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White Spot Development, Incubation and Hatching Success of Leatherback Turtle (*Dermochelys coriacea*) Eggs from Rantau Abang, Malaysia

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White spots developed after 4–5 d of incubation in fertile eggs of the leatherback turtle, *Dermochelys coriacea* from Rantau Abang, Malaysia. Hatch rates of eggs handled during the first 5 d of incubation and after the appearance of the white spot were not impaired if care was exercised in handling to prevent horizontal and vertical rotation of eggs. Selection of fertile eggs for hatchery work based on the development of the white spot appears promising. Hatch rates in eggs incubated in the beach hatchery were generally lower than for eggs incubated in styrofoam boxes kept indoors. Incubation times in the beach hatchery ranged from 55–59 d, while in the styrofoam boxes it ranged from 63–82 d.

NOT all sea turtle eggs are fertile. Hughes et al. (1967), Hughes (1970) and Ehrhart (1981) reported that fertility rates may range from 80–90%. In Rantau Abang, Malaysia, the infertility rate among clutches of leatherback eggs was reported to be 5.6% in 1967 (Balasingam, 1967) while Chan et al. (1985) found that it could reach 22%. Egg infertility has implications on the economics of hatchery practices in Rantau Abang, where leatherback eggs for incubation have to be purchased for MR $1.60 (U.S. $0.60) each.

An attempt was thus made to develop some criteria for selection of fertile and viable clutches for hatchery work. The criterion used was that fertile or viable eggs will develop a white spot on the top surface of the shell during the first few days of incubation (Yntema, 1981). This white spot will enlarge during incubation until the entire shell become opaque and white. In-

fertile eggs on the other hand, will fail to develop the white spot but instead will remain a creamy beige color throughout incubation. Yntema (1979), Ewert (1979) and Blanck and Sawyer (1981) attributed the formation of the white spot to the adherence of the vitelline membrane to the inner shell surface during early embryological development. Thomson (1985) reported that the white spot in *Emydura macquarii* is formed by partial drying of the shell and shell membranes adjacent to the underlying extra-embryonic membranes and suggested that the drying facilitates exchange of respiratory gases. Webb et al. (1987) provide a detailed explanation for the spotting process.

The experiments described in this paper were designed to determine: a) when the white spot appears on leatherback eggs; b) whether handling of the eggs after the development of the white spot has any effects on hatch rates; and c) how incubation and hatch rates in styrofoam boxes and in a beach hatchery differ.

Materials and Methods

The experiments were conducted in the Rantau Abang Turtle Hatchery and Universiti Pertanian Malaysia in Mengabang Telipot, 100 km north of Rantau Abang, from 21 July–12 Oct. 1986. Eggs from four natural nests (designated clutch 1 of 75 eggs, clutch 2 of 69 eggs, clutch 3 of 109 eggs and clutch 4 of 81 eggs) were collected immediately after laying and brought to the hatchery in covered pails. Only normal-sized yolked eggs were taken while the undersized yolkless eggs were discarded. Experimental eggs were assigned to their specific experimental procedures at about 0700 h, with careful handling to prevent horizontal rotation and reorientation of their vertical axes (vertical rotation). Seventy-five eggs were taken from each clutch, except for clutch 2 where only 69 eggs were available. Eggs from each clutch were divided into three lots, A, B, and C of 25 eggs each (23 each for clutch 2). There were hence three treatments with four replicates each. "A" eggs were incubated in sand-nests, 60 cm deep, in the open-air beach hatchery described in Siow (1982). "B" and "C" eggs were placed in styrofoam boxes following the method described by Pritchard et al. (1983). The styrofoam boxes were transported to Universiti Pertanian Malaysia the same morning.

Eggs in the "B" lots were examined once daily in the morning for the appearance of the white spot. When it appeared, all the eggs from the box were carefully removed and handled as described earlier to enable counting of eggs with the white spots. After counting, they were carefully replaced in their respective boxes. Eggs without the white spots were placed on the top to enable subsequent examination without moving them. These eggs were observed for a further 3 d, after which the nest was left to incubate undisturbed.

