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The developmental regulation of mass cultures of free-living conchocelis for commercial net seeding of *Porphyra leucosticta* from Northeast America

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Abstract

The developmental regulation of mass cultures of "free-living" conchocelis (suspension cultures) of *Porphyra leucosticta* from Groton, CT (USA) has been studied in laboratory culture. The conchocelis filaments were vegetatively propagated and maintained in 15 l volumes at 15 °C, 40 μmol m^{-2} s^{-1} and 16 L:8 D. Conchosporangia formation was induced after four weeks by increasing the temperature up to 20 °C, maintaining a photon fluence rate of 40 μmol m^{-2} s^{-1} and decreasing the photoperiod to 8 L:16 D. Conchosporangial filaments were vegetatively propagated and maintained at these conditions for up to 24 weeks. Suspension cultures of conchosporangial filaments were induced to form and release conchospores (after 6–10 days) by decreasing the temperature to 15 °C, increasing the photon fluence rate to 60–100 μmol m^{-2} s^{-1} and lengthening the photoperiod to 12 L:12 D. Conchosporangial formation was found at all photoperiods, however, the ratio of conchosporangia to vegetative conchocelis increased as the photoperiod decreased. With higher photon fluence levels, conchospore release time was decreased, whereas at a temperature of 25 °C spore germination decreased. At their peak release, the quantity of conchospores increased from 7.14 to 18.3 million per gram of conchosporangia with a decrease in conchosporangia density from 1.582 to 1.125 mg ml^{-1}, respectively. On the average, one gram (dw) of free conchosporangia could release about 20 million conchospores at the peak period. These released conchospores were able to attach, germinate and develop into juvenile blades on the synthetic twine (3–5 mm in diameter) of standard nori nets (1.5×18 m). A total of 16 standard nets and eight small nets (2.0×2.5 m) were seeded by fixing the culture nets over a rotary wheel in a 2.5×2.5×0.5 m^{-3} tank containing the mature conchospore inoculum from the free-living conchosporangia cultures. Four seeded standard nori nets were transferred to the sea for nursery culture in Long Island Sound (USA). Conchosporeling densities from 255 to 325 conchosporelings cm^{-1} were produced. After 43 days of nursery culture, the blades grew to 1.49±0.14 cm in length. Our results indicate that the use of "free-living" conchocelis suspension cultures may be an effective alternative technology in the commercial production of the *Porphyra*.

Keywords: Free-living conchocelis; Conchosporangia; Developmental regulation; Seeding; *Porphyra* aquaculture

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1. Introduction

Porphyra are important economic seaweeds in Asia and become a big food industry with a production of over 1.01 billion metric tons wet weight (FAO, 2003) and a value of U.S. $ 1.8 billion dollars (Yarish et al., 1999). For the *Porphyra* tissues contained higher nitrogen and phosphorus contents than other seaweeds and their cultivation could uptake much nutrients out of seawater, now the *Porphyra* were considered to be good bioremediation candidates for marine eutrophication as well as integrated finfish/seaweed recirculating aquaculture system (RAS) (Neori et al., 2004; Zhang et al., 2005). To meet the RAS development, lots of seaweed sporelings need to be provided in any time of the year. *Porphyra* breeding using mollusk shells, established in Asia in the 1960's after the life cycle of *Porphyra* was described (Tseng et al., 1980; Ma and Cai, 1996), still remains the primary method being used in the *Porphyra* commercial culture in Asia (Fei, 1999; Sahoo and Yarish, 2005). In addition to being very time consuming and labor intensive, there has been a steady decrease in the availability of mollusk shells especially with the increase in production in China (He and Wu, 2003; Yang and He, 2004). New breeding technologies are being developed either by using somatic cells (He and Wang, 1994) or with free-living conchocelis (Sun and Tseng, 1996; Fei, 1999).

