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ELEMENTAL AND ULTRASTRUCTURAL CHARACTERISTICS OF THE EGG CAPSULES OF NAUTILUS POMPILIUS

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Abstract

Six pearly nautili (Nautilus pompilius) raised in captivity produced nonviable egg capsules which were examined using scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDS). Macro and microscopic observations revealed two distinct and separate walls encircling the yolk sac. Both walls exhibited a porous appearance in cross-section. Protein analysis demonstrated the proteinaceous nature of nidimental gland secretions and egg capsule walls.

EDS analysis of each wall shows the elemental composition to be identical in both, with each wall containing similar proportions of S, Cl, Mg, Na, K, and trace amounts of Ca. X-ray mapping of Na and Cl along each wall surface suggests similar construction of both walls. EDS analysis of the nidimental gland secretions of the adult female nautilus have shown a composition similar to that of the egg capsule.

KEY WORDS: X-ray Microanalysis, Scanning Electron Microscopy, Egg Capsules, Protein Analysis, Nautilus pompilius

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Introduction

The presence of a single, hard egg case is reported for a variety of animals, e.g., insects, some chondrichthyans (Raja, Scyliorhinus), molluscs (Busycon), birds, reptiles, and primitive mammals (platypus and echidnas). Nautiloids are unique in that their egg yolk is enclosed in two discrete, hard, layers. We report here the results of scanning electron microscopy (SEM) on the inner and outer egg case layers, and of energy dispersive x-ray spectrometry (EDS), protein, and chitin analyses on the egg case and its reputed source, the nidimental gland. Reproduction and egg laying are poorly understood in Nautilus (12). The length of the reproductive period is uncertain, with reproduction and mating possibly occurring at night during the summer months (1,5,8). Presumably it's reproductive strategy is suited to deepwater forms (11). Coincidently, catches of adults by fishermen in the nearshore areas increase in June; in subsequent months small juveniles appear in these areas (1,2).

A brief description of copulatory and spawning behavior of Nautilus was given by Mikami and Okutani (8). During copulation, the tentacles of both sexes entangle with each other; the male spadix contacts the female at the buccal area, after which the female is found to carry a string-like mass, presumably a sperm bulb. The female manipulates the egg capsule with the labial tentacles and attaches the egg capsule to some suitable underwater object.

The egg capsules are apparently soft when laid, and are found attached to underwater objects. Mikami and Okutani (8) have observed egg laying in aquaria, but the only details they reported concerned the manipulation of the egg by the female's labial tentacles. In this same article they hinted that the capsules are soft upon extrusion and harden shortly thereafter. However, no viable eggs were reported in their study. Captive rearing of the eggs of Nautilus has not been reported. It is not known how, or if, egg capsules of presumed non-viable eggs laid in captivity differ from those of viable eggs. Descriptions of the early stages of Nautilus after hatching are sketchy. To date all eggs laid in captivity have been either infertile or aborted, including those in the present study (6,7,8). However, Stenzel (12) presumed that, like other cephalopods, the embryo of Nautilus probably does not enter planktonic stages but rather emerges from the egg as a miniature adult.

Materials and Methods

Six live Nautilus pompilius were captured April 30, 1978, off Cebu Island in the Philippines, and have been kept alive at the New York Aquarium in a semi-open natural seawater, 1000-gallon aquarium tank. Two of the nautiloids were still alive at the time of this writing (March 1985) (This represents a captivity record for N. pompilius of 6 years and 10 months. Water density was maintained at 1.024-1.026, temperature at 18 C, and pH at 8.0. Photoperiod was held constant at 11 hrs. light (with a red filter) and 13 hrs. dark. The nautiloids' diet during this period consisted of shrimp, clam, and smelt. After 18 months of captivity 5 egg capsules were found adhering to the aquarium walls and substrate. Three egg capsules were retrieved, washed with distilled water and prepared for microscopic and elemental analyses. Light microscopy (LM) samples were washed with distilled water, air-dried, broken, and/or petrographically sectioned. For ultrastructural analyses, samples were prepared similarly to LM samples but mounted on SEM stubs and coated with 75 nm of carbon and 100 nm of gold in a Denton vacuum evaporator and viewed in a Cambridge S-250 with EDS or in an ARL SEMQ Microprobe (also with EDS) or AMRay 1600T SEM. For EDS analysis, broken shell fragments, petrographic sections and nidimental gland secretions were mounted on ultrapure carbon stubs, coated with 100 nm of ultrapure carbon and examined with an EDS device attached to the SEM, protein analyses after Packard et al. (10) were done on nidimental gland fluids obtained from freshly dead specimens and egg capsule walls.