Eggs in the "C" boxes were left to incubate undisturbed after they were brought back to the laboratory. Boxes 1B, 1C, 2B and 2C were kept in a well-ventilated shed while boxes 3B, 3C, 4B and 4C were kept in an enclosed laboratory. Mercury thermometers (−10 to 50 C) were inserted horizontally into the center of the nest through a hole made on the side of the box to monitor incubation temperatures in the "B" boxes during the first 8 d of incubation and in the "C" boxes throughout the incubation period.

The moisture in the boxes was maintained by sprinking tap water on the sand daily. The muslin cloth covering the top layer of eggs was removed after about 50 d of incubation, but the sand was retained. After 50 d of incubation, nests in the beach hatchery were each surrounded by chicken mesh to restrain the hatchlings for counting. The number of hatchlings emerging from the experimental nests in the beach hatchery and styrofoam boxes were recorded and the respective hatch rates calculated. A two-way ANOVA was performed on arcsine transformed percent hatch rate data for clutch and experimental effects.

In addition to the treatments described above, the remaining 34 and six eggs from clutches 3 and 4, respectively, were placed in containers of sand in an oven maintained at 30.3–31 C for monitoring the development of the white spot at this temperature. Hatch rates for these eggs were not monitored as samples were progressively taken for embryological studies.

Results

White spot development.—White spots developed in all four clutches of eggs used in the experiments. The timing of the appearance of the white spot was similar in all the "B" boxes as well as in the oven. The white spot first appeared faintly on the fourth day of incubation.

TABLE 1. HATCH RATE AND INCUBATION TIME FOR EGGS INCUBATED IN SAND-NESTS IN THE BEACH (A) AND IN STYROFOAM BOXES (B AND C). Number in parentheses for the "B" boxes indicates the number of eggs which developed white spots.

Clutch	No. of eggs incubated	No. of hatchlings emerged	Incubation time (days)	% Hatch rate
1A	25	8	58	32
2A	23	3	58–59	13
3A	25	18	56	72
4A	25	23	55–59	92
1B	25 (22)	18	75–77	72
2B	23 (21)	16	77–79	70
3B	25 (25)	25	63–65	100
4B	25 (23)	21	63–65	84
1C	25	16	76–79	64
2C	23	12	79–82	52
3C	25	25	63–65	100
4C	25	24	63–65	96

