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Utilization of the light density to reduce the development of *Lyngbya* sp. and their growth on *Caulerpa lentillifera* J. Agardh in a recirculating aquature system

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Abstract
The release of pollutants is endangering ecosystems, biodiversity and seafood. Therefore, it is of the requirement to create innovative methods in seafood production. *Caulerpa lentillifera* J. Agardh is a well-known seaweed for its properties and edible. We cultured *C. lentillifera* in a recirculating aquaculture system under laboratory conditions. However, after 7 days the culture was invaded by epiphyte algae *Lyngbya* sp. This experiment was designed to remove those algae by using light density as a treatment. Two irradiances were tested including 20 µmol photons $m^{-2}.s^{-1}$ (low light density) and 40 µmol photons $m^{-2}.s^{-1}$ (initial light density). Every week we measured the stolon length, thallus weight, and calculated the specific growth rate. Results showed that after 30 days *C. lentillifera* under low light density regained their healthy green color and *Lyngbya* sp. was no longer present. Meanwhile, in the initial light density (40 µmol photons $m^{-2}.s^{-1}$) *Lyngbya* sp. covered almost all thalli of *C. lentillifera*, and half of them were dead. The measured results of specific growth rate ($\%d^{-1}$) and weight (g) of *C. lentillifera* in low light density conditions were better than those of *C. lentillifera* in the initial condition. The light density, therefore, might be used as a treatment to remove *Lyngbya* sp. from *Caulerpa lentillifera* in aquaculture.

Keywords: *Caulerpa lentillifera*, *Lyngbya* sp., light density, recirculating aquaculture system, seaweed culture.

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INTRODUCTION

The current global warming, caused by greenhouse gas emission, results in the rise of ocean temperature and CO_2 contribution to ocean acidification. Besides, heavy metals are absorbed by the seafood [1] and directly involved consumers [2, 3]. Otherwise, sustainable fisheries production is a major challenge for the aquaculture industry. In particular, there is the intensive use of commercial feeds which, in turn, contributes to an important issue in the open water system, water pollution [4].

It is of necessity to develop novel methods in seafood production to mitigate the negative effects of effluents. Integrated systems might be an approach to tackle these issues [4]. By associating fish production, whose nutrients are used to grow seaweeds cleaning the water and producing biomass. Hence, wastes are transformed into resources in those integrated systems [5].

Caulerpa lentillifera J. Agardh could be used in such a method. This seaweed, also known as "sea grape", is edible and commonly consumed in a salad of vegetables. *C. lentillifera* contains high amounts of minerals, proteins and balanced amino acid (ref). It is also notably rich in iodine phosphorus, calcium, magnesium, copper; thereby highlighting its role as an ingredient with high nutritional values and as a healthy food for human diets [6]. Other studies showed that the seaweed might even be used in medicine as an anti-diabetic agent [7] and dye industries [8, 9]. Besides those properties, *C. lentillifera* has shown the potential ability to remove nutrients from aquaculture effluent [10–13] and heavy metals from wastewater [9, 14]. However, it has been documented that their culture in ponds might become a problem because they absorb heavy metals [3] and for this reason, it might be interesting to culture them in a controlled system.

To determine the best environmental conditions for culturing this seaweed, the growth of *C. lentillifera* was observed under laboratory conditions. We tested a condition (photoperiod of 10 h:14 h (light:dark) with 40 μmol photons $m^{-2}.s^{-1}$ at 27.5°C), described as optimal for the asexual propagation of *C. lentillifera* J. Agarhd by Guo [12]. In the first days, the seaweed adapted quickly and was healthy. Almost every thallus had a new branch and rhizoids, their stolon became longer. However, despite the controlled conditions, *Lyngbya* sp. appeared, developed on it, and quickly grew in a few days. Their development had reduced the growth of *C. lentillifera*. In nature, the problem caused by algae seriously affects the yield of seaweed. It is a constraint of seaweed farming, for example, the epiphytism (*Lyngbya* sp.) had completely covered the raft of *Kappaphycus alvarezii* farming in Tamil Nadu, India [15]. The *Lyngbya* sp. is a benthic cyanobacterium that is common and prevalent in the shallow water of tropical and subtropical marine areas including atolls, salt marshes and estuary water [16].

