Antheridium development and spermatozoid release in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner

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In *Sargassum*, specialized fertile, lateral branches or receptacles bear flask-shaped, unisexual cavities or conceptacles. In the male conceptacle, the antheridia arise from any of the wall cells that line the conceptacle. Antheridium development is accompanied by changes in the wall composition. Spermatozoid release in *Sargassum vulgare* and *S. johnstonii* is effected by the combined efforts of the two wall layers and associated cytoplasmic polysaccharides. During the spermatozoid release, the outer layer of the antheridial wall ruptures at the apical region and the cytoplasmic polysaccharides generate pressure releasing spermatozoids. Differences and similarities that exist between the spermatozoid and the oogonial release are highlighted.

Key Index Words: antheridium—Fucales—polysaccharides—*Sargassum*—spermatozoid.

Sargassum vulgare C. Agardh bears androgynous receptacles where each receptacle possesses male and female conceptacles. In *S. johnstonii* Setchell & Gardner, however, three types of plants exist that bear either (a) androgynous (b) female or (c) male receptacles. In these two taxa both the antheridium development and spermatozoid release are highly synchronised and programmed phenomena. In *S. johnstonii*, occasionally the male conceptacles bear underdeveloped oogonia. The present paper deals, in these two taxa, with the histochemical changes that occur during progressive stages of antheridium development and spermatozoid release.

Materials and Methods

The plants of *Sargassum vulgare* and *S. johnstonii* were collected from Port Okha, Gujarat, during the low tide periods during the years 1988 and 1990. Selected portions of vegetative and reproductive thalli were fixed in 10% aqueous acrolein, post-fixed in 1% mercuric chloride and processed for light microscopic studies (Feder and O'Brien 1968). Two micron thick sections were cut with glass knives, using a locally made adaptor that fits to the rotary microtome. The sections were stained with Periodic-Acid Schiff Reagent (PAS) and Feulgen reagent (Vijayaraghavan and Shukla 1990); 0.05% Toluidine Blue 0 (TBO) at pH 4.4 (McCully, 1966); Alcian Blue (Parker and Diboll 1966) and Coomassie Brilliant Blue (CBB) (Weber and Osbörn 1975). Photomicrographs were taken on ORWO B/W film using Carl Zeiss photomicroscope.

Results

The thalli of *Sargassum vulgare* and *S. johnstonii* during the reproductive phase, bear many receptacles, that are borne in the axils of the leaves (Figs. 1, 2). The receptacles bear unisexual conceptacles which develop and mature between October and February. The distribution of the oogonial and antheridial conceptacles in a receptacle is examined. Amongst several female conceptacles only 2 or 3 male conceptacles are seen (Figs. 3, 5). Rarely, however, a greater number of male conceptacles is also encountered (Fig. 4).