In China, the breeding with free-living conchcelis has been trying in recent years. Chen (1980) made the first attempt using free-living conchocelis of *P. haitanensis* in 250 ml glass bottles with six months, and conchospores were then seeded on nori nets (about 6 ha) and cultivated in the sea. Fei (1999) also reported on field trials using free-living conchocelis of *P. haitanensis*. However, until now this method has not been applied in the *Porphyra* production (Yang and He, 2004). For it was difficult to control the schedule of massive conchospores release. The *Porphyra* seeding usually takes place during the third week of September and continues for up to two weeks in China. Too early or too late was unfavorable in the *Porphyra* aquaculture (Fei, 1999). It is critical to have developmental regulation of the conchosporangia to release enough conchospores for commercial seeding operations. In this study, *Porphyra leucosticte*, one of the most important *Porphyra* species in USA, was applied to study on the developmental regulation of free-living conchocelis as an effective alternative technology in the commercial production of the *Porphyra* conchospores. The environmental control of the formation, maturation and development of the free-living suspension cultures of conchosporangia and the subsequent release of commercial quantities of conchospores will be described. The new breeding technology with free-living conchosporangia was developed.

2. Materials and methods

2.1. Free-living conchocelis culture

A monoecious plant of *Porphyra leucosticta* was collected from the coast of Groton, CT, March, 1997. The thallus surface was gently brushed with wrapped cotton ball and cleaned with autoclaved seawater in the laboratory. Small quadrate pieces of reproductive tissues (measuring about 25 mm^2) were excised from single blades. Conchocelis cultures (strain CT 23-1a) were established from zygotosporangia and maintained in the Marine Biotechnology Laboratory at the University of Connecticut, Stamford, CT, USA. The culture method of free-living conchocelis followed the descriptions of Zhu et al. (1997) and Yarish et al. (1998). Free-living conchocelis filaments were cultured in 1000 ml flasks with 800 ml of von Stosch's enriched (VSE) seawater medium (as cited by Ott, 1965) at 15 °C, 40 μmol m^{-2} s^{-1} and at 16 L:8 D with gentle aeration.

2.2. Induction and mass culture of conchosporangia

For mass culture of conchocelis, 7.5±2.5 g (wet weight) of conchocelis was cultured initially in 15 l Pyrex glass jars (with 10 l of VSE seawater media) at 20 °C, 16 L:8 D, 40 μmol $m^{-2}\cdot s^{-1}$ and with gentle aeration. The medium was changed weekly. After 4 weeks, the vegetative conchocelis filaments were cut up with a Waring blender at the highest speed (for 15 s) to produce conchocelis fragments. The conchocelis fragments were filtered through a 55 μm nitex mesh netting mounted in a 15×3 cm PVC pipe. The tissue was backflushed and rinsed with the VSE medium three times into the blender and refiltered to get a uniform mixture of conchocelis fragments (50–70 μm). The conchocelis fragments were collected and then cultured in the 20 l Pyrex glass jars with 15 l of VSE medium, at 8 L:16D, 40 μmol $m^{-2}\cdot s^{-1}$ and with gentle aeration. Media was changed weekly.

The effects of photoperiods (16 L:8 D, 12 L:12 D, 8 L:16 D) on conchocelis filaments maturation into conchosporangia were evaluated at 20 °C, 30 μmol $m^{-2}\cdot s^{-1}$ and with gentle aeration. The effects of temperatures (10, 15, 20, 25 °C) on conchocelis maturation into conchosporangia filaments at 8 L:16 D, 40 μmol $m^{-2}\cdot s^{-1}$ and with gentle aeration were also evaluated. The number of conchocelis with conchosporangia,

within the first 100 randomly observed conchocelis filaments in each culture, was recorded after 25 days of culture. The percentage of reproductive conchocelis was calculated for each of the triplicate 15 l cultures.

2.3. Environmental regulation for the mass release of conchospores

Triplicate cultures of mature conchosporangial filaments were grown in 250 ml Erlenmeyer flasks, with 200 ml of VSE and gentle aeration, under 40, 60, 80 and100 μmol $m^{-2}\cdot s^{-1}$ at 15 °C, under a photoperiod of 12 L:12 D, to evaluate the effects of photon fluence rate on conchospore release. Released conchospores were counted daily during the 6 day period. In another set of experiments, triplicate cultures of mature conchosporangial filaments were grown in 250 Erlenmeyer flasks, with 200 ml of VSE (with gentle aeration) under 40 μmol $m^{-2}\cdot s^{-1}$ at 15 °C, under a photoperiod of 12 L:12 D. The effects of conchosporangia density (1.125 and 1.582 mg ml^{-1}, dry weight) on conchospore release were then evaluated.

