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Developmental and histochemical studies on antheridium formation and spermatozoid release in *Turbinaria conoides* (Phaeophyta)

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The receptacles in *Turbinaria conoides* (Fucales, Sargassaceae) are bisexual with the antheridia generally occurring at the upper end of the conceptacle. Antheridia are either sessile or stalked and the number of stalk cells varies from one to three. The young antheridium (uni/binucleate stage) has one wall layer whereas those with eight or more nuclei have two. Critical staining shows that wall layers contain alginic acid and sulphated polysaccharides. In mature antheridia the inner wall layer has a greater deposition of sulphated polysaccharides than the outer. The lumen between the two wall layers is filled with sulphated polysaccharides. As the antheridia mature two zones of polysaccharides and a change in the metachromasy of its cytoplasm can be recognised. At the time of spermatozoid release the cytoplasmic zonation degenerates. The outer wall layer lyses at or near the apex of the antheridium and the spermatozoids are discharged enclosed in sulphated polysaccharides.

Key Index Words: alginic acid—cytoplasmic zonation—metachromasy—sulphated polysaccharides—spermatozoids.

The mature antheridium in the Fucales has two wall layers (Fritsch 1945). On the basis of histochemical reactions, however, McCully (1968) suggested that *Fucus distichus* subsp. *edentatus* (Pyl.) Powell has four wall layers. Levring (1952) discussed metachromasy in the antheridial wall of *Ascophyllum nodosum* (L.) Le Jol. and *Fucus serratus* L. but did not elucidate the number of wall layers.

In the Fucaceae the male cells enclosed by the inner wall layer are extruded as oblong packets embedded in mucilage through the ostiole (Fritsch 1945), but the mechanism of spermatozoid release is not entirely clear. In *Laminaria* spermatozoid release is mediated by a pheromone that causes the rupture of antheridial wall. The mucilage in which the spermatozoids are enclosed at the time of release consists of fucoidin (Maier 1982). The present study of *Turbinaria conoides* (J. Ag.) Kütz. was undertaken to examine the nature of materials associated with antheridial wall layers and their role in spermatozoid release.

Material and methods

Turbinaria conoides was collected from Port Okha at low tide periods during the months of December 1981, October 1982, December 1982, June 1983, and September 1983. Port Okha is situated 22°28′N, 69°05′E on north Gujarat (India) coast and is bordered by the Arabian Sea. Receptacles at progressive developmental stages were fixed in 10% acrolein and post-fixed in 1% mercuric chloride to stabilize polyphenols. Dehydration, infiltration and embedding were carried out according to Feder and O'Brien (1968). Sections of 2 μm thickness were cut using glass knives on a Spencer AO rotary microtome with locally-designed adaptor. The plastic sections were either stained with 0.05% toluidine blue O (TBO) prepared in benzoate buffer at pH 4.4 (McCully 1966) or by periodic acid Schiff's reagent, PAS, (Feder and O'Brien 1968). Aldehyde groups

introduced by acrolein fixation were blocked before PAS reaction by treating the slides with chlorous acid (RAPPAY and VAN DUIJN 1965) or with 0.5% aqueous dimedone. The slides were also stained with 0.5% Alcian blue at pH 0.5 for sulphated polysaccharides (PARKER and DIBOLL 1966), and coomassie brilliant blue method for proteins (WEBER and OSBORN 1975). Controls were performed to check the specificity of various histochemical reactions.

Results

Morphology

Turbinaria conoides is a compact, radially organised cone-like plant. The thalli are firmly attached to the substratum by means of attaching discs and the spreading branches of haptera. The main axis is upright, cylindrical and densely covered by leaves (Fig. 1A). The receptacles are branched (Fig. 1B) and axillary. Both antheridia and oogonia are borne on the same conceptacles (Fig. 1C). Spermatozoids are shed en masse whereas the oogonia are attached to the inside of the conceptacle by mesochiton stalks.