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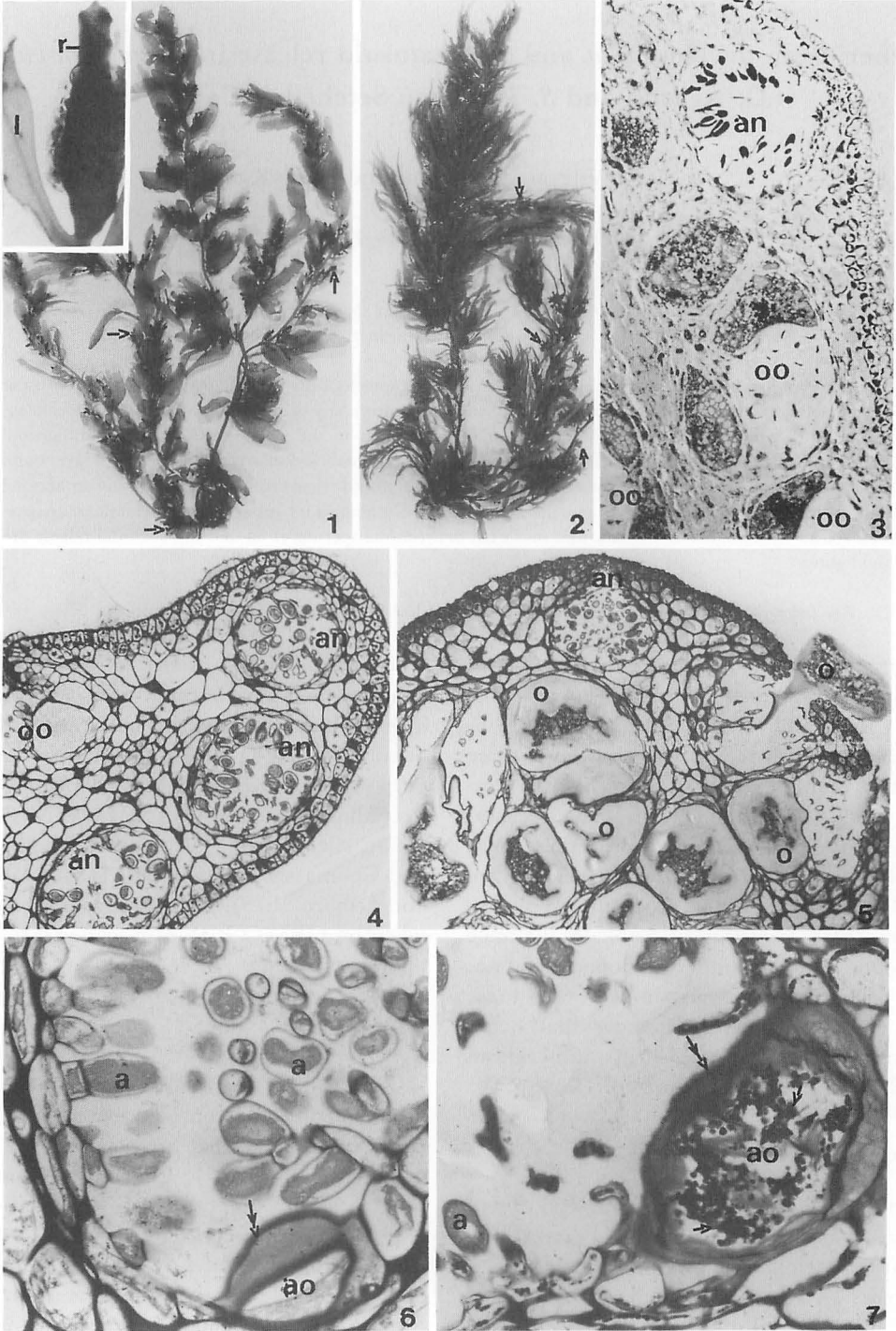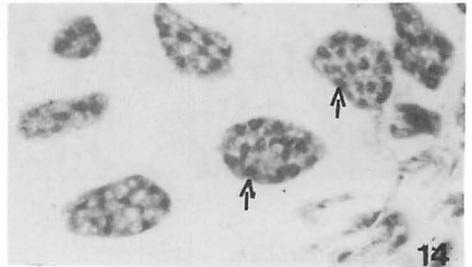