2.4. Attachment and germination of conchospores

For checking temperature effect, one piece-(1×2 cm^2) of mesh (55 μm nitex) netting was placed in each 250 ml culture flask, with 200 ml VSE medium (with gentle aeration) containing 2800 released conchospores ml^{-1}. The effects of temperature (5, 10, 15, 20 and 25 °C) on the attachment and germination of conchospores at 40 μmol $m^{-2}\cdot s^{-1}$, under a photoperiod of 12 L:12 D, was evaluated for nine grids (200×200 $\mu m^2\cdot grid^{-1}$) in the nitex mesh netting at 100 magnifications for each of the five replicates. In another series of experiments, four pieces (1×2 cm^2) of 55 μm nitex mesh netting was placed in each 250 ml culture flask, with 200 ml VSE medium (with gentle aeration) containing 2800 released conchospores ml^{-1}, one piece mesh was took out for checking in every 15, 30, 60 and 180 min. The effects of photon fluence rate (60 and 100 μmol $m^{-2}\cdot s^{-1}$) and time (15, 30, 60 and 180 min) on conchospore attachment and germination at 15 °C and 12 L:12 D was also evaluated for nine grids (250×250 μm^2) in the nitex mesh netting at 100 magnifications for each of the five replicates.

2.5. Seeding conchospores on nets and net cultivation

The mature conchosporangial fragments were cultured in the 20 l Pyrex glass jars with 15 l of VSE medium, at 20 °C, 40 μmol $m^{-2}\cdot s^{-1}$, 8 L:16 D, from July 2001. The mass cultures were aerated. Media was changed weekly. Three seedings on nets with conchospores from free-living conchosporangia were done on November 1, November 17, 2001 and March 7, 2002, respectively. Prior to seeding, free-living mature conchosporangial filaments were cultured at 15 °C, 70± 10 μmol $m^{-2}\cdot s^{-1}$, 12 L:12 D for 6–10 days. When the conchosporangia began to release conchospores, they were transported to the seeding tank.

The indoor seeding system had one and two bundles of four standard nori nets (1.8×18 m) fixed to a rotary wheel in a seeding tank ($2.5\times2.5\times0.5$ m^3) containing the mature conchospore inoculum from the free-living conchosporangia culture. In addition, eight Chinese (2×2.5 m^2) nets were also seeded. The seeding tank contained 0.3 μm filtered seawater (gently aerated by 3 parallel airlines the length of the long axis of the tank) maintained at 13.5±1.5 °C by a chiller (1/2 H. P, AE5D-A, Aquanetics Co, San Diego, CA, USA.). Illumination of 160±40 μmol $m^{-2}\cdot s^{-1}$ was provided by three high pressure sodium vapor lamps (1000 W, DM1000, Hydrofarm Co., Bristol, PA, USA.) under a photoperiod 12 L:12 D.

The density of spores and sporelings attached on the nets were determined in the third day after seeding. Eight seeded standard nori nets were transferred to the sea for nursery culture in Long Island Sound (Bridgeport, CT, USA.) from Nov. 21, 2001 to Jan. 7, 2002. The density on each of 4 randomly selected nori nets (triplicate observations for each standard nori nets), and the size (length × width) of 75 sporelings on each of these 4 randomly standard nori nets were measured

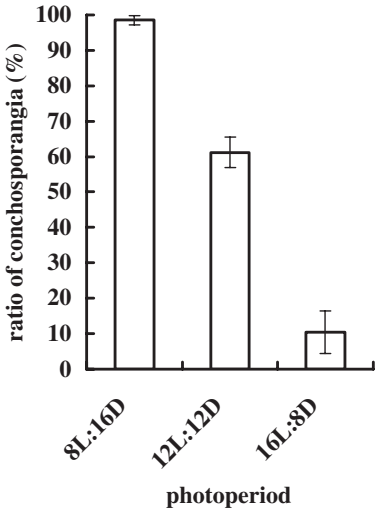

Fig. 1. Effect of different photoperiods on conchosporangia filament formation. The conchocelis filaments were cultured at 20 °C, 40 μmol $m^{-2}\cdot s^{-1}$ for 25 days. Data represent average of triplicate samples.