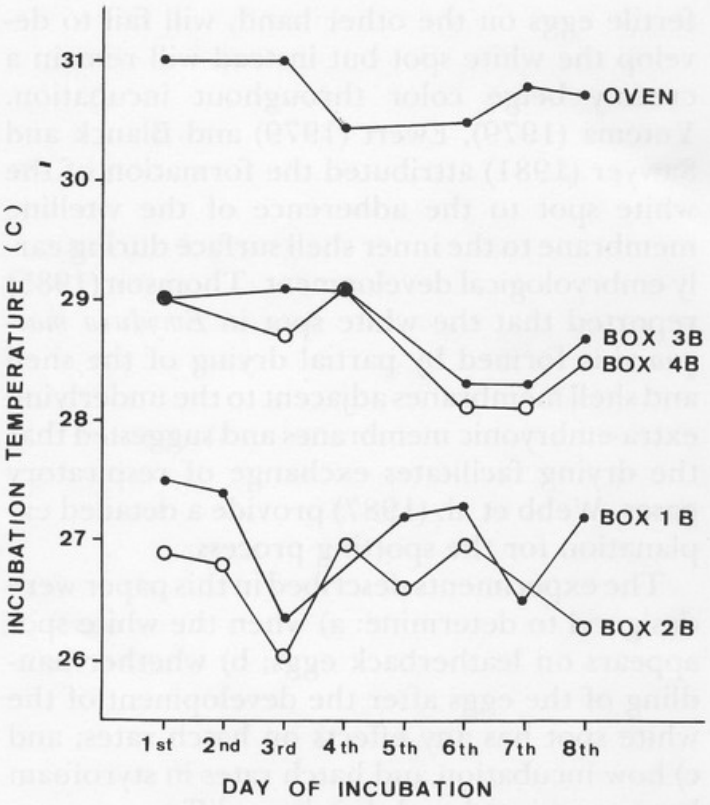

Fig. 1. Incubation temperature of eggs monitored for the development of the white spot. Refer to text for explanation of treatments.

By the fifth day, it was clearly discernable as an opaque white patch of diameter 1–1.5 cm. The number of eggs developing white spots on the fifth day of incubation were 22, 15, 25 and 23 for boxes 1B, 2B, 3B and 4B, respectively (Table 1). The remaining few eggs without white spots in boxes 1B and 4B never developed the white spot. On the sixth day of incubation, six more eggs from box 2B developed white spots (total now 21).

The nest temperatures for the "B" eggs as well as eggs placed in the oven during the first 8 d of incubation are shown are Figure 1. The higher temperatures in boxes 3B and 4B and in the oven did not seem to hasten the development of the white spot.

Incubation and hatch rates.—The hatch rates and incubation periods for all the experimental batches are shown in Table 1. In general, clutches 1 and 2 had poor hatch rates which appeared more pronounced in the beach hatchery. Hatch rates in the "B" boxes were quite good despite the handling of eggs during the first 5 d of incubation. To confirm this, a two-way ANOVA was performed which showed significant differences in hatch rates between clutches (confidence level of 99.08%) but the differences among the treatments were slight (confidence level of 95.78%). However, when the multiple range Student-Newman-Keul's test was performed for treatment effects, differences could not be detected at 95% confidence level but could be detected at 90% confidence level. At this confidence level, the hatch rates in the beach ("A" eggs) were generally lower while hatch rates in the styrofoam boxes ("B" and "C" eggs) were similar. This suggests that careful handling of eggs during the first 5 d of incubation to monitor the development of the white spot did not impair hatch rates.

The incubation time for nests incubated in the open-air beach hatchery ranged from 55–59 d for the four clutches. Eggs from clutches 1 and 2 incubated in styrofoam boxes kept in a well-ventilated shed where incubation temperatures ranged from 25–30 C took 75–82 d to hatch while clutches 3 and 4 kept in an enclosed laboratory where incubation temperatures ranged from 27–32 C took 63–65 d to hatch.

DISCUSSION

The first appearance of the white spot has frequently been reported to occur within a day or less after oviposition (Yntema, 1964, 1979; Blanck and Sawyer, 1981). Ewert (1979) re-

ported that the white spot will appear in eggs between a few hours to 3 d old. In a later review, however, Ewert (1985) reported that for some freshwater turtles, onset of spotting can range from 1–42 d. Our study shows that it takes about 4–5 d of incubation before the white spot will develop on eggs of *Dermochelys*. Whitmore and Dutton (1985) observed that, while relocating "doomed" leatherback eggs within 24 h after oviposition, none of these eggs had developed the white spot. The rate of development of the white spot did not appear to be influenced by temperature. Eggs held at 26–27.5 C, 28.1–29.1 C and at 30.3–31 C developed the white spot at the same rate. However, this will need to be investigated further by monitoring the eggs at shorter time intervals because in the present study, the eggs were monitored only once daily.