Developmental studies

The antheridial mother cell is distinguished by dense cytoplasm and a prominent nucleus. Uninucleate (Fig. 1D) and binucleate antheridia possess only a single wall layer. Antheridia with four nuclei could not be observed but those with eight nuclei show two distinct wall layers. The nuclei in such antheridia are small, round and possess chromocentres. The initial nuclear division in the antheridium is meiotic and subsequent divisions are mitotic giving rise to 64 spermatozoids.

Antheridia are either stalked or sessile and when stalked the number of stalk cells varies from 1-3 (Fig. 1E). Sessile antheridia are formed directly on the germinal epithelium. Mature antheridia have two wall layers and the lumen is filled with polysaccharide materials (Fig. 1E-H). In the stalk cell the longitudinal wall is thicker than the tangential wall. The former shows two layers whereas the latter has only single layer. The stalk cell contains a small nucleus, phenolic bodies, a few polysaccharide granules and chromatophores. As the antheridium matures, its cytoplasm gradually becomes granular and shows two zones, the outer containing free granules and the inner aggregates (Fig. 1I). Spermatozoids remain in the inner zone. A few antheridia show linearly-arranged granules (Fig. 2B). In some antheridia the spermatozoids are sequestered by thin walls and each compartment contains a nucleus along with a portion of cytoplasm (Fig. 2C).

