RESEARCH ARTICLE

How Do Hatcheries Influence Embryonic Development of Sea Turtle Eggs? Experimental Analysis and Isolation of Microorganisms in Leatherback Turtle Eggs

JOURNAL OF EXPERIMENTAL ZOOLOGY
PART A
ECOLOGICAL GENETICS AND PHYSIOLOGY
A Journal of Integrative Biology

JUAN PATINO-MARTINEZ[1,2]*, ADOLFO MARCO[1], LILIANA QUIÑONES[1], ELENA ABELLA[1], ROBERTO MURIEL ABAD[1], AND JAVIER DIÉGUEZ-URIBEONDO[3]

[1]Estación Biológica de Doñana, CSIC, Department of Biodiversity Conservation and Ethology, Seville, Spain
[2]ARANZADI Zientzia Elkartea Society of Science, Donostia, San Sebastián
[3]Real Jardin Botánico CSIC, Mycology Department, Madrid, Spain

ABSTRACT

Many conservation programs consider translocation of turtle nests to hatcheries as a useful technique. The repeated use of the same incubation substrate over several seasons in these hatcheries could, however, be harmful to embryos if pathogens were able to accumulate or if the physical and chemical characteristics of the incubation environment were altered. However, this hypothesis has yet to be tested. We conducted two field experiments to evaluate the effects of hatchery sand and eggshell decay on the embryonic development of leatherback sea turtle eggs in Colombia. We identified the presence of both fungi and bacteria species on leatherback turtle eggs. Sea turtle eggs exposed to previously used hatchery substrates or to decaying eggshells during the first and middle third of the embryonic development produced hatchlings that were smaller and/or weighed less than control eggs. However, this did not negatively influence hatching success. The final third of embryonic development seems to be less susceptible to infection by microorganisms associated with decaying shells. We discuss the mechanisms that could be affecting sea turtle egg development when in contact with fungi. Further studies should seek to understand the infection process and the stages of development in which the fungi are more virulent to the eggs of this critically endangered species. *J. Exp. Zool. 317:47–54, 2012.* © 2011 Wiley Periodicals, Inc.

How to cite this article: Patino-Martinez J, Marco A, Quinoñes L, Abella E, Abad RM, Diéguez-Uribeondo J. 2012. How do hatcheries influence embryonic development of sea turtle eggs? experimental analysis and isolation of microorganisms in leatherback turtle eggs. J. Exp. Zool. 317:47–54.

Anthropogenic exploitation of sea turtles, for food (eggs and meat), medicine (including aphrodisiacs), and shells has been ongoing for hundreds of years in almost every major ocean basin in which they occur (Fretey, 2001; Spotila, 2004; McClenachan et al., 2006). Although such harvest has now largely stopped in all but a few nations (Bell et al., 2006), commercial and artisanal fishing has caused the accidental death of hundreds of thousands of individuals (Lewison et al., 2004; Peckham et al., 2007). These impacts together have caused a decline in the majority of nesting

Grant Sponsor: BBVA foundation and Consejería de Medio Ambiente de la Junta de Andalucía; Ministerio de Ciencia e Innovación of Spain; Grant number: CGL2009-10032.

*Correspondence to: Juan Patino-Martinez, Estación Biológica de Doñana, CSIC, Department of Biodiversity Conservation and Ethology, Av Américo Vespucio S/N 41092 Seville, Spain. E-mail: juanpatino@ebd.csic.es

Received 5 November 2010; Revised 22 August 2011; Accepted 29 August 2011

Published online 21 October 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jez.719

populations worldwide (Seminoff and Shanker, 2008). In some cases these declines have been alleviated, for example by reduction of incidental captures by fishing boats (Ferraroli et al., 2004; Hays et al., 2004), the restoration or conservation of critical habitats (Troeng and Rankin, 2005), the protection of females and nests (Eckert and Eckert, '90; Pilcher and Enderby, 2001; Baptistotte et al., 2003; Sarti et al., 2007), awareness campaigns to reduce consumption of meat and eggs (Troeng and Rankin, 2005), and greater regional cooperation between countries (Fossette et al., 2008).

At the nesting beach, one of the most common practices among conservation programs consists of transferring complete clutches to supervised and protected hatcheries, which helps to mitigate losses due to poaching, attacks by predators, and natural flooding or erosion (Chan et al., '85; Garcia et al., 2003; Baskale and Kaska, 2005). Such egg translocation programs can have positive impacts on conservation efforts (Wyneken et al., '88; Garcia et al., 2003; Chacón-Chaverri and Eckert, 2007; Clusella Trullas and Paladino, 2007) and have additional benefits for spreading awareness campaigns among the public (Pike, 2008). However, on some nesting beaches, the use of protective hatcheries is considered unnecessary (Lum, 2005; Patino-Martinez et al., 2008), or even counterproductive to conservation aims (Chan and Liew, '96; Kamel and Mrosovsky, 2004; Mrosovsky, 2006, 2008).

