

Egg Failure in Natural and Relocated Sea Turtle Nests

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ABSTRACT.—Eggs of the loggerhead sea turtle, *Caretta caretta*, often fail to hatch. Infertility and embryonic mortality were investigated as sources of egg failure, showing that standard methods of estimating infertility of eggs produce overestimates. High species diversity of bacteria within eggs or the occurrence of the same bacteria in both females and their eggs was correlated with lower hatching success. Comparisons of hatching success were made among natural nests and two commonly used methods of egg relocation. Eggs relocated to polystyrene incubators had higher hatching success than did eggs reburied in other beach sites. Eggs in undisturbed natural nests had lower hatching success than relocated eggs. Thus, egg relocation is an effective conservation method, provided sites are chosen carefully.

During the past several decades there has been an overall reduction in all sea turtle populations (Rebel, 1974; Pritchard, 1980; Ehrenfeld, 1982) attributed directly and indirectly to habitat destruction, including commercial development of nesting beaches for human habitation. There also is evidence that heavy predation on young, poaching of eggs and adults, pollution, and unsound fishing practices have contributed significantly to observed population declines (Pritchard, 1980; Witham, 1982; Mrosovsky, 1983).

The early stages of embryonic and hatching life represent a crucial period in the life history of sea turtles when mortality levels are extremely high (Richardson and Richardson, 1982; Stancyk, 1982). Analyses of nests have shown that many eggs fail to hatch (Bustard, 1972; Fowler, 1979; Whitmore and Dutton, 1985). Protection techniques focus primarily on artificial rearing of the eggs and/or hatchlings. Egg protection strategies include covering nests with screens to reduce predation and relocating eggs for incubation under (a) "natural" conditions (protected areas where eggs are reburied in the sand above the anticipated spring high tide level), or (b) artificial conditions using expanded polystyrene incubators or other nonmetal containers. Such relocation often may result in decreased hatching success when compared with undisturbed, natural nests.

Some workers have suggested that be-

cause egg failure occurs in undisturbed natural nests, some eggs are infertile (Bustard, 1972; Fowler, 1979; Stancyk et al., 1980). Infertility is often assumed when no embryo is found upon inspection. Since it is standard procedure not to remove unsuccessful eggs until hatching and emergence are complete, 55-70 days after the clutch is deposited, early embryonic death may be masked by decomposition and may not be distinguishable from true infertility.

This paper defines the potential causes of hatching failure in marine turtle eggs by examining potential pathogens, infertility, and methods of egg manipulation intended to promote survivorship. We also correlate the recovery of potentially pathogenic bacteria from the interior contents of unhatched eggs with reduced hatching success. We seek to answer the following questions: (1) Does egg relocation affect hatching success? (2) Are differences in hatching success associated with incubation method? (3) Are unhatched eggs with no obvious signs of development actually infertile? (4) Are potentially pathogenic bacteria associated with unhatched eggs and what might be their sources?

METHODS

Comparisons Between Reburied and Incubator Nests.—In 1983, all *Caretta caretta* nests (N = 53) located on Jekyll Island, Georgia, were relocated because under natural con-

ditions these nests are usually destroyed (by predators, storm tides, and poachers). Relocation was complete within four hours of egg deposition. Eggs were collected either during oviposition (by allowing them to fall to the bottom of the nest and then removing them for transport) or by excavation after they were covered by the female. All clutches were placed in cotton bags for transport. Fifty clutches were "reburied" in hand-dug nest holes in the hatchery (a fenced area of sand, free of vegetation, located in the secondary dune layer). The remaining three clutches were placed in glass-fronted, polystyrene incubators (38 × 38 × 19 cm) for observation and comparison of hatching success with that of the reburied clutches. The incubation temperature of 5 randomly selected hatchery nests averaged 30°C (range of 26°–34°C) over the incubation period. The three polystyrene incubators averaged 32°C (range of 29°–34°C). Field temperature data were obtained by checking mercury thermometers enclosed in porous PVC tubes that had been buried next to the clutches.

Following emergence and release of the hatchlings, each relocated nest was excavated to determine hatching success. Pipped eggs were not counted as hatched. Dead hatchlings free of the egg shell, or nearly free, were counted as hatched. Hatching success was compared by chi-square tests corrected for continuity (Zar, 1984).

