Scientific Research

Understanding the Mechanism of Gamete Release in *Sargassum vulgare* C. Agardh

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ABSTRACT

Sargassum vulgare C. Agardh shows androgynous receptacles, each bearing on an average 12 unisexual conceptacles which open outside by ostiole, and wherein gametangia (antheridia or oogonia) lie interspersed with paraphyses. Since out-put of eggs is extremely low, 4 - 6 per female conceptacle, *Sargassum* sp. ensures its survival under all eco-physiological conditions. The released oogonium is "wrapped" in sulphated polysaccharide-rich wall layer known to provide protection against desiccation. Oogonia after being "extruded" out of ostiole, are "incubated" on receptacle, where they grow into eggs that are easily contacted by spermatozoids. Gamete release is synchronous and almost simultaneous ensuring high rates of fertilization. The release occurs on days falling near a full moon or new moon, during low tides when conceptacles lie exposed. Gamete release occurs first from upper conceptacles, which "house" mature gametangia while lower ones are still developing. This results in gamete release over an extended period of time. The zygote dispersal and propagule recruitment also show adaptations selectively advantageous for the alga.

Keywords: Gamete Release; Mesochiton Stalk; Oogonium Incubation; Propagule Dispersal; *Sargassum vulgare*

1. Introduction

Several reviews over the years have discussed various factors affecting the developmental stages of algal strands including gamete release, fertilization, dispersal period, settlement, attachment, recruitment and subsequent growth [1-4]. Amongst marine algae, Fucales have evoked a lot of interest in phenomena related to gamete release, zygote formation and germling dispersal [5-8]. Significant progress has been made in understanding the mechanisms that increase fertilization success of fucoids inhabiting intertidal zones marked with periods of high water velocities [9]. Biological and environmental factors affect the intertidal populations, and amongst all, wave action has received much attention over the last 20 years [10,11]. Direct effects of wave forces on intertidal organisms include damage, detachment and displacement [12]. Reproductive periodicity in fucoid algae, can be correlated with lunar or tidal cycles as in *Silvetia compressa* [13], *Fucus distichus* [14], *Fucus vesiculosus* [15] and *Sargassum vestitum* [16]. High water motion can inhibit gamete release in *Fucus vesiculosus*, *F. distichous*, *Pelvetia fastigiata* and result in low fertilization success as not all conceptacles expel eggs at the same time [17]. This mechanism allows cross fertilization and prevents potential inbreeding that result in selfing. Reproductive timing and synchronized reproduction itself may increase fertilization success. This synchronous gamete release (spawning) integrates various environmental signals [18, 19]. Inspite of detailed account of various aspects of gamete release, the comparisons between the male and female conceptacles is lacking. In this research paper besides giving a detailed account of mucilage associated with gamete release the authors bring out certain features of differences in male and female conceptacles.

2. Material and Methods

The plants of *Sargassum vulgare* C. Agardh a brown seaweed (Phaeophyceae, Fucales) were collected during January (2005) from Port Okha (22°28.528'N, 069° 04.322'E), Gujarat (India). Plants were washed with seawater to remove debris and brought to laboratory in air tight plastic bags. Selected portions were fixed in 10% aqueous acrolein, post fixed in 1% mercuric chloride for 24 h to stabilize polyphenols, thereafter rinsed with distilled water and processed for light microscopic studies [20]. The tissue was dehydrated at 4°C with three successive changes in 2 methoxyethanol for 24 h each, followed by 100% ethanol and propanol for 24 h and finally two successive changes were given with n-butanol for 24

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h. The dehydrated material was infiltered and finally embedded in glycol methacrylate. Sections (2 µ thick) were cut with glass knives using a rotary microtome. The sections were stained with Periodic Acid Schiff reagent and 0.5% Toluidine Blue O at pH 4.4 [21]. Total proteins were localized using Coomassie Brilliant Blue [22]. Alcian blue was used to stain sulphated polysaccharides [23].