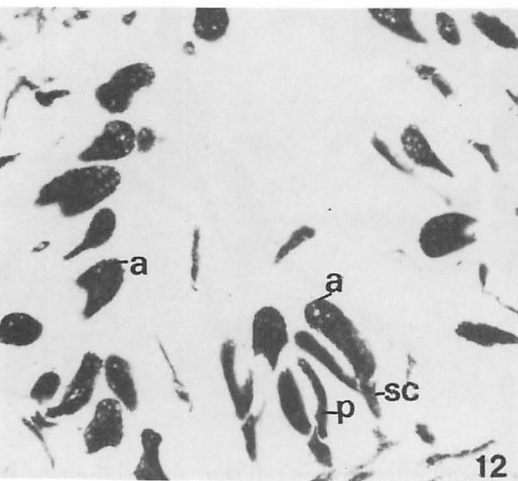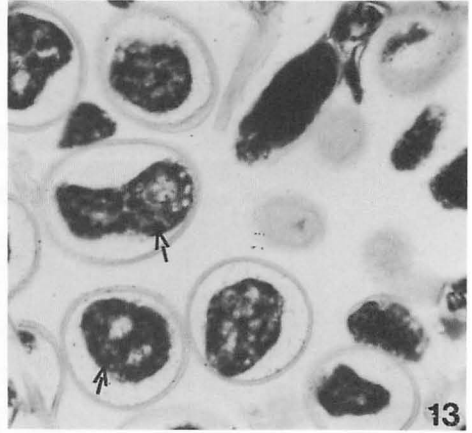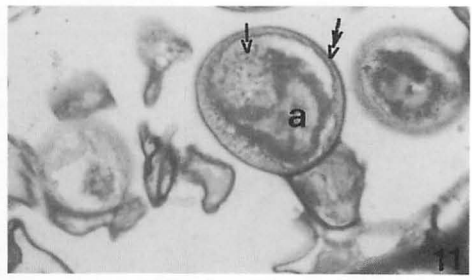