HERPETOZOA 22 (3/4): 137 - 143
Wien, 30. Dezember 2009

Structure and chemical composition of shells of abnormal eggs of Olive Ridley Turtles, *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) (Testudines: Cheloniidae)

Struktur und chemische Zusammensetzung der Schalen abnormer Eier der Bastardschildkröte *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) (Testudines: Cheloniidae)

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KURZFASSUNG

Die Autoren untersuchten die chemische Zusammensetzung und morphologische Struktur abnorm großer und kleiner Eier in Gelegen der Bastardschildkröte *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). Hinsichtlich Größe und Aggregatbildung (zwei bis fünf miteinander verbundene) abnorme Eier sind bei dieser Art nicht ungewöhnlich; sie zeigen keine Anzeichen sich entwickelnder Embryonen, sind also steril.

Der Kalziumgehalt der Schalen besonders großer (8,5–12,0%) und kleiner (3,1–6,0%) Eier war deutlich niedriger als von Schalen normal großer Eier (21%). Im Anteil anderer chemischer Elemente an der Schale waren die abnormen und normalen Eier einander ähnlich. Bei bei den abnormen Eitypen besaßen die Schalen eine un-vollständige Kalkschichte in Form miteinander verschmolzener Körnchen ohne klare Struktur. Die Faserschicht bestand nahezu nur aus einer einzigen Lage von Fasern, die in Schalen abnorm großer Eier als regelmäßiges Flechtwerk, in Schalen abnorm kleiner Eier aber unregelmäßig angeordnet waren. Abnorme Eitypen werden vermutlich bei Kalziummangel und unter unausgewogenen physiologischen Bedingungen gebildet.

ABSTRACT

We studied the chemical composition and morphological structure of abnormally large and small eggs in clutches of the Olive Ridley Turtle, *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). Eggs of abnormal size and aggregation (two to five eggs joined together) are not uncommon in this species. Such eggs are infertile and do not show any sign of embryonic development.

The calcium content of shells of both abnormally large (8.5–12.0%) and small (3.1–6.0%) eggs was clearly lower than in shells of normal sized eggs (21%). The proportion of other chemical elements in shells of such eggs was comparable to the normal condition. In both abnormal egg types, the shells had an incomplete calcareous layer of fused granules without defined structure, and the fibrillar stratum was made up of a single layer only; in shells of abnormally large eggs the fibers were disposed in a trellis pattern; whereas, they were irregularly arranged in shells of small eggs. Such shell types are probably formed under calcium deficiency or imbalanced physiological conditions.

KEY WORDS

Reptilia: Testudines: Cheloniidae; *Lepidochelys olivacea*, Olive Ridley Turtle, abnormal egg, eggshell, calcium, ultrastructure, shell unit, fibers, eggshell chemical composition, Gahirmatha, Orissa, India

INTRODUCTION

The eggshell has several functions, such as protection from desiccation, representing a link between external environment and egg content. (PACKARD 1994; PACKARD & PACKARD 1991; SCHLEICH & KÄSTLE 1988) and a calcium reserve for the developing embryo (SAHOO et al. 1998). The structural details of a large number of reptilian eggshells was reported by SCHLEICH & KÄSTLE (1988) and reviewed by PACKARD (1994) and PACKARD & DEMACRO (2004). Scanning electron microscopic studies of eggshells of Olive Ridley Turtles *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) from Gahirmatha, Orissa, India have been reported by SAHOO et al. (1996a, 1996b) and that from Phuket, Thailand by WANGKULANGUL et al. (2000). However, nothing is known about the structure of shells of abnormal eggs of any of the chelonians.

Although most of the eggs are normal in most of the clutches, abnormalities in one or other form are not very uncommon. Abnormality of the eggs may occur in size (large or small), as two or more eggs joined together, shells with multiple layers, shell-less eggs, etc. Eggs more than 15% larger or smaller in diameter than the average were considered abnormally sized eggs. There are several reports on the occurrence of abnormal eggs in Olive Ridley Turtles (PRITCHARD 1966; SILAS & RAJAGOPALAN 1984; DASH & KAR 1990; MOHANTY-HEJMADI 1990). Such eggs do not hatch or show any indication of development (e.g., a white spot on the shell) and are treated as infertile (SILAS & RAJAGOPALAN 1984).

