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Morphogenesis and growth in the early life stages of *Sargassum oligocystum* Montagne from fertilized eggs to juveniles examined in culture

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Abstract: Species of *Sargassum* are widely distributed along the coasts of Thailand. *Sargassum oligocystum* Montagne is a dominant species consisting of *Sargassum* beds, playing an important ecological role in a marine ecosystem along the east coast of the Gulf of Thailand. However, there is little information available on the early life stages of *S. oligocystum* . To fill the gap in this ecological knowledge, fertilized eggs obtained from the receptacles of wild matured individuals were cultured and morphogenesis in the early life stages of *S. oligocystum* due to their development was observed through laboratory culture. A fertilized egg divided transversely into one large cell and one small cell. The latter gradually induced rhizoidal cells after several divisions and many rhizoidal cells came out at the basal part of germling in 3 day culture. Finally, they became the holdfast of germling. In the large cell, cell divisions occurred and apical part came out in 1 day culture. It developed into the first cauline leaf in 7 day culture and the fourth cauline leaf was appeared in 30 day culture, which were lanceolate. Cauline leaves were lanceolate to spatulate in 60 day culture and broad spatulate in 90 day culture. Three-month-old juveniles of *S. oligocystum* were cultured in two 500 L fiberglass tanks set outdoor under a roof with translucent windows, and one was filled with seawater and another was filled with seawater with urea dissolved at a concentration of 4 g t^{-1}. When juveniles cultured in two different conditions for five weeks, the growth rate of the germlings of *S. oligocystum* cultured in seawater was always higher than that of culture in seawater with urea dissolved. The results suggest that *S. oligocystum* has a potential to adapt to grow under lower nutrient environment.

Keywords : *Sargassum oligocystum,* morphogenesis, early development and growth, culture

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1. Introduction

Sargassum C. Agardh is one of the largest genus of brown algae and the most important seaweed both ecologically and economically. The *Sargassum* plants are distributed all over the world, especially in tropical and temperate regions (YOSHIDA, 1983). Seaweed beds consisting of *Sargassum* species influence the dissolved oxygen content in seawater (hereafter, this is referred to as DO) through photosynthesis (KOMATSU 1989; MURAOKA, 2004; MIKAMI *et al.*, 2007) and consequently the pH value by CO_2 absorption through photosynthesis and release through respiration (KOMATSU and KAWAI, 1986). They support biodiversity and habitat for marine organisms (KOMATSU *et al.*, 1982; KOMATSU, 1985; KOMATSU *et al.*, 1990; KOMATSU and MURAKAMI, 1994; KOMATSU *et al.*, 1995; KOMATSU *et al.*, 2007; KOMATSU *et al.*, 2008). *Sargassum* species comprise bioactive compounds such as vitamins, carotenoids, dietary fibers, proteins, and minerals, and biologically active compounds, like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol and pheophytin (LUCAS and SOUTHGATE, 2012). *Sargassum* species are used as human foods, especially by people living in coastal areas (*e.g.* KIRIMURA, 2007). There are many reports on the bioactive substances extracted from seaweeds, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-diabetic, antioxidant, and cytotoxic substances (*e.g.* ZANDI *et al.*, 2010; TAJBAKHSH *et al.*, 2011; YENDO *et al.*, 2014; MEHDINEZHAD *et al.*, 2015). *Sargassum* plants also play an effective bio-absorption role to remove nutrients (FEI, 2004) and heavy metals such as cadmium ion (Cd^{2+}), copper ion (Cu^{2+}), and mercuric ion (Hg^{2+}) dissolve in seawater. Therefore, this function of *Sargassum* species is focused from the environmental and economic aspects (RAMAVANDI *et al.*, 2015; DELSHAB *et al.*, 2016).