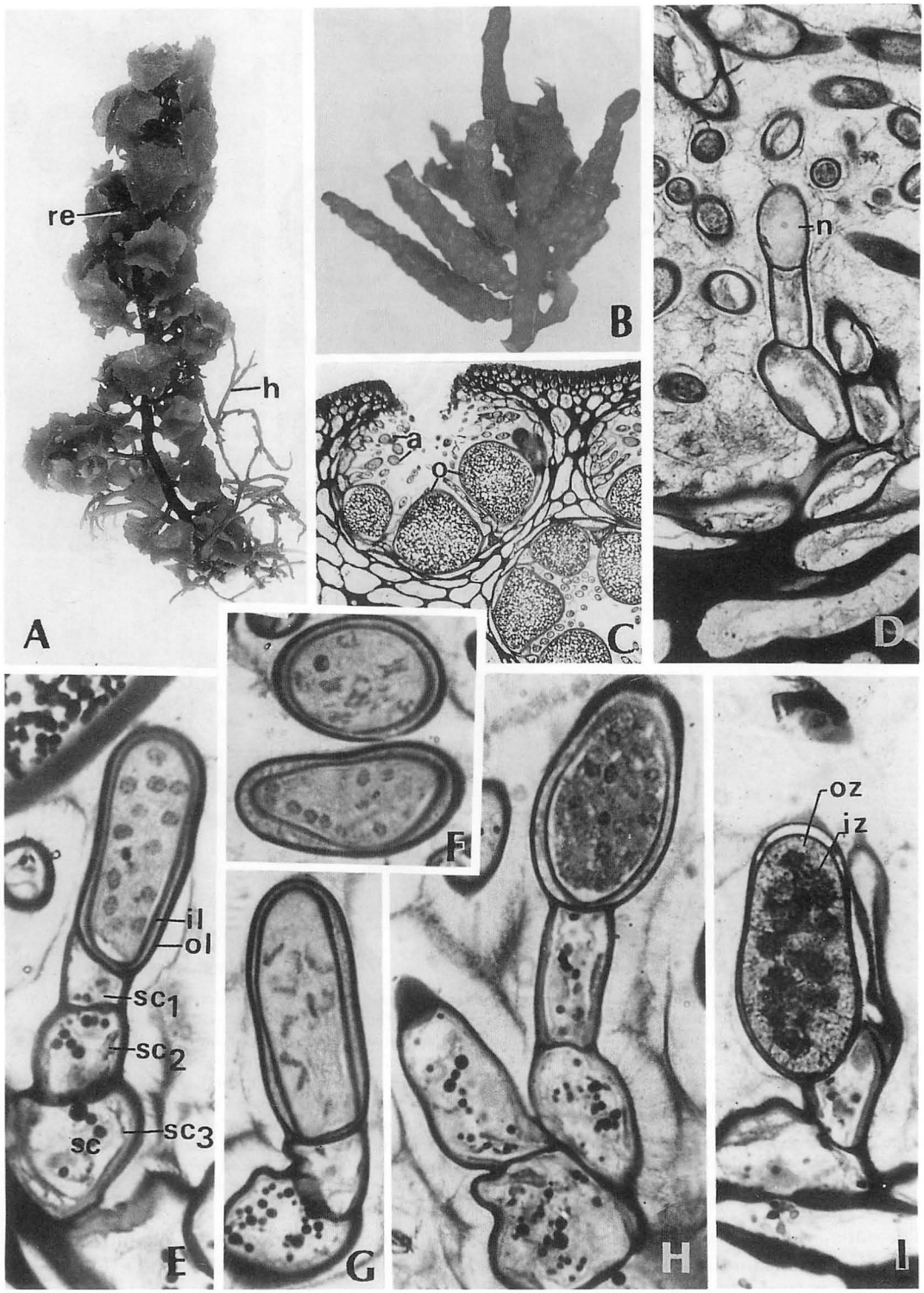
Antheridial release

In an antheridium ready for release the space narrows between the two wall layers, except in the upper region (Fig. 2D). The inner wall layer extends at the proximal end forming a small stalk (Fig. 2E). The spermatozoids enclosed in the polysaccharide material are released in a mass (Fig. 2F) and are gradually pushed through the ostiole.

Histochemical studies

Insoluble polysaccharides: In uninucleate and binucleate antheridia the wall layers stain reddish-violet with TBO and moderate magenta with PAS. In antheridia with eight nuclei the wall is two-layered and each wall layer shows different staining intensities.