For scanning electron microscopy the plants were fixed in 6% Glutaraldehyde, post fixed in OsO_4. The portions were then dehydrated in ascending acetone series, ranging from 10 through 90% followed by two changes in absolute acetone for 10 min. The material was later passed through dry acetone (absolute acetone in $CuSO_4$) followed by critical point drying at 20°C and 800 lb/square inch with pressure for 30 min. This temperature and pressure were raised to 35°C and 1200 lb/square inch respectively. These were then coated with gold (100 Å thickness) under vacuum on sputter coating unit (Edward coating unit) for 2 min to eliminate charging. The coated material was examined and photographed with LEO 435 VP, variable pressure scanning electron microscope at an accelerating voltage of 15 KV.

3. Results

Sargassum vulgare has a perennial existence in intertidal pools where the plants undergo a period of active vegetative growth from July to October and reproductive growth from October to February. When the temperature dips and illumination is low during November, the plants enter a period of sexual reproduction which culminates in gamete release and zygote formation in the month of February. The germlings are dispersed around February and March. After an active reproductive phase, the fronds shows senesce and die back leaving only perennial holdfast which regenerates in the month of May giving rise to a stipe and leaves.

3.1. Gametogenesis

During fertile phase, plants bear abundant androgynous receptacles (**Figure 1(A)**). This unisexual conceptacles are initiated in the cortical region of receptacles (**Figure 1(B)**).

Amongst several female conceptacles only 3 or 4 male conceptacles are seen in a single receptacle (**Figure 1(C)**). The young conceptacle is oval to pear-shaped with a narrow neck that opens outside through ostiole (**Figure 1(D)**). Inside the conceptacle, differentiation of oogonia, (or antheridia) and also the associated paraphyses takes place. With further development, ostiole narrows down while the base widens to accommodate developing gametangia (**Figures 1(E), (F)**) and thus the mature conceptacle assumes a spherical shape. The cells of conceptacle

floor wall are the progenitors of gametangia and associated paraphyses. The oogonia arise only from the conceptacles cells whereas, antheridia are produced on paraphyses from all over the conceptacle lining.

Male gametangium or antheridium is unilayered when young but, at maturity acquires a second layer (**Figure 1(G)**). Later, a space develops between two layers which
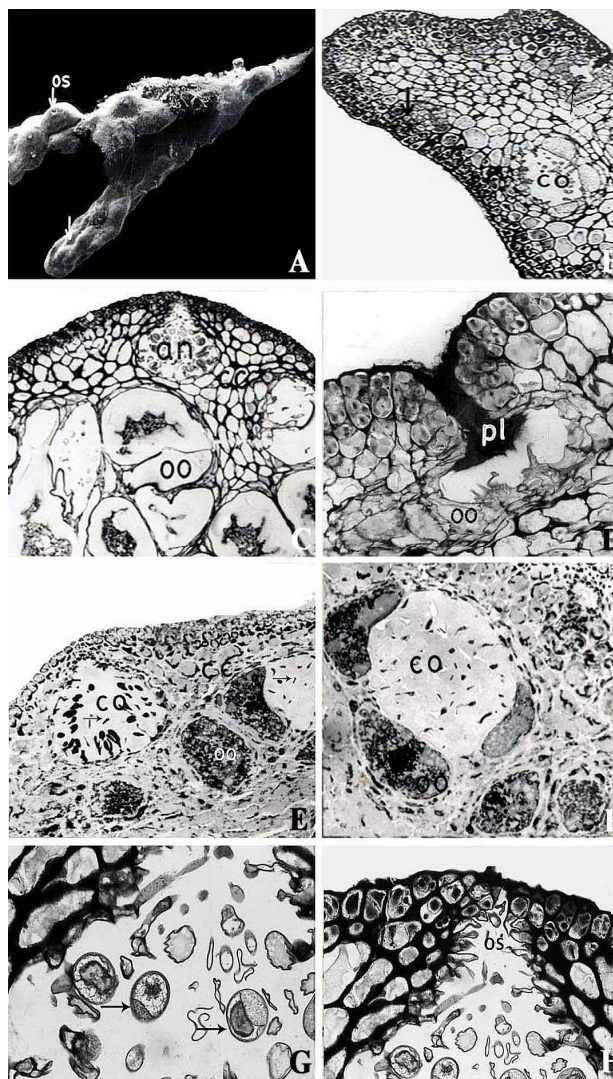