Many reports exist concerning the early development stages of *Sargassum* species such as *S. micracanthum* and *S. ringgoldianum* (OGAWA, 1974), *S. muticum* (NORTON, 1977; HALES and FLETCHER, 1989; UCHIDA *et al.*, 1991; KERRISON and LE, 2016), *S. horneri* (NANBA, 1993; UCHIDA, 1993; YOSHIDA *et al.*, 1995; YOSHIDA *et al.*, 1999; CHOI *et al.*, 2008), *S. filicinum* (YOSHIDA *et al.*, 1999), *S. confusum* (KAWAGOE *et al.*, 2005), *S. vachellianum* (YAN and ZHANG, 2013), *S. thunbergii* (ZIGUO *et al.*, 2008; YONGZHENG *et al.*, 2015), *S. echinocarpum* (HAMZA *et al.*, 2016) and *S. swartzii* (KAVALE and VEERAGURUNATHAN, 2016). In addition, there are reports on the technical development for artificial seed production in *S. fulvellum* (HWANG *et al.*, 2006, 2007) and *S. thunbergii* (ZHANG *et al.*, 2012). However, there is not any available information on the embryo release and early development of *S. oligocystum* which is one of the most common and abundant species in tropical waters of the western Pacific Ocean.

There are some extensive researches on fertilizer application in seaweed cultivation (AMANO and NODA, 1987; BRAULT and QUÉGUINER, 1989; PHILLIPS and HURD, 2003; TYLER *et al.*, 2005; MANSILLA *et al.*, 2007; KIM and YARISH, 2014; MIKI *et al.*, 2016). Urea is an organic compound with the chemical formula of $CO(NH_2)_2$ and is widely used as a fertilizer for nitrogen source. Urea has the highest nitrogen content of all solid nitrogenous fertilizers in common use and can get anywhere at a reasonable price. The standard crop-nutrient rating (NPK rating) of urea is 46-0-0, and it is also used in many multi-component solid fertilizer formulations for land plants (WIKIPEDIA, 2016). However, it is unknown on the effect of urea on the growth of *S. oligocystum*.

Seaweed culture techniques have been developed by researchers to observe the early development of seaweeds. Unfortunately, we have no detailed studies on Thai *Sargassum* species until

Fig. 1 Mature thalli of *Sargassum oligocystum* observed around Samaesarn Island.

now. The objective of this study is to present the morphogenesis and growth in the early life stage of *S. oligocystum* from the fertilized egg stage to the juvenile stage, and to test an effect of urea on the growth of its juvenile plants. Materials were cultured under laboratory and outdoor conditions. Results were served for the objectives of this study.

2. Materials and methods

2.1 Laboratory culture of fertilized eggs

Mature *S. oligocystum* plants were collected in the intertidal zone of Samaesarn Island, Chon Buri Province, Thailand (12° 31′21.37″N, 100° 57′ 25.12″E) in April 2014 (Fig. 1). The plants were cleaned to eliminate epiphytes and rinsed thoroughly with sterilized seawater. Receptacles were examined to check whether fertilized eggs were released and the eggs had attached to their surface or not. The fertilized eggs were removed from the receptacles by brush and rinsed several times with sterile seawater. Plant Nutrition + liquid (Tropica, Aquacare) was used as a culture medium and renewed once a week. Culture conditions were as follows: a salinity of 30, a water temperature of 25℃ and photosynthetic active radiation (PAR) of 85 μmol photons m^{-2} s^{-1} with the use of cool daylight fluorescent tubes (Philips, TL-D 18W/54-765 1SL, Thailand) for a 12 h: 12 h (L:D) (Figs. 2a, b). PAR was measured with a light meter (LI-250A, LI-COR, USA). Growth and development from the fertilized egg stage to the juvenile stage for 90 d were observed. Juvenile thalli cultured for 90 d were used for an outdoor tank culture experiment.