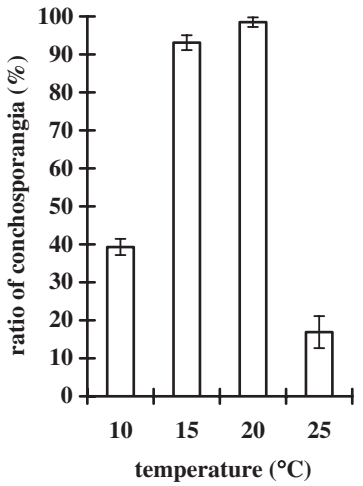

Fig. 2. Effect of different temperatures on conchosporangia filament formation. The conchocelis filaments were cultured at 40 μmol $m^{-2}\cdot s^{-1}$ and 8 L:16 D for 25 days. Data represent an average of triplicate samples.

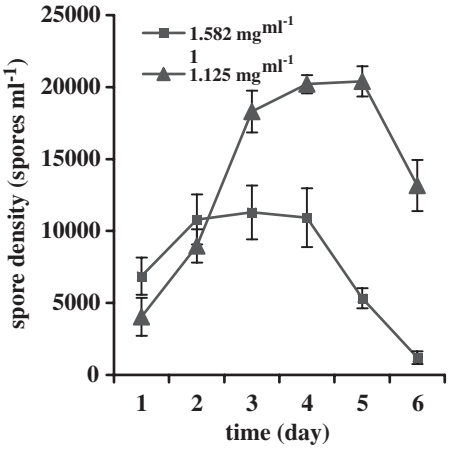

Fig. 4. The quantity of conchospores released from free-living conchosporangia. In 1.582 mg ml^{-1} conchosporangia density group, the quantity of released spores on the peak day was 10,933 spores ml^{-1}; In 1.125 mg ml^{-1}conchosporangia density group, the quantity of released spores on the peak day was 20,400 spores ml^{-1}. Values were the mean±SD, $n=3$.

weekly. The other nets were cultured in the seeding tank for 40–50 days with above same culture conditions, but the illumination of 80±20 μmol $m^{-2}\cdot s^{-1}$ was provided by two high pressure sodium vapor lamps (1000 W, DM1000, Hydrofarm Co., Bristol, PA, USA) under a photoperiod 12 L:12 D. 10 ppm of nitrogen and 1 ppm of phosphorus were applied in the seawater. Seawater was changed every two weeks.

All data are presented as the mean±standard deviation (SD).

3. Results

3.1. Induction and mass culture of conchosporangia

Photoperiod and temperature were key factors on the induction and formation of free-living conchosporangia

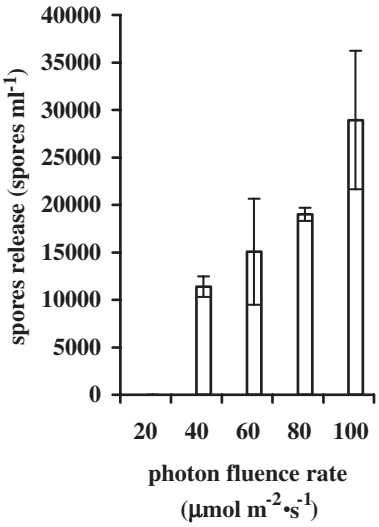

Fig. 3. Effect of photon fluence rate on the release of conchospores from free-living conchosporangia filaments. The conchosporangia branches were cultured at 15 °C and 12 L:12 D. The conchospores were counted on the 6th day. Data represent an average of triplicate samples.