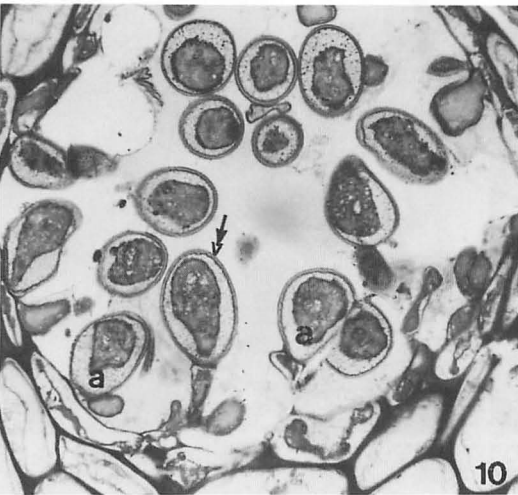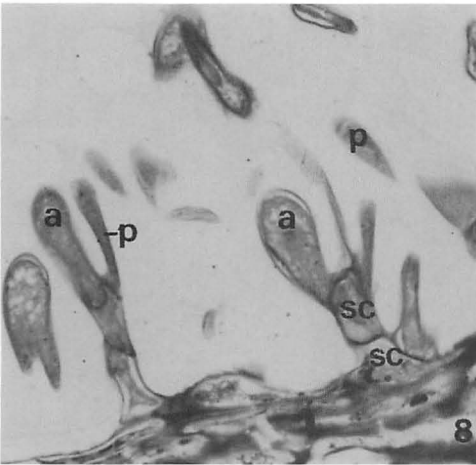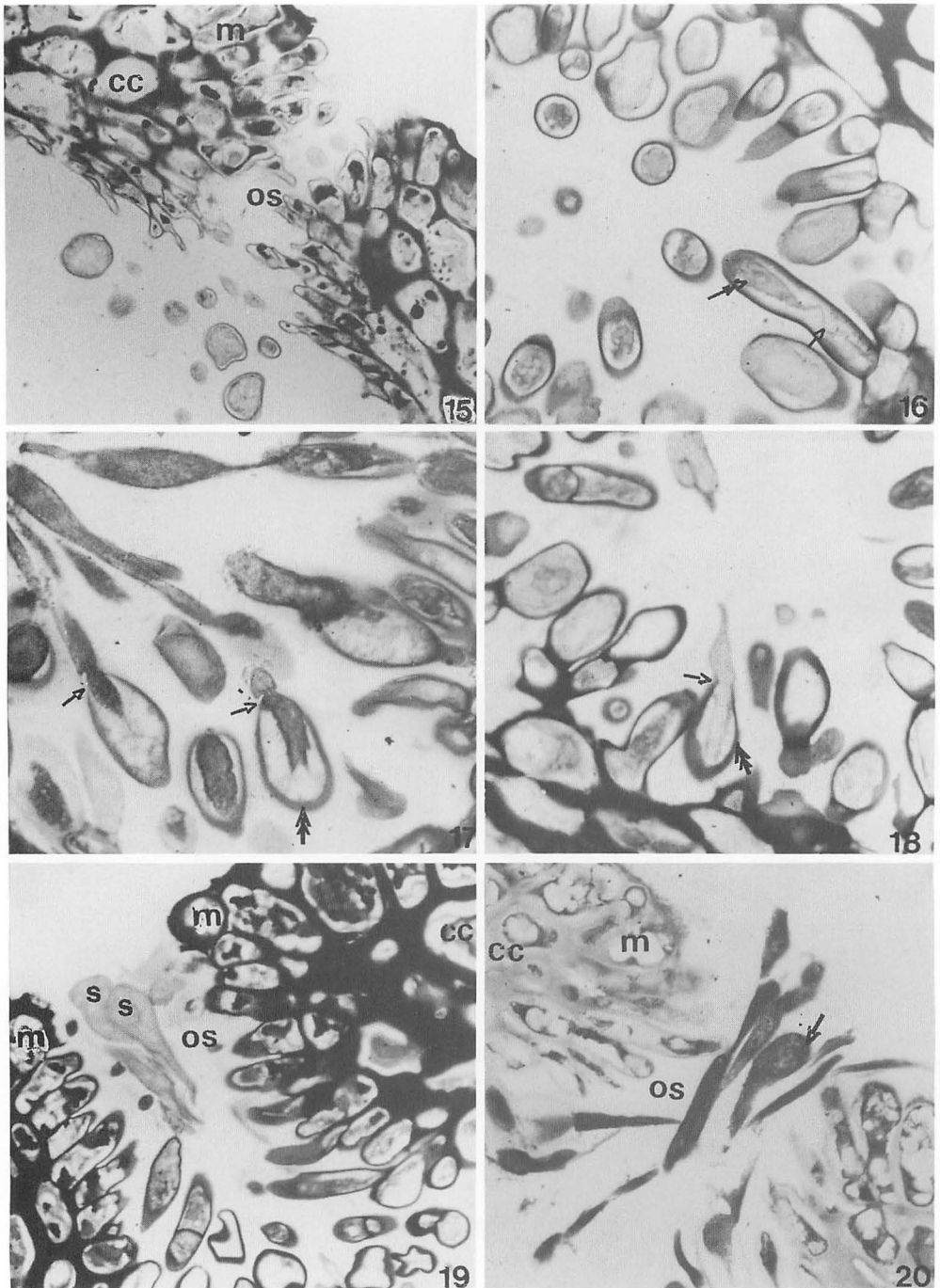
Development of antheridium

