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Contrasting ultrastructures in the eggshells of olive ridley turtles, *Lepidochelys olivacea*, from Gahirmatha, Orissa

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Normal and abnormal sized eggshells of olive ridley turtles, *Lepidochelys olivacea*, from Gahirmatha have been studied for ultrastructural characteristics. Fresh eggshells exhibit nodular shell units on the upper surface followed by a membranous network of fibres and a thin shell membrane. These structures are not seen in hatched eggshells as the calcium is re-sorbed during embryonic development. Abnormal shells do not show any such structure excepting disoriented fibres in its lower surface due to inadequate calcification.

THE eggshell is a mediating boundary and together with

other features it contributes to the isolation of the embryo from the nesting environment. Modifications of the shell structure have probably evolved for functional smoothness and evolutionary adaptations. It might reasonably be anticipated that the eggshells of marine turtles would show even greater structural variation concomitant with the laying habits, temperature and so on. The marine turtle eggshells fall within the category of 'softshelled' eggs^{1,2}. Extensive studies have been carried out on the ultrastructure of green^{3,4}, leatherback^{5,7}, loggerhead² and Kemp's ridley¹ sea turtles. Although olive ridley, *Lepidochelys olivacea*, is the most abundant sea turtle and Gahirmatha (Orissa) is the world's largest rookery of this species⁸, no report on its ultrastructural morphology is available in the literature. Here we describe briefly the eggshell ultrastructures of these turtles.

Eggshells from normal (both fresh and hatched; circular, 36.1–39.5 mm diameter) and abnormal (oblong, very small: 8.5 × 11.5 mm, 3.26 g; and very large: 46.5 × 80.0 mm, 58.32 g) eggs were collected from the Gahirmatha nesting rookery during the 1993 nesting season and were subjected to XRD (Philips PW-1700) and SEM (JEOL, JSM 35 CF) studies.

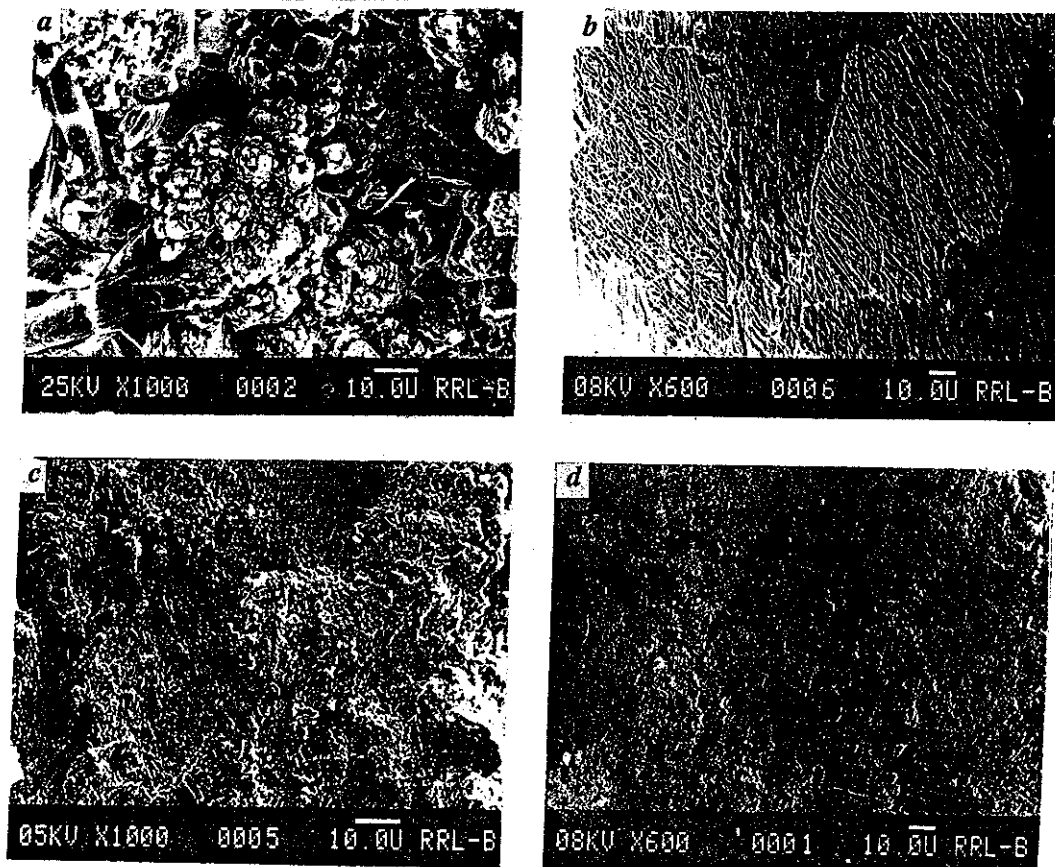


Figure 1a–d. Electron micrographs of normal eggshells of olive ridley turtles. a, Upper surface of fresh eggshell showing loosely arranged crystallites and spicules. b, Lower surface of fresh eggshell showing the fibrillar network and a thin shell membrane covering it. c, Upper surface of hatched eggshell. d, Lower surface of hatched eggshell.

