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Effects of Handling on Hatchability of Eggs of the Leatherback Turtle, *Dermochelys coriacea* (L.)

E.H. CHAN, H.U. SALLEH and H.C. LIEW
Faculty of Fisheries and Marine Science,
Universiti Pertanian Malaysia,
Mengabang Telipot, Kuala Terengganu, Terengganu, Malaysia.

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ABSTRAK

Kadar ketidaksuburan di antara kumpulan telur penyu belimbing (Dermochelys coriacea) *yang telah dieramkan di Pusat Penetasan Penyu di Rantau Abang, Malaysia, dianggarkan sebanyak 22%. Varians penetasan di antara kumpulan telur yang subur berjulat antara 35.7 – 96.7% ($\bar{x}$ = 78%) bagi telur yang telah dikendalikan dengan penuh teliti. Untuk telur-telur ini kadar penetasan tidak menunjukkan korelasi masa di antara peneluran dan menimbus semula. Telur-telur penyu belimbing hanya boleh menahan pengendalian secara kasar selama 5 jam selepas oviposisi. Selepas dari tempoh ini cara penjagaan yang teliti untuk menahan daripada penggulingan, pelanggaran, pusingan dan penyahorientasi paksi tegak dapat meninggikan kadar penetasan.*

ABSTRACT

The infertility rate among clutches of leatherback sea turtle (Dermochelys coriacea) *eggs incubated in the hatchery at Rantau Abang, Malaysia is estimated at 22%. Variance in hatchability among fertile clutches ranges from 35.7 – 96.7% ($\bar{x}$ = 78%) for eggs handled with maximum care. For such eggs hatchability did not show a correlation with the time between oviposition and reburial. Leatherback eggs can tolerate rough handling only up to 5 hours after oviposition. Beyond this threshold, careful handling to prevent rolling, bumping, rotation and disorientation of the vertical axis will enhance hatch rates.*

INTRODUCTION

Hatchery operation is an important tool in sea turtle conservation. Protected incubation under natural conditions, coupled with release of hatchlings after emergence is considered by turtle conservationists as a safe and effective conservation technique (Ehrenfeld, 1981). In Rantau Abang, Malaysia, natural hatching is nonexistent because of 100% commercial harvesting of eggs of the leatherback turtle *Dermochelys coriacea.* A hatchery is critically important. The one described by Siow (1982) was set up by the Terengganu State Fisheries Department in 1961 and since then has released more than 500,000 hatchlings into the sea.

One of the major problems in hatchery work is the inconsistency of hatch rates. Siow and Moll (1981) reported that annual hatching rates in the Rantau Abang Turtle Hatchery have fluctuated from 32% to 71% between 1961 and 1978, with an overall average of 51.6%. In Puerto Rico, *in situ* hatching success of natural clutches of leatherback eggs averages 72% (Tucker & Hall, 1984). Low hatch success of hatchery eggs has been attributed to movement induced mortality (Bustard, 1972; Limpus *et al.*, 1979; Parmenter, 1980). Blanck and Sawyer (1981) suggested that the most critical period in the handling of sea turtle eggs occurs from 2 days to 2.5 weeks. In *Chelonia mydas,* transportation of eggs to the final destination within 3 hours after oviposition would enhance hatching rates. The most sensitive period was reported to occur from 1 – 7 days after oviposition (Parmenter, 1980). Sensitivity to movement has not been reported for leatherback turtle eggs. This paper discusses the effects of different handling methods and duration the eggs were held after oviposition.

MATERIALS AND METHODS

The experiments were conducted in the Rantau Abang Turtle Hatchery from 29 July 1984 to 13 October 1984. All experimental eggs were collected from 29 July to 6 August 1984. Eggs from each nesting or clutch were collected immediately after oviposition and divided into 3 lots prior to transportation to the Hatchery, about 1 km from the collection site. Number of eggs per lot ranged from 20 – 36, depending on the clutch size. These lots were designated A1, A2 and A3.

A1 eggs were held in plastic pails filled with about 4 cm of sand. There was very little rolling of eggs during transportation. When ready for replanting, these eggs were removed singly from the pail and placed carefully in the sand-nests to maintain their vertical orientation.