Unhatched eggs from 20 reburied nests were opened carefully and examined for the presence of embryos to estimate fertility levels of each clutch and to look for probable causes of embryonic death. The more traditional criteria were applied here to familiarize ourselves with the strengths and weaknesses of the technique. Eggs were considered to be infertile if there was an absence of any sign of an embryo or blood (e.g., Fowler, 1979; Whitmore and Dutton, 1985 for their 1981 field season).

Comparisons Between Natural and Relocated Nests.—These studies were conducted in 1984 on a 15 km section of Canaveral National Seashore (CANA), Volusia County, Florida. Thirty-one nests were marked, screened to eliminate predators, and left

as natural controls on the site. Ten other nests, located in high risk areas (from predation or tidal inundation), were relocated. Eggs were collected either as they were released by the female or immediately after they were deposited in the nest pit. All were placed in clean plastic bags and transported in a well-padded bucket. Five clutches were reburied within 3 h of oviposition in hand-dug nest holes at the north end of the site. Five more were buried in 35–40 cm of unsterilized beach sand in glass-fronted polystyrene incubators. These incubators were used so that nonviable eggs failing during the first three weeks of development could be identified and removed. Removal was done with extreme care so that no other eggs in the clutch were traumatized or rotated. Nonviable eggs were opened and cultured (see below). The top eggs were covered by 10–12 cm of sand and kept moist with distilled water when the top 5.0 cm layer became dry. A polystyrene sheet was taped to the glass front when eggs were not under observation. Temperatures averaged 28°C (range of 26°–31°C) over the period of incubation.

Bacterial analyses were conducted by culturing samples of nest sand, cloacal fluid, and unhatched eggs. No viable eggs were sampled. Cloacal samples were obtained from 20 females and their nests during oviposition. To collect cloacal samples, sterile swabs were briefly inserted 15 cm into the cloaca. A sample of the sand walls of each of 10 natural nests was also taken with a separate sterile swab. The swabs were then placed in transport media. Each sample was streaked on blood-agar plates within 3 h of collection and incubated at 30°C. Numbers of eggs which did and did not hatch were tallied for the above nests.

Nonviable eggs, identified by their yellow or beige (rather than white) color, were removed from polystyrene incubators during the third week and at the completion of incubation. At the completion of incubation, nonviable eggs were also recovered from 4 natural and 5 reburied nests. To assure that no bacteria were introduced into the contents from the shells, all eggs were disinfected externally by rinsing in dis-

tilled water to remove sand and then swabbed liberally with povidone iodine followed by 70% isopropyl alcohol. Each was then opened with a sterile scalpel blade. The contents of each egg and, particularly, any embryonic remains were sampled with sterile swabs and cultured.

To test the effectiveness of our method of egg shell disinfection, 4 unhatched eggs were disinfected then touched to blood agar plates prior to opening with a sterile scalpel blade. Three more unhatched eggs were disinfected, their shells scraped in a "shaving" motion with a sterile scalpel blade, and the scraped material cultured. New sterile blades were used to open these eggs and the contents of each were cultured to compare bacteria found with those cultured from the shells.

Bacterial isolates (based on colony morphology) from all sources were inoculated into Stuart transport media and sent to the University of Illinois Veterinary Diagnostic Laboratory for further isolation and identification to genus and, for some, species.

To estimate fertility levels, two eggs from each relocated nest (20 eggs total) were removed during oviposition, incubated for 16–18 h at room temperature (20°C), and opened with a scalpel blade to determine if an embryo was present. If it was, the viscous albumen was removed, exposing the embryonic disc, which was then fixed, removed from the yolk by dissection, and preserved in 10% buffered formalin. A second estimate of fertility was obtained after emergence by examining the contents of unhatched eggs for the presence of embryonic remains or blood and adding these to the total number hatched. The two estimates of fertility were compared. The proportions of unhatched egg with embryos occurring in natural, reburied, and incubator nests were also compared.