Results/Discussion

The overall morphology of the egg capsule can be seen in Fig. 1. An accurate and detailed description of which was first given by Willey (13). The ovum is enclosed by a double casing, the inner casing being completely closed and the outer more or less open at one end. The egg capsule is ovoid or thumb-shaped and is attached vertically to the substrate via a reticulate area at its base. The capsule is hard and retains its shape when dry. Microscopic observations showed that the inner and outer walls of the capsules are rough, and have a striated appearance (Fig. 2). SEM cross-sections of the egg capsules showed that the walls were studded with ovoid holes (Fig. 3), the long axis of which tends to be parallel to the wall surface. Both inner and outer walls exhibit the same degree of porosity.

EDS analysis of the inner and outer walls (Fig. 4a and b) revealed the presence of Na, Mg, S, Cl, K, and Ca. The nidimental gland secretions (Fig. 4c) showed a similar elemental composition except that this gland contained amounts of P and higher amounts of Ca than the walls. We speculate that P in the nidimental gland secretions suggest that a phosphate removing enzyme or catalyst is involved in solidification of the secretions when the egg case is laid. It should be noted that attempts at experimental hardening of the nidimental gland secretions in both air and sea water did not result in hardening of the material to the consistency of the normal egg capsule. In contrast, adult Nautilus shell is almost wholly composed of Ca. Protein analysis reveals that both layers of the egg case have a similar protein concentration (840.2 ± 47 mg/g wet weight of protein). The protein concentration of the nidimental gland is slightly greater than that of the walls (1014 ± 27 mg/g wet weight of protein). The egg yolk fluid, contained much larger amounts of protein (3144 ± 84 mg/g wet weight of protein. Chitin could not be detected using the technique of Gatenby and Beams (3).

In an attempt to determine the source of the egg capsule material, we examined the nidimental gland, which has been implicated in egg production. Griffin (4) and later Haven (5) reported that this gland is present only in female Nautilus. Griffin (4) then went on to describe this gland in his extensive anatomical studies on female Nautilus pompilius. He noted that the gland was only beginning to differentiate in half-grown female animals. It has been suggested by Owen (9) that folds in the oviduct serve to conduct the nidimental gland fluids nearer to the opening of the oviduct. It is therefore apparent that this gland has a role in the female reproductive effort. The similarity of the nidimental gland secretions and the walls of the egg capsule is interesting. Both contain a high concentration of protein and have similar elemental composition, which indicates a link between this gland and egg capsule production, the mechanism of which awaits further study.

Conclusions

The most likely sequence of events in egg case formation is as follows: the inner wall is laid down around the yolk and egg, in or near the ovary/oviduct area, the inner wall hardens, and the outer layer is then laid down over the inner layer shortly before or as the egg is extruded. The egg capsule with the still soft outer layer is then attached to a nearby object with the labial tentacles of the female, whereupon the outer wall hardens. Since at least the outer wall is soft when it is attached to an object, it molds itself to the surface topography of that object.

Acknowledgements

We thank E. Dols, for his assistance in the present study. In addition to Elizabeth Pugh for her typing skills. We would like to thank the New York Aquarium for making specimens available to us.

