Macrothink Institute™

Aquatic Science and Technology
ISSN 2168-9148
2016, Vol. 4, No. 2

Observation on Developmental Changes during the Sexual Reproductive Period of *Sargassum thunbergii*

Ningning Xu

School of Marine Life Sciences, Ocean University of China, Qingdao, Shandong, China

E-mail: 241807505@qq.com

Haili Tan (Corresponding author)

School of Marine Science and Technology, Harbin Institute of Technology at Weihai, Weihai, Shandong, China

E-mail: tanhaili@hitwh.edu.cn

Received: March 7, 2016 Accepted: May 13, 2016 Published: May 14, 2016

doi:10.5296/ast.v4i2.9470 URL: http://dx.doi.org/10.5296/ast.v4i2.9470

Abstract

The growth, development, and sexual reproduction of *Sargassum thunbergii*, which collected from Dongtou, Zhejiang Province were studied under controlled lab conditions, and its early developmental process were recorded. Results showed that: (1) The first appearance of receptacle on *S. thunbergii* in the present study was in the mid-May and became matured in about late of June, and the eggs and sperms were realeased from female and male receptacles respectively. The released eggs attached to the surface of female receptacles and were fertilized by sperms to form zygotes. A clearly correlationship between the length of receptacle and its maturing level was observed, which was helpful for the artificial breeding of *S. thunbergii*. Moreover, temperature and salinity had obviously effect on the growth of receptacles, and temperature seemed more essential as compared to the salinity. The optimal condition combination basing on the orthogonal analysis for the receptacle's growth was 22 ℃ and salinity 30. (2)The fertilized eggs began its first cell division at 1 hour after fertilization and the continuous 2 times of divisions would result in a four-cell structure. Cellular division occured every 2~3 h thereafter and finally formed the multi-cell germling. The germlings would stick to the substrates for adherence, and their lengths increased steadily with some side heaves observed within 23 days. They grew well with a coarse surface and darkened color. Results in the present study inferred that the combination of germling breeding and sexual reproduction for the artificial cultivation of *S. thunbergii* was feasible.

Keywords: Sexual reproduction, Development, Receptacle, *Sargassum thunbergii*

1. Introduction

Sargassum C. Agard (Phaeophyceae, Fucales, Sargassaceae) used to be widely spread in both tropical and warm temperature regions and formed the kelp beds in the intertidal zone which played such an essentially ecological role as primary producer, spawning, nursery and feeding ground etc. (Zhang and Sun, 2007). However, the global changes and the increase of multiple anthropogenic stressors have driven the degredation of the macroalgae bed forming by *Sargassum* species, and it is even suggested that the natural populations of *Sargassum* species along the northern coastal China have been depleted (Zhao et al., 2007, 2008). Setting up effective method that is helpful for the macroalgae restoration is of great concern.

In Sargassum genus, *Sargassum thunbergii* is a common but a useful species for its distinct feature of high ecological values and pharmaceutic values (Yuan, 2005; Wu et al., 2010). It is valuable bait for sea cucumber and now, it is especially important as a candidate for the restoration of intertidal seaweed beds (Chu et al., 2012a, b; Yu et al., 2012). However, the current degradation actuality could not meet the great demands for this species of macroalgae (Yuan, 2005) and therefore, artificial breeding would be benefit for the sustainability of the natural population. The reproductive and developmental processes of *S. thunbergii* have been well understood now. It's dioecious, and two reproductive strategies are involved in its life history: sexual one and asexual one, and the first one usually play the dominant role. Regarding to sexual reproduciton, *S.thunbergii* produces numerous receptacles along the branches of thalli (Zhang et al., 2009) from where the eggs and sperms are respectively derived during the reproductive period which is about late June to early July every year. The released eggs and sperms combine together to form fertilized eggs, which firstly stick to the surface of the thalli and then drop by gravity to the surface of the rock or the oyster shells and begin to grow. With sexual process moving on, the young germlings are observe to development from the zygot and new macroalgae forms (Zhao et al., 2008).

