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Reproductive Biology of *Sargassum thunbergii* (Fucales, Phaeophyceae)

Zhourui Liang, Feijiu Wang*, Xiutao Sun, Wenjun Wang, Fuli Liu

Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China
Email: *wangfj@ysfri.ac.cn

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Abstract

***Sargassum thunbergii* is an economically important brown alga that is used as a perferred food for sea cucumber in China. However, reports on the reproductive biology of *S. thunbergii* are limited. This study observed the characteristics of mature receptacles. The effects of different temperatures, light intensities, and photoperiods on the egg release of mature *S. thunbergii* receptacles were investigated. A liquid-phase oxygen electrode system was used to obtain light saturation and light compensation points of egg and young thalli of *S. thunbergii*. Results showed that temperature was the key factor for the egg release of mature receptacles. The conditions most conducive to egg release were 20˚C to 23˚C temperature and 50 μmol photons $m^{-2}·s^{-1}$ to 200 μmol photons $m^{-2}·s^{-1}$ light intensity. In addition, the light saturation and compensation points of unfertilized eggs or young thalli at 2 d or 3 d postfertilization ranged from 90 μmol photons $m^{-2}·s^{-1}$ to 120 μmol photons $m^{-2}·s^{-1}$ and 14 μmol photons $m^{-2}·s^{-1}$ to 22 μmol photons $m^{-2}·s^{-1}$, respectively. The combined results provide a reference for the sporeling culture of *S. thunbergii*.**

Keywords

***Sargassum thunbergii*, Reproductive Biology, Temperature, Light**

1. Introduction

Sargassum thunbergii (Mertens ex Roth) Kuntze of the class Phaeophyceae, order Fucales, and family Sargassaceae is a commercially important seaweed with excellent properties. *S. thunbergii* is the best natural food for the sea cucumber *Apostichopus japonicus* (Selenka) in China because of its nutritional value [1] [2]. The de-

*Corresponding author.

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mand and economic values of *S. thunbergii* have rapidly increased in recent years because of the increasing development in sea cucumber culture. *Sargassum* resource is heavily destroyed and scarce in Northern China because of the massive harvest of natural populations.

S. thunbergii production has two pathways, namely, sexual reproduction and vegetative reproduction via rhizoid. Sexual reproduction is the primary approach to resolve the sporeling resource shortage of *S. thunbergii*. Therefore, studies on the reproductive biology of *S. thunbergii* are indispensable to rehabilitate the natural resource and promote the sporeling production of this seaweed in China [3]. Previous studies on *S. thunbergii* mainly focused on ecology, active ingredient, taxonomy, resource investigation, nutritional analysis, and population genetic structure [4]-[11]. Only a few experiments were carried out on the early development of germling [12] [13]. Moreover, little is known about the reproductive biology of *S. thunbergii* at the very early sexual developmental stages. Specific issues that need investigations include the characteristics of receptacles and conceptacle ostiole, the influencing factors of egg release, and the light saturation and compensation points of fertilized eggs of *S. thunbergii*.

The present study observed the detailed reproductive characteristics of mature receptacles of *S. thunbergii* and investigated the effects of environmental factors on the egg release of mature receptacles. This study aims to provide theoretical support for the sporeling culture of *S. thunbergii* (**Figure 1**).

2. Materials and Methods

2.1. Field Sampling and Microscopy

S. thunbergii samples were collected from an intertidal zone in Taiping Bay (36˚05'N, 120˚35'E), Qingdao, China on 10 July 2011. Real-time observation to the production and development of *S. thunbergii* was performed all year round. Parental male and female plants were packed in plastic bags and transported in ice to the laboratory to evaluate receptacle maturity under a microscope. Mature receptacles were characterized by the presence of eggs on the receptacle surface. The size and characteristics of male and female receptacles, the sperm release, and the egg release were observed and photographed. The number of conceptacles was counted, and the size of egg and sperm was measured. Tissue slices of male and female receptacles were microphotographed.