The present paper deals with the structural details of the shells of abnormally small and large eggs of *Lepidochelys olivacea* from Gahirmatha rookery, Orissa along the east coast of India. These Olive Ridley Turtles lay, on an average, 112±23 (90-145) spherical, leathery eggs with a diameter of 36.2±1.4 (34.5-39.0) mm and mass of 32.8±1.3 (23.5-34.1) g (DASH & KAR 1990).

MATERIALS AND METHODS

Materials for the present study were collected from the Gahirmatha rookery, Orissa, India during observations on annual mass nesting in 1993-94. Abnormally small and large eggs were found in and collected from seven nests out of 20 nests examined after completion of the hatchlings' emergence (Table 1).

The eggs were collected from the nests, cleaned in the laboratory, measured and weighed. The shells were separated from the egg contents (yolk+albumen), air-dried and then subjected to compositional and structural analyses. The shell samples were dried to constant mass at 80 ºC and digested following the procedure of GIESEY & WEINER (1978). From the digestate, calcium content was determined by complexometric titration (EDTA) using thymolphthalexone as indicator (VOGEL 1978). Magnesium, potassium, phosphorus and sulphur were determined by atomic absorption spectrophotometry (Varian® 1475); flame photometry (Systronics® III); and UV–visible spectrophotometry (Chemito® 2500), respectively. Phosphorus content was below detection limit (20 µg/l in UV–visible spectrophotometry – CLESCERI et. al. 1998). Ultrastructural studies were carried out using a JEOL® scanning electron microscope (JSM® 35 CF) with working voltage ranging from eight to 25 kV. For this purpose, air-dried eggshells were cut into small pieces (2 mm x 2 mm). Samples from the outer surface, radial section and inner surface (surface facing the egg contents) of the eggshells were mounted on stubs with double-sided adhesive carbon tape, and without further preparation coated with gold for two minutes by an ion sputter (JFC® 1100) and then observed under SEM. Three pieces each (outer surface, cross section and inner surface) from all the seven large and six small eggs were subjected to SEM analysis. For comparative data on the structure of normal egg shells see (SAHOO et al. 1996a, 1996b; WANGKULANGUL et al. 2000).

RESULTS, DISCUSSION AND CONCLUSION

Frequency of abnormal eggs

During the examination of individual nests (n = 20) 13 eggs of abnormal size (seven very large and six very small) were found in seven nests (Table 1). Aggregates of multiple eggs were not found in the clutches. The frequency of abnormal eggs is highly variable: LUTHER (1959) reported an abnormal (twin) egg in *L. olivacea* in a clutch of 120 eggs from Gulf of Mannar and PRITCHARD (1966) mentioned such eggs in three nests. SILAS & RAJAGOPALAN (1984) recorded six cases of fusion of three eggs, eight twin eggs and one abnormally large egg out of 120 eggs in beaches of Madras. Greatest variation in shape, size and mass of

Table 1: Particulars of abnormal and normal sized eggs of *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) from Gahirmatha, India.

Tab. 1: Angaben zu abnorm und normal großen Eiern von *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) aus Gahirmatha, Indien.

