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Effects of water levels and water exchange rates on growth and production of sea grape *Caulerpa lentillifera* J. Agardh 1837

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Abstract
Study on cultivation of sea grape (*Caulerpa lentiliifera*) in the 1 m^2- lined ponds, a 2×2 factorial experiment with two water levels (40 and 80 cm) and two water exchange rate (25% and 50%/week) was randomly designed in triplicate. Sea grape was cultivated at salinity of 30 ppt with continuous aeration, initial stocking rate of 1 kg /m^2 and fishmeal was used as nutrient source for sea grape, and cultivation lasted for 60 days. Results showed that a significant interaction effect ($p<0.05$) between water level water exchange rate was only observed for the growth rate of sea grape after 45 and 60 days of cultivation. Water level significantly influenced ($p<0.05$) frond (edible portions) production of sea grape, while the effect of water exchange rate on frond production was not significant ($p>0.05$). Water level and water exchange rate did not affect morphometric frond properties (commercial frond length, ramuli density and ramuli size) and sensory properties. It can be suggested that cultivation of sea grape *C. lentillifera* at water level of 80 cm combined with 50% water exchange per week could be the appropriate factors to obtain highest growth rate (1.95%/day) and frond production (12.21 kg/m^2).

Keywords: Water level, water exchange rate, growth rate, frond production

1. Introduction

The edible sea grape (*Caulerpa lentillifera* J. Agardh 1837) is rich in protein, minerals, dietary fibers, vitamins, unsaturated fatty acids as well as bioactive compounds that act as anti-cancer, anti-oxidative, anti-diabetic, and also help with cholesterol reduction, prevents cardiovascular diseases (Matanjun *et al*., 2010; Saito *et al*., 2010; Paul *et al*., 2014; Sharma and Rhyu 2014) [13, 16, 15, 17]. Therefore, this seaweed has been considered to be beneficial in various help issues and called green caviar, one of the favored species of *Caulerpa* due to its soft, succulent texture and refreshing taste. It is a popular seafood delicacy eaten as raw dip in vinegar or used in fresh salads with other seafood and vegetables (Paul *et al*., 2014; Chen *et al*., 2019) [15, 2]. Sea grape *C. lentillifera* is naturally distributed in tropical and subtropical regions, such as Southeast Asia, Japan, Taiwan and Oceania (Shokita 1991; Paul *et al*., 2014) [18, 15]. This species has high economic value and high biomass production potential in monoculture, and has been cultivated in some Asian countries in for a long time (Shokita 1991; FAO 2003; Paul *et al*., 2014; Chen *et al*., 2019) [18, 6, 15, 2].

In Vietnam, sea grape *C. lentillifera* was transplanted from Okinawa island, Japan, successfully cultivated in the central areas in recent years and has been considered high economic product (Dai *et al*., 2006, 2009; Minh *et al*., 2019) [3, 4, 14]. This seaweed has been commonly cultivated in ponds, open lagoons or in cages, but these methods are greatly affected by the weather conditions as they bring low productivity and uncontrolled quality of product. Previous studies found that sea grape *Caulerpa* spp. cultivated in tanks gave high productivity, free from pollution sources and the products of this seaweed satisfy the criteria for food hygiene, safety and high nutrition (Paul *et al*., 2014; Zuldin *et al*., 2018) [15, 23]. Growth rate and production of *C. lentillifera* were not only affected by environmental conditions such as salinity, temperature, light intensity (Shokita 1991, Wang 2011; Guo *et al*., 2015a; Chen *et al*., 2019) [18, 21, 8, 2] but also by technical factors and culture conditions (Paul *et al*., 2014; Wang et al., 2017; Minh *et al*., 2019) [15, 20, 14]. The aim of this study was to determine the suitable water level and water exchange rate for cultivating sea grape.

C. lentillifera in tank. These works could provide practical information for cultivating this species at commercial scale to provide a healthy vegetable for human consumption.

2. Materials and Methods

2.1. Experimental materials

Sea grape *C. lentillifera* stocks were purchased from a private *Caulerpa* farm in Ninh Thuan province, Vietnam, and acclimated to adapt to the experimental salinity (30 ppt) in a 2 m^3 tank with continuous aeration for 3 days. The healthy thalli of sea grape, which consists of creeping stolons, erect branches and rhizoids were selected for cultivation.