In the present study, the whole process of the early development of *S. thunbergii* was recorded from the receptacles, to eggs and sperms, and then the fertilized eggs till the end of the germling formation. Moreover, the effects of environmental changes, such as temperature and salinity, were estimated simultaneously. Results of the present study would bring light on the artificial breeding of *S.thunbergii* and provide useful information to develop strategies to restore intertidal seaweed beds.

2. Methods and Materials

2.1 Macroalgae Samples

The male and female *S. thunbergii* at reproductive phase with well-developed receptacles were collected from Dongtou Couty, Zhejiang Province from late May to early July, 2012. The ratio between male and female ones was 1:10. Samples were collected and immediately transported to the Artificial Breeding Ground in Lidao, Rongcheng, Shandong Province. These macroalgae were prepared for the artificial breeding under controlled conditions.

The chemical-fiber cloth curtain (2m×0.4m), the seedling curtain made by coil rope (1m×0.5m) and the scallop shell were used as the attaching substrates. The first two substrates were completely dipped into fresh water for some time to remove the potentially harmful substances in the certain, while the shells should be dipped into fresh water for a relatively long time and were put into $KMnO_4$ at a concentration of 200×10^{-6} g L^{-1} for half an hour thereafter. These substrates were rinsed with autoclaved seawater for several times to make them clean enough for the equirement of artificial breeding.

2.2 Observations on the Early Development of S. thunbergii

2.2.1 Observation on the Development of Receptacles

20 receptacles on the male and female macroalgae were picked up randomly. The time was 5, 10, 13, 16, 18, 20, 22, 24, 25, 26, 27 and 28 day (every five days at the beginning; with the increasing of receptacles maturith, we picked up randomly every 2 days, and at last once a day). The lengths were recorded. Method of hand-cut section was applied and the receptacles were cut into thin slices for microscopic observation, and the developmental level of receptacle and the maturing degree of oocytes were determined. Moreover, the diatom of the genital pore and the maturing rate were calculated. The maturing rate was calculated as the following:

$$\text{mauring rate (\%)} = \frac{\text{the number of matured receptacles}}{\text{the total number of the selected recptacles}} \times 100\%$$

2.2.2 Observations on the Development of Eggs

Male and female macroalgae were cultivated separately at room temperature at about 20-21 ℃ under natural light conditions of 60 μmol photon m^{-2} s^{-1} under a 12h: 12h light-dark cycle in illuminating incubator. The initial salinity was about 32. The sizes of the new-releasing eggs were estimated under microscope, and time of release was recorded. The released eggs were collected and the whole process of their early development, including the formation of lateral branch, was observed.

2.3 Effeccts of Salinity and Temperature on Development of Germling

Basing on the sample prepation and method described in 1.2.2, the effects of salinity and temperature changes were analyzed. A 2-factor, 3-level orthogonal experiment was set up. The temperature was set as18℃, 20℃, 22℃ and salinity was 28, 30, 32, respectively. The length and width of the germling were calculated. The favarable combination of temperature and salinity for the early development was determined. Software package of spss17.0 was applied and the realtionship between the relative growth rate of germlings and the environmental changes (temperature×salinity) was analyzed. The decisive factor and the optimal combination of the factors were also estimated.

Table 1. Experimental design of the orthogonal test

group	salinity	Temperature (℃)	RGR（%）
1	1(8)	1（18）	1.52
2	1(28)	2（20）	1.57
3	1（28）	3（22）	1.6
4	2（30）	1（18）	1.6
5	2（30）	2（20）	1.65
6	2（30）	3（22）	1.79
7	3（32）	1（18）	1.57
8	3（32）	2（20）	1.68
9	3（32）	3（22）	1.81

The relative growth rate (RGR, % per day) of germling was calculated as the following formula(Lignel and Pedersén,1989):

$$\text{RGR}=\frac{\ln R_f - \ln R_i}{\Delta t}\times 100\%$$

R_i denoted the initial size of the germling and R_f denoted the final size of it, and the size of the germling was determined as $\text{lenth}_{max} \times \text{width}_{max}$

2.4 Statistics

All data were analyzed using SPSS for Windows 17.0. Prior to all statistical analyses, the homogeneity of variances was verified with Levene's test. Tukey's tests were used for the post hoc comparisons. The differences were considered to be statistically significant if the probability value was less than 5% ($p < 0.05$).

