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Effect of nutrient media and initial biomass on growth rate and nutrient uptake of *Sargassum spinuligerum* (Sargassaceae, Phaeophyta)

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Abstract: The *Sargassum* species are prospective candidates for marine culture, but there are a limited number of reports on their nutrient requirements and optimum initial stocking biomass, and nothing is published for *Sargassum spinuligerum.* This study investigated the effects of three commercially available fertilizers (Hortico, Seasol, and Aquasol) and four initial stocking biomass levels of *S. spinuligerum* on the growth rate and nutrient uptake capacities for 7 weeks. The results showed that *S. spinuligerum* could be grown under outdoor conditions with the optimum initial stocking biomass of 15.35 g per 113 L. The different commercial fertilizers significantly influenced the specific growth rate and nutrient uptake rate of *S. spinuligerum*. Aquasol resulted in a higher specific growth rate than the other commercial fertilizers, with the relative growth rate fluctuating between 0.42 and 1.70 (% per day). Aquasol is recommended as a nutrient supplement to enhance the specific growth rate of *S. spinuligerum.*

Key words: *Sargassum spinuligerum*, marine biomass, nutrients, specific growth rate, nutrient uptake rate

1. Introduction

The genus *Sargassum* belongs to the family Sargassaceae (Phaeophyta), and is one of the most diverse genera in the family, with over 336 species (Guiry and Guiry, 2014). *Sargassum* is distributed globally in both temperate and warm waters, and is most abundant in subtidal areas of the Indo-West Pacific and Australia (Tseng et al., 1985). In Western Australia (WA), there are 46 *Sargassum* species (Huisman and Walker, 1990). The *Sargassum* species are a great source of polysaccharides and phenolic compounds (Keusgen and Glombitza, 1997) for cosmetics, pharmaceutical, and biofuel extraction industries (Hanisak and Samuel, 1987; Murase et al., 2000; Pang et al., 2009).

Temperature, light intensity, nutrient availability, salinity, and initial stocking biomass (ISB) are the most important environmental factors to ensure a high growth rate of culturing macroalgal species (Friedlander and Ben-Amotz, 1991). Nutrient uptake capacity is one of the vital parameters to accelerate macroalgae growth and increase the productivity of *Sargassum* spp. Several studies have shown that the nutrients, particularly nitrogenized forms, are essential for algae growth (Troell et al., 1997; Chow et al., 2001; Marinho-Soriano et al., 2002; Yang et al., 2005). The macroalgae can use the nutrients in the water to increase photosynthetic ability and then convert them into productivity (Marinho-Soriano et al., 2009).

Global production of macroalgae occurs mainly in marine and brackish waters (Muñoz et al., 2011; Food and Agriculture Organization of the United Nations (FAO), 2012). The volume of farmed macroalgae production has increased from 3.8 million tons in 1990 to 19 million tons in 2010 and 26.1 million tons in 2013. However, only 4.5% of the total macroalgae production in 2010 came from cultivation; the remaining production was harvested from the wild (FAO, 2012). According to FAO (2014), there are a few dominant macroalgae species that constitute 98.9% of the world production: *Laminaria japonica*, *Eucheuma* sp., *Gracilaria* sp., *Porphyra* sp., and *Undaria pinnatifida*. China, which produces the largest amount of cultivated macroalgae, manufactured one million tons in 2013 (FAO, 2014). *Sargassum* still remains a small percentage of total macroalgae production and is considerably lower than its potential production and market demand.

To date, the *Sargassum* species have been proposed as a potential candidate for marine culture in large scale macroalgae farms for bioethanol, biohythane, and biofuel products (Gao and Hua, 1997; Pang et al., 2009; Costa et al., 2015; Oliveira et al., 2015; Shao et al., 2015; Soto et al.,

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2015; Terasaki et al., 2016). However, there are a limited number of studies on the cultivation conditions and ecology of *S. spinuligerum* (Hanisak and Samuel, 1987, Tin et al., 2016). The objectives of this study were to investigate the effect of different commercially available fertilizers and different quantities of ISB on the growth rate and nutrient uptake of *S. spinuligerum* in outdoor cultivation conditions.

2. Materials and methods

2.1. Plant collection and preparation

S. spinuligerum was collected from Point Peron, Shoalwater Islands Marine Park, WA (32°16.32′S, 115°41.25′E) in September 2013. Specimens were collected using free-diving techniques. All holdfasts of macroalgae were collected, immediately transferred into sampling buckets with fresh seawater, and then relocated to the Curtin Aquatic Research Laboratory (CARL) within 2 h of collection.

In the CARL, the samples were well rinsed with filtered seawater to remove all epiphytes such as diatoms, red algae (*Gracilaria* sp.), detritus, and any decapods or snails attached to the receptacles (Hanson, 1977; Muñoz and Fotedar, 2010). The samples were then stored in the stocking tanks for acclimation with similar salinity to that of seawater (35 psu) for 2 days. Four ISBs were used for culture as four different treatments from ISB_1 to ISB_4 with mean values of 15.35 ± 1.05, 18.77 ± 1.04, 28.07 ± 1.37, and 40.91 ± 2.25 g (n = 48, F = 51.56, P < 0.05), respectively. The selected ISBs were based on the initial preliminary trials.