RESULTS

Hatching Success of Reburied vs. Incubator Nests.—Of the 50 clutches reburied at Jeckyll Island, 9 were partially destroyed by ghost crab (*Ocypode quadrata*) predation, cold weather, or drifting sand. The remaining 41 nests had an overall hatching

success of 71% (3608 of 5100 eggs). In incubators, it was 83% (135 of 163 eggs). These differences were significant ($\chi^2 = 11.13$, $P < 0.001$, 1 df). None of the 28 failed eggs in the incubators showed a discernable embryo. Twenty-five reburied nests (3024 eggs) were examined thoroughly to determine the fates of the eggs. Of these, 2078 (69%) hatched normally while 946 failed to hatch. Embryos were found in 434 of the 946 unhatched eggs (46%). The total number of clearly fertile eggs was 2512, or 83%, identical to the percentages hatching in incubators.

Several embryos dissected from the non-viable eggs showed extensive fungal invasion (possibly *Mucor* spp.). Deformed embryos showed a variety of forms (including cyclopia, monorhina, acephaly, cranioschisis, twinning, and amelanization) and accounted for eleven embryos.

Hatching Success in Natural and Relocated Nests.—Of the 31 natural nests, six were lost because vandals removed markers and one was destroyed by tidal inundation. The 24 remaining natural and 10 relocated nests were excavated following hatchling emergence. In natural nests the shells of hatched eggs along with unhatched eggs were counted. In relocated nests unhatched eggs were counted and compared with the already known clutch size to determine hatching success. The position of nonviable eggs in each nest was noted. In natural nests at the CANA site 2400 of 2796 eggs (87%) hatched, while in the 10 relocated nests, 1054 of 1151 (92%) of the eggs hatched. Hatching success for relocated eggs was significantly greater ($\chi^2 = 24.26$, $P < 0.001$, 1 df). Analyses of each of the two relocation methods showed that they did not differ in their success rates. Eggs in polystyrene incubators had 91% hatching success (511 of 563), while reburied eggs showed 92% hatching success (543 of 588; $\chi^2 = 3.60$, NS).

Fertility Levels.—The 20 eggs (two from each of 10 females taken while they oviposited) all contained viable embryos. When unhatched eggs ($N = 396$) from natural nests were examined after emergence, 110 (28%) had embryos, while in reburied nests 12 (29%) of 45 unhatched eggs con-

tained embryos. Unhatched eggs from incubators totalled 52, of which 24 (46%) had embryos. A comparison between the two types of relocated nests indicated a higher (though not statistically significant) proportion of eggs contained embryos in the incubators ($\chi^2 = 3.60$, $0.05 < P < 0.10$, 1 df). Proportions of embryos in incubators also were higher than in natural nests ($\chi^2 = 6.64$, $P < 0.01$, 1 df), but reburied and natural nests showed no statistically significant difference ($\chi^2 = 0.03$, NS).

For incubator nests, 535 (511 emerged hatchlings plus 24 embryos) of 563 eggs or 95% of all eggs, were clearly fertile. Among reburied nests, there were 543 hatched plus 12 embryos, or 555 of 588 eggs (96%). In natural nests, the total was 2510 of 2796, or 90% of all eggs. Fertility levels, defined as the number of hatchlings plus the number of embryos, did not differ between incubator and reburied nests ($\chi^2 = 0.15$, NS). Fertility levels were statistically different between natural and relocated nests ($\chi^2 = 24.47$, $P < 0.001$, 1 df).

Nonviable eggs were removed from incubators after three weeks of development and again after emergence (9 weeks). Of the 11 eggs removed at three weeks, 7 (64%) contained embryos. At 9 weeks, 41 eggs were nonviable, of which 17 (41%) contained embryos. These differences were not significant ($\chi^2 = 1.04$, NS).

Bacterial Analyses.—Tests of the egg shell disinfection technique showed no bacterial species in common between egg shells and egg contents. We therefore conclude that the disinfection method is reliable.

The bacteria found in the cloacal fluid of female turtles, the nest sand, and unhatched eggs are listed in Table 1. Ten genera were identified; one (*Proteus*) was exclusively found in sand, two others (*Serratia* and *Klebsiella*) were confined to eggs, and a third (non-hemolytic *Staphylococcus*) was found only in females. The 6 remaining genera could be transmitted to eggs either by the mothers (*Acinetobacter*, *Moraxella*), or by both the mother and the nest sand (*Aeromonas*, *Enterobacter*, *Pseudomonas*, *Vibrio*).