One of the main problems associated with these programs is the intra- and interannual fluctuation in egg-hatching rates (Piedra et al., 2007). In many cases, successful hatching is lower among transferred clutches than in natural in situ nests that have not been manipulated (Eckert and Eckert, '90; Baptistotte et al., 2003; Ozdemir and Turkozan, 2006). The causes for reduced success seem to include embryonic mortality induced by movement of the eggs (Limpus et al., '79) and the greater risk of contamination by microorganisms because of the higher density of nests in hatcheries (Shanker et al., 2003; Ozdemir and Turkozan, 2006), affecting the quality of the incubation substrate, including the organic matter and microorganisms present in the sand (Clusella Trullas and Paladino, 2007).

Although natural fungal growth in natural leatherback sea turtle nests is well known, whether there is greater contamination of eggs in clutches transferred to hatcheries is not well understood (Chan and Solomon, '89; Phillott and Parmenter, 2001a). Likewise, it is unknown whether fungal infections colonize developing eggs, or grow on those that are already dead. To prevent the accumulation of microorganisms in the nest and the decrease risk of egg infection, some authors have recommended the use of different hatchery sites between nesting seasons (Shanker et al., 2003). This may involve moving protective hatchery perimeter walls or other infrastructure each year, which may be costly, complex, or unfeasible. It also does not prevent the fallow hatchery site from being nested by turtles selecting sites there. Maintaining hatcheries in the same place thus has multiple economic and logistical benefits, simplifying the translocation of nests and their surveillance, and reproducing shade levels and successful hatching. It is therefore crucial to evaluate the relationship between the continued use of the incubation substrate in hatcheries and the possible increase in embryonic mortality due to fungal contamination, and, if they were related, to investigate ways to reduce or eliminate these effects. Our study: (1) evaluates the effect of hatchery sand on embryonic development, hatching success, and hatchling phenotypes, (2) evaluates whether there is a period of the embryonic developmental stage that is particularly sensitive to microbial infection, and (3) details the microbiota and bacteria associated with leatherback turtle eggs in Colombia.

MATERIAL AND METHODS

We conducted two experimental studies in the south-western Caribbean sea on the border between Colombia and Panama (Playona beach. 8°43′N, 77°32′W, Fig. 1), where Leatherback turtles (*Dermochelys coriacea*) nest between February and June. This area constitutes the third largest nesting ground for leatherback turtles in the Caribbean and the fourth largest in the world (Patino-Martinez et al., 2008).

Experiment 1: Substrate Experiment

The first of our two experiments studied the embryonic development and hatching success of eggs incubated in three different substrate treatments of sand from the same beach: (i) hatchery sand, (ii) natural sand, and (iii) sterile sand. The first treatment used sand taken from a hatchery after three consecutive years of use. The second treatment used sand from the middle zone of a natural nesting area of the beach. This area does not suffer tidal flooding and is not vegetated, and is located far from the hatchery with no remains of previous sea turtle nests. The third treatment used sand that had been disinfected for 24 hr before eggs burial, using a 5% solution of sodium hypochlorite (Phillott et al., 2002). Each treatment was replicated three times in plastic containers (0.32 m × 0.20 m × 0.21 m, 15 L).

The eggs were collected from three different females on the night in April 2006. The eggs were collected directly from the cloaca of the female using different sterile gloves for each nest. Thus, eggs did not come into contact with any sand until the beginning of the experiment. Three eggs from each of the different females were randomly selected and placed into each of the nine plastic containers (three treatments × three replicates). The eggs were completely covered with sand that had been previously moistened to 6% (volumetrically) with sterile mineral water; the layer of sand covering the eggs was 1 cm deep. Each container was partially filled up to the same level, always leaving a 2-cm empty space with air below the cover. The sealed containers were buried to a depth of 60 cm in the hatchery. The containers were opened weekly to allow gas exchange.