Kien Giang fishmeal (grade 1) contained 9.65% N and 0.84% P in dry weight basis, which was supplied from CATACO Company, Vietnam.

2.2. Experimental design and management

Experiment was performed at Vinh Chau Station (Soc Trang province) belonging to Can Tho University, Vietnam. A two-factorial design was conducted with two water depths (40 cm and 80 cm) combined with two water exchange rates (25% and 50% per week). The experiment followed a completely randomized design in triplicate ponds for a period of 60 days.

The rearing system consisting of twelve lined ponds (1 m x 1 m x 1.2 m) were set up under a transparent roof with a natural photoperiod, and provided continuous aeration. The sea grape thalli were spread over a 1 m^2 -net tray at initial biomass of 1 kg/m^2, covered with a large mesh size net to fix them in the culture tray, and then each culture tray was placed close to the pond bottom. Fishmeal was used as a nutrient source for sea grape and applied every three day; the amount of fishmeal was adjusted from 35 to 70 g/m^3 with the increase of sea grape biomass in the culture tanks (Anh *et al*., 2016) [1]. Water exchange was done weekly based on the allocated treatments.

3. Data collection

3.1. Physico-chemical water parameters

Daily water temperature and pH was recorded at 7:00 and 14:00 h using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius); the concentration of NO_3^-, NH_4^+/NH_3 (TAN) and PO_4^{3-} was monitored weekly. Water samples in the seaweed ponds were taken before water exchange and analyzed by test kit (Sera, Germany). Diurnal changes in surface PAR (light intensity) during daytime were determined every three-day using light meter (Extech EA31, Taiwan).

3.2. Growth rate and production

Biomass and growth rate of sea grape was evaluated at 15 day- intervals. Production of frond and frond properties were recorded after 45 and 60 days of cultivation. These parameters as follow:

- Total production yield of frond (erect branches with spherical tips, edible portion)
- Production yield of commercial frond size (frond length >5 cm)
- Frond proportion (percentage of fronds per total harvestable biomass)
- Morphometric characteristics of fronds such commercial frond length, ramuli density (number of spherical tips (ramuli)/cm of frond, and ramuli diameter)

Frond length was measured by a caliper and ramuli diameter was measured with a pre-calibrated microscope. To estimate the specific growth rate (SGR) of sea grape, the following formula recommended by Shokita (1991) [18].

SGR = [(log final weight − log initial weight)/days of cultivation] x 100.

3.3. Sensory evaluation of fronds (edible portions)

The sensory properties of sea grape fronds (edible portion) such as color, appearance, odour, taste and sweetness of the experimental fronds were assessed by panelists (Minh *et al*., 2019) [14]. The panel members were briefly trained about the tested products, and nine panelists assessed the sea grape frond samples in terms of color, appearance, odour, taste, sweetness and general acceptance using descriptive and 9-point Hedonic scale (1 = dislike extremely, 9 = like extremely).

3.4. Statistical analysis

A two-factor ANOVA test was used to find significant interactions between water level and water exchange rate. For all treatments, the results were analyzed statistically using a one-way analysis of variance (ANOVA) to determine the overall effect of the treatment. A Tukey test was applied to identify significant differences between the mean values at a significance level of $p<0.05$ (SPSS, version 16.0). All percentage values were normalized through an arcsine transformation prior to statistical analysis.

4. Results and Discussion

4.1. Physico-chemical water parameters

The average daily water temperature and pH in seaweed tanks fluctuated in the ranges of 26.8 - 29.9ºC and 7.9 - 8.5, respectively (Table 1). Light intensity during daytime ranged from 55 to 403 µmol photons $m^{-2}s^{-1}$, of which the highest value was observed in early afternoon (13:00 h). Several investigations have found that temperature, pH and irradiance are important environmental factors affecting growth rate of *C. lentillifera* (Dai *et al*., 2006; Wang 2011; Guo *et al*., 2015a; Chen *et al*., 2019) [3, 21, 8, 2]. In this study, temperature, pH and light intensity did not differ greatly among treatments and all were in a suitable range for *C. lentillifera* growth (Shokita 1991; Dai *et al*., 2006; Wang 2011; Chen *et al*., 2019) [18, 3, 21, 2].