Of the 20 nests originally used for cloacal bacterial samples, complete data were

TABLE 1. Summary of bacterial culture results. +, genera present; —, not found in the source.

	Culture source		
	Female turtle	Nest sand	Within nonviable eggs
<i>Acinetobacter</i>	+	—	+
<i>Aeromonas</i>	+	+	+
<i>Enterobacter</i>	+	+	+
<i>Klebsiella</i>	—	—	+
<i>Moraxella</i>	+	—	+
<i>Proteus</i>	—	+	—
<i>Pseudomonas</i>	+	+	+
<i>Serratia</i>	—	—	+
<i>Staphylococcus</i> (non-hemolytic)	+	—	—
<i>Vibrio</i>	+	+	+

obtained for 12 nests (Table 2). These contained 1414 eggs, of which 1308 (93%) hatched. Hatching success among nests was compared using a 12×2 contingency table. Differences were significant ($\chi^2 = 51.69$, $P < 0.001$, 11 df), though no nests had extraordinarily low hatching success (range of 81–98%). To determine if differences in hatching success related to the number of bacterial species present, average hatching success was considered and found not to differ among nests at either of two levels (0–1; 2–4) of maternal (cloacal) bacterial shedding ($\chi^2 = 0.17$, NS). The 12×2 contingency table was then subdivided (Zar, 1984) and the χ^2 values (not Cochran-corrected) thus obtained were subtracted from the total χ^2 (51.69). These analyses showed that the number of bacterial species present in the cloacal fluids ($\chi^2 = 0.52$; NS) or sand ($\chi^2 = 0.06$; NS) made no significant contribution to the observed differences in hatching success. However, nests containing high bacterial diversity (4–7 species) in nonviable eggs, or those bearing one bacterial species in common with their mothers, showed significantly reduced hatching success and did account for some, but not all, of the differences between nests ($51.69 - 22.77 = 28.92$, $P < 0.001$, 10 df and $51.69 - 11.22 = 40.47$, $P < 0.001$, 10 df, respectively). The Cochran-corrected χ^2 values showing the significance levels for each of these tests alone are presented in Table 3.

TABLE 2. Summary of complete bacterial data and hatching success for 12 nests at CANA. Where no bacteria are listed (none), no potential pathogens were cultured. When a genus is listed twice (e.g., P₁, P₂), the sample contained more than one species. Abbreviations: NN—natural nest; INC—incubator nest; RB—reburied nest; A—*Aeromonas*, Ac—*Acinetobacter*; Al—*Alcaligenes*; E—*Enterobacter*; Ec—*Escherichia coli*; K—*Klebsiella*; M—*Moraxella*; P—*Pseudomonas*; Pr—*Proteus*; S—*Serratia*; St—*Staphylococcus* (non-hemolytic); V—*Vibrio*; O—unidentified genera not included in the above.

Nest no.	Nest category	No. hatched	No. not hatched	Percent hatching	No. bacterial species in females	No. bacterial species in eggs	No. bacterial species common to females and eggs	No. bacterial species in sand
1	NN	152	9	94	2 P ₁ , V	3 Al, P ₂ , Pr	none	2 P, Ac
2	NN	90	8	90	2 Ac ₁ , Ac ₂	3 P ₁ , P ₂ , Ac	1 Ac	none
3	NN	122	6	95	4 P, St, M, O	2 P, S	1 P	none
4	INC	101	13	89	1 E	4 E, P, Al, O	1 E	—
5	INC	99	17	85	1 P	6 E, P ₁ , S, P ₂ , M, O	1 P	—
6	INC	110	2	98	1 P	2 P, Al	1 P	—
7	INC	91	6	94	1 O	6 P ₁ , P ₂ , E O, O, M	none	—
8	RB	96	22	81	3 P, M, E ₁	7 V, P, Al, E ₂ , Pr, Ec, O	1 P	—
9	RB	116	4	97	2 P, M	2 E, Pr	none	—
10	RB	86	2	98	none	5 P ₁ , P ₂ , E, Pr, Ec	none	—
11	RB	102	12	89	1 St	5 E, A, Al, P, K	none	—
12	RB	143	5	97	1 P	2 V, A	none	—