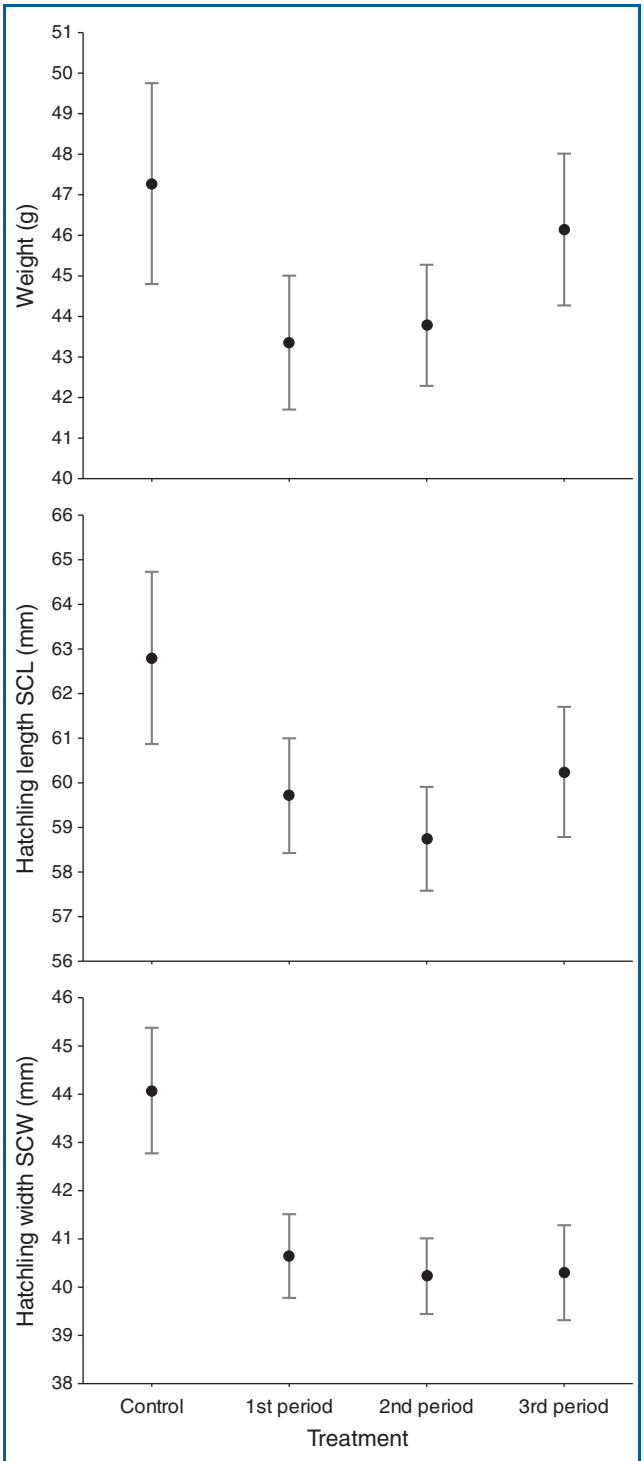

Figure 1. Biometric data of hatchlings in experimental treatments of inoculation with decaying eggshells contaminated by fungi. (SCW = straight carapace width; SCL = straight carapace length, weight in grams; first, second, and third periods inoculated at 0, 20, and 40 days of incubation, respectively, control = no inoculation).

Experiment 2: Decaying Eggshell Experiment

In the second experiment, eggs in different phases of embryonic development were exposed to decaying eggshells that were observed to have the typical coloring associated with the presence of fungi. Fungal and bacterial infection of the eggshells was later verified by molecular analysis in the laboratory.

The eggs were collected from five different females on the same night in April 2007. As in the first experiment, eggs did not come into contact with the sand at laying. The total time that it took from the laying of the eggs to the complete assembly of each experiment did not exceed 4 hr.

The sensitivity of the developing embryos to contamination by decaying eggs was determined by dividing the approximately 60 days of incubation into three equal periods and applying an inoculation treatment at the beginning of each period: (i) day 0–19 of incubation or first period, (ii) day 20–39 of incubation or second period, and (iii) day 40 until hatching or third period. An additional control treatment in which eggs were not exposed to hatched eggshells was set up.

Decaying eggshell samples were taken from nests that had hatched during the same study season as well as from nests that had hatched in the hatchery during the previous reproductive season. The samples from each year were divided into small parts and placed in different plastic containers, with a cover and a base of natural moist sand.

Each inoculation treatment was applied to individual eggs (four from each female), located in individual sterilized (5% solution of sodium hypochlorite) cylindrical containers (8.0 cm × 11.5 cm in height). Each treatment contained 20 experimental units per treatment and 80 experimental units in total. Each egg was inoculated with contaminated eggshells from the current study season as well as those from the previous year by incubating the eggshell fragments with the study eggs.

Contamination of the eggs by microorganisms that could have been present in the sand was prevented by incubating eggs in vermiculite sterilized in an autoclave for 24 hr (Astell AMA262BT, 100–138°C; water potential of −650 kPa). Vermiculite was hydrated with sterile mineral water at a water potential of −150 kPa (1 g vermiculite = 1.24 g of water).

All the sealed containers were buried in the hatchery to a depth of 60 cm. Each container was partially filled up to the same level, always leaving a 2-cm empty space with air below the cover. The containers were opened every 20 days to allow gas exchange.

In both experiments, incubation temperatures were recorded using dataloggers (Hobo StowAway TidbiT v2 Onset, www.onsetcomp.com, ±0.2°C accuracy, measuring 3.0 cm × 1.7 cm in height), which were randomly placed inside two containers. The dataloggers were programmed to record temperature every 30 min. In addition, the control temperature of the beach (without eggs) was recorded at a depth of 60 cm in two different places.