2.2 Outdoor tank culture of juveniles

Three-month-old *S. oligocystum* juvenile thalli were cultured in outdoor tanks of 500 L made from fiberglass, set under a roof with translucent plastic windows (Fig. 2c). To know the nutritional effects for the growth of *S. oligocystum* juvenile, three hundred juvenile thalli were cultured in a tank filled with seawater and also a tank filled with seawater with urea fertilizer dissolved at a concentration of 4 g t^{-1} (hereafter, this is referred to as seawater with urea dissolved for simplicity) (Fig. 2d). The culture mediums in both tanks were renewed once a week. Two replicates were used for each treatment. At intervals of 7 d during 35 d of culture, fifteen young thalli were randomly selected to measure the size of juvenile thalli under each treatment for examination of their growth (Fig. 2e). In an outdoor tank, we measured eight environmental parameters such as water temperature, PAR (HOBO Pendant UA-002-64, USA), pH (Mettler Toledo pH Five Go, Switzerland), salinity (ATAGO 508 IIW, Japan).

2.3 Growth rate and data analyses

A growth rate of a thallus was estimated from an increase in size of thallus. A specific growth rate for *S. oligocystum* was obtained with the formula proposed by Luhan and Sollesta (2010):

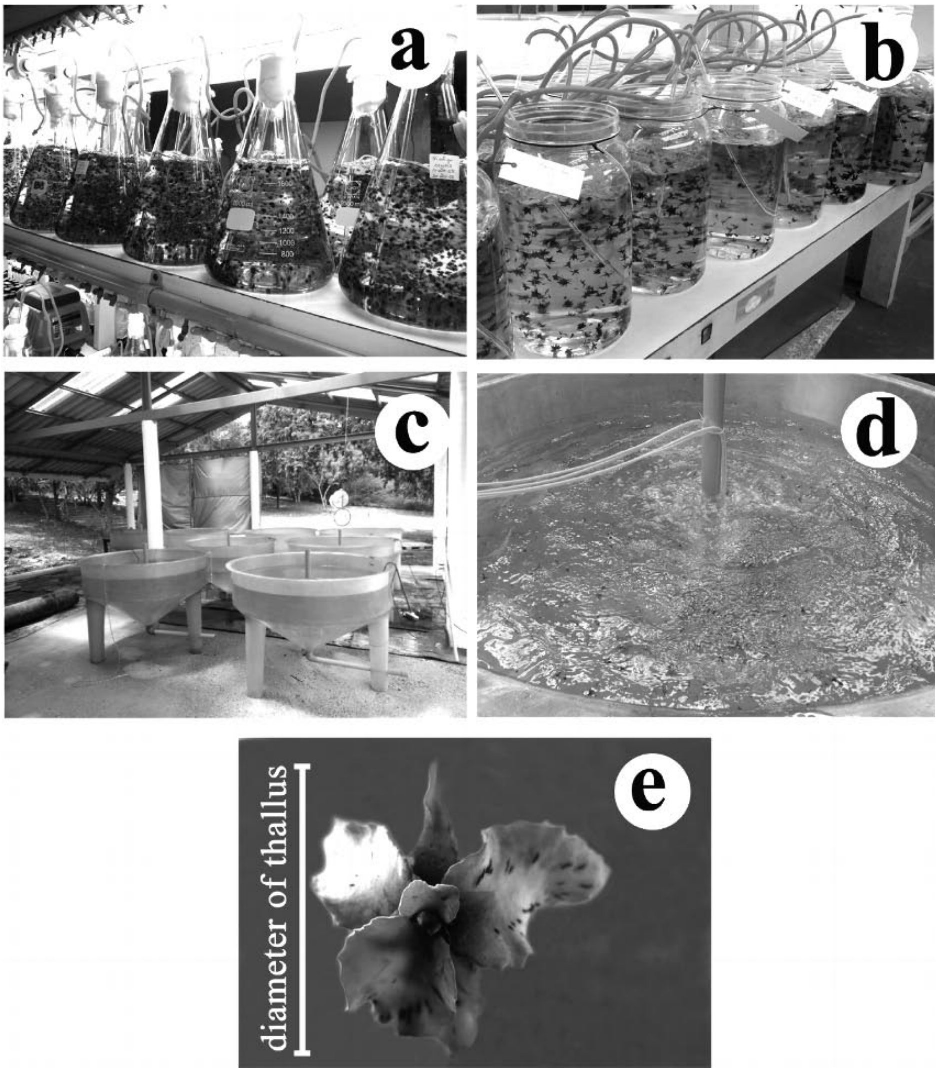

Fig. 2 Pictures showing laboratory cultures of *Sargassum oligocystum* germlings (a and b); juveniles in outdoor tanks (c and d) in Samaesarn Island and the diameter measurement of the thallus (e).

$$SGR = \frac{100\ (\ln W_t - \ln W_0)}{t} \qquad (1)$$

where SGR, t, W_0 and W_t are specific growth rates, time of day after the start of outdoor tank culture, an initial size of thallus (mm) on the first day of culture and a size of thallus (mm) at t, respectively. The first day t and a size W_t on the first day of each week were set as 0 and W_0 because measurements were conducted at intervals of 7 d for 35 d. Differences in specific growth rates of *S. oligocystum* thallus per week were examined between those cultured in seawater or seawater with urea dissolved, and comparing the eight environmental parameters between the two different mediums.