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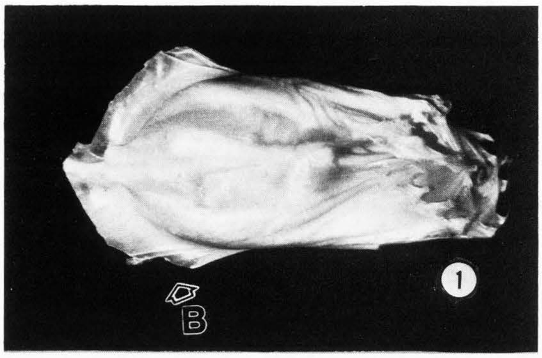
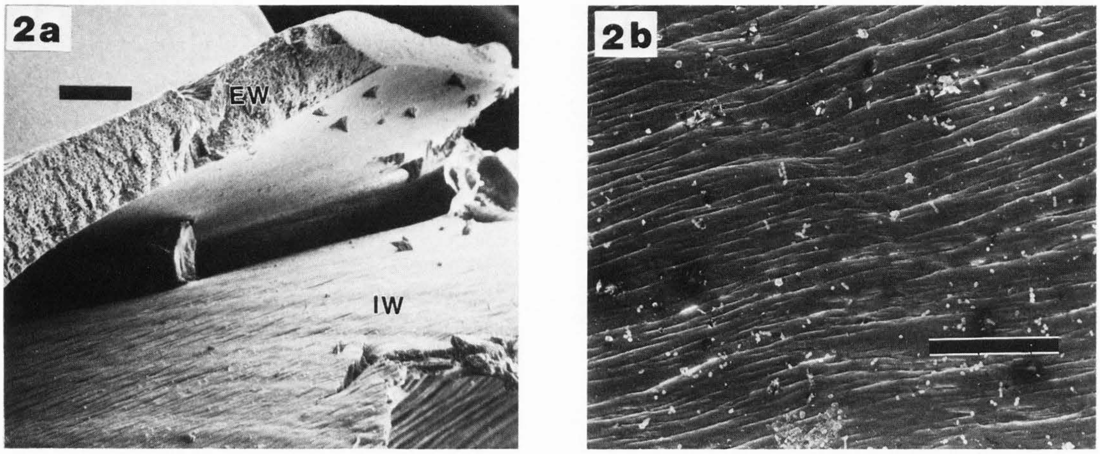


Fig. 1. Exterior, dorsal view of Nautilus pompilius egg capsule showing the outer wall structure: (B) depicts the attachment region (the enlarged area at the bottom of the capsule). The top part of the tapered end was always oriented towards the surface after attachment to the substrate (length is 4 cm).



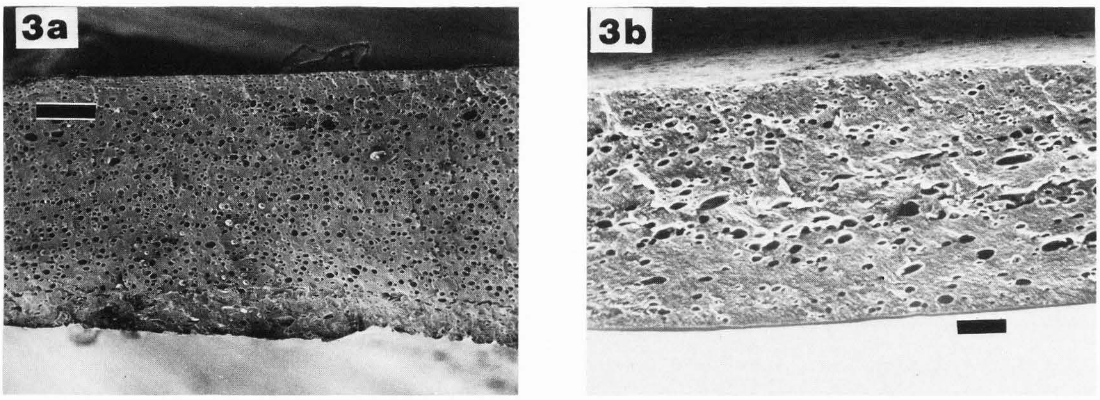


Fig. 2. Egg capsule walls of Nautilus pompilius. (a) Scanning electron micrograph of a cross-section of N. pompilius double walled egg capsule illustrating the juxtapostion of the walls; EW depicts the outer wall; IW depicts the inner wall (bar indicates 400 μm). (b) Scanning electron micrograph of the outer surface of the outer wall of N. pompilius illustrating the striated nature of the wall (bar represents 100 μm).

Fig. 3. Cross-sectional views of egg capsule walls of N. pompilius illustrating ovoid holes. (a) SEM photo of the outer wall. Holes near wall edges tended to be more compressed (bar represents 100 μm). (b) SEM photo of inner wall, note the similarity to the outer wall. The texture of both walls is very granular and amorphous, studded with NaCl crystals, (bar represents 1 μm).