Thus, the aquatic industrial and seaweed farmer usually face benthic cyanobacterial blooms. They lack information about the treatment to limit the development and to remove the *Lyngbya* sp. Jones (1990) showed that *Lyngbya* sp. is incapable of nitrogen fixation under dark conditions [17], while *C. lentillifera* is able to develop under low light density [12]. Thus, we hypothesized that the *Lyngbya* sp. can be treated under low irradiance. This study aims to test our hypothesis by using the low light density to treat and remove the *Lyngbya* sp. from the seaweed culture in laboratory conditions. To test this hypothesis, we set up two conditions in different light densities: The initial irradiance (40 μmol photons $m^{-2}.s^{-1}$) as a control experiment and 20 μmol photons $m^{-2}.s^{-1}$ as a treatment condition. Our experiment expects to find a solution to the problem caused by benthic cyanobacteria without losing the production of *C. lentillifera*.

MATERIAL AND METHODS

Material

Fresh *Caulerpa lentillifera* J. Agardh (Chlorophytes) were sampled at a seaweed pond culture in Ninh Hoa dist., Khanh Hoa prov. in the Central Vietnam. The seaweeds were transported by airplane (5 hours), stored in an insulated container under the temperature range from 23–25°C in a cool box. The seaweed samples were kept in an aquarium

tank (running water cycle) for 2 days to adapt to the laboratory condition. The seawater (30.0 ± 1.0‰ sanity) was filtered by the phytoplankton net before use.

Methods

Green healthy seaweeds with a frond of 4 cm were selected and cut with 2 cm of stolon (fig. 1) (N = 40).

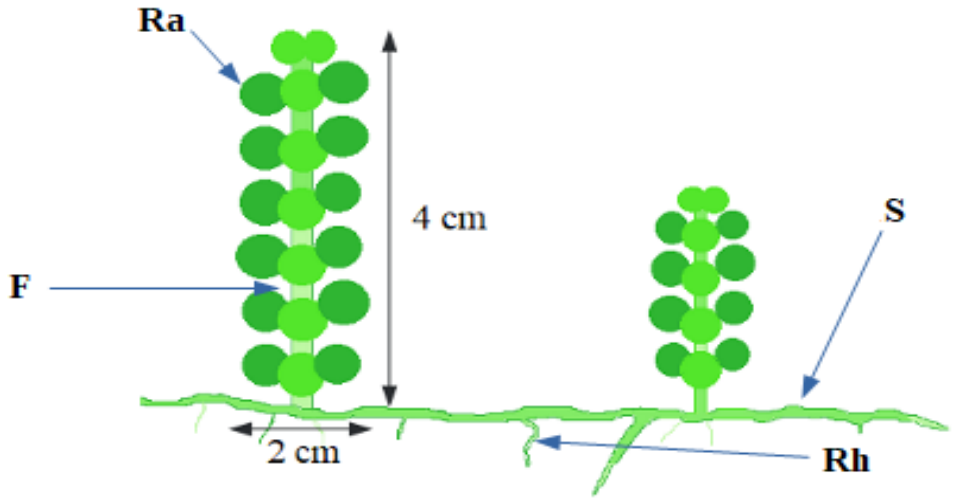

Figure 1. Diagram of *C. lentillifera* and the initial dimension of the thalli chosen for the experiment. F = fruit; Rh = root; S = stolon; Ra = branch

They were disposed in a bottle with 4 liters of seawater under a photon flux density of 40 µmol photons.m^{-2}.s^{-1} and a photoperiod of 10 h:14 h (light:dark) at 27–27.5°C. There were 5 samples per bottle. Seawater was changed once a week. Measures were done daily. Irradiance was measured with a waterproof light density recorder HOBO data logger Pendant UA Temp/Light; pH was measured daily with a probe–pH (Okaton pH 11 series, Singapore).