2.2. Experiment protocol and enriched cultivation media

2.2.1. Cultivation nutrient medium

The seawater was collected from Hillary Harbor, WA (31°49′35S, 115°44.16′E) and then transferred to the CARL by truck. Seawater was filtered through a 5-µm filter to eliminate phytoplankton and organic suspended materials. Sixteen 113-L round plastic tanks (40 × 60∅ cm) were used as containers with aeration. The cultured nutrient media was randomly enriched at the experiment tanks with 1325 mL of three dissolved commercial available fertilizers Hortico (Yates, Padstow, NSW, Australia), Seasol (Scotts Miracle-Gro Company, Marysville, OH, USA), and Aquasol (Yates). The initial nutrient concentration of cultured media was based on established requirements for *Gracilaria cliftonii* (Kumar et al., 2011).

2.2.2. Experimental procedure

The experiment was conducted in the CARL in Perth, Australia, from 21 September to 6 November 2013. These cultured tanks were maintained at outdoor field trial area conditions with an average temperature of 22 °C, salinity at 36 psu, and natural sunlight with a 12 h light and dark cycle (sunrise/sunset: 0620/1805) (Hanisak and Samuel, 1987).

2.3. Data collection and analysis

2.3.1. Environmental parameters

Water temperature (WT), salinity, dissolved oxygen (DO), conductivity, and pH were measured weekly. WT was automatically monitored and recorded every hour using a submerged HOBO Pendant Temperature Data Logger 64K (OneTemp, Adelaide, SA, Australia) for 7 weeks. Salinity was maintained between 35 and 36 psu, and regularly checked by a handheld refractometer (RHS-10ATC; Atago, Kobe, Japan). DO was measured with a DO meter (YSI 55; Perth Scientific, Perth, WA, Australia). Conductivity and pH were determined with a digital pH meter (Cyber scan, pH 300; Eutech Instruments, Singapore).

2.3.2. Nutrient concentration

Nutrients in the cultivation media were analyzed fortnightly. Nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) were determined using AQUANAL test kits (Sigma-Aldrich, Munich, Germany): nitrate (NO_3^-) 1–50 mg/L, nitrite (NO_2^-) 0.005–0.1 mg/L, and phosphate (PO_4^{3-}) 0.02–0.4 mg/L, respectively.

2.3.3. Specific growth rate

The fresh weight, length, and number of lateral branches were measured at the commencement, the middle (3 weeks), and the end of the experiment (after 7 weeks). Then samples were dried at 95 °C for 48 h in an oven to obtain dry weights. From the initial time, the specific growth rate (SGR, %) was calculated using Eq. (1) (Hanisak and Samuel, 1987; Mai et al., 2010).

$$SGR = (100 \ln(W_t/W_o))t - 1, \quad (1)$$

where SGR is specific growth rate (% g/day), W_o is ISB fresh weight, W_t is the final weight of macroalgae after the experiment, and t is cultivation time in days.

Main and lateral thalli growth rates (%/day) were measured to determine apical growth rate using Eq. (2):

$$AGR = (\ln(L_f) - \ln(L_i))/t \times 100, \quad (2)$$

where AGR is apical growth rate of main and lateral thalli (% per day), L_f is final length of the thalli, L_i is initial length of the thalli, and t is cultivation time in days.

2.3.4. Nutrient uptake rate

Nutrient uptake rate (NUR) was calculated using Eq. (3) (Ryther et al., 1981; Fan et al., 2014).

$$NUR = (C_0 - C_t)V/DW/t, \quad (3)$$

where NUR is the nutrient uptake rate of *Sargassum* (mg nutrient/g *DW*/h); C_0 and C_t are the nutrient concentrations at the beginning and at time (t) of the experiment (mg/L), respectively; t is time between two measures (days); V is the water volume (L); and *DW* is the *Sargassum* dry weight (g).

2.4. Statistical analysis

The data were statistically analyzed using IBM SPSS Statistics 22 for Windows (IBM Corporation, Chicago, IL, USA) and Microsoft Excel 2013. One-way analysis of

variance (ANOVA) was employed to test the significance of the variance between treatments. The multiple comparisons, least significant difference (LSD) post hoc test was also implemented to test for statistical significance among treatments. The statistical significance level was set at 0.05, and the results are presented as means ± SE (standard error) unless otherwise stated.

3. Results

3.1. Water quality parameters

The water quality was fairly constant during the experimental period. The averaged DO, WT, and pH were 6.13 ± 0.12, 21.5 ± 0.3 °C, and 8.28 ± 0.04, respectively. Conductivity and salinity were –93.5 ± 1.87 and 36.2 ± 0.8 psu, respectively. There were significant differences during the experimental period in terms of DO (df = 107, F = 5.08, P = 0.002), pH (df = 108, F = 22.67, P < 0.05), and conductivity (df = 107, F = 16.15, P < 0.05). However, there were no significant differences in temperature and salinity during the experiment period (Table 1).