3. Results

3.1 Development of Receptacles on Male and Female Macroalgae

The first appearance of receptacle on *S. thunbergii* was in the mid-May and became mature in about late of June. The whole frond was covered with receptacles when it completely matured and the airbags and leaves were comparatively decreased. The colour of the male macroalga was relatively light, and the receptacles on it were bright yellow at a length of 1.5-2.5cm. Differently, the colour of female one was dark and the receptacles were about 0.9-1.5cm long (Fig.1).

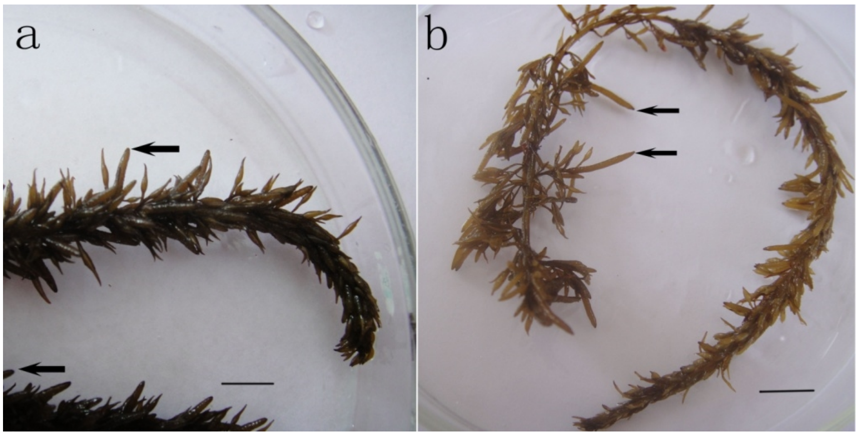

Figure 1. The pictures of *S. thunbergii*

a. female macroalga; b. male macroalga. The arrow in the figure indicates the receptacle (1bar=1cm)

The development of eggs in the conceptacle was recorded (Fig. 2a). It was found that the conceptacle opened steadily with the maturing degree of receptacle and became visible when it completely opened (Fig.2d). The diameters of the conceptacles in male and female receptacles were about 150μm and 180μm respectively, and the maturing degree of the receptacle usually decreased steadily from those at bottom of macroalgae to those on the top. Therefore, the release of the eggs and sperms were firstly observed at the bottom of the receptacles. The release of the oocytes and sperms were usually consistent, while the peak of egg-release was 4-5 days earlier than that of eggs.

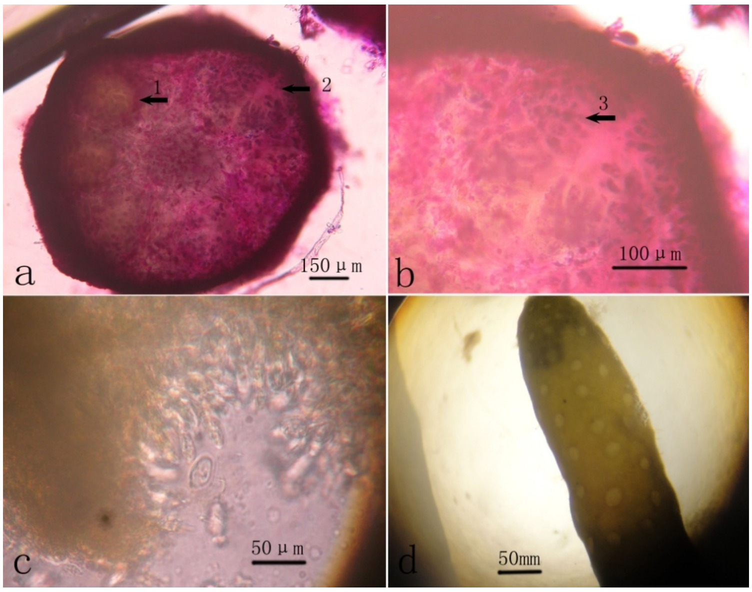

Figure 2. The historical observation on the reproductive organ of *S. thunbergii*

a Transection of female receptacles, ×200 (arrow 1：un-matured oocyte; arrow 2:matured oocyte); b. Transection of female receptacles, ×1000 arrow 3:matured oocyte); c. the opened conceptacle; d. the overall appearance of a matured receptacle

The released eggs would stick to the surface of the receptacle which was about 130μm length (Fig.3a). Egg with good quality was found to have evenly distributed cytoplasm and have good light-admitting feature (Fig. 3b).