Table 1: Average temperature, pH and nutrient contents during experimental period

Treatment	Temperature (ºC)		pH		TAN (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)
	7:00 h	14:00 h	7:00 h	14:00 h			
40 cm-25%	26.8±0.5	29.7±1.0	8.1±0.2	8.5±0.4	0.19±0.11	5.19±0.91	0.72±0.37
40 cm-50%	27.0±0.5	29.9±1.1	8.0±0.3	8.5±0.3	0.32±0.14	5.73±0.94	0.84±0.39
80 cm-25%	26.9±0.6	29.0±1.2	7.9±0.2	8.4±0.4	0.45±0.13	7.21±1.19	1.24±0.57
80 cm-50%	27.0±0.5	28.9±1.3	8.0±0.3	8.5±0.3	0.52±0.21	8.19±1.12	1.38±0.58
Light intensity	7:00 h	9:00 h	11:00 h	13:00 h	15:00 h	17:00 h	
(µmol photons $m^{-2}s^{-1}$)	82±12	169±68	284±70	403±109	224±50	55±19	

Data are means ± SD of three replicates.

The mean concentration of nutrients (TAN, NO_2^-, NO_3^-, and PO_4^{3-}) in the treatment with high water level (80 cm) and exchange rate (50%/week) was relatively higher than that of the low water level (40 cm) and exchange rate (25%/week). This indicated higher water level combined with high exchange rate created more nutrients available in the seaweed ponds. Like other seaweeds, sea grape *C. lentillifera* uptakes nitrogen (N) and phosphorus (P) in habitat which are essential nutrient for their development (Deraxbudsarakom *et al*., 2003; Guo *et al*., 2015b; Liu *et al*., 2016; Wang *et al*., 2017) [5, 9, 12, 17]. The appropriate concentration of nutrient for the optimal growth rate of *C. lentillifera* were 0.5 mmol/L NO_3-N, 0.1 mmol/L PO_4-P and N:P ratio of 5:1 (Guo *et al*., 2015b) [9].

4.2. Biomass and growth rate of sea grape

Figure 1 shows the changes in the average fresh biomass of *C. lentillifera* during cultivation. After 15 days of cultivation, the biomass of sea grape in all treatments increased compared to the initial weight, varying from 4.7 to 5.6 kg/m^2, and then progressively increased until day 60, of which the 80 cm treatments had much higher biomass (14.5-14-7 kg/m^2) than the ones in the 40 cm treatments (10.1-11.7 kg/m^2).

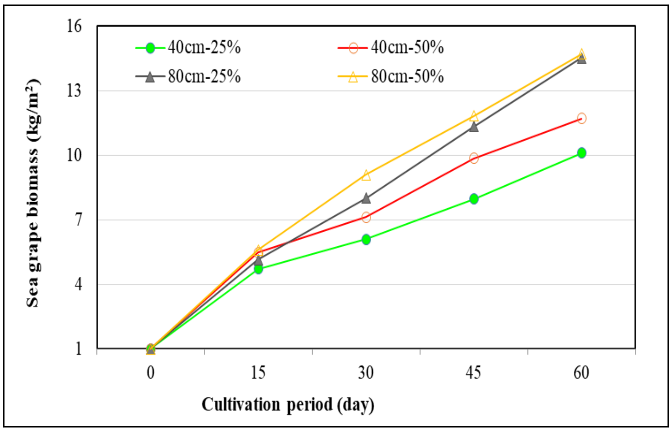

Fig 1: Change in fresh biomass of sea grape *C. lentillifera* during cultivation period

The effects of different water levels and water exchange rate on the specific growth rates (SGR) of sea grape *C. lentillifera* are presented in Table 2. According to the analysis of the water level as the main effect, the SGR of sea grape at day 15 was not affected (p>0.05) by this factor. However, water level greatly affected the SGR from day 30 onwards (p<0.001).