The culture room temperature was controlled at approximately 20˚C. The culture solution was composed of filtered natural seawater enriched with NO_3^--N and PO_4^{3-}-P at 3 and 0.3 mg·L^{-1}, respectively (the same as the solution below), and was changed at an interval of 2 d. The experiments were carried out in culture chambers under the following constant conditions: photoperiod of 12:12 h and light intensity of 50 μmol photons m^{-2}·s^{-1}.

2.2. Influencing Factors of Egg Release

Well-developed mature male and female receptacles were placed into 250 mL beakers with 200 mL of culture solution to observe the egg release. The total number of female receptacles was 60, and the ratio of male and female receptacles was 1:6. The number of days required for the receptacles to release eggs and the percentage of receptacles releasing eggs at the beginning under different environmental factors were investigated. All measurements were carried out on three parallel tests in the following several experiments.

Figure 1. The whole plant picture of *S. thunbergii*.

The temperature test was performed by incubating the receptacles at 13, 16, 20, and 23˚C in a temperature-controlled photoincubator (Jiangnan, Co. Ltd., China) at an irradiance of 50 μmol photons $m^{-2}·s^{-1}$ (12:12 h light:dark cycle). For the light intensity test, the receptacles were incubated at 200, 100, and 50 μmol photons $m^{-2}·s^{-1}$ in a photoincubator at 16˚C (12:12 h light:dark cycle). Photoperiod test was carried out by incubating the receptacles at 15:9 h (15L:9D), 12:12 h (12L:12D), and 9:15 h (9L:15D) light:dark cycles in photoincubators at an irradiance of 50 μmol photons $m^{-2}·s^{-1}$ and a temperature of 20˚C.

2.3. Light Saturation and Light Compensation Points of Egg and Young Thalli

Light saturation and light compensation points of egg and young thalli were measured using a Clark-type oxygen electrode (Hansatech Oxygraph, England) [14]. The light was provided by a halogen lamp. An illuminometer (HANNA, Italy) was used to measure the light intensity, and the temperature was maintained constantly by a cooling circulator (F12-ED Refrigerated/Heating Circulator, Germany). Zero oxygen was used in the oxygen electrode chamber with $Na_2S_2O_4$.

Unfertilized eggs and young thalli were collected and seeded into the beakers. A 2 mL aliquot of the cell solution containing eggs or young thalli was transferred to the oxygen electrode chamber, which was magnetically stirred at a speed of 100 $r·min^{-1}$. Approximately 200 μL of algal solution was removed from the oxygen electrode chamber at a rotor-stirred condition for microscopic counting and length measurements. The number of eggs or young thalli was controlled at approximately 1000 individuals in the chamber.

Photosynthetic rates were measured at 21˚C. The light transmittance of the chamber wall was measured to calibrate the light intensity. The samples were conditioned at a certain light intensity for approximately 60 min to achieve stability before measurement. Each measurement was accomplished within 10 min, and the linear oxygen evolution versus time was recorded. The light saturation and light compensation points of the eggs were measured. The light saturation and compensation points of the young thalli were measured at 2 and 3 d postfertilization. Three measurements were performed each time, and the readings were averaged.

2.4. Statistics

The data were expressed as means ± SD. T-test was used to compare the difference between the control and the treatments at each time point. One-way ANOVA was used to compare the difference between treatments, and multiple comparisons were performed with Student-Newman-Keuls (SNK) method. The significance level was set at 0.05.