Incubation duration was considered as the time elapsed from egg laying to the hatching of each egg (observation of the head of each hatchling emerging from the egg shell). The number of successfully hatched eggs was recorded for each treatment. The hatchlings were monitored from hatching and we recorded their mass (Balance PK-202 Denver instrument; $d = 0.01$ g; Max = 200 g), straight carapace length (SCL) and width (SCW) (Digital caliper Cen-Tech; $d = 0.01$ mm; Max = 150 mm). Their physical condition was also assessed using a righting response test: the hatchlings were initially placed on their backs and we recorded the time it took them to return to their normal position (three times per hatchling). After assessing their physical condition, the hatchlings were immediately released into the sea.

All unhatched eggs were dissected to assess the stage of embryonic development reached and classified according to the final size of the dead embryos. Each dissected egg was assigned a development value in accordance with the criteria defined by Bilinski et al., 2001 (Table 1).

Microbial Analysis

Three samples of decaying eggshells were randomly selected from each container (from the same and previous season) and were analyzed in the laboratory to identify the fungal and bacterial biota before inoculation, and three more samples were analyzed upon completion of the experiment. The samples were transported in sealed plastic containers containing sterile cotton moistened with sterile mineral water to the culture library of the Royal Botanical Garden in Madrid, Spain. The phylogenetic analysis of the isolated strains used sequences of the Internal Transcribed Sequence region of nuclear DNA. The isolation was performed in accordance with the protocol described by Cerenius et al. ('88). Potato dextrose agar was used for the cultures and they were stored in.

Data Analysis

All analyses were performed using the SAS statistical package (SAS Institute Inc., Cary, NC). The analysis of hatching success (yes/no) was performed using mixed-model analyses (GLIMMIX Procedure) with the experimental treatments (Experiment 1—hatchery, beach, and sterile sands; Experiment 2—control, first, second, and third periods) treated as fixed effects. According to the dependent variable used in this model, the binary error distribution and the logit link function were used. We defined the female from which each clutch originated as a random effect (the subject term).

The response variable "embryonic development" showed a strong positively skewed distribution because many eggs did not show any sign of development. Therefore, the model was fitted to a Poisson distribution with the log link function, which was supported by a value of the scaled Pearson statistic close to one.

The statistical significance of the differences among experimental treatments in hatchling biometric data were tested by fitting generalized linear models (proc GLM) to results from the substrate and decaying eggshells experiments.

All variables related to hatchling physical conditions (weight, length, and width) reasonably satisfied the requirements of normality. Normality distribution of residual was checked by graphical and statistical methods.

Models were tested with F statistic for fixed effects and χ^2 statistic for random effects. Tukey post hoc test with Kramer's adjustment for unbalanced designs were used for pair-wise comparisons among levels when a categorical factor showed significant effect. All tests were two tailed and statistical significance was set to $P<0.05$. Values reported refer to mean $\pm$ 1SD.

RESULTS

Experiment 1: Substrate Experiment

The mean incubation temperature of eggs was 29.75 $\pm$ 0.9°C, and was positively and significantly correlated with the temperature of the sand on the beach at a depth of 60 cm (29.85 $\pm$ 0.8°C; $R = 0.92$; $P<0.0001$).

The hatching success of the eggs incubated in different substrates showed no statistical differences (hatchery 16% of eggs hatched successfully, beach 11% and sterile sand 26%; $F_{2,74} = 1.01$, $P = 0.368$). However, there was a significantly higher proportion of DV (Table 1) eggs in sterile sand ($F_{2,74} = 3.65$, $P = 0.030$) than in hatchery or natural sand. Covariance component associated with the random factor

Table 1. Categories used to classify embryonic development.

Development value (DV)	State of development	Definition
0	No development	No visible sign of development
1	Initial embryo death	White shell discoloration (CAM) or embryo length <20 mm
2	Intermediate embryo death	Embryo length >20 mm and <40 mm
3	Embryo death at the end	Deaf-pigmented embryo >40 mm in length
4	Hatched	Living or dead hatchlings

Modified from Bilinski et al. 2001. CAM, chorioallantoic membrane, which produces a typical shell discoloration and indicates the beginning of embryonic development.

"female" was significantly different from zero, which shows a relevant maternal effect on the egg development and subsequent hatching success.

The physical characteristics of the hatchlings (length, width, and weight) were not statistically different among treatments (weight: $F_{2,9} = 2.04$, $P = 0.185$; length: $F_{2,9} = 0.20$, $P = 0.819$; width: $F_{2,9} = 3.44$, df = 9, $P = 0.077$); however, multiple comparisons with the Tukey–Kramer test showed that carapace width was significantly higher in the sterile treatment than on the beach (sterile SCW = 40.02 ± 0.7 mm, beach 36.64 ± 1.1 mm, Tukey–Kramer $P = 0.031$). These comparisons also showed a tendency toward significant differences in weight (sterile = 41.42 ± 2.8 g, beach = 36.69 ± 3.4 g, Tukey–Kramer $P = 0.090$).