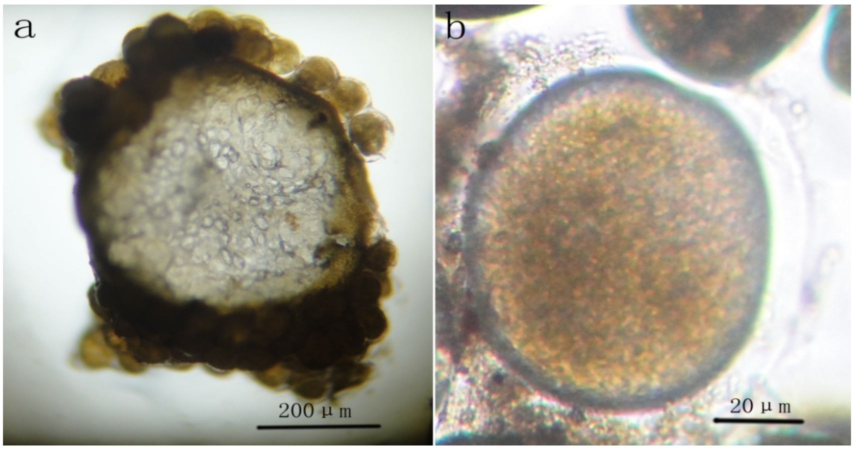

Figure 3. Eggs sticking on the female receptacle of *S. thunbergii*

a. Receptacle with eggs sticking on it; b. good egg

The relationship between the maturing degree and the length of receptacles were estimated. The receptacle grew fast at the first 24d after collection, but the increase of maturing rate was relatively slower. However, the increase of receptacle growth slowed down while the maturing rate increased quickly (Fig.4). Result of SPSS analysis showed an abnormal distribution between them, and the Spearman correlation coefficient was 0.938, inferring that the length of receptacle and its maturing rate has higher correlation. Results also suggest that the matured receptacles could be selected directly by the mesurement of the receptacle's length instead of using the method of hand-cut section. This method would bring light on reduction the costs that spend on transferation of male and female macroalgae in situ to the artificical breeding factory.

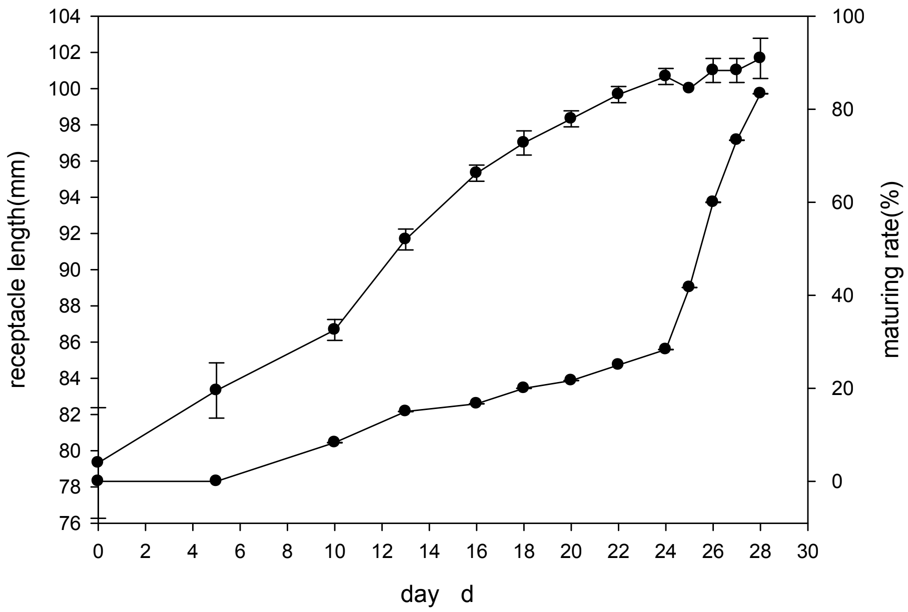

Figure 4. Relation between the length of the receptacle's length and its maturing rate

