasions, females nest in areas close to these muddy areas, laying their eggs on substrates with varying amounts of silt and clay. This study evaluates the impact of clay and silt on embryonic development in the loggerhead turtle (*Caretta caretta*). The specific objectives of this experimental study are to evaluate: 1) the impact of clay/silt on eggs that have been naturally stained with clay/silt, 2) the effect of the substrate with clay/silt on the incubation of the entire nest, 3) the effect of clay/silt covering different percentages of the eggshell, 4) the effect of clay/silt covering the eggshell area where the embryo is attached, 5) the response of clay/silt stained eggs to two substrates with different moisture contents, and 6) the combined effect of salt and clay/silt on embryonic mortality, on weight loss in the eggs, the hatchling mass, size and fitness. Understanding the critical levels of silt and clay that eggs can be tolerated during incubation can be very important to the management of endangered turtle rookeries.

2. Methodology

This study was carried out on the island of Boa Vista, Republic of Cape Verde (West Africa, 16,18°N 22,92°W), during the 2005 and 2006 nesting seasons (from late June to early October). Fourteen samples of substrates from beaches with different levels of clay and silt were analyzed in 2005 following standard methods for soil analysis (Page et al., 1982; ASTM, 2007). Substrate electric conductivity was measured using a conductivimeter (HANNA Instruments, accuracy of 0.01 μS/cm). The calibration was made at the same temperature than soil solutions were measured. For the mineral particle size analysis, the substrates samples were dried up in an oven at 100 °C. First grinding the soil to disaggregate it and then passing it through a 2 mm sieve eliminated gravel. What remains in the sieve was weighed and the coarse, medium and fine sand as well as clay and silt were separated using the correspondent sieves. Mesh size to separate clay and silt was of 0.0625 mm and to separate clay was of 0.0039 mm. Clay and silt were separated by sedimentation of a solution made of clay and silt in water (Gee and Bauder, 1986).

2.1. In-situ nests

Physical characteristics of loggerhead nests were studied during the 2005 season in the Reserva Natural da Tartaruga, the main nesting area on Boa Vista Island that hosts around 75% of the nests laid on the island (Marco et al., 2012). This field study was conducted from Ponta Medronho (16.005° N, 22.760 W) to Ponta do Roque (16.085° N, 22.667° W) on 8 beaches including 5 with the highest nesting density: João Barrosa (2700 m), Ponta Cosme (1800 m), Ervatão (670 m), Calheta (375 m) and Lajedo Teixeira (150 m) (Fig. 1). All of these beaches are pristine, deserted, and without any significant human influences. The dune area is reduced and features low halophytic shrubs and a high density of ghost crabs (*Ocypode cursor*) that heavily depredate loggerhead nests (Marco et al., 2015). Using stratified sampling that considered both the spatial and temporal variability on the 8 beaches, 1550 nests (around 30% of the total number of nests laid in the area during that season) were selected in. The presence of significant amounts of clay and silt in the incubation chamber was determined in all studied nests. Experienced observers visually determined whether a nest was rich in clay and silt during the nest excavation process by the female based on how dark the color of silt and clay is in the area compared to the light color of the sand.

Moreover, during 2005 and 2006, another 29 in-situ nests were marked and monitored throughout the incubation period on the beaches of Ervatão (N = 7), Ponta Cosme (N = 15) and Benguinho (N = 7). The size of the clutch and the nest depth were measured during oviposition. The exposure of nests to high levels of humidity or flooding was determined by visual observation of the nest surface during the daily nest monitoring throughout the entire incubation period. Sand surface of these flooded locations was flat and compacted. The substrates that showed clay and/or silt in the incubation chamber were classified as "Clay"; the substrates composed mainly of sand, showing a relevant absence of silt and clay and located in non-flooding zones, as "Sand"; and the sandy, humid substrates, due to frequent flooding, as "Flooding". The nests remained on the beach in their original location during the whole incubation period until they hatched or until 70 days after they were laid. At day 45 of incubation, a round plastic mesh (diameter: 45 cm, height: 50 cm) enclosure was placed on the surface of the sand around the nest to hold the hatchlings as they emerged. After day 45 of incubation, each nest was checked at night and at dawn to record hatchling emergence. Nests were excavated 5 days after the last recorded emergence. The excavated nest contents were classified into hatched egg shells, dead hatchlings, dead pipped hatchlings, live hatchlings, live pipped hatchlings, eggs without apparent development and unhatched eggs in order to calculate the emergence success of the nest. In some instances, when emerged hatchlings escaped from the plastic enclosure, the emergence success was calculated using the number of complete empty eggshells collected during the nest excavation or the number of emerged hatchlings, depending on which of the two parameters was greater.

The incubation temperature was recorded in 27 natural nests using automatic Stow Away TidbiT Onset data loggers with an accuracy of ±0.3 °C. They were programmed to record a temperature every 30 min. In order to minimize nest manipulation, temperature loggers were placed at night, while the turtles were laying the eggs, approximately in the middle of the egg-laying (egg no. 40), according to the average size of the egg-laying of this population (Varo-Cruz et al., 2007). In order to detect possible deviations in the temperature measurements, the loggers were compared to each other before and after the processes. This was done by keeping the temperature loggers together during 24 h, before and after the data collection. The mean of all thermometers was calculated for each data collection and then the deviation of each thermometer with regard to the collective mean was obtained. Although no thermometer deviated >0.3 °C (device error), these deviations were added or subtracted to the temperature data recorded by the temperature loggers in order to correct the records.

2.2. Hatchery experiments

2.2.1. Experiment 1. Effect of eggs having the eggshell covered by clay and silt on incubation in a sandy substrate

From August 6th to October 15th of the 2005 nesting season, 50 loggerhead nests with a low probability of survival (flooded by the tide or deposited in areas with clay and silt) were relocated to an on-beach hatchery (50 m × 15 m × 0.5 m). 25 of them were removed from natural clay/silt substrates and eggs were totally stained with clay and silt. The