A2 and A3 eggs were transported in sugar sacks which is the normal practice in the Hatchery. The vertical orientation of the A2 eggs was carefully maintained when replanted in sand-nests. A3 eggs, the control lot, were rolled onto the sand by inverting the sugar sack and then placed in the sand-nests. These eggs were handled quite roughly and their vertical axis was not maintained. This is the usual practice in the Hatchery.

Clutches were held for varying times in their containers before replanting to determine the threshold of sensitivity to rough handling. These time treatments were: –

B1 : replanted within 1 hour of oviposition

B2 : replanted at 3 hours after oviposition

B3 : replanted at 5 hours after oviposition

B4 : replanted at 7 hours after oviposition

B5 : replanted at 9 hours after oviposition

B6 : replanted at 11 hours after oviposition

There was a total of 18 treatments (Table 1). Eggs from a total of 18 nesting turtles were used. The three lots from each clutch or turtle were held for the same time before reburial. Each combination of handling and time was replicated three times.

In the Hatchery each lot was incubated in an individual open-air sand-nest at a depth of about 70 cm. The incubation period ranged from 54 to 62 days with most of the hatchlings emerging between 56 to 58 days.

RESULTS

Infertile Eggs and Variation in Hatchability Among Clutches

Of the 18 clutches of eggs used for the experiments, four clutches were found to produce zero hatch rates. Two of these clutches had been reburied within one hour of oviposition while the other two had been reburied 11 hours after oviposition (Table 1). When these unhatched eggs were excavated at the end of the experimental period, i.e. after more than 70 days of incubation, they were found to be in good condition with no signs of moulding on the external surface of the egg-shell. On opening, no embryonic stages were detected. The eggs yolks were intact and surrounded by thick albumin as in fresh eggs. These clutches with zero hatch rates were probably infertile. If this is true, the infertility rate of clutches in Rantau Abang can be estimated to be 22%.

Different clutches of eggs produced different hatch rates. By considering only A1 lots where eggs were handled with maximum care and the vertical orientation eggs was maintained, variance in hatchability among lots ranged from 0 – 96.67% (Table 1). Hatchability did not show a correlation with the time between laying and reburial for the A1 lots. The average hatching success for all A1 eggs was 59%. If infertile

TABLE 1
Summary of data and results for treatments.

Time treatment	A1			A2			A3			Replicate	Date of nesting
	Total no. planted	Total no. emerged	% hatch rate	Total no. planted	Total no. emerged	% hatch rate	Total no. planted	Total no. emerged	% hatch rate		
B1	29	0	0	26	0	0	26	0	0	1	4.8.84
	22	0	0	20	0	0	20	0	0	2	4.8.84
	34	31	91.18	35	31	88.57	36	29	80.56	3	6.8.84
B2	21	11	52.24	20	13	65.00	22	14	63.64	1	29.7.84
	30	29	96.67	30	27	90.00	30	24	80.00	2	30.7.84
	30	21	70.00	25	20	80.00	26	20	76.92	3	31.7.84
B3	34	27	79.41	35	29	82.86	34	27	79.41	1	29.7.84
	33	19	87.88	30	21	70.00	30	17	56.67	2	31.7.84
	24	16	66.67	22	9	40.91	21	16	76.19	3	1.8.84
B4	31	26	83.87	30	29	96.67	31	21	67.74	1	29.7.84
	21	17	80.94	22	17	77.27	22	15	68.18	2	5.8.84
	—	—	—	—	—	—	—	—	—	*3	4.8.84
B5	28	10	35.71	28	8	28.57	26	7	26.92	1	31.7.84
	32	30	93.75	27	24	88.89	27	20	74.07	2	4.8.84
	—	—	—	—	—	—	—	—	—	*3	30.7.84
B6	30	25	83.33	31	26	83.87	28	22	78.57	1	5.8.84
	30	0	0	30	0	0	32	0	0	2	5.8.84
	32	0	0	36	0	0	25	0	0	3	6.8.84

*Rejected replicates because of errors in treatment assignment.

clutches were excluded, hatchability ranged from 35.7 to 96.7% ($\bar{x}$ = 78.16%). Thus if infertile clutches can be identified before replanting, hatchery productivity may increase by about 20%. This is significant considering that eggs are purchased at $1.50 per piece for hatchery work.

Handling and Time Effects

There was a very large variance among clutches. The hatch rate data was neither normally distributed, nor did it satisfy the assumption of homogeneity of variances. Parametric statistical analysis was inappropriate.