■: the length of receptacle; ▼: the mauring rate of receptacle

3.2 The Development of the Fertilized Eggs of S. thunbergii

The matured eggs (Fig.5a) would be fertilized with the sperms, forming the fertilized eggs and began to divide thereafter. The cell wall forming during the first dividing process was parallel to the long axis of the fertilized egg (Fig.5b), and double-cell structure was observed after the first division. The cell at the bottom developed into the rhizoid later, and the other one would prepare for the second division. The second division plane was parallel to the first one, and three-cell structure formed thereafter. The middle cell in the three-cell structure initiated the third division, which was observed to perform from the direction parallel to the longitudinal axis, and the four-cell structure was formed. Cellular division occurred every 2 to 3h thereafter and finally, the multi-cell germling was observed (Fig.5-c, d, e).

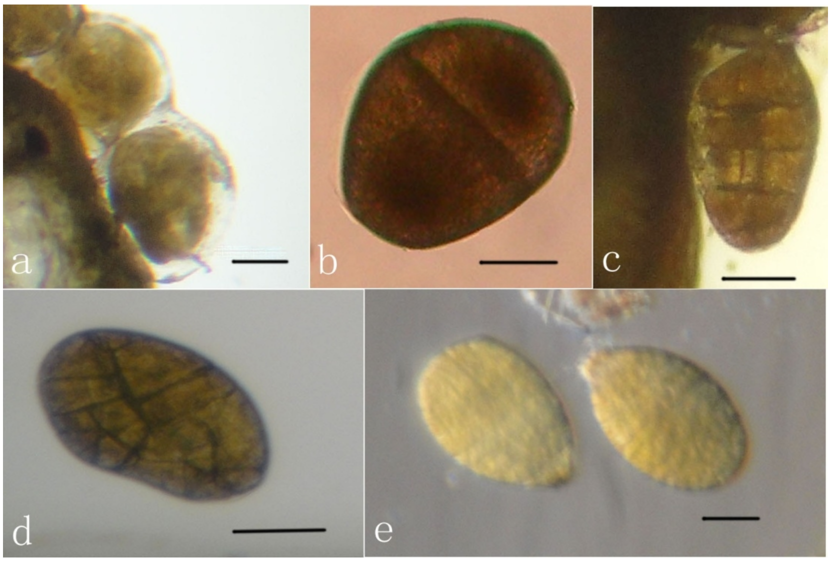

Figure 5. Division and development of the fertilized egg in *S. thunbergii* (1bar =50μm)

a. fertilized eggs; b. two-cell structure after the first division; c, d, e: multi-cell structure

Some words should be emphasized on the formation of rhizoid. At about 25h after the fertilization of the egg, the cell at the bottom began to form 4-8 heaves (Fig.6a). The rhizoid grew very fast and rhizoid at the back of the germling could be observed at 32h after fertilization (Fig. 6b). The rhizoid gradually adhered to the basement at 48h after fertilization, and at 52h, the length of rhizoid was almost equal to that of the thalli and the beard-like rhizoid began to scatter (Fig.6c). With the development of rhizoid, its length exceeded that of the thalli and the numbers increased simutaneously (Fig. 6d). Observation on this process indicated that this period of time was benefit for the growth and increase of rhizoid but had little effect of the growth of thalli, and this feature was helpful for the adherence of germling.