Experiment 2: Decaying Eggshells Experiment

The inoculation of decaying eggshells did not cause any differences in the hatching success between different stages of embryonic development ($F_{3,72} = 0.42$; $P = 0.738$).

The contamination of the eggs did not affect either the duration of the incubation period ($F_{3,36} = 0.580$; $P = 0.632$) or the stage of embryonic development reached for failed eggs ($F_{3,72} = 0.25$, $P = 0.859$). However, there was a negative effect on the weight and size of the hatchlings compared with control treatments (weight $F_{3,27} = 3.81$, $P = 0.021$; SCL $F_{3,27} = 4.66$; $P = 0.009$; SCW $F_{3,27} = 10.02$; $P < 0.0001$, Fig. 1). The righting time in the physical tests was highly variable between treatments (control = 24.8 ± 22 sec; first period = 1.5 ± 0.6 sec; second period = 31.1 ± 10.7 sec, third period = 2.8 ± 0.3 sec. $F_{3,24} = 3.293$; $P = 0.038$).

Microbial Analysis

The microbial analysis revealed both bacteria and fungi in all samples. The phylogenetic analysis of the strains isolated from eggs of Colombian leatherback turtles 004FUST2Co, 006FUST3-Co, and 006FUSJ1Mo revealed that they were similar to Genbank sequences corresponding to *Fusarium solani* and *Fusarium oxysporum*. There were two anastomosis groups among the *Fusarium solani*, that is, compatible strains whose hyphae merged and interchanged cytoplasm and genetic material (teleomorph *Nectria haematococa*, 004FUST2Co anastomosis group I, 006FUST3Co anastomosis group II). All the bacteria (*Acinetobacter lwoffi*, *Acinetobacter baumannii*, *Bordetella bronchiseptica*, *Brevundimonas vesicularisand*, *Corynebacterium aquaticum*, and *Pseudomonas stutzeri*) and fungi isolated were saprophytes, except for *F. solani*.

DISCUSSION

Although it has been demonstrated that fungal growth exists both in natural and translocated sea turtle nests (Phillott and Parmenter, 2001a; Sarmiento-Ramírez et al., 2010), it has not been firmly established whether greater contamination occurs in the eggs of nests transferred to a hatchery. Experiment 1 failed to provide evidence for a negative influence of used hatchery substrate on hatching success in comparison with other substrates from the same beach. On the other hand, the greater embryonic development reached in the treatment with sterile sand and the marginal differences in hatchlings weight and SCW between beach and sterile sand suggest a benefit to eggs incubating in substrates completely lacking microorganisms. However, hatching success was very low compared with natural nests, and thus other mortality factors were likely present.

The leatherback turtle eggs that were in direct contact with decaying eggshells had similar hatching success rates to natural nests and other experimental control treatments (30–70%) (Whitmore and Dutton, '85; Leslie et al., '96; Bell et al., 2004; Piedra et al., 2007). There was no effect on the embryonic development of these eggs or a significant effect on hatching success. However, there was a significant negative effect on hatchling size and weight. Embryos exposed during the initial and second period of their development were more sensitive to contact with contaminated eggshells, resulting in smaller hatchlings with lower body masses than the control treatment. The final third of embryonic development seems to be less sensitive to the impact of exposure on microorganisms from decaying eggshells perhaps as the microorganisms would have had less time to grow and compete for resources with hatchlings.

This is the first study to isolate fungi and bacteria from leatherback turtle nests in Colombia. The contamination of the eggs by *F. solani* and *F. oxisporum* could occur in the oviduct, in a similar way to other species of sea turtles of the Cheloniidae family, where various strains have been isolated in the cloaca of breeding females (Phillott et al., 2002). The possible routes of entry of the microorganisms into the cloaca could be during copulation with males and through contact with spores present in the natural nesting substrate (Phillott and Parmenter, 2001a; Phillott et al., 2002). The fungi of the *Fusarium* species are widely distributed throughout the world and have a very high survival rate in soil, even for decades without any modification of their morphological structure (McKeen and Wensley, '61). It seems possible that fungi may affect leatherback hatchling phenotype (including body size) through a variety of mechanisms:

Competition for Resources Between the Embryo and Fungi

Infertile, undeveloped or dead eggs in nests may provide an entry point for fungal infections, growing on their resources and spreading throughout the nest (Phillott and Parmenter, 2001a). The fungal hyphae could then spread to healthy developing eggs where strong proteolytic and lipolytic activity could allow the fungi to penetrate the eggs to compete for yolk resources (Phillott, 2004). The yolk sac of developing bird embryos has also proved to be highly suitable for the propagation of pathogenic fungi (Brueck and Buddingh, '51).