3. Results

3.1. Morphologic Observation

The mature female and male receptacles of *S. thunbergii* were dumpy and acerose, respectively (**Figure 2**). The male and female receptacles had indistinguishable shape in some cases; thus, an immature thallus is impossible to be identified. Conceptacles were distributed in each receptacle. **Figure 3** and **Figure 4** show the tissue slice and microphotograph of male and female receptacles of *S. thunbergii*, respectively. The female conceptacle was characterized by its large, bulb-shaped oogonia with egg or egg progenitor cells inside (**Figure 4**). Matured egg and sperm discharged outside the frond through the conceptacle ostiole. Eggs adhered to the outer side of the conceptacle ostiole awaiting fertilization, and zygotes fell off the receptacle and developed into young sporophytes. **Figure 5** shows a mature female receptacle with unfallen eggs. Different sizes of conceptacle ostiole appeared in mature female conceptacle. The top of an ostiole was smaller than other parts with a size between 130 and 155 μm. The size of a mature male conceptacle ostiole also varied (approximately 84 μm to 123 μm), with small size at the top and large size at the mid-bottom. Although the female and male conceptacles were dumpy and acerose, respectively, an obvious difference existed among the same sex (**Table 1**).

3.2. Influencing Factors of Egg Release (Table 2)

3.2.1. Temperature

Temperature treatments of 16˚C and 13˚C were significantly different compared with those of 20˚C and 23˚C ($P < 0.05$). The temperature range conducive to egg release was 20˚C to 23˚C. One-way ANOVA showed that the

Figure 2. Morphological comparisons on the twigs with different sections of trees of male and female *S. thunbergii*. (1, 2: Receptacles; 3: Pneumathode; 4: Leaf).

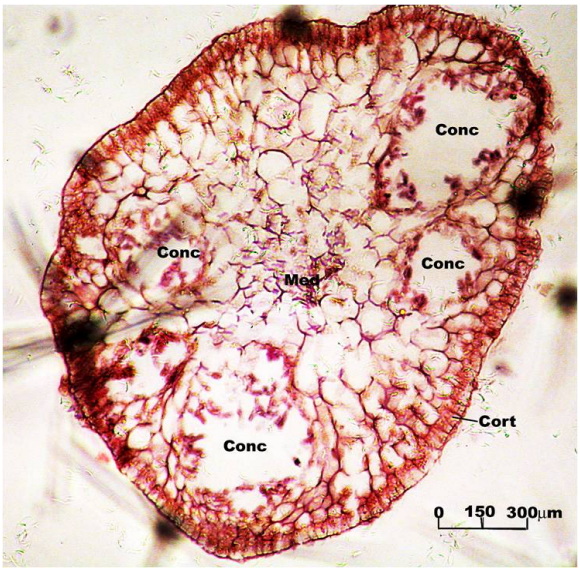

Figure 3. Tissue slice and microphotograph of male receptacle of *S. thunbergii* at 100 times magnification. (Conc: Conceptacle; Cort: Cortex; Med: Medulla).

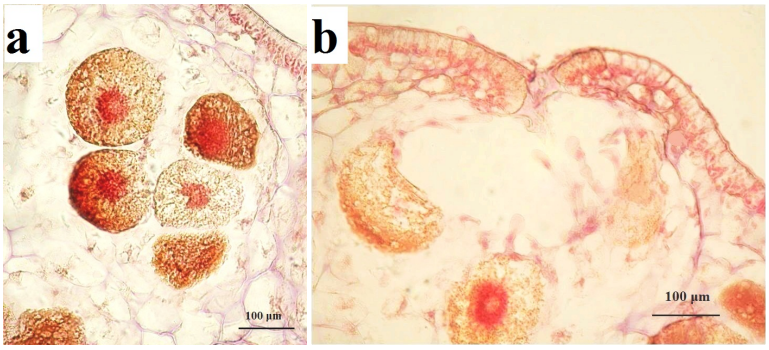

Figure 4. Tissue slice and microphotograph of female receptacle of *S. thunbergii* at 400 times magnification (a, b).

Figure 5. Female receptacle of *S. thunbergii* with eggs.

Table 1. Characteristics of the receptacle, conceptacle ostiole, egg, sperm, and rhizoid of *S. thunbergii* (n = 50).