Figure 1. (A) Scanning electron micrograph showing a receptacle with raised regions that correspond to conceptacles whose ostioles (os) are seen as pores; (B, C) L.S. of receptacles showing conceptacles (co) at various stages of development (arrows). Number of female conceptacles (oo) is greater than male (an) conceptacles in (C). TBO stained; (D) Young female (oo) conceptacle with a narrow ostiole and associated plug (pl) material. TBO stained; (E, F) CBB stained sections showing paraphyses (arrows) in oogonial (oo) conceptacle; In (F) three oogonia (oo) are at different stages of development; (G, H) Male conceptacles showing mature antheridia with two wall layers (arrows) and a sulphated-rich pad like structure. Ostiole remains closed by compactly arranged cells in (H). TBO stained.

gets filled with sulphated polysaccharides. The cytoplasm encloses 64 nuclei that correspond to the number of spermatozoids. The ostiole remains closed due to compactly arranged meristoderm cells, until antheridia mature (**Figure 1(H)**). In *S. vulgare* approximately, 64 spermatozoids are produced per antheridium and many antheridia per conceptacles, while only one egg per oogonium and 4 or 5 oogonia per conceptacle are produced (**Figure 1(F)**).

The young oogonium has an alginate-rich single layered wall known as exochiton (**Figure 2(A)**). During further development two more layers namely mesochiton rich in sulphated polysaccharides and endochiton, a mix of sulphated and carboxylated polysaccharides are formed. Period of mesochiton formation is the most active stage when oogonial nucleus is prominent and nuclear membrane is irregular in outline (**Figure 2(B)**). Nuclear blebs are formed which establish a "cross talk" between cytoplasm and nucleus. The perinuclear region is full of physodes (**Figure 2(C)**). The conceptacle opening remains occluded with a polysaccharide-rich plug **Figure 2(D)**).

3.2. Gamete Release and Role of Mucilage

Sargassum vulgare releases its gametes profusely in packets on a full moon and new moon day during low tides. The oogonia are released while they are "wrapped" in mucilage (**Figure 2(E)**). The mesochiton, which acts as a protective covering at this stage shows a granular texture (**Figures 2(F), (G)**). A maximum of two oogonia may be released through the ostiole at a given time. This is because the released oogonia are held back to the conceptacle floor by a mesochiton stalk and the narrow ostiole which can dilate only a little cannot accommodate more than two stalks (**Figure 2(H)**).

On the contrary, the spermatozoids are released "en masse" through ostiole where the meristoderm loosens and the gametes "wrapped" in mucilage are released. The conceptacle cavity shows scanty mucilage (**Figures 3(A), (B)**). The antheridial contents take up a tadpole-like shape which facilitates their exit through the narrow ostiole (**Figure 3(C)**).

There is copious amount of mucilage in the conceptacle cavity which houses oogonia in contrast to male conceptacle (**Figure 3(B)**). The paraphyses which coexist with oogonia (**Figure 3(D)**) possess mucilage with staining properties similar to plug material (**Figures 3(E), (F)**) and are therefore act as a source of polysaccharides.