After 10 days of development, *Lyngbya* sp. was present on each thallus, mainly on the branches of *C. Lentillifera* (fig. 2). Some thalli of *C. lentillifera* became weak.

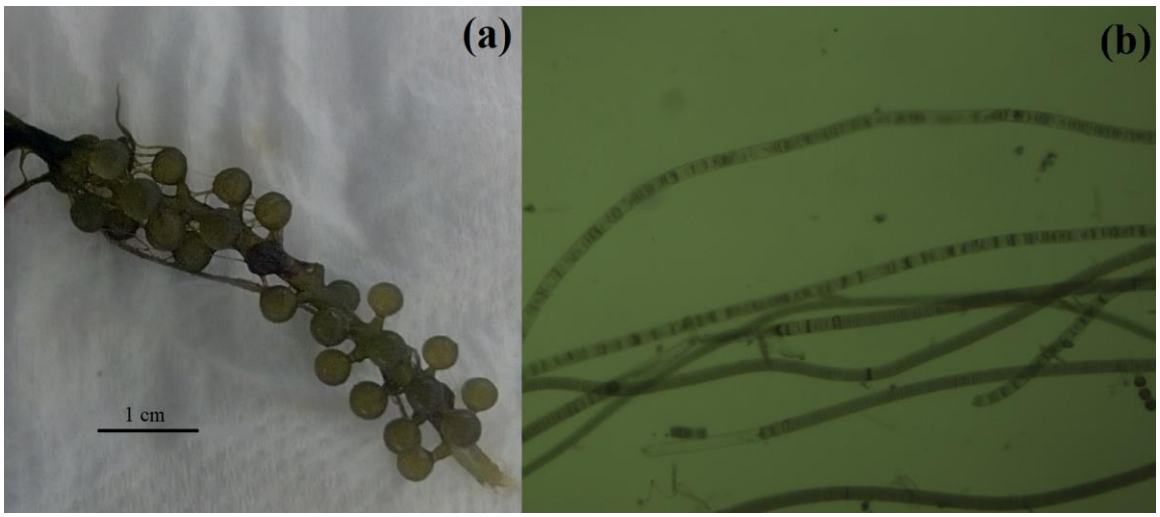

Figure 2. C. lentillifera was attacked by *Lyngbya* sp., (a) a branch of *C. lentillifera* after 10 days of the experiment; (b) structure of *Lyngbya* sp. under a microscope

We randomly distributed all the samples (N = 40) under an irradiance of 20 µmol photons $m^{-2}.s^{-1}$ (low light density) for a treatment condition and 40 µmol photons $m^{-2}.s^{-1}$ (initial light density) with 5 samples per bottle.

We measured every 7 days the length of the stolons, the weight of each sample and calculated the specific growth rate (SGR) with the following formula:

$$SGR = \ln\left(\frac{W_2}{W_1}\right) \times \frac{100}{t} \quad (1)$$

Where: W_2: The weight measured; W_1: The previous weight measured; t: The difference of time between the two measures (= $t_2 - t_1$).

Note: Before every measure of weight, the water attached on the seaweed surface was removed with absorbent paper.

The percentage of *Lyngbya* sp. attached on *Caulerpa lentillifera* was calculated by the ratio between the number of branch and root of *C. lentillifera*, which were attached by *Lyngbya* sp. and the total of branch and root of each sample.

Statistical analysis

The data were tested with an unpaired two-sided t-test. For each test, we compared the mean of the samples under the two light density conditions. At the end of the experiment, the data were tested with a paired two-sided t-test in order to compare the initial and final measures of each group. If the assumption of normality of the sample mean in both groups could not have been made, we have used the non-parametric equivalent of the two-sample t-test: The Wilcoxon rank-sum test.

Differences obtained at a level of $P < 0.05$ indicated that it is significant. Analyses were done using the software R (3.6.0).