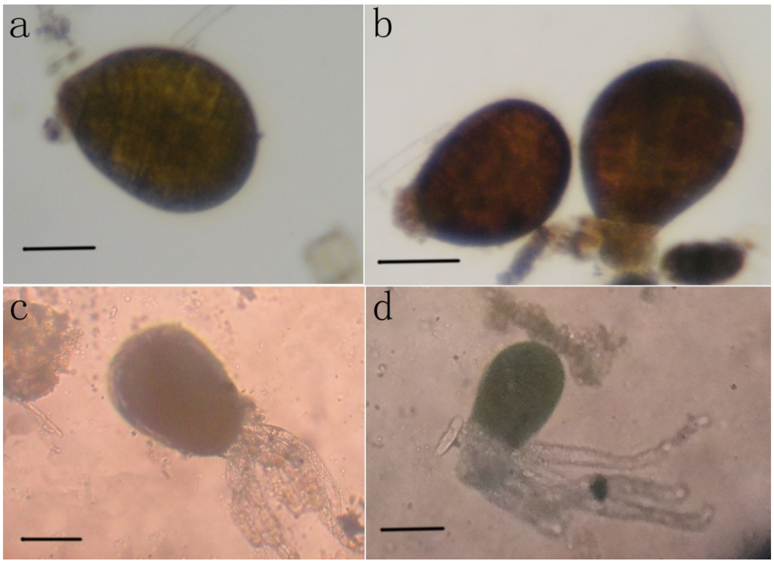

Figure 6. Early development of rhizoid of *S. thunbergii*

a- d: germling with rhizoid (1bar=100μm)

After the germling formation, it would stick to the substrates for adherence. The length increased to above 1000μm at 15d after adherence, which makes it strong enough to resisting the flushing of the tide (Fig.7a). On 23d, the length of the germling grew to above 2000μm and some side heaves were observed (Fig.7b).

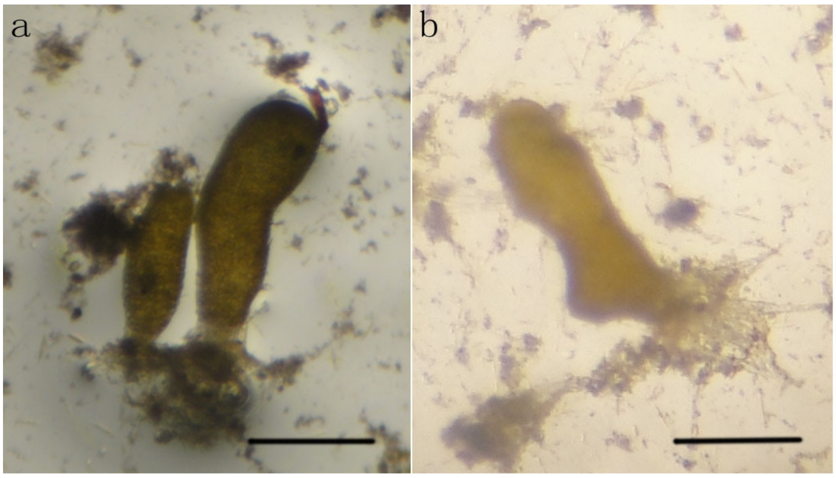

Figure 7. Early development of sporophyte of *S. thunbergii* (1bar=100mm)

a. germling; b. germling with side heave

Effects of temperature and salinity on growth of germlings were estimated and the results showed in Tab.2. The environmental changes exerted relatively obvious effects on the grwoth and great significance was observed between the treated groups and the control, and temperature had more obvious effect than that of the salinity ($R_T > R_S$). The optimal combiniation of growth condition was when the temperature was 22℃ and the salinity was 30 according to the changes of RGR.

Table 2. Results of the orthogonal experiment

	RGR	
T value	Temperature (T)	Salinity (s)
T1	4.69	4.69
T2	4.9	5.04
T3	5.2	5.02
t1	1.56	1.56
t2	1.63	1.68
t3	1.73	1.67
Range (R)	0.17	0.11
Optimal level	t3	s2
Effective level	T>s	

4. Discussion

4.1 Why We Chose S. thunbergii from Dongtou, Zhejiang Province?

In the present study, the matured receptacle on the macroalgae were about 9-15mm, which were different from the previous study of Zheng (1993) in East China Sea which was about

7.4mm and Zhan et al. (2006) in North Yellow Sea which was about 4.5mm. These reports indicated that the receptacles collected from different sites differ obviously. On the other hand, the time of the sexual reproduction of *S. thunbergii* at different places changed a lot too. The reproduction period in coastal Liaoning Province is from August to October, from April to June in Hainan Province and is from June to August in Shandong Province. *S. thunbergii* used in the present study were obtained from Zhejiang Province and its reproductive period is from late May to early June when the temperature of the seawater is 18-20℃. It suggests that the peak of the reproduction of *S. thunbergii* closely relate to its location, and the maturing time would postphone from North to South in China. Ma (2013) found the effect of temperature on the special growth rate of *S. thunbergii* germlings was hihly significant ($P<0.05$). Wu (2015) used the Box-Behnken experimental design and response surface method to find the optimal condition for the growth of *S. thunbergii.* Our results of orthogonal analysis in this study also showed that the early development of germlings were affected greatly by the salinity and temperature, and temperature played a more essential role as compared to that of salinity.