Abnormal eggs / Abnorme Eier Nest No / Nest Nr.	Clutch size / Gelegegröße	*n*	Abnormally large eggs Egg size (mm) / Abnorm große Eier Größe (mm)	Mass (g) / Masse (g)	*n*	Abnormally small eggs Egg size (mm) / Abnorm kleine Eier Größe (mm)	Mass (g) / Masse (g)
1	155	2	83 x 46	48.8	1	17 x 13	18.4
			69 x 44	42.0			
2	121	-	-	-	1	21 x 14	21.8
3	138	-	-	-	2	14 x 10	16.2
						23 x 16	23.6
4	87	1	77 x 51	46.2	-	-	-
5	117	3	65 x 44	43.2	-	-	-
			59 x 41	41.1			
			72 x 48	50.5			
6	164	1	60 x 43	42.3	1	22 x 17	23.2
7	129	-	-	-	1	18 x 16	19.7
Normal eggs (*n* = 280) from (*n* = 28) clutches; means ± standard deviation (according to DASH & KAR 1990) / Normale Eier (*n* = 280) aus (*n* = 28) Gelegen; Mittelwerte ± Standardabweichung (nach DASH & KAR 1990)							
	112±23		36.2±1.4	32.8±1.3			

abnormal Olive Ridley eggs was reported by DASH & KAR (1990).

A few authors studied intraspecific trends in the occurrence of abnormality in sea turtle eggs: CHAVEZ et al. (1968) recorded abnormal eggs from four different nests of Kemp's Ridley *Lepidochelys kempii* (GARMAN, 1880), on the coast of Tamaulipas, Mexico. Such eggs have also been reported for Leatherback Turtles *Dermochelys coriacea* (LINNAEUS, 1766), by CARR & OGREN (1959) nesting in Costa Rica (66 normal + 38 abnormal, 45+7, 73+34, and 80+41); by PRITCHARD (1966) nesting in Guiana (59+

Table 2: Chemical composition (%) of egg shells of *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) from Gahirmatha, India.

Tab. 2: Chemische Zusammensetzung (%) der Eischalen bei *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829) aus Gahirmatha, Indien.

Shell mass (g) / Schalenmasse (g)	$CaCO_3$	Ca	Mg	S	K
Shells of 7 abnormally large eggs / Schalen 7 abnorm großer Eier					
0.63	30.10	12.04	0.027	1.905	0.045
0.50	24.35	9.74	0.027	0.772	0.056
0.54	21.78	8.71	0.032	0.998	0.049
0.58	28.77	11.51	0.025	0.859	0.053
0.48	21.32	8.53	0.029	0.934	0.049
0.60	27.47	10.99	0.030	1.301	0.058
0.55	22.02	8.81	0.025	1.028	0.055
Shells of 6 abnormally small eggs / Schalen 6 abnorm kleiner Eier					
0.09	14.00	5.60	0.018	1.048	0.051
0.08	15.05	6.02	0.021	1.021	0.046
0.05	9.24	3.69	0.027	1.027	0.031
0.10	7.83	3.13	0.018	1.018	0.042
0.10	13.25	5.30	0.024	0.923	0.048
0.08	12.28	4.91	0.019	1.014	0.041
Shells of 18 normal sized eggs / Schalen 18 normal großer Eier					
0.76±0.05	52.79±1.26	21.10±0.50	0.056±0.005	1.143±0.174	0.049±0.004

40, 69+11, 70+1, 83+23, 100+35, 101+39, 120+12, and 126+40) and by HUGHES et al. (1967). It is quite clear from the above that abnormality in eggs is not uncommon at least in two species of Ridley Turtles and the Leatherback. Abnormal eggs may be laid at the beginning, middle or end of a clutch and are invariably infertile (DASH & KAR 1990).

Eggshell chemical composition

The chemical composition of shells of eggs of abnormal and normal size is presented in Table 2. Wide variation was observed in the calcium level of the various types of abnormal eggshells. The calcium content in the shells of seven abnormally large eggs ranged from 8.53% to 12.04%; and from 3.13% to 6.02% in the shells of six abnormally small eggs. These values were clearly lower than those of normal eggshells (21.10%). However, the level of other elements like magnesium, sulphur, phosphorus and potassium were more or less similar to those in normal sized eggs.

Calcium is a significant constituent of eggshells of Olive Ridleys (SAHOO et al. 1998) and Green Turtles *Chelonia mydas* (LINNAEUS, 1758) (SOLOMON & BAIRD 1976). Other elements are present in trace amounts. Low level of calcium in the shells of either normal or abnormal eggs makes them incapable to complete their embryonic development. All abnormal eggs investigated in the present study were infertile. According to SILAS & RAJAGOPALAN (1984), none of the abnormal eggs hatch indicating thereby that they are all infertile. However, the fertility status of the abnormal eggs needs more detailed examination.