Each antheridium develops from the con-
ceptacle wall cell. The antheridial initial
emerges as a papilla, divides by a transverse

wall into a lower cell that acts as the first stalk
cell and the upper antheridial mother cell.
The latter divides again forming the outer
antheridium and the second stalk cell (Fig.

8). The number of stalk cells ranges between 1 or 2 (Fig. 8; SC). Occasionally, sessile antheridia are also encountered where the antheridial initial directly acts as the antheridial mother cell. The antheridium during early ontogeny is unilayered. It possesses dense cytoplasm (Fig. 9) and a centrally located nucleus. The mature antheridium has two conspicuous wall layers (Figs. 11, 13) that enclose cytoplasm containing 64 nuclei. During spermatozoid release, the outer layer lysis occurs and the spermatozoids enclosed within the inner layer are released *en masse* (Fig. 17). Many paraphyses coexist with antheridia.

Histochemistry of antheridium

Wall layers: The antheridium during early stages of development is covered by a single layered wall that stains moderate violet with TBO (Fig. 9); turquoise with Alcian Blue; light magenta with PAS reagent (Fig. 8). These histochemical staining procedures indicate abundant sulphated polysaccharides in the wall layer. The second wall layer becomes distinct at about the 16 nucleate stage, and has a mixture of sulphated and carboxylated polysaccharides. At maturity, however, the inner layer shows abundant sulphated polysaccharides whereas the outer layer shows the dominance of alginic acid. The space between the two wall layers is filled with granular, sulphated materials that reveal metachromasia and stain pink with TBO and turquoise with Alcian Blue.
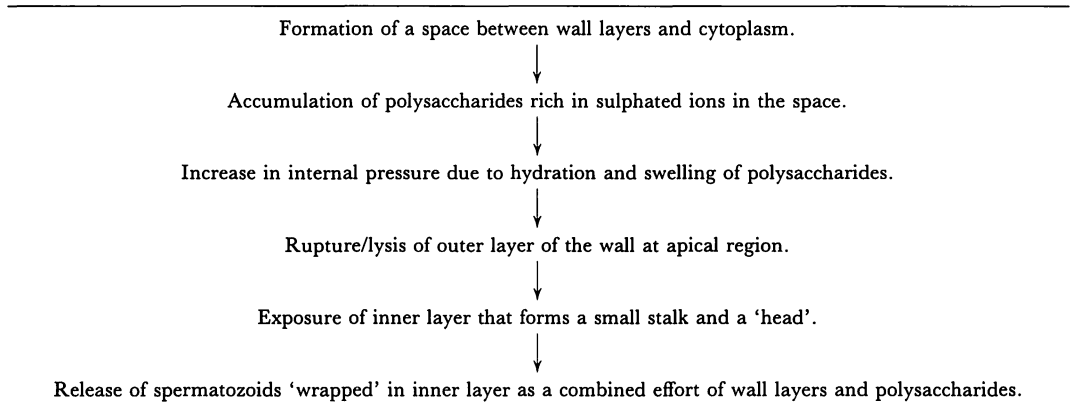
Cytoplasmic inclusions: The cytoplasm of a young antheridium has many vacuoles and a few sulphated polysaccharides that stain light magenta with PAS reagent (Fig. 8) and turquoise with TBO (Fig. 9). With progressive antheridial development, vacuoles are retained but the cytoplasm shows two distinct zones. The inner zone stains turquoise with Alcian Blue and pink with TBO indicating abundant sulphated polysaccharides. The outer zone stains purplish-pink with TBO; magenta with PAS reagent and feebly with Alcian Blue revealing dominance of alginic acid (Figs. 10, 11). As the antheridium develops further, the cytoplasm retracts from the wall and creates a space which is filled with grains that stain for sulphated groups (Figs. 11, 13).

During antheridial development (Fig. 12)

Figs. 1–7. 1, 2. *Sargassum vulgare* and *S. johnstonii*, mature plants showing branches, leaves, bladders and receptacles (arrows). × 0.45. The inset in 1 shows a receptacle (r) in the axil of a leaf (l). × 0.9. 3. *S. vulgare*, longitudinal section of an androgynous receptacle to show one antheridial (an) and three oogonial conceptacles (oo). × 360 (CBB) stained. 4. *S. vulgare*, androgynous receptacle where the number of antheridial conceptacles (an), occasionally, exceeds the oogonial conceptacles (oo). × 225 (TBO stained). 5. *S. vulgare*, 2 or 3 oogonia (oo) are produced per conceptacle. Number of antheridia in a conceptacle (an) is however large. × 225 (TBO stained). 6, 7. *S. johnstonii*, longitudinal sections of antheridial conceptacles to show underdeveloped oogonia (ao) that coexist with the functional antheridia (a). In such oogonia the usual wall differentiation is lacking (double arrow) and the cytoplasm is gorged with physodes (arrows). × 1418 (TBO stained).

Figs. 8–14. *Sargassum vulgare*. 8. Transverse section of an antheridial conceptacle to show paraphyses (p) and antheridium (a) bearing two stalk-cells (sc). × 1575 (PAS stained). 9. Uninucleate antheridia (a) showing numerous small vacuoles. × 1575. (TBO stained). 10, 11. Mature antheridia (a). The two wall layers (double arrows) are prominent. The cytoplasm retracts from the wall and the space, thus, created is filled with granular sulphated polysaccharides (arrows in 11). 10. × 1575; 11. × 2000 (TBO stained). 12. Transverse section of a male conceptacle to show protein-rich cytoplasm of paraphysis (p), antheridium (a) and stalk-cell (sc). × 1575 (CBB stained). 13, 14. Antheridia at 16 nucleate stage, where all the nuclei (arrows) stain well with CBB and Feulgen reagent, respectively. 13. × 2000; 14. × 1575.