Fig. 1A–I. *Turbinaria conoides*. A. Reproductive thallus, at the base of axis are spreading branches of haptera (h) that anchor the plant to the substratum. The receptacles (re) are borne in the axil of the leaves. ×1.5. B. A branched receptacle. ×5.3. C. A portion of mature receptacle to show oogonia (o), antheridia (a) and paraphyses. ×115. D. Magnified view of a young antheridium surrounded by single wall. A large nucleus (n) is present. ×1100. E. Mature antheridium with the two wall layers, the outer wall layer (ol) and inner wall layer (il). The lumen between the two wall layers contains polysaccharide material. Three stalk cells (sc 1–3) are also present. ×1100. F and G. Antheridia at different divisional stages. ×1100. H. A mature antheridium enlarged to show granular polysaccharide in the cytoplasm. ×1100. I. Same, showing two zones of polysaccharides in the cytoplasm. The outer zone (oz) consists of aggregated and intensely stained granules. ×1100.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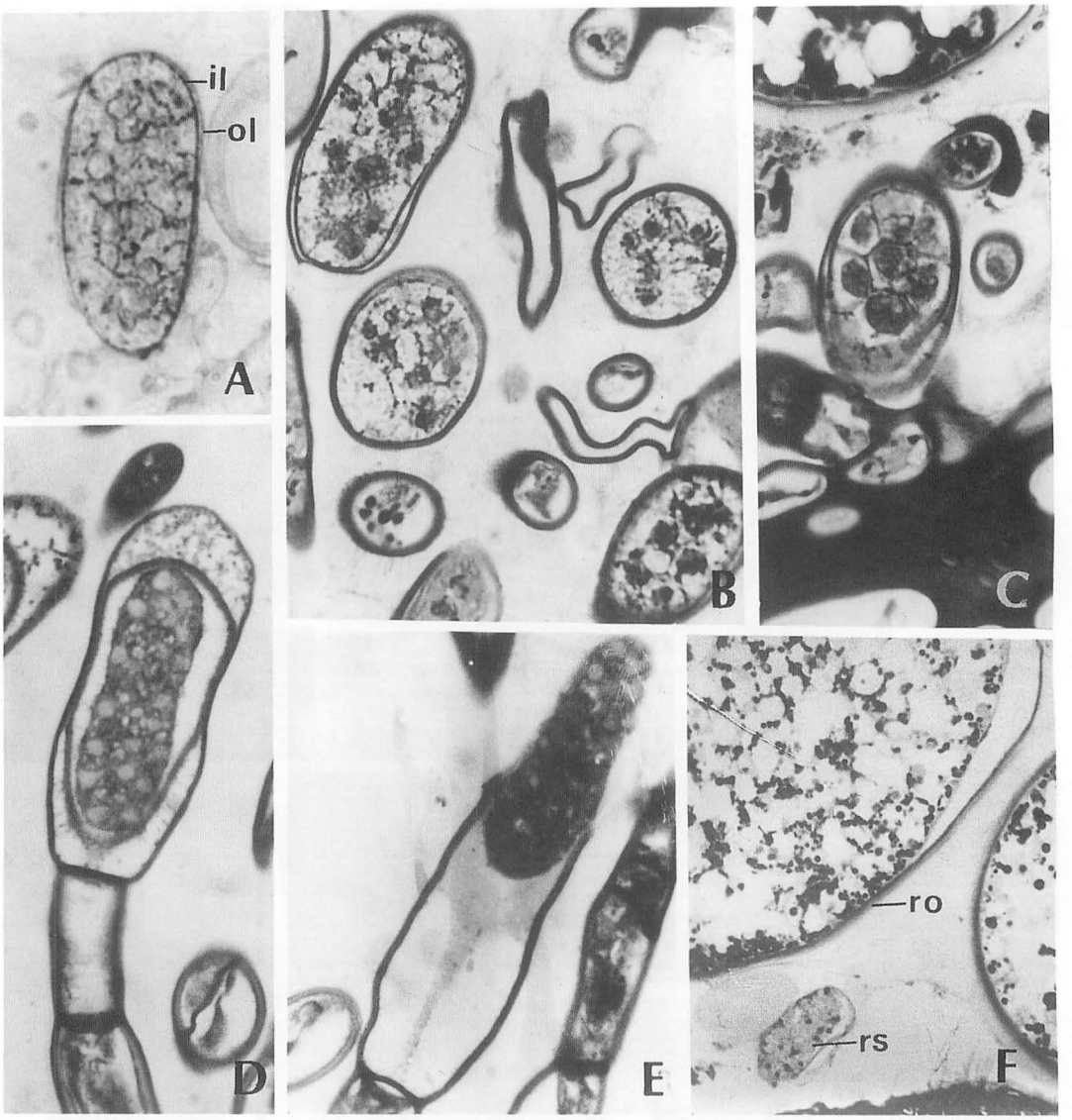

Fig. 2A–F. *Turbinaria conoides*, localization of polysaccharides. A, stained with Alcian blue and B–F, with TBO. A. Mature antheridium to reveal more sulphated polysaccharides in the inner wall layer (il) than in the outer layer (ol). The cytoplasm contains a mixture of large and small granular polysaccharides. The former are intensely stained and aggregated whereas the latter lightly stained and dispersed in the cytoplasm. ×1000. B. Mature antheridia enlarged to show linearly arranged polysaccharide granules (arrows). ×1000. C. An antheridium magnified to show thin partition walls (arrow). Each compartment encloses a nucleus along with a portion of the cytoplasm. ×1100. D and E. Antheridia magnified showing *en masse* release of spermatozoids. In D, the space between the two walls has decreased except at the apical and lower end. In E, the outer wall layer (ol) has ruptured at the apical end for the release of the spermatozoid. ×1400. F. Released oogonium (ro) and spermatozoids (rs). The spermatozoids are ensheathed in the polysaccharide matrix. ×426.