RESULTS

SGR and weight

From the 21st day of culture until the end, there was a significant difference among the SGR of the group at light densities of 20–40 ($W = 212$, $P < 0.05$ and $W = 124$, $P < 0.05$) and this showed that the SGR of *C. lentillifera* was better under a light density of 20 µmol photons $m^{-2}.s^{-1}$. The same results were observed with the weight, and the weight of *C. lentillifera* under a light density of 20 µmol photons $m^{-2}.s^{-1}$ is greater ($t = 2.8158$, $P < 0.05$ and $t = 5.0571$, $P < 0.05$). We also observed a difference between the initial and final weights for both groups and it showed that the *C. lentillifera* under a light density of 20 µmol photons $m^{-2}.s^{-1}$ is heavier ($W = 78$, $P < 0.05$) while the other group is lighter ($t = 5.5196$, $P = 0.05$).

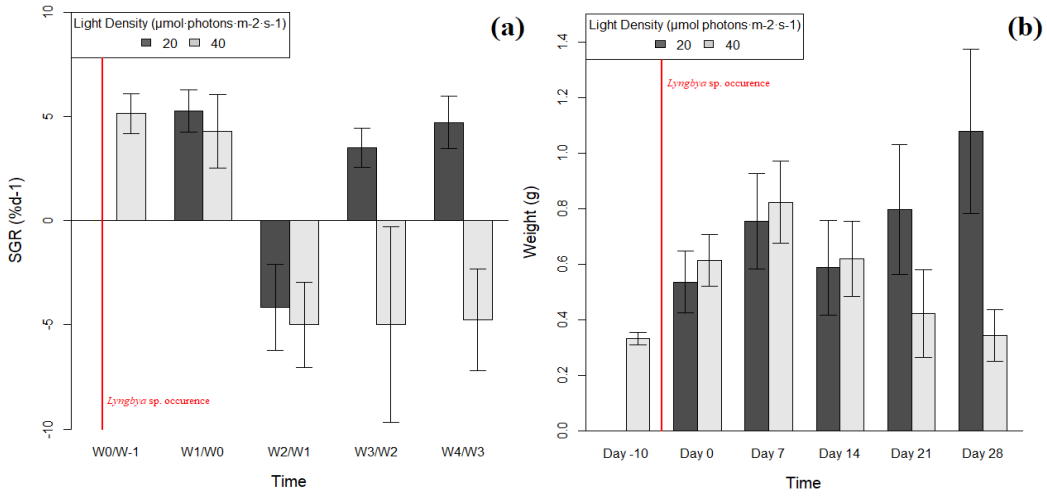

Figure 3. Evolution of (a) specific growth rate (%d^{-1}) and (b) weight (g) of *C. lentillifera* cultivated in different irradiance (20 and 40 µmol photons.$m^{-2}.s^{-1}$) conditions for 28 days. Error bars represent 95 % confidence intervals. N = 40 at (Day 10) and N = 20 for each condition at the beginning of the experiment (Day 0)

We can distinguish 2 phases during their growth: After one week of culture, both groups studied still had a positive SGR around 4.5%d^{-1}, and then a negative SGR after the second week (–4,5%d^{-1}). Some branches from both groups turned white and the growth of *Lyngbya* sp. was more important. From this point the groups of *C. lentillifera* had 2 different behaviors: the weight of the group under a light density of 20 µmol photons $m^{-2}.s^{-1}$ increased, and obviously, the SGR also increased and had approximatively the same values as initially. Meanwhile, the weight of the group of seaweeds under the light density of 40 µmol photons $m^{-2}.s^{-1}$ has continued to decrease and the SGR stayed negative around -5%d^{-1}. After 21 days, new green and healthy branches were emerging from the seaweeds of the first group. No *Lyngbya* sp. was observed developing on it. For the second group, most of the algae were covered with *Lyngbya* sp., except the stolons. After 28 days, *C. lentillifera* growing under a light density of 20 µmol photons $m^{-2}.s^{-1}$ was free of *Lyngbya* sp. For the second group, half of the seaweeds were dead, and the remnant branches were white.

Length

After 4 weeks, the *C. lentillifera* stolon length under the two light density is significantly different (t = 5.1181, P < 0.05) and it showed that the *C. lentillifera* stolons are longer under a light density of 20 µmol photons $m^{-2}.s^{-1}$.