3.2. The effect of cultured nutrient media on the specific growth rate

The SGR of *S. spinuligerum* was influenced by cultured nutrient media. *S. spinuligerum* had the highest growth rate in all cultured nutrient media during the first three weeks of the experiment. Of those, the SGR of Hortico treatment reached the highest percentage value at 1.61 ± 0.38% per day. Meanwhile, the main thallus reached the highest value in the Aquasol treatment with a value of 0.82 ± 0.16% per day. There were significant (P < 0.05) differences between the cultured nutrient media treatments regarding main thallus growth. However, there was no statistical difference in the SGR and lateral thallus of *S. spinuligerum* during the cultivated period.

At the end of the experimental period (after 7 weeks), there were significant differences in the growth rate of *S. spinuligerum* main thallus (df = 58, F = 5.41, P = 0.002). However, there was no difference between the cultured nutrient media treatments in the SGR and lateral thallus. The average growth rate of their highest SGR in the Aquasol reached 1.31 ± 0.31% per day and the lowest value in Hortico treatment reached 0.54 ± 0.09% per day (Figure 1a). The main and lateral thalli reached the highest value in the Aquasol treatment with a value of 0.59 ± 0.11% and 1.46 ± 0.39% per day, respectively (Figures 1b and 1c).

3.3. The effect of initial stocking biomass on the specific growth rate

There was a significant difference (P < 0.05) between ISB treatments with respect to SGR of *S. spinuligerum*, and SGR reached the highest value in the ISB_4 treatment: 1.70 ± 0.26 (% per day) after 3 weeks of cultivation (Figure 2a). Meanwhile, the main and lateral thalli reached the highest values in the IBS_1 treatment: 0.75 ± 0.16 and 2.37 ± 0.84 (% per day), respectively (Figures 2b and 2c). However, there was no significant difference (P > 0.05) between ISB treatments in main and lateral thalli growth rates.

After 7 weeks of culture, the SGR and main thallus were significantly different (P < 0.05) among the ISB treatments. The entire SGR, main, and lateral thallus reached the highest value in the ISB_1 treatment at 1.54 ± 0.19, 0.49 ± 0.11, and 1.19 ± 0.30 (% per day) (Figure 2).

3.4. The effect of different nutrient supplies on nutrient uptake rate

During the first two weeks of the experiment, there was a significant difference (P < 0.05) in nitrate concentrations between the cultured nutrient media treatments but no statistical differences (P > 0.05) in phosphate and nitrite among treatments.

There were significant differences (P < 0.05) in NO_3^- uptake rates between the cultured nutrient media treatments over the cultivation period. For PO_4^{3-}, there were significant differences between the cultured nutrient

Table 1. Overall means of water parameters of different nutrient media during a 48-day trial.

Parameters	Enriched nutrient treatments/cultured media				P-value
	Seawater	Hortico	Seasol	Aquasol	
DO (mg/L)	6.13 ± 0.12^{a}	6.34 ± 0.11^{a}	6.83 ± 0.13^{b}	6.46 ± 0.14^{a}	0.002
Temperature (°C)	21.5 ± 0.3^{a}	21.6 ± 0.3^{a}	22.1 ± 0.3^{a}	21.2 ± 0.3^{a}	0.194
pH	8.28 ± 0.035^{c}	8.25 ± 0.03^{c}	8.69 ± 0.04^{a}	8.41 ± 0.05^{b}	<0.005
Conductivity	–93.54 ± 1.87^{cb}	–89.23 ± 3.38^{c}	–114.10 ± 2.38^{a}	–100.11 ± 2.85^{cb}	<0.005
Salinity (psu)	36.2 ± 0.8^{a}	36.8 ± 0.9^{a}	35.5 ± 0.9^{a}	36.6 ± 0.9^{a}	0.697

Values represent mean ± SE (standard error) of three replicates per treatment.
Same alphabetical superscript letters (a, b, c) in the same row are not significantly different at the P = 0.05 level using the LSD test.