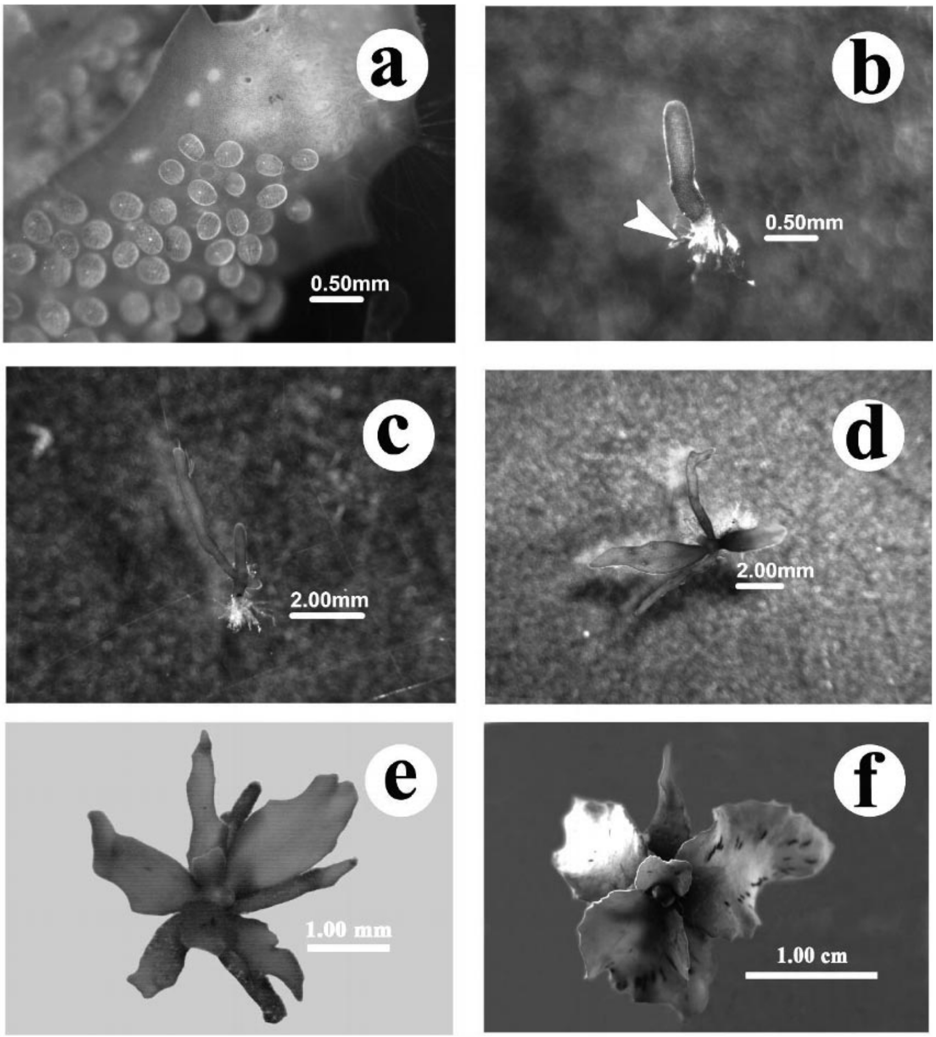

Fig. 3 Embryo development of *Sargassum oligocystum* in a container filled with a culture medium of Plant Nutrition^{+} liquid (Tropica, Aquacare) under a salinity of 30, a water temperature of 25℃ and PAR of 85 μmol photons m^{-2} s^{-1}. Pictures of fertilized eggs in a receptacle on the 1st day (a), a germling on the 3rd day with an arrow showing the rhizoidal cell (b), a germling on the 7th day (c), a juvenile on the 30th day (d), a juvenile on the 60th day (e) and a juvenile on the 90th day (f).

3. Results

3.1 Field observation and embryo culture in a laboratory