Egg Defence Mechanism

Immunological resilience of the developing embryos to infection, preventing fungal spread and perhaps reducing hatchling mortality could, however, be comparatively expensive, and thus affect embryonic growth (Phillott and Parmenter, 2001a).

Interference Gas and Water Exchange Activities Between the Egg and the Embryo

Fungal hyphae have been observed to cover egg shell pores and cause mortality, depending on the percentage of the shell that is covered (Phillott and Parmenter, 2001b). In the case of larger eggs, such as the leatherback's, this ''smothering'' may not be influential enough to cause mortality, but could limit the normal growth of the embryos.

Reduced Calcium Absorption

Calcium is obtained from the eggshell by developing embryonic chelonians (Packard, '94). Healthy developing eggs normally take up to 43% of the egg shell calcium for osteogenesis (Bilinski et al., 2001). There are indications that eggs with fungal infections and without any embryonic development (hatchling death) still undergo a calcium loss, that can only be attributed to the presence of fungi (Phillott et al., 2006). Therefore, although the effects on embryonic development of this calcium reduction in the shell have not been sufficiently studied, it seems reasonable to assume that reduced calcium availability could result in lower hatchling growth rates.

Based on these results, it is evident that it is not only important to evaluate hatching success but also necessary to assess sublethal growth-related effects and study the later survival of the hatchlings in order to gain a full understanding of the effect of fungal nest infection on sea turtle reproductive success.

This study reinforces previous evidence that *Fusarium sp.* fungi are present in reptile eggs in general (Moreira and Barata, 2005), as well as in those of sea turtles in particular (Phillott et al., 2006; Sarmiento-Ramírez et al., 2010). The majority of studies considering the ''fungus–egg'' relationship in sea turtles have taken place in Loggerhead (*Caretta caretta*) (Guclu et al., 2010; Sarmiento-Ramírez et al., 2010), Green (*Chelonia mydas*) (Phillott, 2004; Elshafie et al., 2007), Hawksbill (*Eretmochelys imbricata*) (Phillott et al., 2004), and Flatback (*Natator depressus*) sea turtles (Phillott and Parmenter, 2001b; Phillott and Parmenter, 2006). However, there has been less research in leatherback turtles, despite being one of the factors that could potentially contribute to the notably low hatching success of this species (Whitmore and Dutton, '85; Bell et al., 2004). Previous studies in leatherbacks have mentioned the presence of fungi and their influence on the general appearance of the egg (Whitmore and Dutton, '85; Chan and Solomon, '89; Eckert and Eckert, '90) and the location of the infections within the nest (Phillott and Parmenter, 2001a).

The results of our study demonstrate that the use of hatcheries may not always compromise hatching success through micro-organism infection of eggs. However, our results show the potential role that pathogenic fungi and other microorganisms associated with eggshells may play in the determination of the quality of the hatchlings, indicating that the scope of the effects could endure in nature and limit the later survival of hatchlings. The infection of eggs inside the nest is likely determined by environmental factors such as temperature, humidity, or stress on the eggs. In order to gain a more complete understanding of the effects of infection to developing sea turtle eggs, future work should (1) detail the biological development of different strains and their virulence in each phase of development of sea turtle eggs; (2) describe the nature of the fungal infection with indirect studies using antifungal agents; and (3) model the infection process to identify risk situations for nests.

ACKNOWLEDGMENTS

We thank our collaborators for help with field work: Alfonso Gutiérrez, Berley Tarrá, Daniel Chaverra, Jaime Olivo, David Naranjo, Diego Chaverra, and Feliciano Chaverra, and to Dr. José Leiva, University of Navarra; Spain, for help in bacterial identification. We also thank the environmental and local authorities, Codechocó, Consejos mayor y menor de comunidades negras in Acandí, for facilitating this study on their territory. J.D.-U. was supported by grant CGL2009-10032 of the Ministerio de Ciencia e Innovación of Spain. The manuscript was improved by David Pike, Lucy Hawkes, and two anonymous reviewers.

LITERATURE CITED

Baptistotte C, Thome JCA, Bjorndal KA. 2003. Reproductive biology and conservation status of the loggerhead sea turtle (*Caretta caretta*) in Espirito Santo State, Brazil. Chelonian Conserv Biol 4:523–529.

Baskale E, Kaska Y. 2005. Sea turtle nest conservation techniques on southwestern beaches in Turkey. Isr J Zool 51:13–26.

Bell BA, Spotila JR, Paladino FV, Reina RD. 2004. Low reproductive success of leatherback turtles, *Dermochelys coriacea*, is due to high embryonic mortality. Biol Conserv 115:131–138.