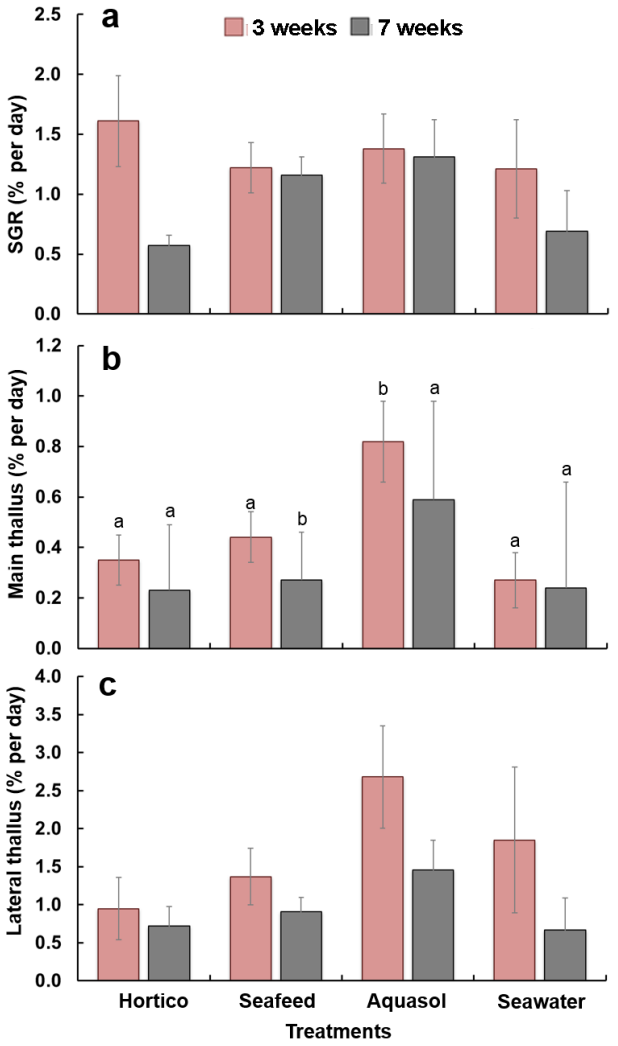

Figure 1. Specific growth rate (SGR), apical growth rate from main (Main), and lateral branches (Lateral) between cultured nutrient media treatments (mean ± SE) in outdoor cultivation conditions. (a) SGR after 3 and 7 weeks of cultivation (% per day); (b) main thallus growth rate (% per day); and (c) lateral thallus growth rate (% per day). Same alphabetical superscript letters (a, b) in the same column (comparisons among cultured nutrient media treatments) are not different at P = 0.05 level using the LSD test.

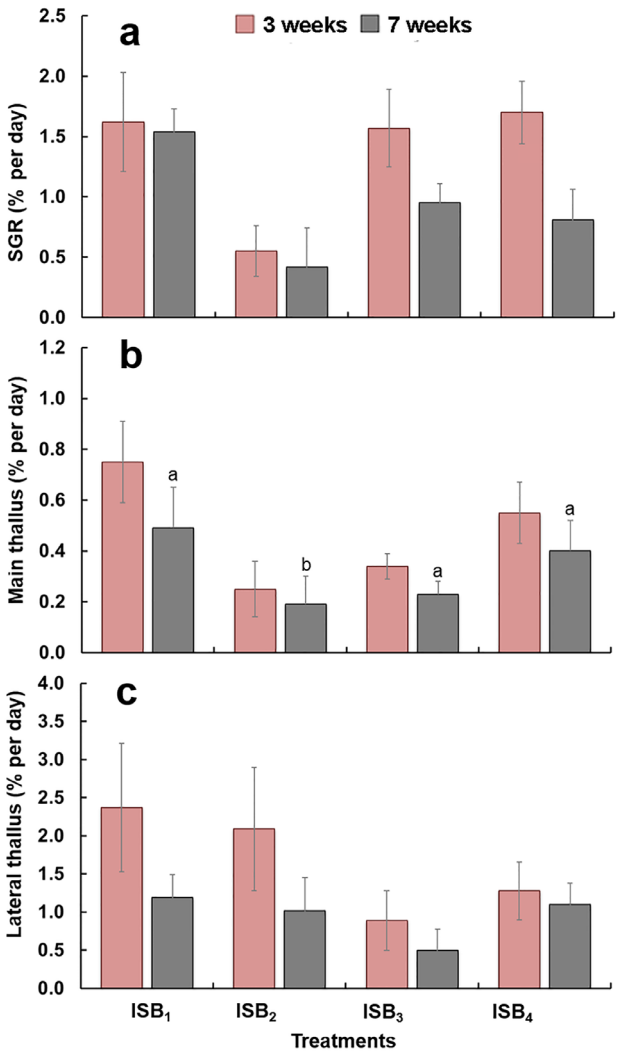

Figure 2. Specific growth rate (SGR), apical growth rate from main (Main), and lateral branches (Lateral) between ISB treatments (mean ± SE) in outdoor cultivation conditions. ISB_1 equivalent with an averaged value of 15.35 g; ISB_2 equivalent with an averaged value of 18.77 g; ISB_3 equivalent with an averaged value of 28.07 g; and ISB_4 equivalent with an averaged value of 40.91 g. (a) SGR after 3 and 7 weeks of cultivation (% per day); (b) main thallus growth rate (% per day); and (c) lateral thallus growth rate (% per day). Same alphabetical superscript letters (a, b) in the same column (comparisons among ISB treatments) are not different at P = 0.05 using the LSD test.

media treatments after the first, second, and fourth weeks, and there was no significant difference in PO_4^{3-} uptake rate after 7 weeks of cultivation (Figure 3; Table 2).