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In general, the olive ridley turtle eggshell contains aragonite (CaCO_3) crystallites. This is in contrast to other marine turtles where traces of calcite and vaterite along with aragonite have been reported. In the case of leatherbacks, all the three crystal forms of calcium carbonate (calcite, aragonite and vaterite)⁵ and in green turtles, both calcite and aragonite are present³. In a normal eggshell of the fresh kind, the outer organic surface exhibits a rough granular structure. The nodular shell units are very distinct which is in contrast to those reported for *Caretta caretta*⁹ or *Dermochelys coriacea*¹⁰ where they are ill-defined. Two types of structures are observed on this surface: micronogules which give the coarse granular appearance; and spicules which vary in size and are also arranged in different planes (Figure 1 a). The arrangement of the shell units is not compact and thus results in pore-like open spaces of varying sizes. Such type of pores are present in Loggerhead⁹ and Kemp's ridleys¹, but are absent in leatherbacks¹⁰. The pores are interrupted by the spicules and are meant for gaseous exchange and water absorption¹¹ which is essential during embryonic development.

The inorganic surface is followed by a layer of interwoven fibrils, which are organic in nature, frequently branched, ramified, exhibit a netted structure (Figure 1 b) and these fibres are similar in composition to avian eggshells¹². Specific positions of these fibres probably act as nucleation sites on which the calcification begins³. These fibres are flexible and stretch when the egg diameter increases during incubation. Below the fibres there is a very thin organic basement membrane through which the internal fibrillar network is visible in ventral view (Figure 1 b). In olive ridley, like other chelonians, the shell membrane is single-layered and not paired as observed in bird's eggshell¹².

The outer surface of a hatched eggshell, even at higher magnification, does not show any organized structure and is rather homogenous with fused granules (Figure 1 c). The surface is obscured by minute spherical or amorphous masses. Partial alteration of hatched eggshells of *Caretta caretta* has also been reported by Schleich and Kastle². This sort of disruption of structures is due to the resorption of calcium from the shell.

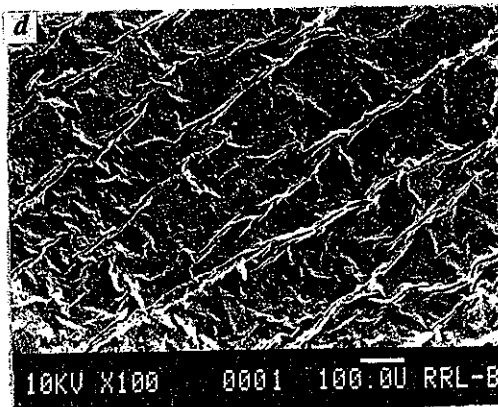
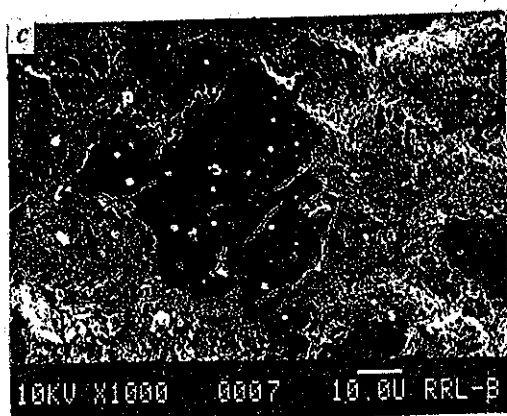
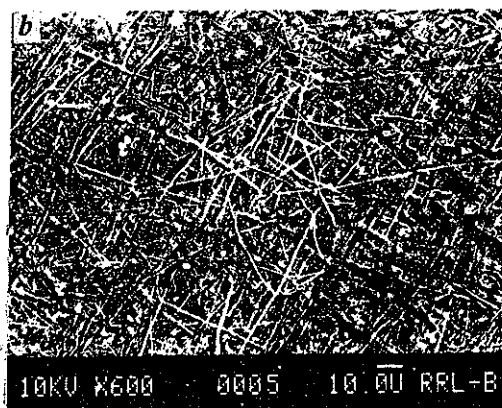
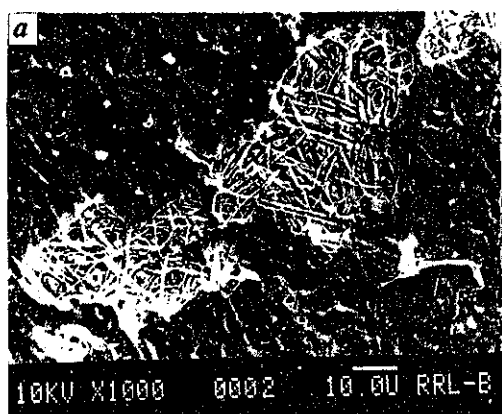