The final stolon length is more important than the initial length in both groups (P < 0.05).

During 28 days, the length of *C. lentillifera* under a light density of 20 µmol photons $m^{-2}.s^{-1}$ increased until reaching a mean of 35.4 ± 7.38 cm (fig. 3), which is nearly 4 times longer than the initial length. On the contrary, the length of the seaweeds under the light density of 40 µmol photons $m^{-2}.s^{-1}$ increased until the 21st day and then decreased and reached a mean length of 16.0 ± 3.62 cm.

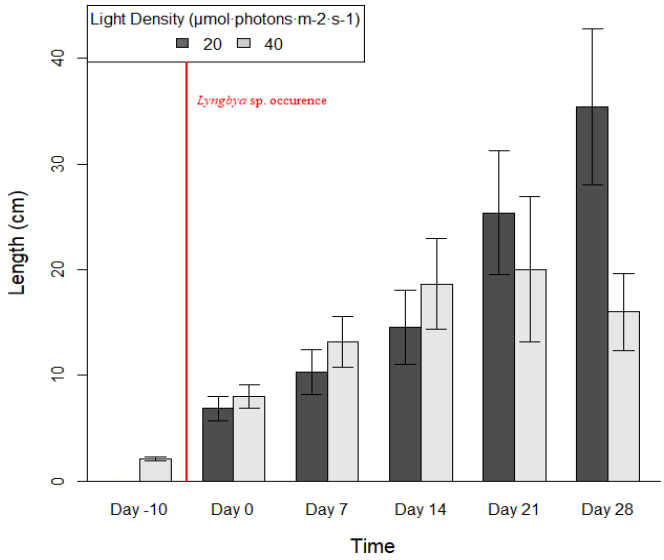

Figure 4. Evolution of length (cm) of *C. lentillifera* cultivated in different photon flux density (20 and 40 µmol photons $m^{-2}.s^{-1}$) conditions for 28 days. Error bars represent 95% confidence intervals. N = 40 at (Day 10) and N = 20 for each condition at the beginning of the experiment (Day 0)

***Lyngbya* sp. Coverage**

Table 1 showed the variation of *Lyngbya* sp. covering *C. lentillifera* during the experiment. The average percentage of *Lyngbya* sp. coverage showed the different tendencies between light densities. The *Lyngbya* sp. coverage increased very fast under 40 µmol photons $m^{-2}.s^{-1}$ condition from

8.3% and 10.8% (day 0) to 90.3% and 99.8% (day 28) on branch and root, respectively. Meanwhile, under lower light condition (20 µmol photons $m^{-2}.s^{-1}$) the *Lyngbya* sp. coverage increased from day 0 to day 14 in both branch and root of *C. lentillifera*. The coverage showed decrease after 21 days under low light conditions. Then the coverage decreased to 12.3% and 15.7% in the branch and root, respectively.

Table 1. Variation of *Lyngbya sp.* coverage on *C. lentillifera* (SD means standard deviation with N = 4 for each condition of each period)

Light density (µmol photons $m^{-2}.s^{-1}$)	Part of *C. lentillifera*	The average percentage of *Lyngbya* sp. coverage (±SD)				
		Day 0	Day 7	Day 14	Day 21	Day 28
20	Branch	8.3 ± 2.5	12.5 ± 5.4	18.3 ± 4.2	15.5 ± 3.8	12.3 ± 2.5
	Root	10.8 ± 4.3	18.6 ± 3.8	20.8 ± 4.3	20.2 ± 6.3	15.7 ± 6.2
40 (initial condition)	Branch	8.0 ± 3.2	15.4 ± 4.6	38.3 ± 3.8	64.3 ± 2.5	90.3 ± 4.7
	Root	10.5 ± 3.6	20.6 ± 4.3	40.8 ± 4.3	80.8 ± 4.3	99.8 ± 4.3

pH

After 7 days, the pH is more important under a light density of 40 µmol photons $m^{-2}.s^{-1}$ than in the bottles under a light density of 20 µmol photons $m^{-2}.s^{-1}$ and this showed that the light density affects the pH (t = -4.737, P < 0.05). The water was then changed, and the same pH evolution was observed in the next weeks. Initial pH was not significantly different (t = 0.25265, P > 0.05).