The outer wall layer stains intensely whereas the inner layer stains lightly with PAS and Alcian blue. With TBO the outer wall layer stains reddish-violet whereas the inner layer stains red. The two wall layers are therefore composed of a mixture of alginic acid and sulphated polysaccharides. The lumen between the two wall layers stains moderately with Alcian blue, light pink with TBO, and is PAS-negative. The lumen thus contains

sulphated polysaccharides. At the antheridium stalk cell junction the wall exhibits intense staining with TBO, PAS and Alcian blue indicating presence of mixture of alginic acid and sulphated polysaccharides. Cytoplasm in the young antheridium stains turquoise with TBO. As the antheridium matures there is a progressive change of

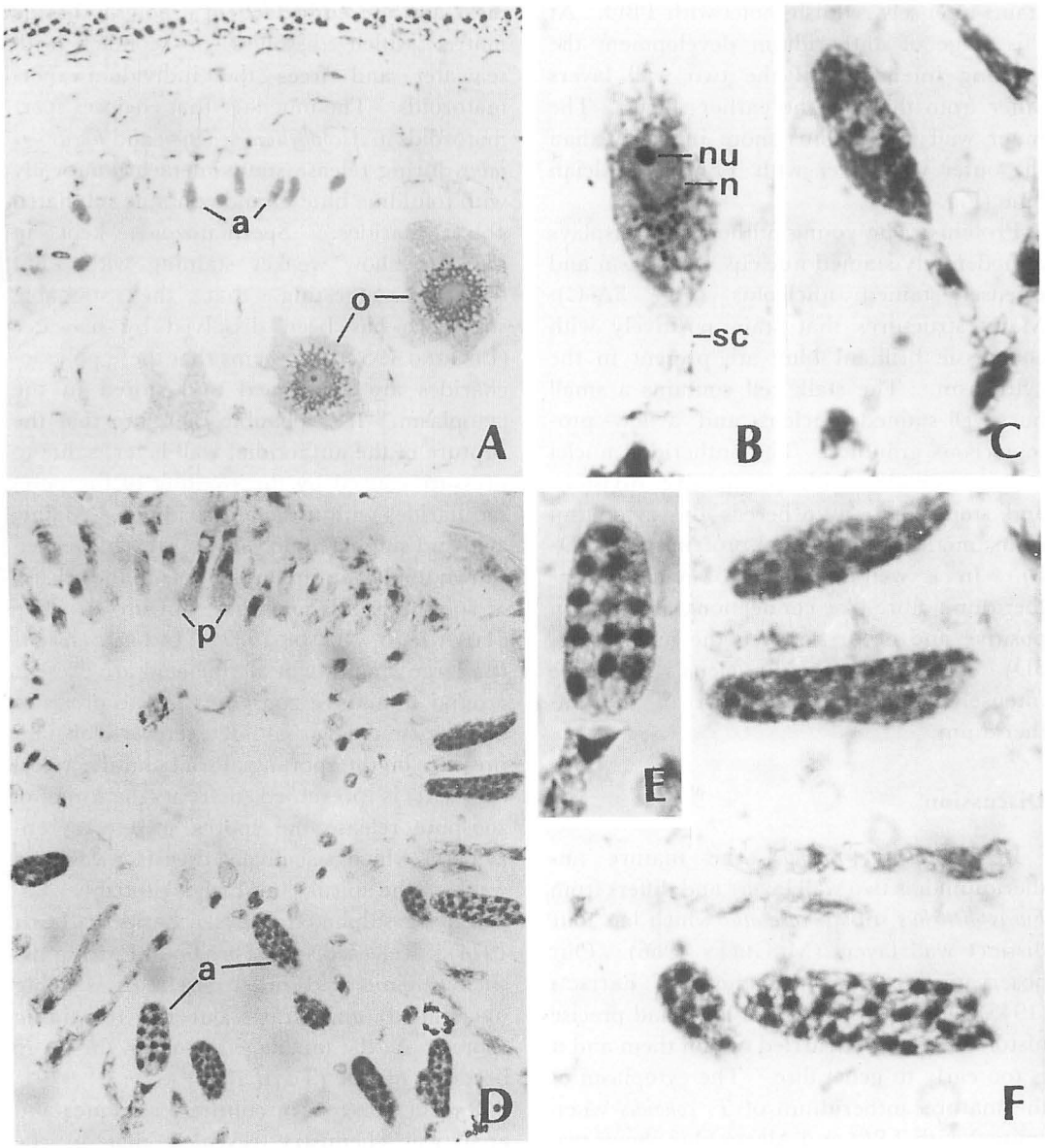

Fig. 3A–F. *Turbinaria conoides*, localization of proteins. A. A portion of transverse section of the conceptacle to show antheridia (a) present at the upper end and the oogonia (o) at the lower portion. ×115. B. A young antheridium enlarged to show a large nucleus (n) and intensely stained nucleolus (nu). Many coomassie brilliant blue positive structures are also present. ×1100. C. An antheridium at later stage of development showing well-defined cytoplasm and chromonemata in the nucleus. ×1100. D. A portion of conceptacle magnified to show paraphysis (p) and antheridia (a) at progressive stages of development. ×115. E. Mature antheridium showing the nuclei that are interconnected by thin and fibre-like structures. ×1100. F. Antheridia at 32-nucleate stage. The size of the nucleus has decreased. The fibre-like structures that interconnect the nuclei are well developed. ×1100.

metachromasy of its cytoplasm which stains reddish-violet with TBO. In the mature antheridium, the outer zone of free and granular polysaccharide stains lightly reddish-violet whereas the inner zone of aggregate granules stains intensely reddish-violet with TBO. At this stage of antheridium development the staining intensities of the two wall layers differ from those of the earlier stages. The inner wall layer stains more intensely than the outer wall layer with TBO and Alcian blue (Fig. 2A).

Proteins: The young antheridium displays a moderately-stained nucleus, cytoplasm and intensely-stained nucleolus (Fig. 3A-C). Many structures that stain positively with coomassie brilliant blue are present in the cytoplasm. The stalk cell contains a small but well-stained nucleus and a few proteinaceous granules. The antheridial nuclei become smaller with progressive divisions and stain intensely whereas the cytoplasm stains moderately for total proteins (Fig. 3D-F). In a well-developed 32-nucleate antheridium fibre-like connections are protein positive and occur between the nuclei (Fig. 3D). The spermatozoid nucleus stains more intensely than the cytoplasm of the antheridium.