To examine correlations between the morphology of unsuccessful eggs and bacterial isolates, nonviable eggs were grouped according to external appearance into the following: (1) turgid, normal color; (2) not turgid, normal color; (3) shell with black or grey spots not penetrating the shell; (4) shell with black or grey bumps penetrating the thickness of the shell; (5) shell collapsed and yellowish-orange; (6) shell not collapsed and yellowish-orange; and (7) shell and/or contents pink. Because most categories were associated with several bacterial genera it was not possible to arrive at meaningful associations with potential pathogens except in category 7. *Ser-*

ratia marcescens was associated with all pink eggs. Eggs containing living but deformed fetuses were restricted to categories 1 and 2. Eggs which hatched late belonged to category 2 alone. Fungal invasion (possibly *Mucor* spp.) occurred in some eggs but was not associated with a particular category.

DISCUSSION

Reburied vs. Incubator Nests.—The effects of the two methods of incubation on relocated egg hatching success differed between the two sites. Eggs relocated to polystyrene incubators did better than those reburied at the hatchery on Jekyll Island. At CANA, the two relocation methods did

not show statistically significant differences but the trend of higher hatching success in incubator eggs was maintained. It is possible that the observed differences at Jekyll Island may be due to sampling error since only 3 nests were placed into incubators. It should be noted, however, that the two nest environments are quite different. Relocated eggs placed in polystyrene incubators are exposed to a more benign and controlled environment (moisture and temperature are stable and optimized). Thus, eggs placed in incubators may do better because of lack of exposure to environmental extremes.

The following factors may contribute to observed differences in reburied nest hatching success: (1) Jekyll Island is located about 2° latitude north of CANA and has a shorter nesting season. We obtained daily average temperatures (NOAA Climatological Data, 1983–1984) for the last month of nesting data collected at each of our two study sites. Nests hatching in October at Jekyll Island were exposed to cooler end-of-the-season average incubation temperatures ($21.77^{\circ}\text{C} \pm 1.58$, $N = 31$ days) than were seen in CANA nests during September ($26.31^{\circ}\text{C} \pm 0.87$, $N = 30$ days; $t = 36.22$, $P < 0.001$, 59 df). Cooler temperatures, in theory, might result in variation in rates of development or reduced ability of late fetuses to complete development, hatch and emerge. (2) Embryos invaded by fungus were relatively abundant at Jekyll Island, while only two were found at the CANA site. Fungal invasion could contribute to the overall lower hatching success at Jekyll Island but would not account for the results specific to each incubation method. (3) The sand is much finer and packs more densely at Jekyll Island. The impact on drainage and oxygen exchange in the nest may result in very different internal nest environments (Ackerman, 1980, 1981a, b), not only at the two sites but between the two incubation methods. (4) The complement of soil microbes may potentially differ between the two sites. The importance of any or all of the above is unknown.

Eggs that failed early in development were permitted to remain in the Jekyll Is-

TABLE 3. Proportions of eggs hatching in relation to the number of bacterial genera present in cloacal fluids, eggs, nest sand, and in both the females and their eggs. χ^2 values are Cochran corrected.

Bacterial genera in:	Number of bacterial genera	No. of nests	Hatch/not hatch totals	χ^2	Prob.
Cloacal fluids	0-1	7	732/57	0.38	NS
	2-4	5	576/42		
Nest sand	0	2	212/14	0.05	NS
	2	1	152/9		
Nonviable eggs	2-3	6	733/34	22.79	0.001
	4-7	6	575/72		
Both cloacal fluids and nonviable eggs	0	6	690/38	11.12	0.001
	1	6	618/68		

land incubators while those in the CANA incubators were removed at 3 weeks into incubation. If diseased eggs infect other eggs, as is suggested by the observed clustering of nonviable eggs, the potential for egg mortality was reduced in the CANA incubators.

Natural vs. Relocated Nests.—Natural nest hatching success was significantly lower than that of relocated nests. While natural nests are subject to the whims of nature and the turtles' choice of sites, relocated nests were placed in the best sites available. Thus, reburied nests were not subject to even incidental wave washover, or burial by collapsing dune scarp or drift sand.