3.3. Propagule Settlement

Mesochiton stalk holds the zygote and initial divisions in germling formation occur while it is retained on receptacle (**Figures 3(G), (H)**). This "parental care" is extended to the germlings that are shed with a well defined rhi-
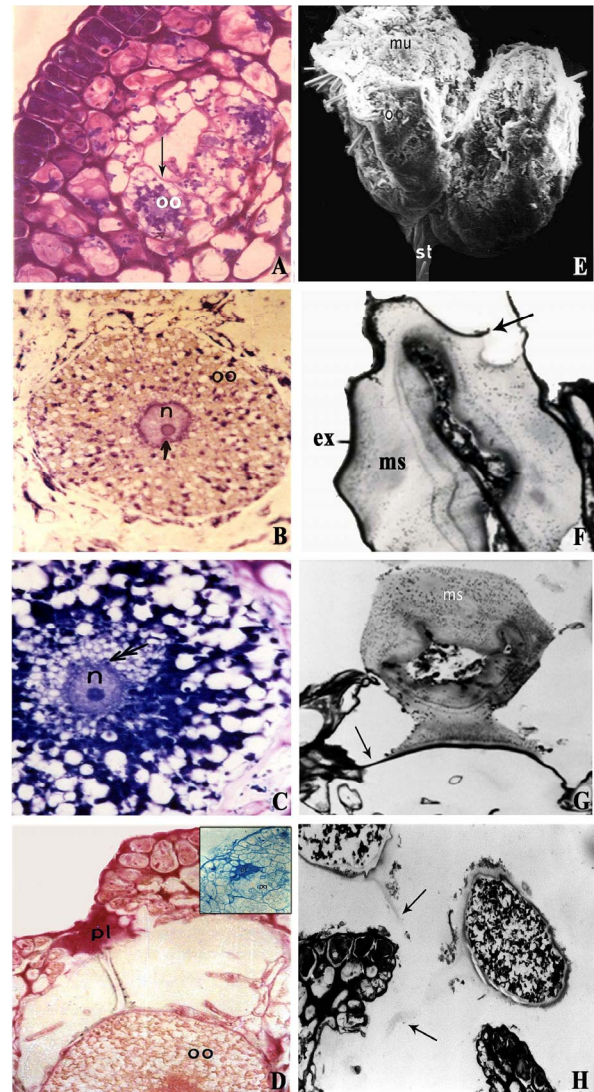

Figure 2. (A) Section through young female conceptacle showing oogonium (oo) with only one layered (exochiton) wall (arrow). TBO stained; (B, C) the nucleus (n) is prominent, with undulated (arrow) envelope and a prominent nucleolus. Nuclear blebs (double arrow) are seen in (C); (D) Plug (pl) material from female conceptacles stains well with PAS and Alcian blue (inset) indicating its complex nature; (E) Scanning electron micrograph showing released oogonia with a mesochition stalk (st) and mucilage (mu); (F, G) Oogonia at the time of release showing ruptured exochition (arrows), thick granular, mesochition (ms) and physode rich cytoplasm. TBO stained; (H) Two oogonia released from a conceptacle with lightly stained mesochiton stalks (arrows). TBO stained.

zoidal end (**Figure 3(G)**).