Bell BA, Blumenthal JM, Austin TJ, Solomon JL, Ebanks-Petrie G, Broderick AC, Godley B. 2006. Traditional Caymanian fishery may impede local marine turtle population recovery. Endangered Species Res 2:63–69.

Bilinski JJ, Reina RD, Spotila JR, Paladino FV. 2001. The effects of nest environment on calcium mobilization by leatherback turtle embryos (*Dermochelys coriacea*) during development. Comp Biochem Physiol A Physiol 130:151–162.

Brueck JW, Buddingh GJ. 1951. Propagation of pathogenic fungi in the yolk sac of embrionated eggs. Proc Soc Exp Biol Med 76:258–261.

Cerenius L, Rennie P, Fowke LC. 1988. Endocytosis of cationized ferritin by zoospores of the fungus *Aphanomyces euteiches*. Protoplasma 144:119–124.

Chacón-Chaverri D, Eckert KL. 2007. Leatherback sea turtle nesting at Gandoca Beach in Caribbean Costa Rica: management recommendations from fifteen years of conservation. Chelonian Conserv Biol 6:101–110.

Chan EH, Liew H-C. 1996. Decline of the leatherback population in Terengganu, Malaysia, 1956-1995. Chelonian Conserv Biol 2:196–203.

Chan EH, Solomon SE. 1989. The structure and function of the eggshell of the leatherback turtle (*Dermochelys coriacea*) from Malaysia, with notes on attached fungal forms. Anim Technol 40:91–102.

Chan EH, Salleh HU, Liew HC. 1985. Effects of handling on hatchability of eggs of the leatherback turtle, *Dermochelys coriacea* (L.). Pertanika 8:265–271.

Clusella Trullas S, Paladino FV. 2007. Micro-environment of olive ridley turtle nests deposited during an aggregated nesting event. J Zool 272:367–376.

Eckert KL, Eckert SA. 1990. Embryo mortality and hatch success in insitu and translocated leatherback sea turtle *Dermochelys coriacea* Eggs. Biol Conserv 53:37–46.

Elshafie A, Al-Bahry SN, Alkindi AY, Ba-Omar T, Mahmoud I. 2007. Mycoflora and aflatoxins in soil, eggshells, and failed eggs of *Chelonia mydas* at Ras Al-Jinz, Oman. Chelonian Conserv Biol 6:267–270.

Ferraroli S, Georges JY, Gaspar P, Le Maho Y. 2004. Endangered species - Where leatherback turtles meet fisheries. Nature 429:521–522.

Fossette S, Kelle L, Girondot M, Goverse E, Hilterman ML, Verhage B, de Thoisy B, Georges JY. 2008. The world's largest leatherback rookeries: a review of conservation-oriented research in French Guiana/Suriname and Gabon. J Exp Marine Biol Ecol 356:69–82.

Fretey J. 2001. Biogeography and conservation of marine turtles of the Atlantic coast of Africa. CMS Technical Series Publication 6, UNEP/CMS Secretariat, Bonn, Germany, p 429.

Garcia A, Ceballos G, Adaya R. 2003. Intensive beach management as an improved sea turtle conservation strategy in Mexico. Biol Conserv 111:253–261.

Guclu O, Biyik H, Sahiner A. 2010. Mycoflora identified from loggerhead turtle (*Caretta caretta*) egg shells and nest sand at Fethiye beach, Turkey. Afr J Microbiol Res 4:408–413.

Hays GC, Houghton JDR, Myers AE. 2004. Endanqered species—Pan-Atlantic leatherback turtle movements. Nature 429: 522–522.

Kamel SJ, Mrosovsky N. 2004. Nest site selection in leatherbacks, *Dermochelys coriacea*: individual patterns and their consequences. Anim Behav 68:357–366.

Leslie AJ, Penick DN, Spotila J, Paladino F. 1996. Leatherback turtle, *Dermochelys coriacea*, nesting and nest success at Tortuguero, Costa Rica, in 1990-1991. Chelonian Conserv Biol 2:159–168.

Lewison RL, Freeman SA, Crowder LB. 2004. Quantifying the effects of fisheries on threatened species: the impact of pelagic longlines on loggerhead and leatherback sea turtles. Ecol Lett 7:221–231.

Limpus CJ, Baker V, Miller JD. 1979. Movement induced mortality of loggerhead eggs. Herpetologica 35:335–338.

Lum LL. 2005. Beach dynamics and nest distribution of the leatherback turtle (*Dermochelys coriacea*) at Grande Riviere Beach, Trinidad & Tobago. Revista De Biología Tropical 53:239–248.

McClenachan L, Jackson JBC, Newman MJH. 2006. Conservation implications of historic sea turtle nesting beach loss. Front Ecol Environ 4:290–296.

McKeen CD, Wensley RN. 1961. Longevity of *Fusarium oxysporum* in soil tube culture. Science 134:1528–1529.

