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Abstract

MICROORGANISM INFECTION OF OLIVE RIDLEY EGGS

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ABSTRACT

Nest success among olive ridley turtles nesting in arribadas in Costa Rica is extremely low, ranging from 4-8%. One of the presumed causes of egg loss is fungal and bacterial infection in the beach. We undertook an extensive study of the role of microorganism infection in natural and artificial nests of olive ridleys to determine if bacteria and fungi are primary or secondary agents in embryo death. We found no correlation between level of bacteria or specific fungi in sand and hatching success in Playa Nancite, Santa Rosa National Park, Costa Rica. Experimental infection of eggs in controlled environment in the laboratory showed no difference in hatching rates between infected eggs and controls. The rates varied from 0 to 100% success, with an average of 52.5%. Our results show that when eggs are well hydrated and in optimum temperatures, they can hatch despite the presence of bacteria and fungi. Therefore these should be considered opportunistic agents in egg death caused by other environmental causes.

INTRODUCTION

Massive synchronous nesting of sea turtles is peculiar only to the genus *Lepidochelys*. This rare phenomenon in nature, known as "arribada", occurs in few places in the world: one site in Mexico, two in India, two in Costa Rica, and a few small arribadas in Nicaragua (Cornelius, 1986). The olive ridley sea turtle, *Lepidochelys olivacea*, nests in large numbers in Costa Rica at Nancite and Ostional beaches. During a 3-7 day period as many as 100,000 turtles may emerge to nest on these sites (Hughes & Richard, 1974). Arribadas occur from July through December, with peak numbers in October.

Although large numbers of olive ridleys nest, recruitment is very low. Hatching success at Nancite and Ostional is only 4% and 8% respectively (Cornelius *et al.*, 1990). The major cause of nest loss is attributed to microorganism contamination (Cornelius *et al.*, 1990).

Several authors have found certain bacteria and fungi to be pathogenic to turtles, although their effect on embryos has not been established (Marcus, 1981; Murphy and Collins, 1980; Hoff *et al.*, 1984; Jacobson, 1981). Egg-borne aspergillosis has been documented in poultry (Eggert & Barhnhart, 1953). *Salmonella* can penetrate turtle eggs, and neoneates emerge from eggs already infected (Murphy & Collins, 1980).

High bacterial species diversity inside eggs and bacteria occurring in both females and their eggs were correlated with lower hatching success in the loggerhead sea turtle, *Caretta caretta* (Wyneken *et al.*, 1987). Embryo mortality was also associated with infection of green turtle (*Chelonia mydas*) eggs by *Aspergillus spp.* (Solomon & Baird, 1979).

The objective of this study was to determine the role of bacteria and fungi in the hatching success of olive ridley eggs. To achieve this goal, a three phase project was conducted. From March though December 1987, we conducted a survey on the microflora found in eggs and sand at Nancite beach and in sand from adjacent Naranjo beach. Naranjo is an 8-km long beach rarely visited by olive ridleys, but a known nesting site for leatherbacks (*Dermochelys coriacea*). From September through November 1987, we artificially incubated turtle eggs in 7 different substrates. From May 1988 through November 1991, with several months of interruption, we experimentally infected eggs with bacteria and/or fungi and artificially incubated them.

METHODOLOGY

Materials and methods used for identification of beach microflora and comparison of 7 different substrates for artificial incubation have been previously described (see Mo *et al.*, 1990).

A complete factorial design with 4 variables at 2 levels was performed with 2 replicates per treatment. The variables were: temperature, humidity, fungi and bacteria (Table 1). The measured response was number of turtle hatchlings. Eggs were collected in a clean plastic bag as they were laid by the turtles and transported to the laboratory within 10 hours of oviposition. Upon arrival to the laboratory, all eggs were cleaned and disinfected by mechanical action combined with a solution of iodine-alcohol, followed by 70% alcohol. They were then put in sterile broth or broth containing a pool of bacteria, in petri dishes, in such a way that only the bottom part of the eggs touched the liquid. After 6 hours all eggs were transferred to large glass jars lined with a foam pad and covered by a double layer of cotton pad lined with gauze. Each jar contained 10 eggs. Broth containing fungi was poured directly onto the eggs in the jars. There were control eggs with only sterile broth or no broth at all. Incubation rooms were kept at the desired temperatures ± 1 C. Neonates were counted as they hatched, and all remaining eggs were opened after 60 days of incubation.

Results were analysed by multi-variate factor and an *a posteriori* Tukey test.

RESULTS AND DISCUSSION

Results of microflora found on the beach and of artificial incubation in 7 different substrates have been described in Mo *et al.*, (1990).

In the experimental infection of eggs, hatching rates varied between 0 and 100%, with no significant difference among the treatments. These data corroborate our earlier findings in natural nests and in artificial nests with substrates from Nancite and Naranjo, in which the presence of high levels of bacteria and fungi were not correlated with hatching rates.

We observed that whenever we achieved an optimum humidity level, fertile eggs were successful in producing a neonate, despite the presence of fungi and/or bacteria. The range of temperatures we chose were within the normal observed during the rainy season in Nancite and Ostional (Cornelius *et al.*, 1985). It is well known that during the dry season no eggs hatch on the beach, due to the extremely high sand temperatures. The eggs literally cook in the hot dry sand.

In earlier trial runs we observed that eggs dehydrated progressively and were subsequently invaded by bacteria and fungi. This was noticeable when the otherwise white color of a healthy egg turns into a yellowish, pinkish, purple or black egg. Hatching success was near zero in such cases. When we devised the best artificial nest system, and achieved an optimum humidity level, hatching success increased to an average of 52.5% among all treatments. If we take into account the normal number of infertile eggs, plus the manipulation in the first 17 h after oviposition, this rate is quite high.

Several authors have established early embryo mortality caused by movement of the eggs in the first 24 or 26 hours (Limpus *et al.*, 1979; Parmenter, 1980; Blanck & Sawyer, 1981; Whitmore & Dutton, 1985).

Our results show that bacteria and fungi naturally found in Nancite sand are not responsible for embryo death, but are opportunistic agents that invade the egg after it has lost its natural defenses, probably due to physical environmental factors. Development of eggs with fungi is known in other species, such as the domestic chicken (Eggert & Barhnhart, 1953), and bacteria can penetrate and be found in successful turtle eggs (Murphy &

Collins, 1980). Further studies of environmental physical factors and also of pollutants may contribute to explain the low hatching success in Nancite and Ostional beaches.

Table 1. Variables and levels of complete factorial design.

Variables	Level+	Level-
Temperature	32.5 C	28.0 C
Humidity	0-160ml/day	0-80ml/day
Fungi*	Present	Absent
Bacteria**	Present	Absent

*Pool of fungi consisted of *Saksenaea, Aspergillus sp1, Aspergillus sp2, Fusarium, Cladosporium, Mucor, Allescheria, Acremonium*, and *Penicillum*, grown for at least 7 days.

**Pool of bacteria consisted of *Acinetobacter, Pseudomonas sp, Pseudomonas aeruginosa, Bacillus, Staphylococcus*, and *Vibrio*, all individually grown to a concentration of 10^5 colonies/ml.

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