4. Discussion

The results of the present study indicate that *S. spinuligerum* could be grown under outdoor conditions with WT ranging from to 25.3 °C (average 21.5 ± 0.3 °C), salinity at 36.2 ± 0.8 psu, pH ranging from 8.25 to 8.69, with the optimum ISB at 15.35 ± 1.05 g per 113 L. Regardless of the cultured nutrient media treatments, ISB_1 (approximately 15 g) had the highest value for *S. spinuligerum* growth. Aquasol resulted in the highest values of SGR, main, and lateral thalli after 7 weeks of cultivation. This combination had the same pattern and results at combination number

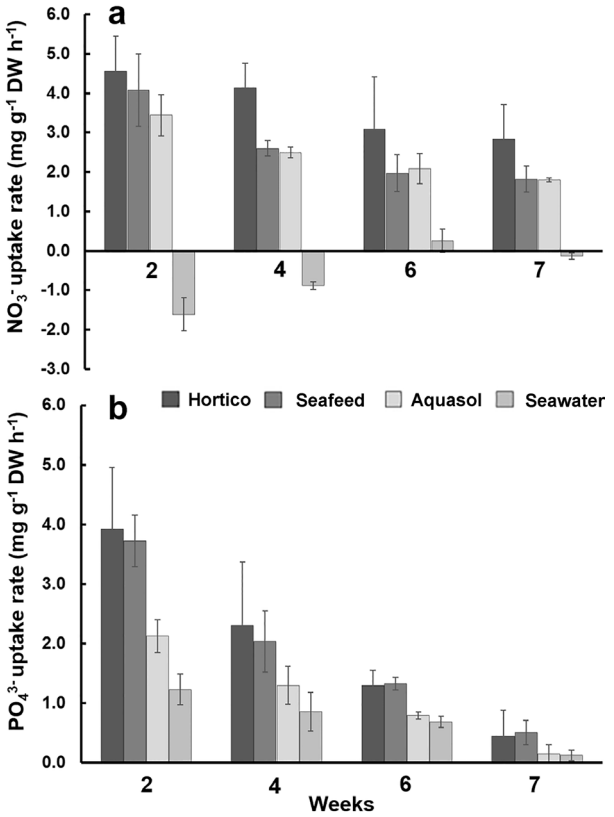

Figure 3. Change in NO_3^- and PO_4^{3-} uptake rates under different nutrient media of *Sargassum spinuligerum* at different treatments and over the different cultivation times. Values are means ± SE (n = 4).

nine when we combined ISB_1 and Aquasol (Table 3). There was a significant difference ($P < 0.05$) between ISB treatments on the SGR of *S. spinuligerum,* and SGR was highest in the ISB_4 treatment at 1.70 ± 0.26 (% per day) after 3 weeks of cultivation. The highest SGR in all of the study experiments was 2.54 ± 0.28 (% per day), which is similar to results found in a study on *S. baccularia* in the central Great Barrier Reef, Australia, where the maximum value reached 2.71 ± 20.76 (% per day) under a continuous nutrient supply (Schaffelke and Klumpp, 1998). Our results were also similar to the SGR of *S. horneri,* with values of 2.7 ± 0.75 (% per day, in length) and 3.28 ± 1.03 (% per day, in fresh weight) after being cultivated for 25 days in an enriched nutrient media of 10 mg KNO_3 + 1 mg KH_2PO_4 (Pang et al., 2009).

Conversely, our SGR results were lower than those of another previous study on integrated macroalgae (*Sargassum*) - prawn culture system (ISP) (Mai et al., 2010). The ISP results showed that after 30 days of cultivation, *Sargassum* sp. grew rapidly in a monoculture medium and integrated with prawn as 5.70 ± 0.82 and 0.74 ± 3.16 (% per day), respectively (Mai et al., 2010). Meanwhile, our SGR results on *S. spinuligerum* were much higher than other published studies on various *Sargassum* species in Florida, USA: *S. cymosum* (0.094 ± 0.011 % per day), *S. filipendula* (0.107 ± 0.003 % per day), *S. fluitans* (0.109 ± 0.003 % per day), *S. natans* (0.073 ± 0.005 % per day), *S. polyceratium* (0.078 ± 0.015 % per day), and *S. pteropleuron* (0.112 ± 0.008 % per day) (Hanisak and Samuel, 1987). One possible explanation for the differences in SGR from the previous studies might relate to different cultural conditions and nutrient media (Pickering et al., 1993; Guimaraens, 1999).