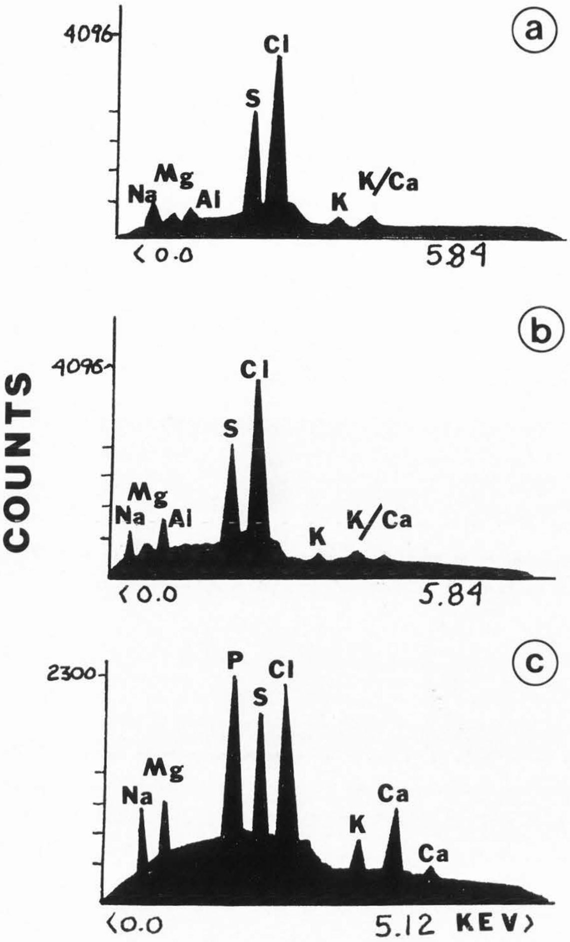


Fig. 4. Energy dispersive x-ray analysis: (a) Outer wall, (b) Inner wall. The presence of Al in (a) and (b) was shown to be an artifact of the instrumentation as wavelength dispersive x-ray analysis with an ARL SEM-Q failed to reveal any Al. (c) Nidimental gland secretions. The gland showed similar elemental composition to (a) and (b) with the exception of large amounts of P and higher amounts of Mg and Ca (see text for explanation).

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Discussion with Reviewers

A.S. Pooley: Is it possible that the sodium, magnesium and chlorine found (in roughly stoichiometric amounts for NaCl and $MgCl_2$, allowing for the much lower efficiency for detection of sodium in the EDS) is simply the dried residue of sea water?
Authors: No, the egg capsules were washed in distilled water to remove extraneous salts from sea water. It is our belief that S, Mg, and Cl represent insoluble components of the egg capsule. From other studies, the efficiency of detection of sodium to chlorine is in a ratio of 1:2 or better.

A.S. Pooley: If sulfur is the only significant insoluble element found in the egg capsule, are the differences between the composition of the egg capsule and the nidimental gland secretion greater than the similarities?
Authors: No, as discussed above, we believe that the S, Mg and Cl noted are insoluble components. The only significant elemental difference is phosphorus. Furthermore, potassium and calcium are found in both the inner and outer walls and nidimental gland secretions.

A.S. Pooley: Could the flattening of the ovoid holes (Fig. 3) be caused by shrinkage in this unfixed material?
Authors: No. Other studies not reported herein on egg capsules fixed with 5% seawater buffered glutaraldehyde showed no difference in hole structures between fixed and unfixed material.

R.K. Batten: A very recent publication has depicted the first known live rearing of chambered nautilus: Carlson BA. (1985). The First Known Embryos of the Chambered Nautilus. Hawaiian Shell News, 336.
Authors: Thank you for your update.

D.B. Spangenberg: What is meant by non-viable egg capsules?
Authors: We presume the eggs were non-viable since there was a white jelly-like substance between the chorion and inner capsule and the chorion itself was transparent.

D.B. Spangenberg: What was the reason for the chosen photoperiod?
Authors: We attempted to duplicate environmental conditions, the red filter was used to darken the overall lighting to simulate deepwater environments.

D.B. Spangenberg: Is anything known about cells which secrete the calcium to make the shell? What

form is the calcium in the shell comprised of?
Authors: We are not aware of information concerning the cells. The shell itself is composed of an outer porcellanous layer containing prisms of calcium carbonate in an organic matrix, along with an inner nacreous layer. We believe that the form of calcium carbonate is aragonite.

D.B. Spangenberg: A very recent article has further discussed live rearing of Nautilus embryos: Arnold JM, Carlson BA. (1986). Living nautilus embryos, preliminary observations. Sci. 232, 73-76.
Authors: Thank you for your update.