Our results show that, at CANA, relocation was not detrimental to hatching success. These results suggest that as long as the eggs are not traumatized, are moved early (prior to the establishment of egg membranes) and placed in "safe" areas, relocating nests is an effective conservation method.

Fertility.—The data (Fig. 1) suggest that manipulation of eggs may contribute to egg failure, and that eggs believed to be infertile may not be. Because the proportion of infertile eggs differs according to incubation methods in our studies and in those of others (Bustard, 1972; Whitmore and Dutton, 1985; Fig. 1), it is likely that the

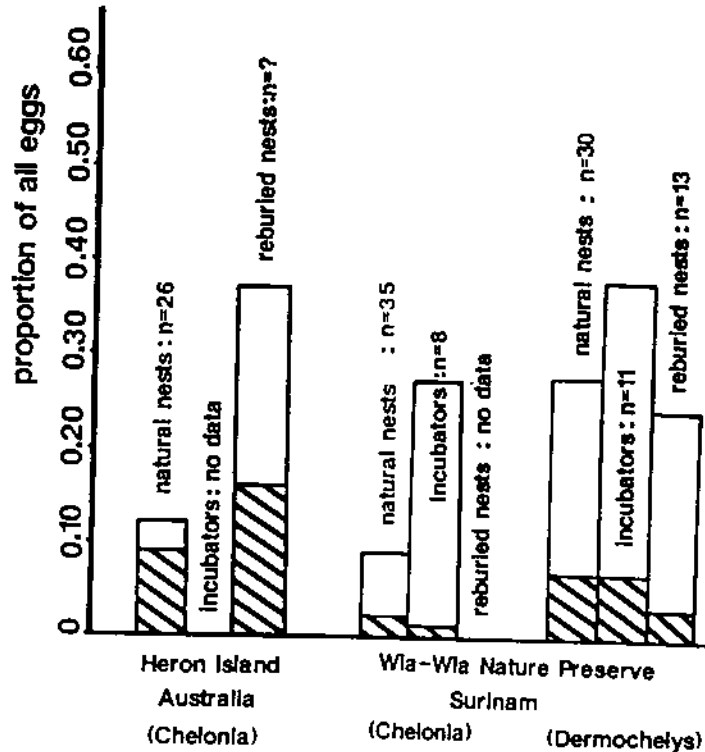


FIG. 1. Proportion of eggs failing to hatch in natural, reburied, and incubator nests at Heron Island, Australia (Bustard, 1972), and in Surinam (Whitmore and Dutton, 1985). Open bars, embryos present; hatched bars, eggs lacking any sign of an embryo; n = number of nests. Bustard's data represent estimates based upon emergence success and proportions of unhatched eggs designated as infertile. Data from Whitmore and Dutton represent subsets of natural nests above high tide (never inundated) and incubator nests placed in polystyrene boxes. Note wide variation in both infertility estimates and total egg failures, but generally lower levels of both categories of failure in undisturbed (natural) nests.

apparent infertility of eggs may be confused with early embryonic death. Small embryos may be missed by workers who examine the foul eggs in haste, or embryos may disintegrate, leading to the erroneous classification of embryonic mortality as infertility. When abnormal eggs were removed from the CANA incubators at 3 and 9 weeks, a higher proportion of nonviable eggs contained embryos at 3 weeks than after emergence. These findings suggest that embryos which died after 3 weeks but which were left in the nest for a longer period may have disintegrated by the time the nest was excavated. Our analysis of eggs sampled at oviposition showed 100% fertility. Others have also shown an increase in fertility levels when another criterion (the establishment of egg membranes) is used (Blanck and Sawyer, 1981; Whitmore

and Dutton, 1985). Unfortunately, this technique is impractical for use with buried eggs because it involves visual inspection at a time when excavation could result in movement-induced mortality. Because there is no a priori reason to expect that eggs which differ in treatment (when collected randomly at a given site) will differ in fertility, the data support the hypothesis that much embryonic death has been misidentified as infertility. Similar conclusions were also reached by Whitmore and Dutton (1985).