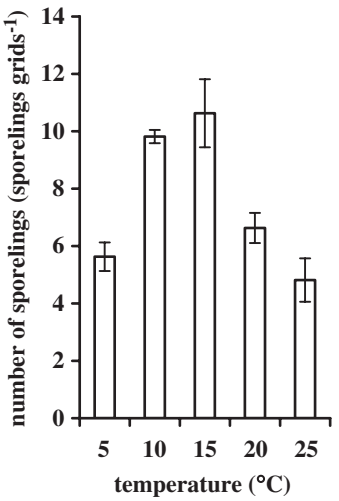

Fig. 5. The effect of temperature on the attachment and germination of conchospores from free conchosporangia. The conchospores were incubated at 30–40 μmol $m^{-2}s^{-1}$ and 12 L:12 D. The attached conchospores were counted on the 2nd day. Data represent the mean of 5 replicate samples.

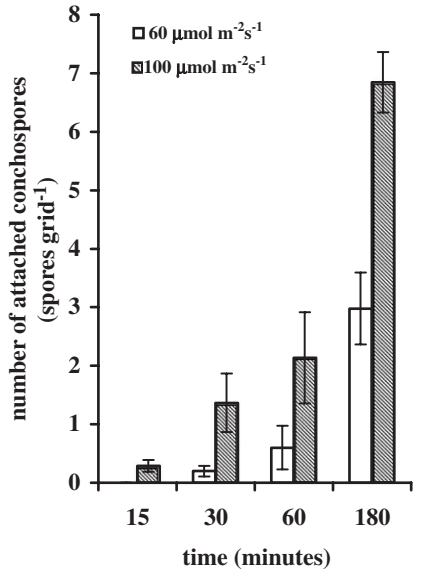

Fig. 6. Effect of the photon fluence rate and time on the conchospore attachment and germination. The conchospores were incubated at 60 and 100 μmol $m^{-2}s^{-1}$ group respectively, with 15 °C, 12 L:12D for 180 min. Data represent average of 5 samples.

filaments from free-living conchocelis filaments. Fig. 1 shows the effect of photoperiod on the conchosporangia formation. The ratio of conchosporangia to conchocelis under a short day length (8 L:16 D), a neutral day length (12 L:12 D) and a long day length (16 L:8 D) was 98.5%, 61.2% and 10.4% respectively, after 25 days of culture. The effect of temperature on the conchosporangia formation is given in Fig. 2. The ratio of conchosporangia to conchocelis at 15 and 20 °C was higher than that at 10 and 25 °C (Fig. 2). At 20 °C, the ratio of conchosporangia to conchocelis was the highest (98.5%).

3.2. Environmental regulation for the mass release of conchospores

The conchosporangia maturation and conchospore release was promoted with the increase of photon fluence rates (Figs. 3 and 4). The free-living conchosporangia density also influenced the release of conchospores. At the lower conchosporangia density of 1.125 mg ml^{-1}, conchospore release was almost twice as much as compared to the high conchosporangia density of 1.582 mg ml^{-1}. In Group A (1.582 mg ml^{-1} conchosporangia density) and Group B (1.125 mg ml^{-1}conchosporangia density), the quantity of released spores in peak day was 10,933 and 20,400 spores ml^{-1} respectively.

3.3. Attachment and germination of conchospores

Temperature and photon fluence rate were important factors for the attachment and germination of conchospores. Conchospore attachment and germination was optimal between 10 and 15 °C (Fig. 5). Fig. 6 shows the effect of different photon fluence rates (60 and 100 μmol $m^{-2}\cdot s^{-1}$) and different release schedules (15, 30, 60 and 180 min) on the attachment and germination of conchospores. At the high photon fluence rate of 100 μmol $m^{-2}\cdot s^{-1}$, more conchospores were attached on the nitex mesh and germinated. The rate of the conchospore attachment was about 19.8 spores 9 $grid^{-1}$ $hour^{-1}$. The results indicated that the conchospores released from free-living conchosporangia were available to attach on the fibers of the nitex mesh within 3 h.