TABLE 2
Normalised and ranked values for handling and time treatments.
Normalised value, $N = \frac{(x - \bar{x})}{S.D}$ for each treatment-time combination.

Ranked values were assigned in ascending order based on the normalised data.

	Handling Treatment					
	A1		A2		A3	
Time Treatment	Normalised value	Ranked value	Normalised value	Ranked value	Normalised value	Ranked value
	—	—	—	—	—	—
B1	—	—	—	—	—	—
	0.80	27.5	0.33	22.0	−1.12	4.0
	−1.15	1.5	0.67	25.5	0.48	23.5
B2	0.93	32.0	0.13	17.0	−1.06	8.0
	−1.10	7.0	0.85	31.0	0.25	18.0
	−0.58	12.5	1.16	36.0	−0.58	12.5
B3	1.04	34.0	−0.10	15.0	−0.95	10.0
	0.30	20.0	−1.12	4.0	0.82	29.0
	0.08	16.0	0.96	33.0	−1.04	9.0
B4	0.83	30.0	0.28	19.0	−1.11	6.0
	—	—	—	—	—	—
	1.14	35.0	−0.39	14.0	−0.75	11.0
B5	0.80	27.5	0.32	21.0	−1.12	4.0
	—	—	—	—	—	—
	0.48	23.5	0.67	25.5	−1.15	1.5
B6	—	—	—	—	—	—
	—	—	—	—	—	—

Hence the percent hatch rates were normalised for each clutch, using the formula $((x-\bar{x})/$ S.D.) and the resulting values were ranked (Table 2). Two replicates were rejected because of errors in treatment assignment. Infertile clutches were also excluded in the analysis. The data were then analysed using the Kruskal-Wallis test (Zar, 1974) to test the hypothesis that the rank of the normalised values was random. To a high degree of probability, it was not. A significant difference was detected between the handling effects ($H = 8.237$, d.f. $= 2$ and $0.025 > p > 0.01$). Using the nonparametric multiple comparison test (Zar, 1974), it was found that the hatch rates for lots A1 and A2 were the same, but different for the A3 lots (A1 = A3, $0.05 > p > 0.025$; A1 = A2, $p > 0.50$; A2 = A3, $p < 0.001$). This indicates that careful handling of the turtle eggs to prevent bumping and rolling and disorientation from the vertical axis as they were placed in the nest had a significant effect on hatchability. There was no difference, however, in hatchability if the eggs were transported in sugar sacks or in pails, provided that they were handled carefully on replanting.

Normalised values for each lot were plotted against the time of planting. *Figure 1* shows that for eggs planted at 1, 3 and 5 hours after oviposition, normalised values for A1, A2 and A3 handling techniques seem to overlap over a wide range. Beyond 5 hours after oviposition, normalised values for all A3 lots separate into a cluster of negative values while A2 and A3 lots continue to overlap in a cluster of higher values. The normalised data were divided into two groups according to time treatments, i.e. B1 to B3 in one group and B4 to B6 in the other. The normalised values were then reranked and the Kruskal-Wallis test performed for the respective groups to test the handling effects. No significant difference was detected for the B1 to B3 group ($H = 1.241$, d.f. $= 2$, $0.75 > p > 0.5$) while a significant difference was found for handling effects in the B4 to B6 group ($H = 9.78$, d.f. $= 2$, $0.01 > p > 0.005$). For the B4 to B6 group, it was found that the relative hatchability of lots A1 equal A2 but do not equal A3 using the nonparametric multiple comparison test (A1 = A3, $0.01 > p > 0.005$; A1 = A2, $0.50 > p > 0.20$; A2 = A3, $0.001 > p$). This shows that the usual practice of rough handling and rolling the eggs into the nest can cause decreased hatchability if eggs are replanted more than 5 hours after oviposition.