To assess the nutrient uptake ability of *S. spinuligerum*, in this study, we reserved the cultivation media from the initial cultivation until the end of the experiment. During the experiment period, we only removed and adjusted salinity by adding fresh water into the tanks. It is thought that different species not only have different growth rates and nutrient requirements but also depend on the cultivation media exchange scheme of the experiment (Pedersen and Borum, 1996). In a study on *S. baccularia* from the Great Barrier Reef in a continuous flow culture system, the growth rate reached the highest values with supplied nutrient concentrations at 208 mg/L and 29.4 mg/L for N–NH_4 and P–PO_4, respectively. However, the growth rate of this species decreased when the nutrient concentration increased (Schaffelke and Klumpp, 1998). N and P concentrations in *S. baccularia* tissue increased when nutrient concentrations increased, but tissue N and P concentrations became saturated when N and P reached around 2.5% and 0.22 % (dry weight), respectively. However, the nutrient concentration of this study was lower than a study on *S. horneri* using 10 mg of KNO_3/L and 1 mg of KH_2PO_4/L. The cultivation media was renewed once every two days (Pang et al., 2009). While previous studies have been conducted on the nutrient requirements of macroalgal, such as *Lamina saccharina*, *Enteromorpha intestinalis* (Kamer and Fong, 2001), and *S. baccularia* (Schaffelke and Klumpp 1998) the cultivation media was changed either weekly or once every two weeks. For instance, the red algae *Gracilaria tikvahiae* can uptake ammonium–nitrogen relatively quickly and can increase the nitrogen content in the tissue within 8 h or less (Ryther et al., 1981). After a 4-week culture, the nutrient uptake capacity of *G. birdiae* for PO_4^{3-}, NH_4^+, and NO_3^- decreases by 93.5%, 34%, and 100%, respectively (Marinho-Soriano et al., 2009). Determining the nutrient uptake and photosynthesis of green algae, *Ulva prolifera*, under different conditions showed that there was a limited nitrate uptake, but an increase in phosphorus. The N/P or NO_3^-/NH_4^+ ratio affects the uptake rate, and at an N/P ratio of 7.5, *U. prolifera* achieves the highest N/P uptake rates (Guimaraens, 1999). However, there are a limited number

Table 2. Mean ± SE up-take rate of NO_3^- and PO_4^{3-} of *Sargassum spinuligerum* in different nutrient media for seven cultivation weeks (n = 4).

Media	NO_3^- (mg/L)				PO_4^{3-} (mg/L)			
	2 weeks	4 weeks	6 weeks	7 weeks	2 weeks	4 weeks	6 weeks	7 weeks
Hortico	4.6 ± 0.89[b]	4.1 ± 0.92[b]	3.1 ± 0.52[a]	2.8 ± 0.42[a]	3.9 ± 1.04[b]	2.3 ± 0.43[a]	1.3 ± 0.28[a]	0.4 ± 0.26[a]
Seasol	4.1 ± 0.61[b]	2.6 ± 0.19[b]	2.0 ± 0.14[b]	1.8 ± 0.10[b]	3.7 ± 1.06[b]	2.0 ± 0.51[a]	1.3 ± 0.32[a]	0.5 ± 0.32[a]
Aquasol	3.4 ± 1.33[b]	2.5 ± 0.46[b]	2.1 ± 0.38[ab]	1.8 ± 0.29[b]	2.1 ± 0.25[b]	1.3 ± 0.10[b]	0.8 ± 0.06[a]	0.1 ± 0.09[a]
Seawater	−1.6 ± 0.88[a]	−0.9 ± 0.33[a]	0.3 ± 0.05[c]	−0.1 ± 0.08[c]	1.2 ± 0.44[a]	0.9 ± 0.20[ab]	0.7 ± 0.15[a]	0.1 ± 0.09[a]

Same alphabetical superscript letters (a, b, c) in the same column are not significantly different at P = 0.05 level using the LSD test.

Table 3. Specific growth rate of *Sargassum spinuligerum* under different combinations of initial stocking biomasses and nutrient media (mean ± SE). Negative growth rate presents mortality.

Combination number	Nutrients	Biomass (g)	SGR	Main	Lateral
1	Hortico	15	0.88 ± 0.09	0.35 ± 0.15	1.20 ± 0.77
2	Hortico	20	0.42 ± 0.08	0.26 ± 0.09	0.45 ± 0.32
3	Hortico	30	0.53 ± 0.24	0.17 ± 0.04	0.28 ± 0.11
4	Hortico	40	0.46 ± 0.18	0.25 ± 0.09	1.00 ± 0.74
5	Seasol	15	1.63 ± 0.27	0.29 ± 0.08	0.70 ± 0.07
6	Seasol	20	0.87 ± 0.09	0.26 ± 0.13	0.87 ± 0.13
7	Seasol	30	0.68 ± 0.25	0.22 ± 0.09	0.82 ± 0.09
8	Seasol	40	1.46 ± 0.31	0.30 ± 0.16	1.26 ± 0.16
9	Aquasol	15	2.54 ± 0.28	1.08 ± 0.22	1.25 ± 0.42
10	Aquasol	20	1.76 ± 0.27	0.29 ± 0.09	2.51 ± 0.80
11	Aquasol	30	1.28 ± 0.35	0.33 ± 0.06	0.71 ± 0.96
12	Aquasol	40	−0.35 ± 0.43	0.68 ± 0.14	1.35 ± 0.94
13	Seawater	15	1.12 ± 0.25	0.26 ± 0.10	1.62 ± 0.92
14	Seawater	20	−1.34 ± 0.48	−0.28 ± 0.00	−1.97 ± 0.00
15	Seawater	30	1.31 ± 0.32	0.20 ± 0.13	0.20 ± 0.38
16	Seawater	40	1.65 ± 0.19	0.41 ± 0.06	0.87 ± 0.53

of studies on the optimum ISB required, nutrient uptake, and optimum cultivation environment of *S. spinuligerum* under laboratory conditions (Hanisak and Samuel, 1987).