Table 2: Effect of water level and water exchange rate on the specific growth rate (SGR, %/day) of sea grape *C. lentillifera* during culture period

Water level	Water exchange	SGR day 15	SGR day 30	SGR day 45	SGR day 60
Individual treatment means					
40 cm	25%	4.46±0.48^a	2.60±0.17^a	2.00±0.09^a	1.68±0.03^a
40 cm	50%	4.92±0.13^a	2.84±0.11^{ab}	2.21±0.02^{ab}	1.78±0.03^b
80 cm	25%	4.74±0.14^a	3.01±0.10^{bc}	2.34±0.05^{bc}	1.94±0.04^c
80 cm	50%	4.99±0.23^a	3.19±0.07^c	2.38±0.06^c	1.95±0.02^c
One-way ANOVA: effect of water level					
40 cm		4.69±0.40^a	2.72±0.18^a	2.10±0.13^a	1.73±0.06^a
80 cm		4.87±0.21^a	3.10±0.13^b	2.36±0.05^b	1.94±0.03^b
One-way ANOVA: effect of water exchange rate					
25%		4.60±0.35^a	2.81±0.25^a	2.17±0.20^a	1.81±0.15^a
50%		4.96±0.17^a	3.02±0.21^b	2.30±0.10^b	1.87±0.10^b
ANOVA, p value					
Water level (1)		0.313	<0.001	<0.001	<0.001
Water exchange rate (2)		0.061	0.014	0.008	0.010
Two-way ANOVA interaction (1) × (2)		0.512	0.737	0.045	0.031

Means within the same column sharing different superscripts are significantly different (p<0.05)

Similarly, water exchange rate significantly influenced (p<0.05) the growth rate of sea grape from day 30 onward. A two-factor ANOVA indicated significant interaction between water level and water exchange rate for the SGR at day 45 and day 60 (p<0.05).

The average SGR at day 15 in the various treatments was no significant differences were seen (p>0.05), and ranged from 4.46-4.99%/day. At day 30, the SGR of the 80 cm-50% treatment was highest, and significantly different (p<0.05) from the 40 cm-25% and 40 cm-50% groups but insignificant difference (p>0.05) with the 80 cm-25% treatment, and similar results were observed for day 45. After 60 days of cultivation, the SGR in the 40 cm-25% treatment was lowest, and significantly different from other treatments (p<0.001). The results suggest that growth rate of sea grape decreased in the following order: 80 cm-50% and 80 cm-25%>40 cm-50%>40 cm-25%. This phenomenon could be due to sea grape cultivated at high water level combined with high water exchange rate created more nutrients available (Table 1) and more space for the development of sea grape that facilitates the growth of biomass.

Previous studies reported that water depth greatly affected the development of *C. lentillifera* (Yusuke and Ayako 2004; Tanduyan *et al*., 2006) [22, 19] and sea grape had higher growth rate when inhabited in rich nutrient water bodies (FAO 2003; Dai *et al*., 2006; Huang 2012; Liu *et al*., 2016) [6, 3, 11, 12].

Notably, the specific growth rate of sea grape in all treatments tended decreased with the culture period this could be due to higher biomass at later stage resulting in restricted living space for sea grape and shading effects that reduce vegetative propagation of the biomass.

4.3. Frond production yield

Results showed that the effect of water level on total frond production and commercial frond yield was very strong (p<0.001) but it did not influence frond proportion (p>0.05) at day 45 and day 60. Moreover, water exchange rate had a significant main effect on the total frond productions (p<0.05) while commercial frond yield and frond proportion were insignificantly affected by water exchange rate (p>0.05). A significant interaction effect (p<0.05) between water level and exchange rate was only observed for the commercial frond yield at day 60 (Table 3).

Table 3: Frond production yield (kg fresh weight/m^2) and frond proportion (%/total harvestable biomass of sea grape)