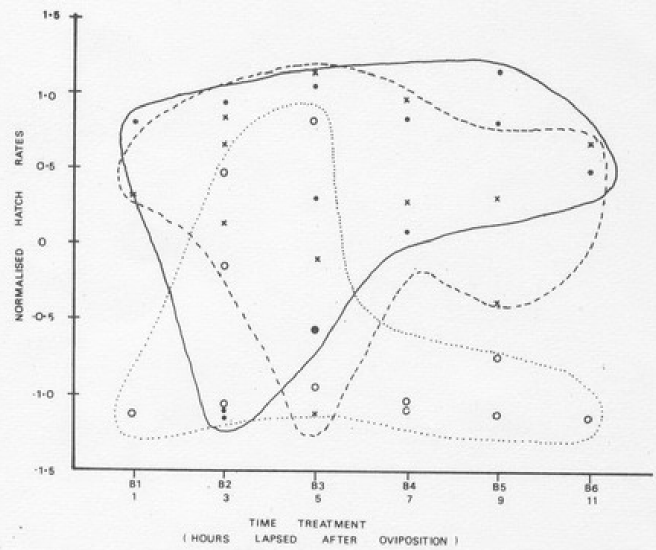

*Fig. 1. **Relationship between normalised hatch rates** and time treatment for A1 (dots bounded by full line); A2 (crosses bounded by broken line) and A3 (open circles bounded by dotted line) handling techniques. See text for explanation of A1, A2 and A3.*

DISCUSSION

It is generally understood that not all turtle eggs deposited on the beach are fertile (Ehrhart, 1981). Fertility rates have been reported to range between 80 − 90% annually (Hughes *et al.*, 1967; Hughes, 1970; Ehrhart, 1981), which is in agreement with the infertility rate of 22% estimated in the present study. Fertility studies on turtle eggs have been neglected. It is still not possible to distinguish an infertile egg from one which had died from early embryonic death (Owens, 1981). Blanck and Sawyer (1981) found that all fertile eggs of *Caretta caretta* develop a white circle on the shell during the first day of incubation. This circle enlarges during incubation until the egg is entirely white. Infertile eggs on the other hand remain a creamy beige colour throughout. The formation of the white ring is related to the adherence of the shell membrane to the shell during early embryonic development and appears to apply to all turtle eggs during incubation (Harless and Morlock, 1979). Further work on leatherback eggs is being conducted to determine when this white patch appears and the possibility of holding the eggs till the patch appears so that those that remain a uniform beige colour can be rejected for hatchery work and returned to egg collectors for reimbursement. Such eggs can subsequently be resold for consumption.

Natural variance in hatchability among clutches has been observed in sea turtles. Siow (1982) examined 100 natural clutches of leatherback eggs in Rantau Abang and found the hatch rate to range from 0 – 95%, with a mean rate of 65.06%. In St. Croix, U.S. Virgin Islands, mean hatching success of leatherback nests surviving to term was 59.1% with a range of 0 – 97.3% (Eckert and Erkert, 1984). Our range of 0 – 96.67% in hatchability for eggs handled with maximum care falls within these values. We still do not understand why this natural variance occurs.

Results from the handling and time-effect experiments show that leatherback turtle eggs can withstand rough handling only up to 5 hours after oviposition. This threshold seems significantly shorter than reported by other workers (Blanck and Sawyer, 1981; Parmenter, 1980). Beyond this threshold, extreme care must be exercised on replanting to prevent undue rotation, rolling, bumping and disorientation of the vertical axis of eggs. The sensitivity of eggs to movement can be explained by events which occur in early embryological development. When an egg has remained stationary for a period after oviposition, the yolk rises through the albumin to a position at the top of the egg (Fisk & Tribe, 1949). The turtle embryo develops at the top of the egg, just underneath the egg-shell. Such an orientation may be crucial for the survival of the developing embryo.

Sensitivity to rolling, bumping and rotation has been attributed to the disruption of some type of early membrane formation. Blanck and Sawyer (1981) found that two extra-embryonic membranes were formed in *Caretta caretta* eggs between 36 hours and 45 days of incubation. These delicate membranes encompass the embryo and attach it precariously to the top of the shell and any tearing would result in death. The timing of extra-embryonic membrane formation may differ from species to species. Hence while Blanck and Sawyer (1981) indicated sensitive periods for *C. caretta* to occur between 36 hours and 45 days of incubation, Parmenter (1980) found that in *Chelonia mydas,* sensitivity was greatest from 1 to 7 days of incubation and did not totally abate till after 20 days. Further, he recommended that *C. mydas* eggs should be transported to the final destination within 3 hours after oviposition.

It is clear that hatchery operation can be optimised only on the basis of an understanding of the fundamental processes of developmental biology of the species concerned. This preliminary work has now prompted us into studies aimed at developing criteria for selection of fertile and viable eggs for hatchery work; normal developmental biology of leatherback turtles; and properties of fresh, developing and unhatched eggs.

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