3.4. Seeding conchospores on nets and cultivation

Large-scale breeding with released conchospores and free-living conchosporangia were carried out in the laboratory from Nov. 1–3, 2001 to March 7–9, 2002. The conchocelis filaments were vegetatively propagated and maintained in 15 l volumes at 15 °C, 40 μmol m^{-2} s^{-1} and at 16 L:8 D. At 20 °C, 40 μmol m^{-2} s^{-1} and 8 L:16 D, conchosporangia formation was induced after four weeks. Almost 100% of the conchocelis filaments developed into conchosporangia filaments when they were cultured for 5–6 weeks. The mature conchosporangia were maintained for 3–6 months (from July 2001 to Mar. 2002). Initial seeding operations

Table 1
Net seeding with conchospores from free-living conchosporangia in *Porphyra leucosticta* (2001-2002)

No	Net number	Wet weight/volume of conchosporangia (g l^{-1})	Photon fluence rate (μmol $m^{-2}\cdot s^{-1}$)	Seawater temperature (°C)	Sporeling density (cm^{-1} rope)
1	8*	42.1/30	120–200	13–14	287.39±57.26
2	8*	18.9/15	150–200	14–15	325.72±129.35
3	8**	39.5/30	100–150	13–14	255.00±111.57

* Standard nets (1.5×18 m^2 in size).
** Chinese nets (2×2.5 m^2 in size).

began in Nov. 1–3, 2001 and continued until Mar. 7–9, 2002 (see Table 1). Prior to the seeding operation, the conchosporangia were cultured at higher photon fluence rates of 70±10 μmol $m^{-2}\cdot s^{-1}$ for about 10 days. As soon as conchosporangia began to release conchospores they were transported to the seeding tank. Following another increase in the photon fluence rate to 160±40 μmol $m^{-2}\cdot s^{-1}$ and a decrease in temperature to 14±1 °C, the conchosporangia began releasing large quantities of conchospores (Table 1). Eight nets were initially put in the seeding tank for up to 3 days and monitored. Conchospores were produced from free-living conchosporangia in this seeding operation. The released conchospores were attached to the fibers of the standard nori nets and germinated into sporelings with 1–4 cells. In the third day, the sporeling density of the initial seeding operation was checked and counted as 287±57 cm^{-1}sporelings in average. Another seeding operation began two weeks later. The initial released conchospores was 170 million and sporeling density on the net fibers was 326±129 sporelings cm^{-1}. A third seeding operation, using specially made nets from China, commenced on March 7–9, 2002. Before the mature conchosporangia began to release conchospores, they were put into the seeding tank. In up to 72 h, conchospores were released and a sporeling density of 255±111 sporelings cm^{-1} was observed on the fibers of the nori nets.

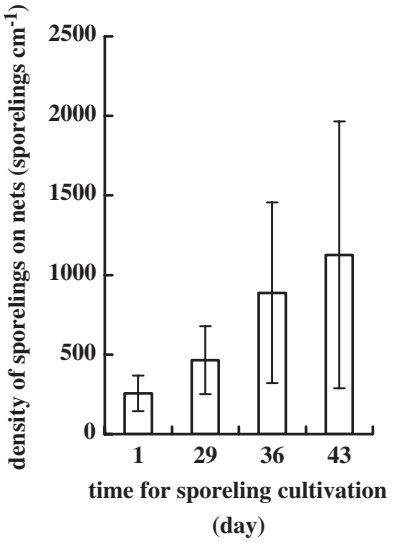

Fig. 7. Density of sporelings on culture Net B cultured at the nori farm in Long Island Sound, Bridgeport, CT, USA, from Nov 21, 2001 to Jan 7, 2002. Increase of sporeling density indicated that the young sporelings released massive archeospores when they cultivated in sea field. Data represent the mean of triplicate samples.