Figs. 15–20. *Sargassum vulgare*. 15. Transverse section of the antheridial conceptacle passing through the ostiolar region (os). The meristoderm (m) and the cortical cells (cc) become lax and make passage for the spermatozoid exit. × 1575 (PAS stained). 16. Antheridium prior to spermatozoid release. The inner wall layer extends into a fragile tail (arrow) whereas the head portion bulges (double arrow) enclosing the cytoplasm with the spermatozoid nuclei. × 1575 (TBO stained). 17, 18. Mature antheridia to show at the apical end ruptured outer wall layer (arrows). Subsequently, the spermatozoid mass is released leaving behind the outer wall layer as empty shell (double arrow). The material that surrounds the spermatozoids is rich in sulphated polysaccharides (arrow). The outer layer (double arrow) is, however, rich in carboxylated polysaccharide. × 2000 (TBO stained). 19, 20. Spermatozoids (s) being released *en masse* through the ostiole (os). Meristoderm (m) and cortical cells (cc) cytoplasm reveal abundant phenolic materials. Spermatozoid nuclei are seen embedded in proteinaceous-matrix (arrows) in Figure 20. × 1575 (19, TBO and 20, CBB stained).

Table 1. Steps involved in spermatozoid release in *Sargassum vulgare* and *S. johnstonii*

Formation of a space between wall layers and cytoplasm.

↓

Accumulation of polysaccharides rich in sulphated ions in the space.

↓

Increase in internal pressure due to hydration and swelling of polysaccharides.

↓

Rupture/lysis of outer layer of the wall at apical region.

↓

Exposure of inner layer that forms a small stalk and a 'head'.

↓

Release of spermatozoids 'wrapped' in inner layer as a combined effort of wall layers and polysaccharides.

the cytoplasm is gorged with proteins but negligible phenolic materials. The nuclei stain well with Feulgen reagent (Fig. 14). The adjoining paraphyses are, however, rich both in proteins and phenolic compounds. The ostiole, in the young conceptacle, always remains closed due to the compact meristoderm (Fig. 5). The plug-material at this end is, however, absent (see Table 2). In the antheridial conceptacle of *S. johnstonii*, an underdeveloped oogonium is occasionally encountered. Such oogonia possess phenolic-rich cytoplasm and unilayered wall (Figs. 6, 7).

Spermatozoid Release

Prior to release, the meristoderm at the ostiole region losens (Fig. 15). The outer layer of the antheridium ruptures/lyses at the apical region (Fig. 17) and due to the pressure generated by sulphated polysaccharides the spermatozoids enclosed in the inner layer are released *en masse* through the ostiole (Figs. 19, 20).

During the spermatozoid release, the inner layer of the antheridial wall assumes a 'tadpole' like shape; where the tail portion forms a small, fragile stalk (Fig. 16) and the distal head portion contains spermatozoids. The head portion is first to emerge out through the ruptured outer wall of the antheridium (Fig. 18; see also Table 1). The outer layer

Table 2. Antheridium/oogonium development and spermatozoid/oogonium release in *Sargassum vulgare* and *S. johnstonii*

	Antheridium	Oogonium
DIFFERENCES:		
Progenitor	Basal and neck region of conceptacle	Only basal region
Number per conceptacle	Many	4–6
Ostiolar plug	Absent	Present
Polarity	No change during spermatozoid release	Inversion occurs; proximal end is extruded first
After release	Spermatozoids are motile	Remains attached through mesochiton-stalk
SIMILARITIES:		
Outer wall layer	Ruptures prior to release	Same
Inner wall layer	Forms a stalk (rudimentary)	Forms a stalk (prominent)
Cytoplasmic polysaccharides	Aid in release	Same

remains behind like an empty shell (Fig. 18).

Discussion

The mature antheridium in *Sargassum vulgare* and *S. johnstonii*, has a double layered wall; the outer layer is rich in alginic acid and inner in sulphated polysaccharides as reported in *Turbinaria conoides* (Sokhi and Vijayaraghavan 1990). In *Fucus edentatus* (McCully, 1968), four distinct wall layers have been reported. In *Ascophyllum nodosum* and *Fucus serratus*, the walls of the antheridium stain metachromatically with TBO and are probably composed of fucoidin (Levring 1952).