Characteristic	Female receptacle	Male receptacle	Female conceptacle ostiole	Male conceptacle ostiole	Egg	Sperm	Rhizoid
Shape	Cylindrical, dumpy	Cylindrical, acerose	Spherical, ellipse	Spherical, ellipse	Spherical	Pear-shaped, with two lateral flagella	Thin strip-shaped
Size/number	Mean length: 9.9 mm (5.6 mm to 14.0 mm) Diameter: 0.97 mm (0.9 mm to 1.2 mm)	Mean length: 15.3 mm (11.0 mm to 22.5 mm) Diameter: 0.88 mm (0.7 mm to 1.0 mm)	Mean diameter: 130 μm to 155 μm (105 μm to 210 μm)	Mean diameter: 84 μm to 123 μm (67 μm to 200 μm)	Mean diameter: 155 μm (127 μm to 178 μm)	Mean length: 18.4 μm Mean width: 14.1 μm	Mostly with number 5 to 8
Color	Brown	Brown	Yellowish brown	Yellowish brown	Greenish brown	Transparent, shiny. Blue-green	Transparent, colorless. A spot of pigment focused at the bottom
Remark	Mature receptacle (containing eggs), excluding the length of receptacle stipe	Mature receptacle, excluding the length of receptacle stipe					Rhizoid formation (early roots)

number of days required to release eggs and the percentage of receptacles releasing eggs at the beginning were both significantly ($P < 0.05$) affected by temperature.

3.2.2. Light Intensity

One-way ANOVA showed that the percentage of receptacles releasing eggs at the beginning was significantly ($P < 0.05$) affected by light intensity. The highest percentage of receptacles releasing eggs at the beginning was observed at the light intensity of 200 μmol photons $m^{-2}{\cdot}s^{-1}$. A significant difference ($P < 0.05$) in the percentage of receptacles releasing eggs at the beginning was observed between light intensities of 200 and 50 μmol photons $m^{-2}{\cdot}s^{-1}$ treatment.

3.2.3. Photoperiod

One-way ANOVA showed that percentage of receptacles releasing eggs at the beginning was significantly ($P < 0.05$) affected by photoperiod. The lowest percentage of receptacles releasing eggs at the beginning was observed at the photoperiod of 9L:15D. This result was significantly different ($P < 0.05$) from that of 15L:9D treatment. However, the same number of days (6 d) was required for female receptacles to release eggs at dif-

Table 2. Number of days required for receptacles to release eggs and percentage of receptacles releasing eggs at the beginning under different environmental factors (n = 3).

Environmental factors		Number of days required for receptacles to release eggs d^{-1}	Percentage of receptacles releasing eggs at the beginning
Temperature	13˚C	26	13%
	16˚C	17	22%
	20˚C	6	47%
	23˚C	5	70%
Light intensity	200 μmol photons $m^{-2}\cdot s^{-1}$	14	85%
	100 μmol photons $m^{-2}\cdot s^{-1}$	16	61%
	50 μmol photons $m^{-2}\cdot s^{-1}$	17	28%
Photoperiod	15L:9D	6	50%
	12L:12D	6	42%
	9L:15D	6	30%

ferent photoperiods in the experiment. This finding suggests that photoperiod was an important but not a key factor that affects the speed of releasing eggs.

3.3. Light Saturation and Light Compensation Points of Egg and Young Thalli

Table 3 shows that light intensity gradually increased with culture time during the 3 d experiment. The light saturation and light compensation points of unfertilized eggs or young thalli at 2 d or 3 d postfertilization ranged from 90 μmol photons $m^{-2}\cdot s^{-1}$ to 120 μmol photons $m^{-2}\cdot s^{-1}$ and 14 μmol photons $m^{-2}\cdot s^{-1}$ to 22 μmol photons $m^{-2}\cdot s^{-1}$, respectively.