The receptacle formation of *S. oligocystum* was observed from February to June around Samaesarn Island, Chon Buri Province, Thailand. Fertilized eggs released from conceptacles attached to their surface. After verifying the start of germination, zygotes were isolated in containers filled with culture medium. The first segmentation in an egg occurred transversely to the longitudinal axis of the egg and divided it into one large cell

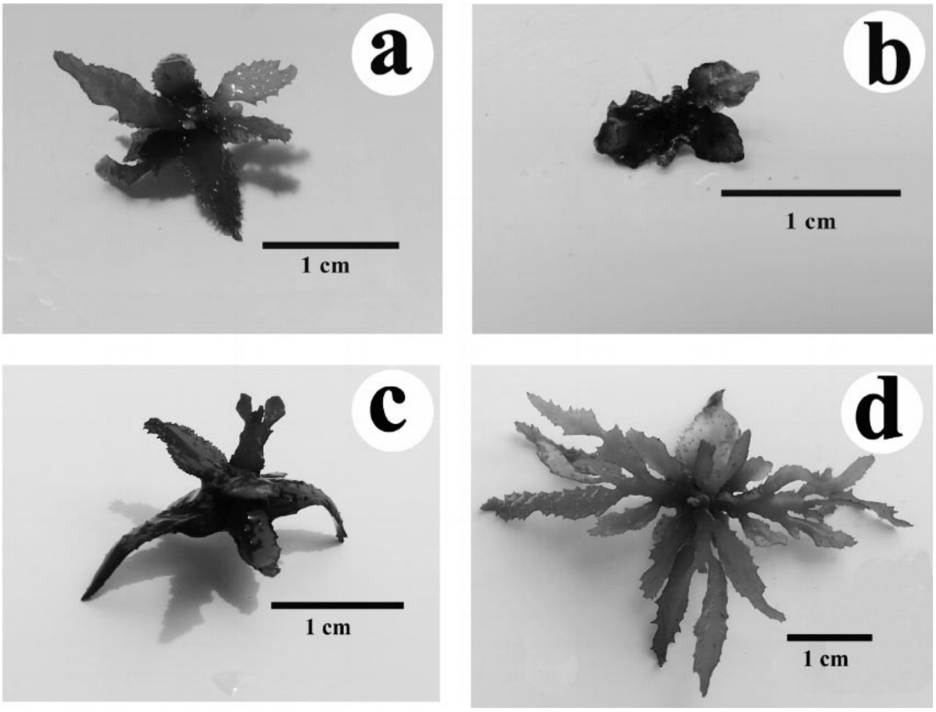

Fig. 4 *Sargassum oligocystum* juveniles in seawater (a) and seawater with urea dissolved at a concentration of 4 g t^{-1} (b) on the 5th day, with lateral branches in seawater on the 7th day (c) in seawater on the 14th day, and (d) from the start of outdoor tank culture.

