

Short note | Nota breve

Environmental and management factors affecting embryonic development in the loggerhead turtle *Caretta caretta* (L., 1758): implications for controlled egg incubation programmes

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This thesis aims to contribute to our knowledge of the reproductive biology of the loggerhead turtle *Caretta caretta* (L., 1758) in general and the environmental factors affecting embryonic development in particular. Ways of improving current techniques for the management and conservation of sea turtles were assessed and new methods, aimed at increasing the productivity of populations, are proposed.

From 2005 to 2008, field and experimental studies were conducted on the nesting population of loggerheads on the island of Boavista, Cape Verde Islands. Objectives were 1) to improve knowledge of the physical-chemical characteristics of loggerhead nests, 2) to improve knowledge of the factors that influence egg incubation and their eco-physiological and biomechanical effects on embryonic

development, and 3) to investigate the effects of egg manipulation on embryonic development. The study also aimed to establish criteria for the technique most suitable for protecting nests, to evaluate the most effective protocols for the implementation of these techniques, and to contribute to the monitoring and conservation of the loggerhead nesting population on Boavista. The thesis has seven chapters, which present the results of an equal number of independent studies.

The island of Boavista, and, in particular, the *Reserva Natural das Tartarugas* (Sea Turtle Nature Reserve), is one the main nesting sites for loggerheads in the Cape Verde Islands, as well as throughout the eastern Atlantic Ocean. The Nature Reserve stretches for 12 km along the southeastern coast of Boavista and is the third most

important site worldwide for loggerhead hatchling production, rendering its protection and conservation essential. On Boavista, the most important area for hatchling production is the ca. 10 km stretch of beach between Ponta do Roque and Ponta Medronho, located within the *Reserva Natural das Tartarugas*. Here, between 200,000 and 300,000 eggs may hatch each year, i.e. 65-70% of the total number of hatchlings on the island. Along the western coast of Boavista few turtle young hatch, while ca. 10% and 15% of young hatch on the northern and eastern beaches of the island, respectively.

The analysis of data from daily counts of nesting activity during the 2005 nesting season showed a great daily variability among different beaches (from João Barrosa to Ladjedo Teixeira) in the number of nests, nesting success and incubation success. It was found that some beaches, such as Ponta Cosme and Curral Velho, where a large number of nests are laid, produced few hatchlings. Calheta and João Barrosa, despite being the beaches with the greatest production of hatchlings, do not host the largest number of nests. Very high densities of nests per linear metre of beach were found in the study area, with a maximum of 2.9 nests at Ladjedo Teixeira beach. This is the highest density of loggerhead nests documented worldwide.

By taking into account the initial clutch size and the number of exhumed nests, it was estimated that the mean emergence success of nests on Boavista, calculated by the number of nests on each beach studied, is 31.3% for the island as a whole. The fertility rate of nests was measured using two different techniques: 1) through observation of the embryonic white spot (animal pole) between 12 and 96 hours after oviposition, and 2) through analysis of the material obtained in exhumed nests. The average fertility rate of loggerhead nests on Boavista is higher than 93% (measured with the white spot technique). It varies between 75% and 100% among nests and does not change seasonally. The results indicate that, at this moment, fertilization of eggs, abundance of breeding males and sexual health are adequate to guarantee an optimal reproductive success. The estimation of fertility rate through the observation of the embryonic white spot is a precise, simple and

relatively unsophisticated method, which can be carried out in the field without affecting emergence success of nests or sacrificing embryos. Estimation of fertility rate through exhumation of nests and assessment of dead eggs has proven to be a very imprecise technique and its application is not recommended. The main causes of nest mortality in the field were natural: tidal flooding, predation by the ghost crab *Ocypode cursor* (L., 1758) and deposition of clay and silt substrates. Nests located in the flooding zone had a mortality of 100%.

The nesting and incubation season (June-October) of the loggerhead turtle in Cape Verde coincides with the warmest period of the year. During the study, incubation temperature of the nesting substrate was recorded at different beaches on Boavista (north, south, east and west) with temperature loggers programmed to record the temperature every 30 minutes. The incubation temperature in natural nests was also recorded. During the nesting seasons 2005 to 2008, the average temperature at incubation depth was 29.7°C. Significant interannual, seasonal and spatial variations were found in incubation temperatures, which complicated the estimation of sex ratio of hatched young. Average temperature during the second third of the incubation period varied between 27.9°C and 31.4°C. The average sex ratio among hatchlings was 78% female, but this value can decrease to 47% in years with low temperatures. The average incubation time was estimated to be 57 days. Apparently, sex ratio on Boavista is more balanced than in other populations in the Atlantic and Mediterranean. However, calculating how incubation temperature would change if environmental temperature increased 1 or 2°C, as has been predicted by some for the next decades, shows that the proportion of females would increase to 89.4% and 95.5% respectively.

Natural nests in substrates with a high content of clay or silt had higher mortality rates than nests in sandy substrates. Experiments showed that clay and silt substrates can cause mass mortality in nests and that those nests relocated to hatcheries with traces of clay or silt had higher mortality rates than nests in substrates without. Eggshells that were exposed to clay and silt in the laboratory suffered from

severe dehydration and high and fast embryo mortality. A higher percentage of clay produced more severe egg dehydration and its influence on embryonic development may have lethal effects. It is therefore recommended to relocate nests laid in a substrate containing significant amounts of clay to sandy non-flooding beach areas. Alternatively, they can be relocated to hatcheries with substrates composed of medium or coarse-grained sand, the temperature suitability of which has been previously analyzed.

Even though the eggs of this marine animal are incubated on the beach, loggerhead eggs are highly vulnerable to salinity levels lower than those of sea water. In laboratory experiments, in which eggs were exposed to different salinity levels, it was estimated that a salt concentration of 15.2‰ had a lethal effect on half of the population (LC50) during the whole length of incubation. Samples of natural incubation substrates from Boavista beaches were analyzed, which did not show salt concentrations toxic to eggs. Sub-lethal salt levels increased the time hatchlings needed to completely reabsorb the yolk after hatching and reduced weight and size of the offspring.

Field experiments were carried out by relocating nests 0 h, 12 h, 24 h, 84 h and 96 h after egg deposition. Results showed that nests can be safely and effectively relocated

at night or during daytime up to 96 hours after being laid without affecting their emergence success or the hatchling's phenotype. However, in case of delayed nest relocation, more attention needs to be paid to egg handling and transporting in order to avoid sudden movements and rotation. By improving nest protection programmes, delayed relocation can help save a higher number of nests at risk that would not be relocated if traditional relocation procedures – which dissuade from relocating nests later than six hours after oviposition – were followed. It can also improve beach monitoring at night and female tagging, which would reduce human interference in the egg-laying of sea turtles.

An experiment was carried out in the nest hatchery by incubating eggs at different depths (35 cm, 40 cm, 45 cm, 50 cm, 55 cm). The incubation depth of a nest affects the number of born hatchlings, their physical condition and sex ratio. Deeper nests show a higher emergence success, a more balanced sex ratio and produce more vigorous hatchlings in comparison with shallower nests. Measurement of depths in natural nests showed that, during her lifetime, a single female buries her nests at different depths, depending on her body length, but also on environmental and behavioral factors. In nest relocation programmes, depth at which a nest is buried is an important factor that must be taken into account and evaluated.

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