Eggshell structure

The scanning electronic microscopic study revealed that the eggshell consists of two layers: an outer calcium carbonate layer and an inner shell membrane adjacent to the egg contents.

S h e l l s o f a b n o r m a l l y l a r g e e g g s : The smooth outer surface of shells of abnormally large eggs had an incomplete

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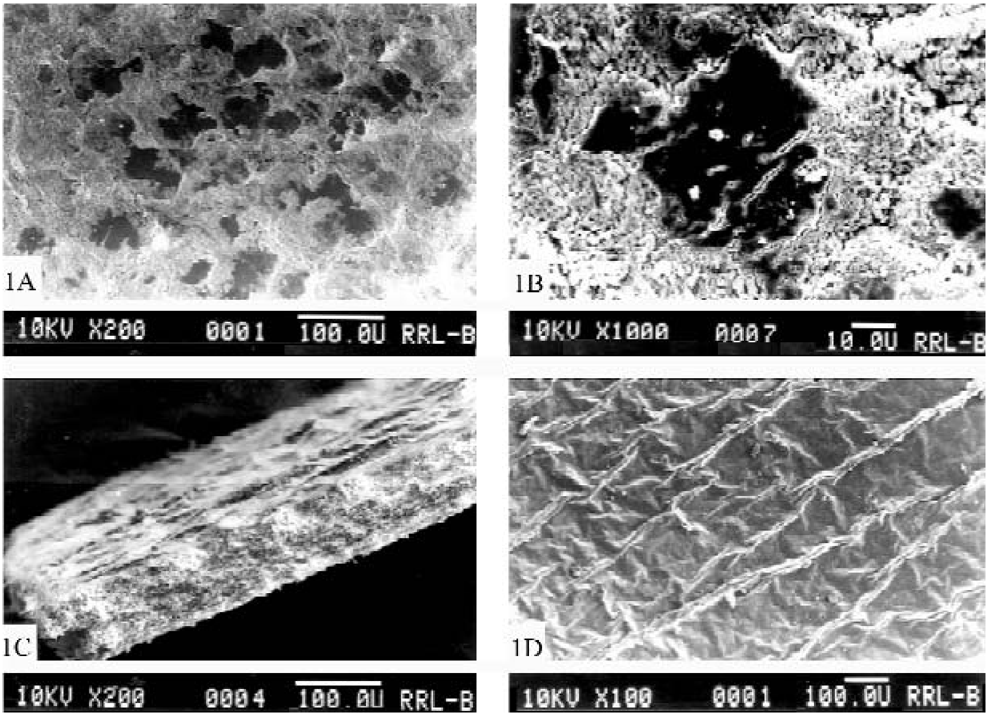
Figs. 1A - 1D (opposite page, top) / Abb. 1A - 1D (gegenüberliegende Seite, oben).
Figs. 2A - 2D (opposite page, bottom) / Abb. 2A - 2D (gegenüberliegende Seite, unten).