Moreira PL, Barata M. 2005. Egg mortality and early embryo hatching caused by fungal infection of Iberian rock lizard (*Lacerta monticola*) clutches. Herpetol J 15:265–272.

Mrosovsky N. 2006. Distorting gene pools by conservation: assessing the case of doomed turtle eggs. Environ Manage 38:523–531.

Mrosovsky N. 2008. Against oversimplifying the issues on relocating turtle eggs. Environ Manage 41:465–467.

Ozdemir B, Turkozan O. 2006. Hatching success of original and hatchery nests of the green turtle, *Chelonia mydas*, in Northern Cyprus. Turk J Zool 30:377–381.

Packard MJ. 1994. Patterns of mobilization and deposition of calcium in embryos of oviparous, amniotic vertebrates. Isr J Zool 40:481–492.

Patino-Martinez J, Marco A, Quinones L, Godley B. 2008. Globally significant nesting of the leatherback turtle (*Dermochelys coriacea*) on the Caribbean coast of Colombia and Panama. Biological Conservation 141:1982–1988.

Peckham SH, Diaz DM, Walli A, Ruiz G, Crowder LB, Nichols WJ. 2007. Small-scale fisheries bycatch jeopardizes endangered pacific loggerhead turtles. PLoS ONE 2.

Phillott AD. 2004. Penetration of the eggshell ano invasion of embryonic tissue by fungi colonising sea turtle eggs. Hepetofauna 34:44–47.

Phillott AD, Parmenter CJ. 2001a. The distribution of failed eggs and the appearance of fungi in artificial nests of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtles. Aust J Zool 49:713–718.

Phillott AD, Parmenter CJ. 2001b. Influence of diminished respiratory surface area on survival of sea turtle embryos. J Exp Zool (Mol Dev Evol) 289:317–321.

Phillott AD, Parmenter CJ. 2006. The ultrastructure of sea turtle eggshell does not contribute to interspecies variation in fungal invasion of the egg. Can J Zoology 84:1339–1344.

Phillott AD, Parmenter CJ, Limpus CJ, Harrower KM. 2002. Mycobiota as acute and chronic cloacal contaminants of female sea turtles. Aust J Zool 50:687–695.

Phillott AD, Parmenter CJ, Limpus CJ. 2004. Occurrence of mycobiota in eastern Australian sea turtle nests. Mem Queensl Mus 49:701–703.

Phillott AD, Parmenter CJ, McKillup SC. 2006. Calcium depletion of eggshell after fungal invasion of sea turtle eggs. Chelonian Conserv Biol 5:146–149.

Piedra R, Velez E, Dutton PH, Possardt E, Padilla C. 2007. Nesting of the Leatherback turtle (*Dermochelys coriacea*) from 1999–2000 through 2003–2004 at Playa Langosta, Parque Nacional Marino Las Baulas de Guanacasta, Costa Rica. Chelonian Conserv Biol 6:111–116.

Pike DA. 2008. The benefits of nest relocation extend far beyond recruitment: a rejoinder to Mrosovsky. Environ Manage 41:461–464.

Pilcher NJ, Enderby S. 2001. Effects of prolonged retention in hatcheries on green turtle (*Chelonia mydas*) hatchling swimming speed and survival. J Herpetol 35:633–638.

Sarmiento-Ramírez, Abella E, Martín MP, Tellería MT, lopez-Jurado LF, Marco A, Diéguez-Uribeondo J. 2010. Fusarium solani is responsible for mass mortalities in nests of loggerhead sea turtle, Caretta caretta, in Boavista, Cape Verde. FEMS Microbiol Lett 312:192–200.

Sarti L, Barragán AR, Garcia D, Garcia N, Huerta P, Vargas F. 2007. Conservation and biology of the leatherback turtle in the Mexican Pacific. Chelonian Conserv Biol 6:70–78.

Seminoff JA, Shanker K. 2008. Marine turtles and IUCN Red Listing: a review of the process, the pitfalls, and novel assessment approaches. J Exp Marine Biol Ecol 356:52–68.

Shanker K, Choudhury BC, Andrews HV. 2003. Sea turtle conservation: beach management and hatchery programmes. Centre for Herpetology/Madras Crocodile Bank Trust,Tamil Nadu, India.

Spotila J. 2004. Sea turtles: a complete guide to their biology, behavior and conservation. Baltimore, Maryland, Press TJHU.

Troeng S, Rankin E. 2005. Long-term conservation efforts contribute to positive green turtle *Chelonia mydas* nesting trend at Tortuguero, Costa Rica. Biol Conserv 121:111–116.

Whitmore CP, Dutton PH. 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. Biol Conserv 34:251–272.

Wyneken J, Burke TJ, Salmon M, Pedersen DK. 1988. Egg failure in natural and relocated sea turtle nests. J Herpetol 22:88–96.