When combined considered the previous reports and results of present study we concluded that: firstly, the artificial cultivation of *S. thunbergii* in Dongtou, Zhejiang Province has reached a considerable scale, and sources of the parent macroalgae are relatively stable. Moreover, the growth of the cultured population is synchronous and the absolut reproductive fecundity is relatively higher. Sencondly, applying the population of artificial breeding would avoid the possible damage on the nature population. Besides, it would provide an effectively compensatory strategy for restoration or harvest of germlings when the parent macroalage are not sufficient or good enough. Thirdly, applying the method of splashing germlings for restoration with high demands for the hydrological conditions, such as no continuous raining, no wind are permitted because the flush of fresh water would significantly decrease the survival rate of the germlings and the wave aroused from the big wind would disturb the muddy seawater, which would result in the increase of sedimentation and bury the germlings. Shandong Province is entering the rainy seaon in mid-June, which is harmful to the seeding of the germlings. Therefore, the collection and transferation of the germlings from Zhenjing Province and seeding in coastal Shandong Province would avoid the disadvantage of hydrological conditions and benefit for the growth of germlings.

4.2 What Had Happened during the Period of Early Development of S. thunbergii?

It was the first time that the relationship between the length of receptacle and its maturity was estimated. We found that the growth of receptacles and their maturity was not consistent, but an obvious relationship between them was observed. Therefore, the maturing level could be judged according to the length of the receptacle. This result could be used in the artificial breeding of *S. thunbergii*. For example, the parent macroalgae could be cultivated under natural conditions for some time and then be transferred into the breeding factory when the receptacles develop to certain lengths. The maturing levels of the receptacles are judged according to their length, which would avoid the complex and professional process of the method of hand-cut section. The working efficiency could be increasing by the application of this convenient method. The observation on the early development in the present study was a little bit late than the previous reports (Pan et al., 2007). For instance, we found the bottom heaves of rhizoid cells only at 25h after fertilization while that was about 24h in the report of Xu (2009). Besides, the similar result was observed on the appearance of the side heaves. The possible explanation might be that the parent macroalgae we used were from South of China, whose developmental time would be earlier than those from North of China. Results further proved that certain differences of receptacle and the germling development between the north China and the south China.

As to the third division of the fertilized egg, two different forms were suggested to be involved in this process: first was that cells on the top divided along with the direction parallel to the longitudinal axis. It meant that four-cell structure would be formed by the longitudinal division. Another division was said to begin from the middle cells, and their longitudinal division would result in the four-cell structure too (Xu, 2009). We only observed the second divisional form in the present study. Moreover, we found that the rhizoid was derived from one cell and this result consisted with the previous reports of Xu (2009) and Wang et al. (2006). We discovered in the present study that the germlings developed into rhizoid period, the rhizoids grew very fast and the germling could be firmly fixed onto the substrates and benefited for the further growth. It was suggested to be an useful strategy for the complex conditions in inter-tidal zone. Temperature played a more essential role in the early development of *S. thunbergii* compared to salinity and temperature control in the artificial breeding would be helpful for the increase of the suvival rates of the germlings.

Acknowledgements

This work was financially supported by Natural Scientific Research Innovation Foundation in Harbin Institute of Technology(no. HIT.NSRIF.2016086) and scientific research fund of Harbin Institute of Technology at Weihai (project number HIT(WH)201202).

Reference

Bao, J., Tian, X., Dong, S., et al. 2008. Effect of temperature, salinity and light intensity on nitrogen and phosphorus uptake by *Sargassum thunbergii. Journal of Fishery Sciences of China, 15*(2), 293-300.