Water level	Water exchange	Total frond yield day 45	Total frond yield day 60	Commercial frond yield day 45	Commercial frond yield day 60	Frond proportion day 45	Frond proportion day 60
Individual treatment means							
40 cm	25%	6.47±0.51^a	8.20±0.53^a	1.93±0.16^a	2.50±0.27^a	81.5±1.6^a	81.2±2.3^a
40 cm	50%	8.10±0.36^b	9.37±0.45^a	2.21±0.25^a	2.94±0.13^a	82.0±1.8^a	80.1±3.9^a
80 cm	25%	9.30±0.72^c	11.97±0.59^b	3.10±0.19^b	3.45±0.10^b	81.9±2.5^a	82.4±2.2^a
80 cm	50%	9.57±0.57^c	12.20±0.36^b	3.17±0.22^b	3.41±0.17^b	81.0±0.7^a	82.9±0.8^a
One-way ANOVA: effect of water level							
40 cm		7.28±0.98^a	8.78±0.78^a	2.07±0.24^a	2.72±0.31^a	81.8±1.6^a	80.7±2.9^a
80 cm		9.43±0.60^b	12.08±0.45^b	3.14±0.19^b	3.43±0.14^b	81.5±1.7^a	82.7±1.5^a
One-way ANOVA: effect of water exchange rate							
25%		7.88±1.65^a	10.08±2.12^a	2.51±0.66^a	2.97±0.55^a	81.7±1.9^a	81.8±2.1^a
50%		8.83±0.91^b	10.78±1.59^b	2.69±0.57^a	3.17±0.29^a	81.5±1.3^a	81.5±2.9^a
ANOVA, p value							
Water level (1)		<0.001	<0.001	<0.001	<0.001	0.775	0.214
Water exchange rate (2)		0.018	0.038	0.174	0.087	0.849	0.852
Two-way ANOVA interaction (1) × (2)		0.066	0.137	0.405	0.046	0.472	0.579

Means within the same column sharing different superscripts are significantly different (p<0.05)

Total frond yield at day 45 and day 60 varied in the ranges of 6.47-9.57 kg/m^2 and 8.20-12.20 kg/m^2, respectively. The commercial frond yields varied from 1.93-3.17 kg/m^2 at day 45 and 2.50-3.41 kg/m^2 at day 60. When comparing individual treatment means the 80 cm-25% and 80 cm-50% were similar and both were significantly higher than the 40 cm-25% and 40 cm-50% treatments (p<0.001). This illustrated sea grape cultivated at higher water level enhanced frond production. However, frond proportion was similar among treatments (p>0.05), ranging from 81 to 82% at day 45, and 80.1-82.9% at day 60 (Table 3).

The study of Yusuke and Ayako (2004) [22] found that production of *C. lentillifera* was significantly affected by water depth. Paul *et al*. (2014) [15] reported that productivity of *C. lentillifera* in a 6-week period yielded on average of 2 kg/week and this species had a higher proportion of fronds to horizontal runners (stolons) and a higher density of fronds per unit area. The studies of Dai *et al*. (2006, 2009) [3, 4] confirmed that fronds are the edible portion of sea grape *C. lentillifera*. Therefore, frond proportion (percentage of frond/thallus) is the important criteria of sea grape productivity; sea grape has high proportion of frond/thallus resulting in high production. Under pond culture conditions, frond proportions varied in the range of 70-80%, which affected by the culture system and cultivation duration (Dai *et al*., 2006; 2009) [3, 4]. Other study reported that *C. lentillifera* cultivated in tanks for 2 months obtained average frond proportion of 62.64%, in which the commercial frond proportion (frown length ≥ 5 cm) was on average of 28.74% (Hoa *et al*., 2013) [10].

4.4. Morphometric frond properties

Considering morphometric frond properties (frond length, ramuli density and ramuli size), water level or water exchange rate did not statistically affect these parameters (p>0.05). In addition, there were no significant interaction effects (p>0.05) on these characteristics.

Table 4: Average frond length (cm), ramuli density* (cell/cm) and ramuli diameter (mm)