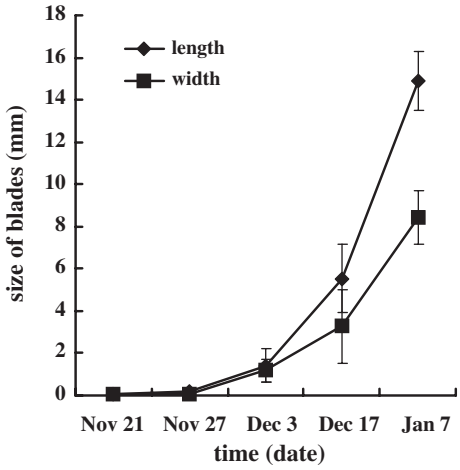

Fig. 8. Growth of sporelings on culture Net B cultured at the nori farm, Long Island Sound, Bridgeport, CT, USA from Nov 21, 2001 to Jan 7, 2002. Data represent the mean of 50–100 samples.

Eight nori nets were initially deployed on to a modified Japanese Ikada nursery cultivation system on the nori farm in Long Island Sound from November 21, 2001 to January 7, 2002. The densities, length and width of conchosporelings attached to one of the culture nets were monitored weekly. After 43 days of cultivation the mean density of sporelings on the one of representative standard nori nets reached 1126.7±839.3 sporelings cm^{-1} (Fig. 7). The increase of sporeling density indicated that the blades were producing archeospores. Fig. 8 shows the increase in length and width density of sporelings cultured at the nori farm. After 43 days of cultivation, blades attained lengths of 1.49±0.14 cm. The other nets were cultured in a floating system or seeding tank for 40–50 days, and their maximal length reached up to 1.0–1.2 cm.

4. Discussion

Breeding with free-living conchosporangia in the *Porphyra* was a system engineering, which including free-living conchocelis amplification culture, induce and formation of conchosporangia from conchocelis, conchosporangia amplification culture and maintenance, massive conchospores release, and seeding spores on nets. Each step would be important in the new breeding technology and needed the strong support from the detail research data of the conchocelis development regulation. Until now the new breeding has not been applied in the *Porphyra* breeding production for poor understanding of exact regulation of whole conchocelis development course and conchocelis massive culture technology (Fei, 1999; Yang and He, 2004). In this paper, a whole

procedure for the new breeding with free-living conchosporangia was presented after studies on the regulation of whole conchocelis development course and breeding practice in *P. leucosticta*. By breeding procedure, it included free-living conchocelis amplification culture, inducement and formation of conchosporangia from conchocelis, conchosporangia amplification culture and maintenance, conchosporangia mature culture, massive conchospores release, seeding spores on nets, and nursery culture of seeded nets in sea field. Especially, the conchosporangia inducement and formation, conchosporangia maintenance and massive conchospores release would be three key procedures during the *Porphyra* breeding production. In previous studies, lot of the conchocelis development research work and breeding tries has been done. Most phycologists noted and worked on only conchosporangia inducement and formation as well as spores release (Avila et al., 1986; Sidirelli-Wolff, 1992; Garguilo et al., 1994; Tang and Fei, 1997; Knight and Nelson, 1999; Notoya and Sugawara, 1999; Orfanidlis, 2001; Pereira et al., 2004), but the conditions for inhibiting spores release and the schedule for sporelings nursery culture at sea field were ignored. Few papers involved on massive conchospores release (Sun and Tseng, 1996; Fei, 1999; Yang and He, 2004) and conchosporangia maintenance. In this paper, 100% conchosporangia was got by controlling environment factors in massive culture for 5–6 months, and the matured conchosporangia could maintain for half year and did not release spores. When they were cultured into a special culture environment, the maintained conchosporangia could release conchospores massively in a short time, 6–10 or 3–6 days (Yang and He, 2004). It means that the conchosporangia development could be regulated exactly and do release spores in any time of the year. It was very important for the new breeding technology would be applied in the *Porphyra* breeding production.