4. Discussion

The maturation of *S. thunbergii* mostly correlated with water temperature. *S. thunbergii* receptacle could develop and grow rapidly at a temperature range of 20˚C to 23˚C. In the Qingdao coast, the high reproductive season of *S. thunbergii* is from late June to July, and the receptacle decomposes when the water temperature exceeds 24˚C. A conceptacle containing egg is a unique characteristic for *S. thunbergii*. Zygotes usually detach from the receptacle within 12 h to 36 h, and some eggs can form embryonic sporeling on the receptacle in still water. For *S. thunbergii* growing in seaside, the eggs can completely detach from the receptacle within 24 h.

In this paper, high light intensity was conducive to egg release for *S. thunbergii*. Some researchers [15] got the similar results for *Sargassum fursiforme*. And they thought that the peak of egg release was at the temperature range of 22.5˚C to 25.5˚C, which was a bit different from that of the paper. For *S. fursiforme*, compared to the short light period, the longer light period had positive effects on the releasing of eggs [16]. The result was same with that of the paper. Light is an important factor for the growth of *S. thunbergii*, and controlling the light intensity at optimal level is necessary for the success of its artificial breeding. From the very early developmental stage, *S. thunbergii* sporeling showed a high light saturation point. This result suggests the well-developed photo-adaptability of the seaweed, which inhabits in a relatively high-light environment. For seed collection during the artificial breeding of *S. thunbergii*, mature *S. thunbergii* were sprinkled on the curtain so that the oosperm may fall off naturally and attach to the substrate. Oosperm photosynthesis may be light limited because of the overlapping of alga thalli. The parental *S. thunbergii* would be removed within 2 d to 3 d; thus, ensuring that most of the young thalli are at optimal level of light intensity in the period is necessary. Moreover, the light saturation and light compensation points of the unfertilized eggs or young thalli must be identified at 2 d to 3 d postfertilization to control the light intensity before the removal of the parental alga. Egg release usually occurred in the early morning. Egg release at a relatively low light intensity can be attributed to the low light compensation point of the *S. thunbergii* eggs.

Table 3. Light saturation and light compensation points of egg or young thalli (n = 3).

	Egg (Figure 6(a))	Young thalli (2 d) (Figure 6(b))	Young thalli (3 d) (Figure 6(c))
Length (μm)	145.5 ± 10.1	155.0 ± 12.3	170.7 ± 14.2
Light compensation point (μmol photons $m^{-2}\cdot s^{-1}$)	14 ± 2	18 ± 3	22 ± 4
Light saturation point (μmol photons $m^{-2}\cdot s^{-1}$)	90 ± 12	100 ± 15	120 ± 14

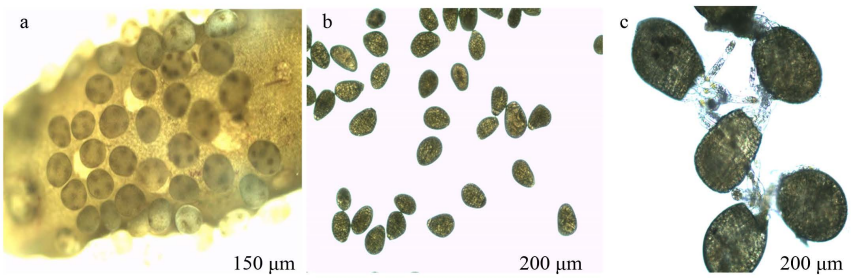

Figure 6. *S. thunbergii*, showing eggs (a), young thalli cultured 2 days (b), and 3 days (c).

In conclusion, temperature was the key factor affecting the egg release of mature receptacles. The conditions most conducive to egg release were 20˚C to 23˚C temperature and 50 μmol photons $m^{-2}\cdot s^{-1}$ to 200 μmol photons $m^{-2}\cdot s^{-1}$ light intensity.

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