Chu, H. C., Zhang, Q. S., Tang, Y. Z., Zhang, S. B., Lu, Z. C., & Yu, Y. Q. 2012. High tolerance to fluctuating salinity allows Sargassum thunbergii germlings to survive and grow in artificial habitat of full immersion in intertidal zone. *Joural of Experimental Marine Biology and Ecology, 412*, 66-71. http://dx.doi.org/10.1016/j.jembe.2011.10.025

Chu, S. H., Zhang, Q. S., Liu, S. K., Tang, Y. Z., Zhang, S. B., Lu, Z. C., & Yu, Y. Q. 2012. Tolerance of Sargassum thunbergii germlings to thermal, osmotic anddesiccation stress. *Aquatic Botany, 96*, 1-6. http://dx.doi.org/10.1016/j.aquabot.2011.09.002

Ma, X., Liang, Z., Liu, F., et al. 2013. Cultural conditions for *Sargassum thunbergii* germling. *Progerss in fishery sciences, 34*(6), 133-137.

Pan, J., Zhang, Q., & Xu, B. 2007. Mrophological observation of sexual reproduction and juvenile sporophyte development in sea weed *Sargassum thunbergii*. *Fisheries Science, 26*(11), 589-592.

Sun, X., Wang, F., Wang, W., et al. 2010. Large scale breeding of Sargassumthunbergii based on sexual reproduction. *Progress in fishery sciences, 31*(3), 84-91.

Wang W. 2003. Investigation and study on Sargassum and Hizikia in Zhejiang Province. *Journal of Shanghai Fisheries University, 12*(3), 227-232.

Wang Z., & Liu J. 2007. The sexual reproduction of *Sargassum thunbergii* and its application in seedling breeding. *Oceanologia et Limnologia Sinica., 38*(5), 453-457.

Wang, F., Sun X., & Li F. 2006. Studies on sexual reproduction and seedling-rearing of

Sargassum thunbergii. *Marine fisheries research, 27*(5), 1-6.

Wu, H., Ding, G., Zhang, Sh., et al. 2015. Optimization of the growth condition of *Sargassum thunbergii* seedlings with response surface method. *Periodical of ocean unitersity of China, 45*(7), 34-38.

Wu, H., Liu, H., Zhan, D., & Li, M. 2010. Research present situation of *Sargassum thunbergii*. *Territory and natural resources study, 1*, 95-96.

Xu, B. 2009. *Studies on the reproductive ecology of Sargassum thunbergii*. The Ocean University of China.

Yuan, C. 2005. Current Status and Development of Feed in Sea Cucumber. *Fisheries science, 24*(12), 54-56.

Zeng, C., & Lu, B. 2000. *Chinese seaweed flora* (pp. 56-58). The Science Publishing Company.

Zhang, Q. S., Li, W., Liu, S., & Pan, J. H. 2009. Size—de—pendence of reproductive allocation of *Sargassum thunbergii* (Sargassaceae, Phaeophyta) in Bohai Bay, China. *Aquatic Botany, 91*, 194–198. http://dx.doi.org/10.1016/j.aquabot.2009.06.003

Zhang, S. Y., & Sun, H. C. 2007. Research progress on seaweed bed ecosystem and its engineering. *Chinese Journal of Applied Ecology, 18*(7), 1647–1653.

Zhao, F. J., Wang, X. L., Liu, J. D., & Duan, D. L. 2007. Population genetic structure of Sargassum thunbergii (Fucales, Phaeophyta) detected by RAPD and ISSR markers. *J. Appl. Phycol., 19*, 409–416. http://dx.doi.org/10.1007/s10811-006-9147-2

Zhao, Z. G., Zhao, F. J., & Yao, J. T. 2008. Early development of germlings of Sargassum thunbergii (Fucales, Phaeophyta) under laboratory conditions. *J. Appl. Phycol., 20*, 475–481. http://dx.doi.org/10.1007/s10811-008-9311-y

Zheng, Y., & Chen, Z. 1993. The seasonal growth and reproduction of *Sargassum thunbergii* (Pheaophyta) in Pingtan Island, Fujian Provine. *Journal of Fujian normal university (Natural science), 9*(1), 81–85.

Copyright Disclaimer