The *Porphyra* conchocelis development could be regulated by controlling culture environmental factors (Dring, 1967; Chiang and Wang, 1980; Avila et al., 1986; Waaland et al., 1987; Sidirelli-Wolff, 1992; Garguilo et al., 1994; Tang and Fei, 1997; Fei, 1999; Knight and Nelson, 1999; Notoya and Sugawara, 1999; Orfanidlis, 2001; Varela-Alvarez et al., 2004). In most previous research work, only the temperature and photoperiod were noted and applied to regulate the conchocelis development as well as the spores release (Tang and Fei, 1998; Yang and He, 2004). The light photon fluence rate was also a very important environment factor for controlling conchosporangia maintenance and massive spores release (Yang and He, 2004). Actually, Waaland et al. (1990) and Orfanidlis (2001) earlier noted the importance of temperature, photoperiod and light photon fluence rate in the *Porphyra* conchocelis development. In breeding practice, the conchocelis development regulation also was influenced by the conchocelis or conchosporangia density and culture scale. Higher density and bigger scale usually would block lights into the culture containers. In addition, the environment conditions for conchocelis development regulation varied in different species and even different strains in same species. So the factors of culture method and environment as well as species would make the conchocelis development regulation become much complex and difficult.

The conchosporangia maintenance and massive conchospores release depended on both temperature and light photon fluence rate. Waaland et al. (1990) considered that photoperiod, temperature and irradiance were all involved in the production and maturation of conchospores in several *Porphyra* species. As for *P. leucosticte*, when the temperature for conchosporangia culture increased from 20 to 5–15 °C, and the photon fluence rate increased from 20 to 40–100 μmol $m^{-2}s^{-1}$, more conchosporangia would be matured and more spores would be released (see Fig. 3).

Developing the technology of conchosporangia long term maintenance would be helpful for the new breeding technology was applied in the *Porphyra* aquaculture production. The conchosporangia could be maintained for several months by controlling the temperature and photon fluence rate. In *P. leucosticte*, the conchosporangia would not release spores when temperature was higher than 20 °C or photon fluence rate was below 15 μmol $m^{-2}s^{-1}$. Only when both temperature decreases below 20 °C (such as 5–15 °C) and photon fluence rate increases up to 17–18 μmol $m^{-2}s^{-1}$ (or 20 μmol $m^{-2}s^{-1}$ in suspension culture), the conchosporangia would release spores. So conchosporangia could be cultured at or above 20 °C and lower photon fluence rate for long term maintenance. In this study, the conchosporangia had been maintained for more than a half year, and they could still release conchospores and were used for net seeding. It would be a benefit to breeding with free-life conchosporangia, for the conchosporangia could be reproduced massively and maintained for a long time, and the maintained conchosporangia could be used to release spores for nets seeding in any time of the year. In previous researches, it was very difficult to inhibit the conchosporangia releasing the spores when conchosporangia became matured or stayed in the season for spore release, or if the matured conchosporangia failed to release spores in the season for spore release, they would become useless after the season (or schedule) for spore release ended.

During breeding course, the culture temperature was easy to control. But when culture scale was amplified or conchocelis density became higher, the photon fluence rate would be a key factor for breeding. Usually, the bioreactors for culturing plant cells were applied to reproduce massive conchocelis or conchosporangia. In a bioreactor system, higher density of conchosporangia would block lights. So photon fluence rate should increase 5–20 μmol $m^{-2}s^{-1}$ when culture scale was amplified or conchocelis density became higher. However, too high photon fluence rates could result in conchosporangia damage and death by photoinhibition (Tseng et al., 1980; Ma and Cai, 1996). When seeding nets were carrying, the bigger shallow tanks were required to get enough lights for spores releasing and germinating. The container size and the density of conchosporangia were the limited factors for big scale breeding.

According to the commercial breeding methods, the conchosporangia in 1 m^2 of shells could provide enough conchospore quantity for about 7 standard nets (Tseng et al., 1980; Fei, 1999). Commercial production of the *Porphyra* about 1000 standard nori nets required about 140 m^2 of shells. According to the experiment in Fig. 4, 1 g (dry weight) of free-living conchosporangia filaments released about 20 million conchospores on peak days for 3 nets seeding. This indicates 1000 standard nori nets needs about 300 g (dry weight) of free-living conchosporangia filaments, which requires about 20 Pyrex glass jars with 15 l of cultured material. Totally a 20–40 m^2 breeding room was enough for the new breeding method. It indicated that the new breeding technology can save both breeding place and breeding time.

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