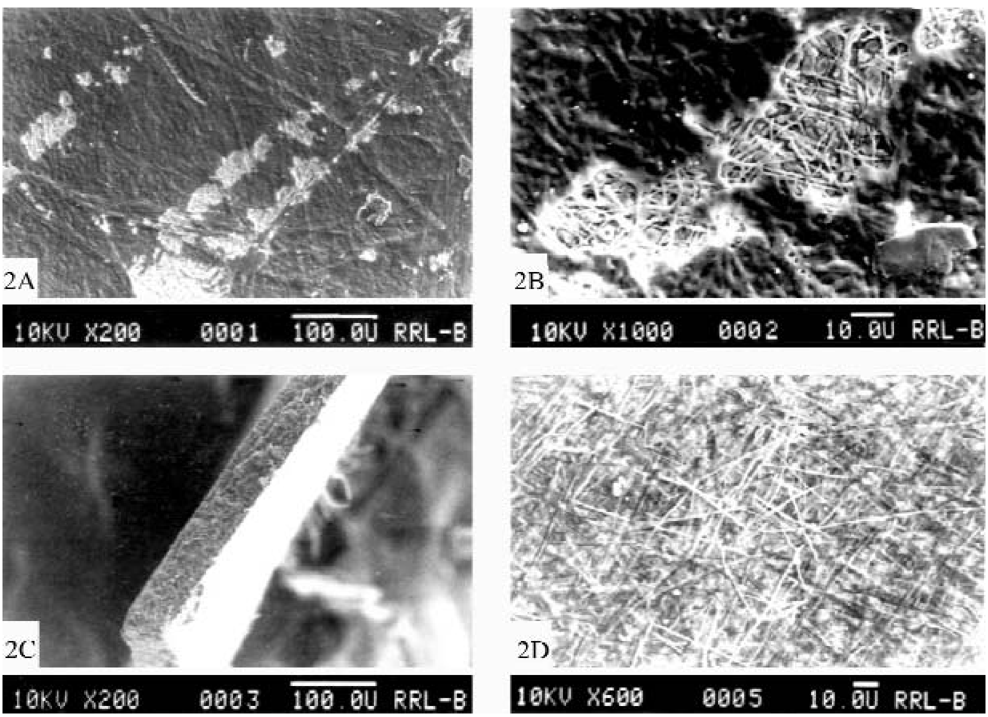
Fig. 1: Scanning electron micrographs of the shell of abnormally large eggs of *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). A - Smooth outer surface. The thin calcareous layer is incomplete with distinct, irregular and large open spaces. B - Enlarged view of A. The membrane fibers are exposed at the open spaces. C - Cross section of the eggshell. The calcareous layer is towards the bottom of the figure. It shows loosely arranged granules without any distinct structure and appears as a spongy mass. D - Inner surface showing the unique fibrillar arrangement. The more or less parallel and bifurcated fibrils are arranged in a trellis pattern. Each fiber consists of two microfibrils.

Abb. 1: Rasterelektronenmikroskopische Aufnahmen der Schalen abnorm großer Eier von *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). A - Die glatte äußere Oberfläche. Die dünne Kalkschicht ist nicht geschlossen und zeigt deutlich unregelmäßige große Fehlstellen. B - Vergrößerung von A. Die Fibrillen der Faserschicht liegen im Bereich der Defekte bloß. C - Querschnitt der Eischale. Die Kalkschicht (unten) besteht aus lose angeordneten Körnern ohne klare Struktur und erscheint als schwammige Masse. D - Die innere Schalenoberfläche zeigt die einzigartige Fibrillenanordnung. Die mehr oder weniger parallelen, gabeligen Fibrillen bilden eine Art Flechtwerk. Jede Fibrille besteht aus zwei Mikrofibrillen.

Fig. 2: Scanning electron micrographs of the shell of abnormally small eggs of *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). A - Smooth outer surface. Observe the very thin calcareous layer, through which fibers of the shell membrane are visible even at lower magnification. B - Enlarged view of A. The calcareous layer is present in the form of a thin covering which is broken at places. C - Cross sectional view which appears amorphous. D - Inner surface showing membrane fibers. Disoriented fibers are of unequal thickness and are associated with tiny globules at few places.

Abb. 2: Rasterelektronenmikroskopische Aufnahmen der Schalen abnorm kleiner Eier von *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829). A - Die glatte äußere Oberfläche. Man beachte die sehr dünne Kalkschicht, durch die hindurch die Fasern der Schalenmembran selbst bei geringer Vergrößerung sichtbar sind. B - Vergrößerung von A. Die Kalkschale ist in Form einer dünnen, stellenweise durchbrochenen Schicht ausgebildet. C - Im Querschnitt erkennt man die amorphe Schalenstruktur. D - Die innere Schalenoberfläche mit den Fasern der Schalenmembran. Die Fasern sind ungeordnet, ungleich dick, an einigen Stellen mit winzigen globulären Strukturen assoziiert.