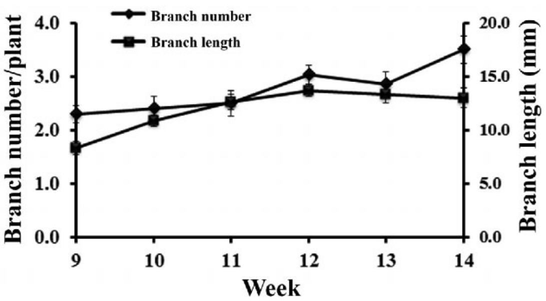

Fig. 5 Branch development of *Sargassum oligocystum* cultured in outdoor tanks from the 9th week to the 14th week.

and one small cell. The latter was gradually induced to rhizoidal cells after several divisions and rhizoids grew out, and became a basal part of the germling for the attachment to substrate. The cells from the former cell became an apex in 1 day culture (Fig. 3a). Germlings produced many rhizoids in 3 day culture (Fig. 3b). They developed the first cauline leaf in 7 day culture (Fig. 3c) and the fourth cauline leaf came out in 30 day culture (Fig. 3d), becoming juvenile. The shape of these leaves was lanceolate. Through the development, cauline leaves were lanceolate to spatulate in 60 day culture (Fig. 3e), and broad-spatulate in 90 day culture (Fig. 3f).

In the seventh week of culture, juvenile thalli of *S. oligocystum* developed a primary branch (Fig. 4c). The average number of branches was 2.3–2.4 with 8.3–10.9 mm in length from the 9th to 10th week, 2.5–3.0 branches with 12.6–13.7 mm in length between the 11th and 12th weeks, and 2.9–3.5 branches with 13.0–13.3 mm between the 13th and 14th weeks (Figs. 4d and 5a).

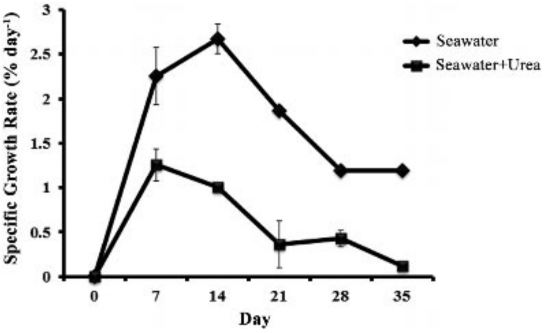

Fig. 6 Growth rates of *Sargassum oligocystum* juveniles (mean ± standard error) at intervals of 7 d, cultured in outdoor tanks filled with seawater (closed circle) and seawater with urea dissolved at a concentration of 4 g t^{-1} (closed square) for 35 d.

3.2 Growth rate of juvenile cultured in outdoor tanks

Three-month-old juveniles of *S. oligocystum* (hereafter referred to as young thalli) were used for a growth experiment cultured in tanks filled with two different mediums: seawater or seawater with urea dissolved. At the end of the experiment on the 35th day, the average size of thalli obtained from the tanks filled with seawater and seawater with urea dissolved were 18.7 ± 0.3 mm and 13.7 ± 0.2 mm, respectively. The highest specific diameter of growth rates for the thallus in the former and latter tanks were 2.7 ± 0.2% d^{-1} on the 14th day and 1.3 ± 0.2% d^{-1} on the 7th day of culture, respectively (Fig. 6). Growth rate for the juvenile thalli of *S. oligocystum* cultured in the latter tanks were decreased because of growth of blue-green algae on the thallus surface (Fig. 4b), while those in the former tanks showed less growth of blue-green algae (Fig. 4a).