Discussion

In *Turbinaria conoides* the mature antheridium has two wall layers and differs from *Fucus distichus* subsp. *edentatus* which has four distinct wall layers (McCULLY 1966). Our observations agree with those of FRITSCH (1945). So far few Fucales have had precise histological studies carried out on them and it is too early to generalise. The cytoplasm of the mature antheridium of *T. conoides* when stained with TBO and Alcian blue shows two kinds of polysaccharide granules: (1) the lightly stained dispersed granules and (2) the intensely stained aggregated granules. BIDWELL *et al.* (1968) reported two kinds of fucoidin in *Fucus vesiculosus* L.: (1) a readily hydrolysed and water soluble component that may serve as a reserve and (2) an insoluble component that acts as an important structural component of the plant. We think that the polysaccharides in the cytoplasm of mature antheridia of *T. conoides* may act as reserve. The spermatozoids are released as a mass and are embedded in a polysaccharide matrix which dissolves on contact with seawater and frees the individual spermatozoids. The mucilage that encloses spermatozoids in *Ascophyllum nodosum* and *Fucus serratus* during release stains metachromatically with toluidine blue O and contains sulphated polysaccharides. Spermatozoids kept in seawater show weaker staining with TBO thereby suggesting that the stainable substance has been dissolved by seawater (LEVRING 1952). It seems that these polysaccharides are preformed and stored in the cytoplasm. It is possible therefore that the rupture of the antheridial wall layer is due to pressure caused by the swelling of the polysaccharides within the antheridium. Alginic acid and sulphated polysaccharides have been shown to play an important role in the release of spermatozoids and spores of brown algae (TOTH 1976, NELSON 1982). In *Chorda tomentosa* large amounts of alginic acid are present around immature zoospores. The presence of this mucilage could exert a constant pressure on the sporangial wall and the apical cap. It is presumed that at the time of zoospore release the spores secrete an enzyme(s) which selectively digests away and weakens the apical cap which is probably composed of sulphated polysaccharides (TOTH 1974). Released spores are bound within the sticky alginic acid and it swells (now being unconfined) and draws out the remaining spores. As the mucilage dissolves, the spore becomes mobile (TOTH 1976).