Careful handling of eggs after the development of the white spot did not seem to impair hatch rates, as shown in the statistical analysis. Hence, selection of fertile clutches based on the appearance of the white spot appears promising. Spotting has been used by Yntema (1981) as an index of egg viability in laboratory experiments and by Whitmore and Dutton (1985) as an index of fertility in the examination of unhatched eggs. However, presence of the white spot does not guarantee hatchability (Table 1). Additionally, it must be cautioned that the eggs must be handled with extreme care to prevent horizontal as well as vertical rotation of eggs. Limpus et al. (1979) and Parmenter (1980) found that inversion and rolling of eggs (vertical rotation) in early post-ovipositional development can significantly reduce hatch rates in loggerhead and green turtle eggs. Later experiments have indicated that mortality can be induced by horizontal rotation as well (Limpus, pers. comm.). Chan et al. (1985) reported that rough handling of eggs, i.e., involving horizontal and vertical rotation beyond 5 h after oviposition can significantly reduce hatch rates of leatherback eggs. However, the embryos of some freshwater turtles may not have a period of sensitivity to turning, or the effects may be much less severe (Ewert, 1985).

In the application of the method to hatchery operation, it is recommended that clutches be replanted as is normally practiced (Siow, 1982). Until we are able to confirm or disprove the discovery of Balasingam (1967) that small clutches of ca 50 eggs give enhanced hatch rates, the normal practice of replanting in clutches of ca. 50 eggs will be maintained. After five or six days of incubation in the sand on the beach, the nest can be excavated and the top layer of eggs examined for white spot development. Clutches which have not developed the white spot can be retrieved. The infertile eggs can either be returned to the egg collectors for reimbursement or sold to consumers. Eggs which have been buried for 5–6 d are still fresh and should be accepted by consumers. In the market, the eggs remain fresh up to 2 wk, after which they are boiled in brine and sold as preserved eggs. Workers in the hatchery also dig up unhatched clutches at the end of the incubation period (usually after more than 70 d) for consumption. The eggs from these 100% infertile clutches appear remarkably fresh, with the yolk intact, and are relished by the villagers.

Currently, about 25,000 leatherback eggs are purchased yearly for hatching in Rantau Abang (Brahim et al., 1987). Assuming that the infertility rate among clutches is 22% (Chan et al., 1985), the savings from practicing egg selection can be substantial.

The increased incubation time for eggs incubated in styrofoam boxes was probably caused by lowered temperatures in the boxes compared to the beach nests. Mrosovsky and Yntema (1980) estimated that a 1 C lowering of temperature is reflected in a 5 d increase in incubation time. In this study, incubation times in styrofoam boxes lengthened from 4–24 d, implying a lowering of incubation temperatures of 0.8–4.8 C compared to nests in the beach. This will have effects on the sexes of the hatchlings produced (Yntema, 1976; Rimblot et al., 1985).

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Appendix 1

Statistical Analysis

Two-way ANOVA on arcsine transformed data of % hatch rate of Table 1. Absolute values of number of hatchlings emerged could not be used because of differences in number of eggs for each treatment-clutch combination.

(Using 95% confidence level, i.e., a = 0.05)

Clutch	Treatment A	Treatment B	Treatment C	Mean per clutch
1	34.450	58.052	53.130	48.544
2	21.134	56.789	46.146	41.356
3	58.052	90.000	90.000	79.351
4	73.570	66.422	78.463	72.818
Mean per treatment	46.802	67.816	66.935	

Analysis of variance

Sources of variation	Sum of squares	df	Mean square	*F*-ratio
Clutch	3049.5410	3	1016.5137	10.104
Treatment	1130.2968	2	565.1484	5.618
Residuals	603.6185	6	100.6031	
Total	4783.4563	11		

For Clutch effect: $P = 0.00923$ (Confidence level = 99.08%), i.e., clutch effects significant.

For Treatment effect: $P = 0.04219$ (Confidence level = 95.78%), i.e., treatment effects significant.

Student-Newman-Keul's test for treatment effects

Source	Diff. means	SE	q-calculated	*P*	q (90%)	Hypothesis
B vs A	21.014	5.015	4.190	3	3.559	difference
B vs C	0.881	5.015	0.176	2	2.748	no difference
C vs A	20.133	5.015	4.015	2	2.748	difference

Hence B = C ≠ A.

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