The averages of environmental parameters are as follows: water temperature ranged from 30.4 to 30.9℃; photosynthetic active radiation ranged from 21.7 to 40.5 μmol photons $m^{-2}s^{-1}$; salinity in tanks filled with seawater and seawater with urea dissolved ranged from 32 to 33.9 and 31.5 to 35.5, respectively; The pH in the former and latter tanks ranged from 8.1 to 8.3 and 8.2 to 8.4, respectively (Fig. 7). There was little difference in environmental parameters between the former and latter tanks.

4. Discussion

The germling development of *S. oligocystum* is similar to that of tropical or temperate species of *Sargassum* such as *S. confusum, S. horneri, S. thunbergii, S. swartzii* and *S. vachellianum* (UCHIDA, 1993; KAWAGOE *et al.*, 2005; ZHAO *et al,* 2008; YAN and ZHANG, 2013; KAVALE and VEERAGURUNATHAN, 2016). The development of embryonic germlings in this species follows the classic "8 nuclei in 1 egg" type, as described for Sargassaceae. Fertilized eggs developed into embryos at the primary-rhizoid stage in 24 h, and the secondary-rhizoid stage in 3 d. The first leaflet of the germling with cylindrical shape was formed in 7 day culture. It is reported that cues on an egg release and the early germling growth of seaweeds were water temperature, irradiance, photoperiod, day length, nutrient, desiccation, thermal and osmotic stress (NORTON, 1977; UCHIDA *et al.*, 2991; NANBA, and OKUDA, 1993; YOSHIDA *et al.*, 1995, 1999; STEEN, 2004; STEEN and RUENESS, 2004; HWANG *et al.*, 2006; CHOI *et al.*, 2008; CHU *et al.*, 2012; YONGZHENG *et al.*, 2015). However, such cues could not be observed through our culture experiment.

The specific growth rates of the juvenile thalli of *S. oligocystum* cultured in the tanks filled with seawater were higher than those cultured in the tanks filled with seawater with urea dissolved. This difference is due to the chemical composition of the nutrient solutions used in this experiment. Urea is an excellent nitrogen source for some seaweeds such as kelps, but others show growth inhibition. For example, BRAULT and QUÉGUINER

(1989) studied the effect of inorganic and organic nitrogen sources on the growth of *Ulva gigantean* and found that ammonium was a better nitrogen source than urea and nitrate. PHILLIPS and HURD (2003) reported on nitrogen ecophysiology of four intertidal seaweeds (*Stictosiphonia arbuscula*, *Apophlaea lyallii*, *Scytothamnus australis* and *Xiphophora gladiata*) from southeastern New Zealand and reported that there is a difference in absorption by nitrogen sources and its seasonality. The order of nitrogen sources well absorbed by seaweeds is $NH4^{+}$> $NO3^{-}$> urea in winter and $NH4^{+}$ = $NO3^{-}$ > urea in summer. MANSILLA *et al.* (2007) reported that *Gigartina skottsbergii* germlings grew more rapidly when they were cultured in solution of Bayfoland 250 SL and Provasoli than the growth rates cultured in solution with urea and superphosphate, which were significantly lower. Nitrogen and phosphorus are limiting nutrients for growth and yield of seaweeds in most natural environment. Physiological and biological factors of seaweeds may have an influence on growth and uptake of nutrients, such as inter-seaweed variability, nutritional history, type of tissue, life history stage, age, surface area to volume ratio of a thallus, and morphology. (HARRISON and HURD, 2001). HARRISON and HURD (2001) mentioned also that epiphytes growing on surfaces of seaweeds can control seaweed growth to a critical level by starving nitrogen uptake for several days. The present study shows that the specific growth rates of *S. oligocystum* juveniles cultured in seawater with urea dissolved were decreased by blue-green algae contamination. It is possible that some attached algae may use the nutrients more efficiently than *S. oligocystum*. It is estimated that *S. oligocystum* succeeds to acquire a great ability to adapt to the low nutrient level in tropical waters, especially in the waters of the east coast of the Gulf of Thailand.

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