Bacterial Correlations.—Egg mortality associated with destruction of natural and relocated nests has been ascribed to erosion, predation, plant root invasion, and tidal inundation (Stancyk et al., 1980; Hopkins and Murphy, 1981). In relocated nests, hypotheses have focused upon the possi-

bility of movement-induced mortality caused by disruption of the egg membranes (Limpus et al., 1979; Limpus, 1980; Parmenter, 1980; Blanck and Sawyer, 1981; Whitmore and Dutton, 1985). Egg failure resulting from other causes has received little attention. Fungal invasion of eggs has been implicated in two cases (Solomon and Baird, 1979; G. Leong, pers. comm.). Bacterial pathogens have not previously been addressed in sea turtle egg failure.

Patterns of egg failure in both relocated and natural nests show that eggs fail in clusters. Because the clusters occur anywhere in the nest regardless of incubation method, egg failure may not be attributed solely to developmental abnormalities, physical stresses, position in the nest, or order of deposition. The clustering of nonviable eggs suggests an infectious etiology, (i.e., if one egg fails, those next to it also become infected and fail). Because more than one bacterial species was associated with each cluster it was impossible to identify a causal organism or seek specific correlations between bacterial species and egg failure. No single species was present in all nonviable eggs, but each had at least one potential pathogen.

Microbial pathogens appear to be implicated in egg failure. Those nests which had the lowest hatching success also had the highest number of bacterial species present. Additionally all bacterial species found represent known reptilian pathogens (Frye, 1981; Marcus, 1981; Jacobson, 1984). There were no statistically significant trends between the numbers of bacterial species in cloacal fluid or in nest sand and a nest's hatching success. However, complete nest sand data were available for only 3 nests.

There was a statistically significant trend for reduced hatching success when a bacterial species was found in both a mother and her clutch. Pathogen transfer from mother to egg may be more frequent than was detected by our sampling procedure. In theory, females may infect their clutches prior to oviposition. Shedding of the pathogen (the time when it can be detected) may occur only periodically. Thus eggs may become infected in the oviduct, but several

days later when nesting occurs, the shedding phase may have ended and no obvious pathogen may be detected in the cloacal fluid.

Other plausible (though untested) explanations for the occurrence of pathogens in the eggs that could not be traced to the mother or sand include the following. (1) Potentially pathogenic organisms may occur in extremely low levels in the mother or nest sand and hence are missed when cultures are taken. Once a nest is established, the warm, very moist environment may be ideal for the growth of such organisms which, in higher densities, may infect eggs. (2) Growth of soil organisms, such as bacteria and fungi, may be enhanced in the nest once it is established. Since a substantial amount of fluid is released by the mother as eggs are deposited and the eggs are relatively warm, the nest environment is very different from that of sand alone.

Analyses of egg failure in other families of turtles have identified *Salmonella* as a pathogen (Ewert, 1979). *Salmonella* and *Arizona* have been shown to be transmitted from mother to offspring through the egg stage in freshwater turtles (Siebeling et al., 1974), though the effect on hatching success was not addressed. The fungus *Fusarium solani* has been implicated as the causal organism in failure of Kemp's Ridley (*Lepidochelys kempi*) eggs incubated in polystyrene containers (G. Leong, pers. comm.). Solomon and Baird (1979) have described the invasion of *Aspergillus* spp. into the egg shell membranes of *Chelonia mydas* eggs and noted the potentially deleterious effects. These studies, along with the results presented above, suggest that further study of potential pathogens may be important in understanding a significant factor affecting hatching success.