Table 2. pH and salinity of the seawater after 7 days of culture of *C. lentillifera* in different photon flux densities (20 and 40 µmol photons $m^{-2}.s^{-1}$). Different letters above bars indicate significant differences. Mean ± 95% confidence intervals. N = 4 for each condition of each period

Light density (µmol photons $m^{-2}.s^{-1}$)	Day	pH
20	0	7.62 ± 0.03^{a}
	7	7.81 ± 0.08^{b}
40 (initial condition)	0	7.61 ± 0.02^{a}
	7	8.06 ± 0.14^{c}

Notes: The letter a, b, c indicates the difference in the statistic. Difference letters mean significant differences.

DISCUSSION

This experiment indicates that the *C. lentillifera* under a light density of 20 µmol photons $m^{-2}.s^{-1}$ had almost no *Lyngbya* sp. present on their branches after 28 days. In addition, new branches were emerging along the stolons. On the opposite, the seaweeds under a light density of 40 were covered by *Lyngbya* sp. and about to die at the end of the experiment. In fact, *C. lentillifera* from the first group was longer, heavier, and had a better SGR than the second group. In particular, during the 4^{th} week, we found an SGR of 4.5%d^{-1}. This result is consistent with the study of Guo et al., [12] for the same temperature. Their final length and weight were greater than the initial ones. Those seaweeds have overcome the presence of *Lyngbya* sp. and will keep growing.

The *Lyngbya* sp. occurred during the time between two measures, so we cannot say whether it had a quantitative effect on the growth of *C. lentillifera* before the beginning of the treatment. Still, the thalli appeared to be weak, some ramuli became white. However, even if the measures were taken just before its occurrence, we might not have been able to observe significant results because *C. lentillifera* is mainly weakened on the long-term aspect.

During the experiment, we observed an increase in the pH, particularly under the light density of 40 µmol photons $m^{-2}.s^{-1}$. This is linked to the fact that *C. lentillifera* and *Lyngbya* sp. are both oxygenic photosynthetic organisms and will use the CO_2 in the seawater to synthesize carbon. The depletion of the cyanobacteria might be explained by the fact

that *Lyngbya* sp. cannot fix N_2 under the dark condition as found by Jones [17].

We also observed different phases of variation of the SGR and obviously the weight. In particular, the SGR was negative at the end of the second week for both groups because the weight decreased. But those observations were not the same for the length, which kept increasing at that moment. One explanation could be the loss of some white-dead branches during the second week, while the stolons kept growing. But this might be not enough to explain this event because we observed an outcome of cyanobacteria in the same period. This phenomenon could not be explained with the data collected.

Nonetheless, this study may be useful in the project of creating a new way of producing seafood with *C. lentillifera* in tanks. If those tanks were to be invaded by *Lyngbya* sp., reducing the light density with some nets might be enough to eliminate those algae without losing the production of *C. lentillifera*. However, it might take some time before witnessing the results.

CONCLUSION

This experiment indicates that the length, weight, and SGR is greater in the case of *C. lentillifera* growing under a light density of 20 µmol photons $m^{-2}.s^{-1}$ after 28 days of experimentation. In addition, qualitative observation showed that after one month *Lyngbya* sp. is no longer present in those seaweeds while the seaweeds growing under 40 µmol photons $m^{-2}.s^{-1}$ are almost dead. For these reasons, we can argue the light density as a treatment to reduce and eliminate the presence of *Lyngbya* sp. However, those results take time and might not be the most suitable. However, in the future, if such a development happens in a tank, reducing the light density might be enough to kill the cyanobacteria. This study also contributes to finding a solution to reduce the acidification issue by using seaweed culture to absorb CO_2 in the seawater. The artificial culture of seaweed is the most important model for the offshore island to provide a fresh vegetable food source which contributes to the sustainable development in the offshore islands.

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