Recent progress in culture techniques and analytical chemistry revealed that in the members of Laminariales, particularly *Macrocystis, Laminaria* and *Chorda*, a volatile compound of low molecular weight secreted from released eggs induces spermatozoid release. The substance was named as lamoxirene (MAIER 1982, MÜLLER *et al.* 1985). In *Laminaria digitata* (L.) LAMOUR., the an-

theridia show a specialized swelling of the cell wall. In the apical region of the antheridium the cell wall is markedly thickened to form a "cap" (MAIER and MÜLLER 1982, MAIER 1987). The spermatozoids are surrounded by copious mucilage and both factors contribute to an explosive bursting of antheridium (MAIER and MÜLLER 1982).

During dehiscence the wall in this region is disintegrated to such an extent that the spermatozoids can rupture it within about 0.3 sec and it forces out antheridium apparently driven by an internal pressure which perhaps is generated by swelling of mucilage (MAIER 1987). In *Scytosiphon* both male and female gametes are released by dissolution of the gametangial wall (CLAYTON 1984). In *T. conoides* the spermatozoids are pushed up gradually by the inner wall layer into the conceptacle cavity and later discharged into the seawater. The manner in which the spermatozoid mass passes from the conceptacle cavity to the external seawater is intriguing and needs further investigations. MANTON and CLARKE (1956) suggested that in *Fucus* sp. the spermatozoids are only released after the mucilage in the conceptacle is extruded and dissolved.

Oogonial release in *T. conoides* appears to be entirely different (SOKHI and VIJAYARAGHAVAN 1986). The released oogonium lies outside the ostiole but remains attached to the exochiton within the conceptacle by means of mesochiton stalk. Prior to release oogonium shows reverse polarity. At the distal end of the oogonium a mesochiton pad remains attached to the exochiton and proximal end is first to be extruded. No such inversion occurs in the antheridium as the small and transistory stalk formed by the inner wall layer in the proximal end pushes the polysaccharide-surrounded spermatozoids. The inner wall layer of the antheridium in *T. conoides* thus performs a dual role: it aids in release and also in the protection of the released spermatozoids.

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SOKHI, G.・VIJAYARAGHAVAN, M. R.：褐藻 *Turbinaria conoides* の造精器形成と精子放出に関する発生学的ならびに組織化学的研究

ラッパモク属の一種 *Turbinaria conoides*（ヒバマタ目ホンダワラ科）の生殖器床は両性で，造精器は通常生殖器巣の上端に形成される。造精器は無柄または有柄で，柄細胞の数は1～3である。若い造精器（1～2核期）は1層の細胞壁をもつが，8核以上の段階になると2層の細胞壁をもつ。染色法により，細胞壁はアルギン酸と硫酸多糖類を含むことを明らかにした。成熟した造精器では，細胞壁の内層は外層より硫酸多糖類の沈着が著しい。内層と外層の間の内腔は硫酸多糖類で満たされている。造精器が成熟するにつれ，2層の多糖類と細胞質のメタクロマジーの変化が認められるようになる。精子の放出時には，細胞質の成層は退化する。外層は造精器の先端またはその近くで分解し，精子は硫酸多糖類に包まれた状態で放出される。(Department of Botany, University of Delhi, Delhi-110007, India)