Figure 2 a-d. Electron micrographs of abnormal eggshells of olive ridley turtles. a, Upper surface of a very small egg shell. Note how a very thin, almost transparent, calcareous layer covers the fibrous network. b, Lower surface of a very small eggshell. Disoriented fibrils with microglobules are seen. c, Upper surface of a very large eggshell. Remnants of calcareous layer (black) is seen over a spongy mass. Pores appear thin and elongated due to interconnection. d, Lower surface of a very large eggshell in which the parallel bifurcated fibrils are arranged in a trellis pattern.

Similar alteration in eggshell ultrastructure in developing embryos due to utilization of calcium reserve has been reported in domestic fowl¹³ and leathery (*Dermochelys coriacea*)¹⁴ and softshell turtles (*Trionyx spiniferus*)¹⁵. The fibrillar network of the inner surface is not visible as it is covered by a dense, structureless thick layer (Figure 1 d).

The abnormality of the eggs is mainly in the form and extreme size, small or large or in the form of two or more eggs joined together¹⁶. These eggs never hatch. There is no report on the structure of abnormal eggshells of any marine turtle. In case of abnormally small eggs, the calcification of the shell is incomplete and present in the form of a very thin layer through which the inner surface is clearly visible (Figure 2 a). No crystal growth is discernible on its upper inorganic surface. The lower organic surface consists of widely spaced fibres and microglobules without the basement membrane. The fibres are of uneven thickness, irregularly arranged and show branching (Figure 2 b). Tiny globules are associated with these fibres and seem to be the nucleation centres for crystal growth. The abnormally large eggshells are also without any crystal or nodular structure on its outer surface. Remnants of a calcareous layer as small patches are seen over a spongy mass (Figure 2 c). Pores are interconnected and appear elongated. The fibrillar network in the lower surface is remarkable. The fibres are bifurcated and run more or less parallel to each other in the form of a trellis pattern (Figure 2 d). These parallel fibres are further perpendicularly crossed by similar fibrils at regular interval.

Thus, the olive ridley eggshells, though appearing alike externally, show contrasting ultrastructures. Dissimilar structure is observed not only amongst normal and abnormal shells but also between fresh and hatched ones. Fresh shells consist of an outer inorganic calcareous layer with nodular units and an inner organic layer which is represented by a profuse mat of fibrillar network.

The eggshells undergo drastic ultrastructural changes during incubation like those of Aves and other turtles as a result of resorption of calcium from the shell. The abnormal eggs do not show any crystalline structure probably due to improper growth and inadequate calcium deposition.

- 1 Hirsch, K. F. *Copeia*, 1983, 2, 382-397.
- 2 Schleich, H. and Kastle, W., *Reptile Egg Shells*, SEM Atlas, G. S. Verlag, Stuttgart, 1988, pp. 119.
- 3 Solomon, S. E. and Baird, T. *J. Exp. Mar. Biol. Ecol.*, 1976, 22, 145-160.
- 4 Solomon, S. E. and Baird, T., *J. Exp. Mar. Biol. Ecol.*, 1977, 27, 83-92.
- 5 Chan, E. H. and Solomon, S. E., *Anim. Technol.*, 1989, 40, 91-102.
- 6 Solomon, S. E. and Watt, J. M., *Anim. Technol.*, 1985, 36, 19-27.
- 7 Solomon, S. E. and Tippett, R., *Anim. Technol.*, 1987, 38, 73-79.
- 8 Bustard, H. R. Tiger Paper, 1976, 3.
- 9 Packard, M. J. and Hirsch, K. F., *Scanning Electron Microscopy* 1986, IV, 1581-1590.
- 10 Chan, E. H., Saleh, H. U. and Liew, H. C., *Pertanicka*, 1985, 8, 265-271.
- 11 Tracy, C. R., Packard, G. C. and Packard, M. J., *Physiol. Zool.*, 1978, 51, 378-387.
- 12 Becking, J. H., *The Ibis*, 1975, 117, 143-151.
- 13 Johnston, P. M. and Comar, C. L., *Am. J. Physiol.*, 1955, 183, 365-370.
- 14 Simkiss, K., *Comp. Biochem. Physiol.*, 1962, 7, 71-79.
- 15 Packard, M. J. and Packard, G. C., *J. Morphol.*, 1979, 159, 131-144.
- 16 Dash, M. C. and Kar, C. S., *Turtle Paradise Gahirmatha*. Interprint New Delhi, 1990, pp. 295.

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