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LITERATURE CITED

- ACKERMAN, R. A. 1980. Physiological and ecological aspects of gas exchange by sea turtle eggs. *Amer. Zool.* 20:575-583.
- . 1981a. Oxygen consumption by sea turtles (*Chelonia mydas*) during development. *Physiol. Zool.* 54:316-324.
- . 1981b. Growth and gas exchange of embryonic sea turtles (*Chelonia*, *Caretta*). *Copeia* 1981: 757-765.
- BLANCK, C. E., AND R. H. SAWYER. 1981. Hatchery practices in relation to early embryology of the loggerhead sea turtle, *Caretta caretta* (Linne). *J. Exptl. Marine Biol. Ecol.* 49:163-177.
- BUSTARD, H. R. 1972. Sea turtles: Their natural history and conservation. Taplinger Publishing Co., New York. 220 pp.
- EHRENFELD, D. 1982. Sea turtle conservation strategy. In K. A. Bjorndal (ed.), *Biology and conservation of sea turtles*, pp. 567-571. Smithsonian Institution Press, Washington, D.C.
- EWERT, M. A. 1979. The embryo and its egg: Development and natural history. In M. Harless and H. Morlock (eds.), *Turtles: Perspectives and research*, pp. 333-413. John Wiley and Sons, New York.
- FOWLER, L. 1979. Hatching success and predation in the green sea turtle *Chelonia mydas*, at Tortuguero, Costa Rica. *Ecology* 60:946-955.
- FRYE, F. L. 1981. Biomedical and surgical aspects of captive reptile husbandry. VM Publishing Co., Edwardsville, Kansas. 456 pp.
- HOPKINS, S., AND T. MURPHY. 1981. The reproductive ecology of *Caretta caretta* in South Carolina. South Carolina Marine Resources. Study Completion Report. Project No. E-1, Study No. VI-A-1. 97 pp.
- JACOBSON, E. R. 1984. Biology and diseases of reptiles. In J. G. Fox, B. J. Cohen, and F. M. Logon (eds.), *Laboratory animal medicine*, pp. 449-476. Academic Press, Orlando, Florida.
- LIMPUS, C. J. 1980. Potential problems in the artificial incubation of turtle eggs. *Herpetofauna* 12:23-24.
- , V. BAKER, AND J. D. MILLER. 1979. Movement induced mortality of loggerhead eggs. *Herpetologica* 35:335-338.
- MARCUS, L. C. 1981. *Veterinary biology and medicine of captive amphibians and reptiles*. Lea & Febiger, Philadelphia. 239 pp.
- MROSOVSKY, N. 1983. *Conserving sea turtles*. The British Herpetological Society, London. 175 pp.
- PARMENTER, C. J. 1980. The incubation of the eggs of the green sea turtle (*Chelonia mydas*), in the Torres Strait, Australia: The effect of movement on hatchability. *Austral. Wildl. Res.* 7:487-491.
- PRITCHARD, P. C. H. 1980. The conservation of sea turtles: Practice and problem. *Amer. Zool.* 20:609-617.
- REBEL, T. P. 1974. *Sea turtles*. University of Miami Press, Coral Gables, Florida. 250 pp.
- RICHARDSON, J. I., AND T. H. RICHARDSON. 1982. An experimental population model for the loggerhead sea turtle (*Caretta caretta*). In K. A. Bjorndal (ed.), *Biology and conservation of sea turtles*, pp. 165-176. Smithsonian Institution Press, Washington, D.C.
- SIEBELING, R. J., P. M. NEAL, AND W. D. GRANBERRY. 1974. Evaluation of methods for isolation of *Salmonella* and *Arizona* organisms from pet turtles treated with antimicrobial agents. *Appl. Microbiol.* 25:240-245.
- SOLOMON, S. E., AND T. BAIRD. 1979. The effects of fungal penetration on the egg shell of the green turtle. *Electron Micros.* 2:434-435.
- STANCYK, S. E. 1982. Non-human predators of sea turtles and their control. In K. A. Bjorndal (ed.), *Biology and conservation of sea turtles*, pp. 139-152. Smithsonian Institution Press, Washington, D.C.
- , O. R. TALBERT, AND J. M. DEAN. 1980. Nesting activity of the loggerhead turtle *Caretta caretta* in South Carolina. II. Protection of nests from raccoon predation by transplantation. *Biol. Conserv.* 18:289-298.
- WHITMORE, C. P., AND P. H. DUTTON. 1985. Infertility, embryonic mortality and nest site selection in the leatherback and green sea turtles in Suriname. *Biol. Conserv.* 34:251-272.
- WITHAM, R. 1982. Disruption of sea turtle habitat with emphasis on human influence. In K. A. Bjorndal (ed.), *Biology and conservation of sea turtles*, pp. 519-522. Smithsonian Institution Press, Washington, D.C.
- ZAR, J. H. 1984. *Biostatistical analysis*. 2nd ed. Prentice-Hall Inc., Englewood Cliffs, New Jersey. 718 pp.

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