This study indicated that the main thallus reached the highest value in the Aquasol treatment at 0.82% ± 0.16% per day. The different cultivation media significantly influenced the growth rate of *S. spinuligerum* during the first three weeks of the experiment. There was a statistical difference between cultivation media treatments on main thallus growth ($P < 0.05$). Our experimental results showed that the P uptake rate steadily decreased over cultivation time, and the concentration of PO_4^{3-} in the cultivate media was reduced to 38.9% and 59.6% after the fourth and sixth weeks, respectively. After the six weeks of cultivation, PO_4^{3-} concentration began to increase in all the experiments due to the mortality of *Sargassum* thalli. Then PO_4^{3-} was released back into the cultivation media, which created a conducive environment for the opportunistic fast-growing *Ulva* sp. (Fan et al., 2014), *Porphyra* sp. (Israel et al., 1999; Liu et al., 2010), *Chaetomorpha linum* (Xu and Lin, 2008), and *Enteromorpha intestinalis* (Kamer and Fong, 2001) attached to the *Sargassum* thallus. In the experiments with lower growth rates or thalli mortality, the growth of *Porphyra* sp. algae was higher than in the other experiments. In comparison with the fast-growing macroalgae species, *Ulva* sp., *Porphyra* sp., and *Enteromorpha* sp. have N

demand per biomass that is 30 times higher than in the slow-growing *Sargassum* species. The reason is that the growth rate of the fast-growing species is ten times faster and their N requirements are three times higher than in the slow-growing species (Pedersen and Borum, 1996).

In conclusion, the different cultivation media significantly influenced growth rates of *S. spinuligerum* in both the SGR and NUR. The study indicated that *S. spinuligerum* could be cultivated for biomass in outdoor conditions with the optimum ISB at 15.35 ± 1.05 g and enriched with Aquasol, which contributed to a higher SGR than other tested commercial fertilizers. Aquasol is recommended to enhance the SGR of *Sargassum* cultivation. Further studies could investigate the growth ability in coastal environments where the effects of river flow could cause eutrophication. Additional research may be required on the physiological response of *S. spinuligerum* when cultivated under changing key environmental parameters such as increasing seawater temperature and ocean acidification.

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References

Costa JC, Oliveira JV, Pereira MA, Alves MM, Abreu AA (2015). Biohythane production from marine macroalgae *Sargassum* sp. coupling dark fermentation and anaerobic digestion. Bioresource Technolog 190: 251-256.

Chow F, Macchiavello J, Santa CS, Fonck E, Olivares J (2001). Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariaceae) as a biofilter in the depuration of effluents from tank cultures of fish, oysters, and sea urchins. J World Aquac Soc 32: 215-220.

Fan X, Xu D, Wang Y, Zhang X, Cao S, Mou S, Ye N (2014). The effect of nutrient concentrations, nutrient ratios and temperature on photosynthesis and nutrient uptake by *Ulva prolifera:* implications for the explosion in green tides. J Appl Phycol 26: 537-544.

FAO (2012). The state of world fisheries and aquaculture. Rome, Italy: FAO Fisheries and Aquaculture Department.

FAO (2014). The state of world fisheries and aquaculture – Opportunities and challenges. Rome, Italy: FAO Fisheries and Aquaculture Department .

Friedlander M, Ben-Amotz A (1991). The effect of outdoor culture conditions on growth and epiphytes of *Gracilaria conferta*. Aqua Bot 39: 315-333.

Gao K, Hua W (1997). In situ growth rates of *Sargassum horneri* (Fucales, Phaeophyta). Phycol Res 45: 55-57.

Guimaraens M (1999). The influence of environmental factors on the seasonal dynamics of *Ulva* sp. and *Sargassum* sp. in the Cabo Frio upwelling region of Brazil. PhD, University of Miami, Miami, FL, USA.

Guiry MD, Guiry GM (2014). AlgaeBase. National University of Ireland, Galway http://www.algaebase.org. Accessed 29 September 2014.

Hanisak MD, Samuel M (1987). Growth rates in culture of several species of *Sargassum* from Florida, USA. Hydrobiologia 151-152: 399-404.

Hanson RB (1977). Pelagic *Sargassum* community metabolism: Carbon and nitrogen. J Exper Mar Bio Eco 29: 107-118.

Huisman JM, Walker DI (1990). A catalogue of the marine plants of Rottnest Island, Western Australia, with notes on their distribution and biogeography. Kingia 1: 349-459.

Mai H, Fotedar R, Fewtrell J (2010). Evaluation of *Sargassum* sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) culture system. Aquaculture 310: 91-98.

Israel A, Katz S, Dubinsky Z, Merrill J, Friedlander M (1999). Photosynthetic inorganic carbon utilization and growth of *Porphyra linearis* (Rhodophyta). J Appl Phycol 11: 447-453.

Muñoz J, Kumar V, Fotedar R (2011). Seaweed culture with special reference to Latin America. In: Fotedar R, Phillips B, editors. Recent Advances and New Species in Aquaculture. Melbourne, VIC, Australia: Wiley-Blackwell, pp. 252-276.

Kamer K, Fong P (2001). Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis.* Mar Ecol Prog Ser 218: 87-93.