1A
10KV X200 0001 100.0U RRL-B
1B
10KV X1000 0007 10.0U RRL-B
1C
10KV X200 0004 100.0U RRL-B
1D
10KV X100 0001 100.0U RRL-B


2A
10KV X200 0001 100.0U RRL-B
2B
10KV X1000 0002 10.0U RRL-B
2C
10KV X200 0003 100.0U RRL-B
2D
10KV X600 0005 10.0U RRL-B

calcium carbonate layer with distinct and irregular pores (25–75 µm in diameter) (Fig. 1A, 1B). Occasionally these pores joined to form larger openings. Unlike normal eggshells where an array of nodules and spicules were present (SAHOO et al. 1996a, 1996b; WANGKULANGUL 2000), the calcareous layer in shells of abnormally large eggs consisted of loosely conglomerated granules. Nodular shell units of normal Olive Ridley Turtle eggshells or those of other marine turtles (SOLOMON & BAIRD 1976; HIRSCH 1983; PACKARD & HIRSCH 1986; SOLOMON & TIPPETT 1987) are not found in shells of abnormal eggs. Shells of abnormally large eggs were thicker (200 µm) (Fig. 1C) than those of normal ones (about 150 µm) (SAHOO et al. 1996a, 1996b). In cross sections (Fig. 1C), the calcareous layer appeared amorphous. Mound-like bulging points were present at regular intervals in the calcareous layer, which appeared to be "incomplete" or represented "just initiated" shell units. The shell units in all probability remain incomplete due to non-availability of sufficient calcium or some physiological disturbance in the oviductal mileu.

The fibrillar network in the shell membrane of abnormal eggs consisted of a single layer of fibers (Fig. 1D), whereas, it was multilayered in normal eggshells (SAHOO et al. 1996a, 1996b). The fibers were bifurcated and ran more or less parallel to each other in the form of a trellis pattern. The parallel fibers were further perpendicularly crossed at regular intervals by similar fibers. Each fiber consisted of two microfibrils and was associated with tiny globules. The interwoven, branched and netted fibrillar arrangement of normal shells (SAHOO at al. 1996a, 1996b) was absent in abnormal shells. The calcareous and fibrillar layers did not have an intimate or continuous association with each other.

Shells of abnormally small eggs: The authors hypothesize that the shells of abnormally small eggs were most likely formed under calcium deficiency. The calcareous layer formed a very thin covering through which the shell membrane fibers were clearly visible (Figs. 2A, 2B). No crystal structure was discernible on the superficial calcareous layer. In cross sections (Fig. 2C) of the shell, inorganic and organic layers could not be distinguished. Total shell thickness was about 50 µm. The granules on the shell membrane were fused and formed larger granules of varied shape and size. The multi-layered shell membrane (Fig. 2D) consisted of widely placed fibers and microglobules. The fibers were of uneven thickness, irregularly arranged and often showed branching. Tiny globules were also associated with the fibers. The overall structure of the shells of abnormally sized eggs was not comparable to that of normal eggshells as reported by SAHOO et al. (1996a, 1996b) and WANGKULANGUL et al. (2000) or to that of contemporary chelonians (HIRSCH 1983).

Abnormal eggs are not uncommon in sea turtles and are probably formed under calcium deficiency or disturbed physiological conditions. The shell forming physiological processes might not be fully operational when the shell is formed for the first few eggs, and gets exhausted towards the end when serial eggs are formed. The shells of such eggs contain lower amounts of calcium, which is also reflected in their ultrastructure. Such eggs are infertile and are not capable of embryonic development.

ACKNOWLEDGMENTS

Thanks are due to the Forest Department, Government of Orissa for permission to work in the Protected Area. Financial assistance from the Department of Ocean Development, Government of India to GS is gratefully acknowledged.

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DATE OF SUBMISSION: November 4, 2008 Corresponding editor: Heinz Grillitsch

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