Water level	Water exchange rate	Frond length day 45	Frond length day 60	Ramuli density day 45	Ramuli density day 60	Ramuli diameter day 45	Ramuli diameter day 60
Individual treatment means							
40 cm	25%	7.98±0.15	10.59±0.22	13.7±0.4	11.3±0.1	2.23±0.05	2.04±0.05
40 cm	50%	8.14±0.18	10.68±0.82	13.9±0.4	11.4±0.2	2.29±0.07	2.05±0.02
80 cm	25%	8.36±0.32	10.95±0.16	13.9±0.2	11.5±0.1	2.28±0.02	2.03±0.05
80 cm	50%	8.25±0.16	11.14±0.06	14.1±0.3	11.2±0.2	2.32±0.08	2.02±0.03
One-way ANOVA: effect of water level							
40 cm		8.04±0.16	10.64±0.54	13.8±0.3	11.3±0.2	2.26±0.06	2.05±0.03
80 cm		8.31±0.21	11.05±0.15	14.0±0.2	11.4±0.3	2.30±0.12	2.02±0.04
One-way ANOVA: effect of water exchange rate							
25%		8.14±0.32	10.77±0.26	13.8±0.3	11.4±0.2	2.26±0.05	2.03±0.04
50%		8.20±0.09	10.91±0.58	14.0±0.3	11.3±0.2	2.31±0.13	2.04±0.03
ANOVA, p value							
Water level (1)		0.053	0.139	0.188	0.875	0.467	0.284
Water exchange rate (2)		0.625	0.573	0.317	0.289	0.436	0.941
Two-way ANOVA interaction (1) × (2)		0.159	0.846	0.946	0.106	0.891	0.607

Main effect means within the same column sharing different superscripts are significantly different (p<0.05)
*Ramuli density expressed as number of ramuli/cm of frond (cell/cm)

The values of individual treatment means for morphometric frond properties did not significantly differ among treatments (p>0.05). The length of frond fluctuated from 7.93 to 8.25 cm and 10.59-11.14 cm for day 45 and day 60, respectively.

Density of ramuli varied in the range of 13.7-14.1 cell/cm at day 45 and 11.2-11.5 cell/cm at day 60. The diameters of ramuli at day 45 were between 2.23-2.32 mm and 2.02-2.05 mm at day 60. Generally, frond lengths at day 60 were longer than at day 45 but ramuli density was relatively lower. Similarly, the size of ramuli seemed to be quite smaller in day 60 than in day 45. These results indicated that prolonging cultivation of sea grape *C. lentillifera* could negatively affect the morphometric frond properties.

According to Paul *et al.* (2014) [15], control of the production cycle including biomass production and product quality of sea grape is practical approach to develop commercial system for sea grape cultivation. Other findings confirmed that technical factors include initial stocking density, water movement, culture method, nutrient supply, which affect growth rate, production, the shape and texture of fronds (Paul *et al*., 2014; Wang *et al*., 2017; Minh *et al*., 2019; Chen *et al*., 2019) [15, 20, 14, 2].

Fig 2: Thallus of sea grape *C. lentillifera* cultivated at water level of 40 cm and 80 cm combine with 25% and 50% water exchange rate at day 60**.**

Figure 2 showed that thallus of sea grape *C. lentillifera* cultivated at water level of 40 cm combine with 25% water exchange rate per week contained less frond (erect branch) compared to other treatments. However, appearance of fronds was not distinctly different among treatments.

4.5. Sensory evaluation of commercial frond (edible proportion)

Sensory evaluation is a scientific method of assessing the consumption quality of food under controlled conditions, and appearance, odor, flavor and texture of product are extremely important for the enjoyment of food (Go *et al*., 2013) [7]. For sea grape *C. lentillifera*, color, appearance, odour, taste and sweetness of fronds (edible portions) are the main criteria in sensory evaluating product quality for human consumption (Minh *et al*., 2019) [14].

Table 5: Sensory score of fronds (edible portions) of sea gape harvested at day 60

Treatments	40 cm-25%	40 cm-50%	80 cm-25%	80 cm-50%
Sensory score	7.18±0.19^a	7.56±0.27^a	7.47±0.35^a	7.67±0.07^a

Mean values with the same superscripts in the same row are not significantly different (*p*>0.05)

Table 5 showed that the average sensory score (color, appearance, odour, taste and sweetness) of fronds (edible portions) in different treatments ranged from 7.18 to 7.67 that indicated these products are highly acceptable to the panelists. There were no significant differences in sensory score among treatments (*p*>0.05). This illustrated that water level and water exchange rate did not affect sensory quality of sea grape fronds.

5. Conclusion

Sea grape (*Caulerpa lentiliifera* J. Agardh, 1837) cultivated in the lined pond at water depth of 80 cm gave higher growth rate and frond production than at the depth of 40 cm regardless water exchange rate.

Morphometric frond properties (commercial frond length, ramuli density and ramuli size) were not significantly affected by water level and water exchange rates.

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