Keusgen M, Glombitza KW (1997). Pseudofuhalols from the brown alga *Sargassum spinuligerum.* Phytochemistry 46: 1403-1415.

Kumar V, Fotedar R, Longbottom S (2011). Effect of nutrient media on the growth, physicochemical and agar properties of *Gracilaria cliftonii* cultured in ocean and inland saline water. J Appl Aqua 23: 317-328.

Liu D, Keesing JK, Dong Z, Zhen Y, Di B, Shi Y, Fearns P, Shi P (2010). Recurrence of the world's largest green-tide in 2009 in Yellow Sea, China: *Porphyra yezoensis* aquaculture rafts confirmed as nursery for macroalgal blooms. Mar Pol Bul 60: 1423-1432.

Marinho-Soriano E, Morales C, Moreira WC (2002). Cultivation of *Gracilaria* (Rhodophyta) in shrimp pond effluents in Brazil. Aquacult Res 33: 1081-1086.

Marinho-Soriano E, Nunes SO, Carneiro MAA, Pereira DC (2009). Nutrients' removal from aquaculture wastewater using the macroalgae *Gracilaria birdiae*. Biomass Bioenerg 33: 327-331.

Muñoz J, Fotedar R (2010). Epiphytism of *Gracilaria cliftonii* (Withell, Millar & Kraft) from Western Australia. J Appl Phycol 22: 371-379.

Murase NO, Kito HI, Mizukami YU, Maegawa MI (2000). Productivity of a *Sargassum macrocarpum* (Fucales, Phaeophyta) population in Fukawa Bay, Sea of Japan. Fis Sci 66: 270-277.

Oliveira JV, Alves MM, Costa JC (2015). Optimization of biogas production from *Sargassum* sp. using a design of experiments to assess the co-digestion with glycerol and waste frying oil. Bioresource Technol 175: 480-485.

Pang S, Liu F, Shan T, Gao S, Zhang Z (2009). Cultivation of the brown alga *Sargassum horneri*: sexual reproduction and seedling production in tank culture under reduced solar irradiance in ambient temperature. J Appl Phycol 21: 413-422.

Pedersen MF, Borum J (1996). Nutrient control of algal growth in estuarine waters - Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar Ecol Prog Ser 142: 261-272.

Pickering TD, Sladden VH, Furneaux RH, Hemmingson JA, Redfearn P (1993). Comparison of growth rate in culture, dry matter content, agar content and agar quality of two New Zealand red seaweeds, *Gracilaria chilensis* Bird, McLachlanet Oliveira and *Gracilaria truncata* Kraft. J Appl Phycol 5: 85-91.

Ryther JH, Corwin N, DeBusk TA, Williams LD (1981). Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26: 107-115.

Schaffelke B, Klumpp DW (1998). Nutrient-limited growth of the coral reef macroalga *Sargassum baccularia* and experimental growth enhancement by nutrient addition in continuous flow culture. Mar Ecol Prog Ser 164: 199-410.

Shao P, Liu J, Chen X, Fang Z, Sun P (2015). Structural features and antitumor activity of a purified polysaccharide extracted from *Sargassum horneri.* Int J Biol Macromol 73: 124-130.

Soto M, Vázquez MA, de Vega A, Vilariño JM, Fernández G, de Vicente MES (2015). Methane potential and anaerobic treatment feasibility of *Sargassum muticum*. Bioresource Technolog 189: 53-61.

Terasaki M, Chikara K, Atsushi I, Hiroko K, Bhaskar N, Masashi H, Kazuo M (2016). Spatial and seasonal variations in the biofunctional lipid substances (fucoxanthin and fucosterol) of the laboratory-grown edible Japanese seaweed (*Sargassum horneri* Turner) cultured in the open sea. Saudi J Biol Sci doi: 10.1016/j.sjbs.2016.01.009 (in press).

Tin CH, Anthony JC, Ravi KF, Michael JO, Michael WL, Shovonlal R (2016). Seasonal changes in water quality and *Sargassum* biomass in southwest Australia. Mar Ecol Prog Ser 551: 63-79.

Troell M, Halling C, Nilsson A, Buschmann AH, Kautsky N, Kautsky L (1997). Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Bangiophyceae) and salmon cages for reduced environmental impact and increased economic output. Aquaculture 156: 45-61.

Tseng CK, Yoshida T, Chiang YM (1985). East Asiatic species of *Sargassum* subgenus *Bactrophycus* J. Agardh (Sargassaceae, Fucales), with keys to the sections and species In: Abbott IA, Norris JN, editors. Taxonomy of Economic Seaweeds with Reference to Some Pacific and Caribbean Species. Eureka, CA, USA: California Sea Grant Program, pp. 1-14.

Xu Y, Lin J (2008). Effect of temperature, salinity, and light intensity on the growth of the Green macroalga, *Chaetomorpha linum.* J World Aqua Soc 39: 847-851.

Yang H, Zhou Y, Mao Y, Li X, Liu Y, Zhang F (2005). Growth characters and photosynthetic capacity of *Gracilaria lemaneiformis* as a biofilter in a shellfish farming area in Sanggou Bay, China. J Appl Phycol 17: 199-206.