In *Sargassum vulgare* and *S. johnstonii*, near 16 nucleate stage, the cytoplasm reveals two zones; the outer zone rich in carboxylated polysaccharides and the inner zone in sulphated. At maturity, the oogonial cytoplasm also reveals a polarised distribution of polysaccharides where carboxylated moeties abound the pericytoplasmic region and the sulphated ones the perinuclear zone (Kaur and Vijayaraghavan 1991).

Numerous vacuoles have also been observed in the cytoplasm of mature antheridium. In *Scytosiphon* sp. vesicles are thought to play a role in spermatozoid liberation (Clayton 1984). The vesicles are the source of enzymes responsible for cell wall lysis and the resultant breakdown products form the hydrated mucilaginous matrix that cause the dehiscence of gametangia. A similar role of vacuoles in *Sargassum* spp. is envisaged.

In *Sargassum vulgare* and *S. johnstonii*, physodes are negligible in the antheridial cytoplasm which may be due to the low number of chloroplasts. The stalk-cell, paraphyses, and conceptacle wall cells are, however, gorged with physodes suggesting a protective function (*see also* Kaur and Vijayaraghavan 1992). It is also possible that the tannins in physodes may serve as herbivore deterrents and thus help to prevent damage to the antheridia (Phillips and Clayton 1991).

Underdeveloped oogonia have been observed in the male conceptacles of *Sargassum*

johnstonii. It is tempting to speculate the significance of these freaks. This reveals that unisexual conceptacles have 'hidden' bisexuality, which, if expressed results in poor development of the oogonium. Taxa with unisexual conceptacles are usually considered more advanced with adaptation for cross-fertilization and reproductive-economy (Dawson 1940).

Reproduction in brown algae is environmentally controlled both for survival and to ensure simultaneous gametic maturity (Clayton 1986). In *Sargassum vulgare* and *S. johnstonii*, spermatozoid release is a highly synchronized phenomenon. Prior to gamete release, a space develops between cytoplasm and the wall layers which is filled with the sulphated polysaccharides. The outer layer lyses only at the apical end. This is followed by ejection of the spermatozoids enclosed within the inner layer through the antheridial orifice.

The inner layer is rich in sulphated polysaccharides of hygroscopic nature. These polysaccharides swell up, generate pressure and the outer layer ruptures. The inner layer undergoes changes in its contours and extends into an anterior end and a posterior portion which trails behind as if the spermatozoid mass enclosed in sulphated polysaccharides is being passively pushed. The spermatozoids remain immobile due to the presence of polysaccharide envelope. Once near the ostiole, polysaccharides contact water, dissolve away and render the spermatozoids free to swim actively towards the mates. Toth (1974) presented histochemical evidence to show that in *Chorda tomentosa* the space is filled with alginic acid and sulphated polysaccharides. The hydration and subsequent swelling of these mucilaginous carbohydrates creates an increase in the internal pressure that leads to the rupture of the sporangial walls.

This investigation supplements our knowledge on gamete release in *Sargassum* spp. (*see also* Kaur and Vijayaraghavan 1991). There is not only a slight difference in the size and number between male and female gametes but conspicuous differences exist in

their behaviour (see Table 2). Thus, it is not only the oogonia which are released by a highly coordinated mechanism but also the spermatozoids. The ultrastructural, biochemical and physiological aspects of this highly programmed process still remain unknown.

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M. R. Vijayaraghavan and Inderdeep Kaur：*Sargassum vulgare* C. Agardh と
S. johnstonii Setchell & Gardner の造精器の発達と精子の放出

造精器は生殖器床の内面の細胞から生ずる。発達初期には硫酸多糖に富む一層の細胞壁が認められ，後にはアルギン酸に富む第二の層が区別される。細胞質には液胞を生ずる。精子放出は二層の細胞壁と細胞質多糖の協調過程によって起こる。造精器壁の外層は頂部が破れ，細胞質多糖が精子放出の圧力を生ずる。精子放出と卵放出の間の差異と類似性を明示した。(Department of Botany, University of Delhi, Delhi 110007, India)