4. Discussion

Sargassum is one of the most widely investigated alga with various events in reproductive phase having been elucidated [24,25]. Great emphasis has been laid down
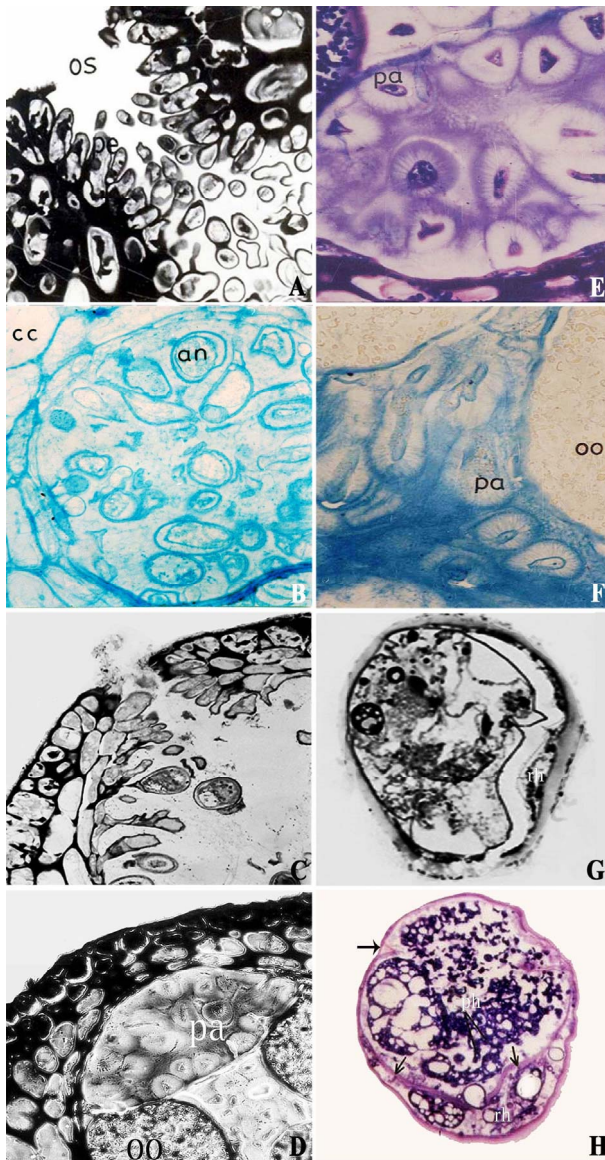
Figure 3. (A)-(C) Sections through male conceptacles where ostiole (os) "gapes" apart in (A); conceptacle has reduced polysaccharides in the cavity (Alcian Blue stained) in (B) and spermatozoids "wrapped" in a mucilage showing their release in (C). (A) and (C) are TBO stained; (D)-(F) Paraphyses (pa) from oogonial conceptacles; (D) phase contrast micrograph; (E) stained with Alcian blue; (F) TBO stained; (G, H) Incubated germlings with a smaller vacuolated rhizoidal (rh) and a larger physode (ph) rich cell. The arrows show walls laid down in the germlings.

on gamete release and post fertilization recruitment stages [2,26,27]. Reproductive events are governed by a number of environmental cues which evolve as a mechanism for populations to increase the probability of fertilization by releasing gametes at the same time. Gamete release at low tide period is stated to be a consequence of mechanism selected to permit successful fertilization. Sexual reproduction is enhanced in habitats or at the

times where water motion is low and water rela- tively calm [26].

According to Pearson and Brawley [14] ability of *Fucus distichous* to synchronise gamete release during periods of low water motion is an extremely valuable adaptation for an organism inhabiting intertidal zone. Low tides provide ideal conditions for making large number of gametes available which increase chances of gamete collision. According to Suto [28], the period of antherozoid motility is short and the adaptations are therefore towards quick encounter with eggs. Another feature which ensures a high success rate of fertilization and establishment is the mucilage around the female gametes and propagules [29].

The present study highlights for the first time, that architecture of male and female conceptacles is fine-tuned for sexual act. The male conceptacle in *Sargassum heterophyllum* is reported to possess an ostiolar plug [29] which plays an important role in determining the time of gamete release. In the present study, male conceptacle in *S*. *vulgare* lacks ostiolar plug and remains closed by tightly fitted ostiolar (meristoderm) cells. On the other hand, female conceptacle has a plug. The present study indicates that these plug helps in isolating the developing structures from outer eco-physical pressures and also provides gametangia insulated environment. This regulates oogonial release by not allowing female to exit till spermatozoids are made available. Removal of such a plug from female conceptacle might take a little longer because it is not only copious in amount but also a mixture of polysaccharides. Thus it needs to be dissolved in order to make way for oogonia being released. Since plug is absent from antheridial conceptacle, the release of spermatozoids is easier and it may have a bearing on temporal difference in gamete release. In such a situation, it is quite likely that gamete release in this androgynous species is protandrous.

One feature reported for the first time is the difference in number of paraphyses in male and female conceptacles. Paraphyses are known to be associated with mucilage secretion that fills the cavity, bathes oogonia and occludes ostiole. The role of paraphysial secretions in early oogonial development is to check desiccation. At maturity, the secretions help in making ostiole slippery hence facilitating gamete release [8]. Whether the ostiolar closure in *Sargassum* conceptacles has any evolutionary bearing on the callose plugs during micro and mega sporogenesis of higher plants, needs further investigation.

5. Acknowledgements

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