Effects of Different Light Intensity Fluctuations on Growth Rate, Nutrient Uptake and Photosynthetic Efficiency of *Gracilaria asiatica*

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ABSTRACT

With the growing demand of seaweed for the food and pharmaceutical industry, this study examine the effects of different light intensities coupled with initial nutrient concentrations on growth rate, nutrient uptake as well as photosynthetic efficiency on *Gracilaria asiatica*. Agarophyte (*Gracilaria asiatica*) obtained from Fujian in China were used for this study. The samples were incubated at temperature 20°C and intensity 90 $\mu$mol m$^{-2}$ sec$^{-1}$ with culture medium, salinity 21‰ of Von Stoch’s modified enriched seawater (VSE) which was added every 3 days and seawater changed every 6 days. The trial lasted for 15 days. The results of the present study revealed that different light intensities affected growth rate of *Gracilaria asiatica* regardless of the initial concentration. Under both high and low nutrient concentrations, the highest growth rate of 18.87% day$^{-1}$ and 10.56% day$^{-1}$, respectively were observed at the light intensity of 90±20 $\mu$mol m$^{-2}$ sec$^{-1}$. Nutrient uptake was influenced by different salinities under both high and low concentrations. The removal efficiency of both NO$_3$-N and PO$_4$-P were higher at low initial concentration compared to treatments with higher initial concentration. The highest photosynthetic yield of 0.63 was recorded at the light intensity of 90±20 $\mu$mol m$^{-2}$ sec$^{-1}$ under high initial concentration whiles that of low concentration was 0.67 recorded at the light intensity of 90±40 $\mu$mol m$^{-2}$ sec$^{-1}$. This study document that for a better growth rate, *Gracilaria asiatica* should be cultured at the light intensity of 90±20 $\mu$mol m$^{-2}$ sec$^{-1}$ regardless of the nutrient concentration.

Key words: Light intensity, growth rate, nutrient uptake, photosynthetic efficiency, *Gracilaria asiatica*

INTRODUCTION

Seaweeds are a very important and commercially valuable resource for the food industry; they also serve as soil conditioners and are used in traditional medicine because of their perceived health benefits (Jiao et al., 2011). Seaweeds used to be harvested from nature but its cultivation has significantly grown rapidly since the early 20th century due to the continuously rising demand for food and industry (Yang et al., 2015).

More than 50% of the seaweeds used for agar production are obtained from *Gracilaria* species (Sousa-Pinto et al., 1999). This is due to the farming of several species of this genus (Santelices and
Gracilaria belongs to the Rhodophyta family (Freile-Pelegrin and Murano, 2005). Wu (1998) and Liu (2001) documented that there are more than 30 species in the genus Gracilaria in China. Nasmia et al. (2014) also documented that the production of seaweed, Gracilaria is estimated to increase concurrent with the increasing demand of seaweed for the food industry, pharmacy and cosmetics.

According to Yang et al. (2015), several key environmental factors have been identified to affect Gracilaria cultivation. These include light, temperature, salinity, nutrients, cultivation depth, water movement and herbivorous fish and epiphytes. Light is an essential physical factor for photosynthesis in seaweed and higher plants since it is used to absorb nutrients into cells, especially nitrogen and carbon.

Carnicas et al. (1999) reported that in tropical zones, when the irradiance reach around 2500 μmol m⁻² sec⁻¹, the maximum photosynthesis of the sun algae is around 500 μmol m⁻² sec⁻¹ and that of shade algae is around 60-150 μmol m⁻² sec⁻¹. The study of Gomez et al. (2005) assessed the effect of photosynthesis under natural solar radiation in river estuary in Chile. The results of their study showed that Gracilaria chilensis is shade-adapted because it has high chlorophyll a and phycobilins and low light saturating point (Ek), between 60 and 170 μmol m⁻² sec⁻¹.

Xu et al. (2009) reported that, the highest specific growth rates of Gracilaria lichenoides (16.26% day⁻¹) and Gracilaria tenuistipitata (14.83% day⁻¹) were recorded at light intensity 287.23 and 229.07 μmol m⁻² sec⁻¹, respectively. The growth rate of Gracilaria increases when light intensity is increased. However, in nature Gracilaria does not live under high light intensity except in summer (Gomez et al., 2005).

The cellular composition of algae is affected by light intensity. Cuhel et al. (1984) reported that green algae, Dunaliela tertiolecta exhibited a decrease in protein content and an increase in the lipid fraction when light intensities were increased up to saturation.

It is known that due to the disruption of the chloroplast lamellae caused by high light intensity (Brody and Vatter, 1959) as well as inactivation of enzymes involved in carbon dioxide fixation (Iqbal and Zafar, 1993) when light intensity is increased above saturating limits it causes photo inhibition (You and Barnett, 2004).

Growth of algae is affected by the intensity of light through its effects on photosynthesis (Stockenreiter et al., 2013). Although growth rate under increasing light intensity is a function of strain and culture temperature, the growth rate of algae is maximal at saturation intensity and decreases with both increase or decrease in light intensity (Juneja et al., 2013). Considering the importance of Gracilaria asiatica to the food and pharmaceutical industries, establishing the optimum light conditions would help increase production. Although, some studies have been conducted on the effects of environmental factors on seaweed, there seems to be little information on how these factors affect Gracilaria asiatica. The objective for this present study was therefore to assess whether different light intensity fluctuations affects growth rate of Gracilaria asiatica. This study also ought to evaluate how light intensities coupled with different nutrient concentrations affect nutrient uptake, removal efficiency and photosynthetic efficiency of Gracilaria asiatica.

MATERIALS AND METHODS
Experimental material: Agarophyte (Gracilaria asiatica) were collected from Fujian, China. The experimental materials were transported to the laboratory where the algal thalli were selected, rinsed and the epiphytes removed using filtered seawater. Prior to the experiment,
Gracilaria asiatica were incubated at a temperature below 20°C and an intensity of 90 μmol m⁻² sec⁻¹ with a 12L:12D in filtered seawater. The culture medium, salinity of 21% seawater (VSE), was added every 3 days and seawater changed every 6 days.

**Experimental treatment and culture condition:** In order to assess the effects of different light intensity fluctuations on Gracilaria asiatica, three different light intensity fluctuations (90, 90±20 and 90±40 μmol m⁻² sec⁻¹) with two different initial concentrations of NO₃-N (50 and 500 μmol L⁻¹) and PO₄-P (3 and 30 μmol L⁻¹) were employed for the study. NO₃-N concentrations were enriched with NaNO₃, while PO₄-P concentrations were enriched with Na₂HPO₄·12H₂O.

The experiment lasted for 15 days. Seaweed biomass was measured at 3-day intervals, and NO₃-N and PO₄-P concentrations were determined by SKALAR Analyzer, the culture medium was changed every 3 days. The photosynthetic efficiency was determined at the beginning and the end of the experiment. The study was conducted in triplicates.

**Growth and nutrient uptake experiment:** Seaweed were put in 250 mL flasks and containing 200 mL of seawater added into the culture medium of Von Stoch’s modified enriched seawater (VSE). The control experiments were constant light intensity treatments (90 μmol m⁻² sec⁻¹) and two light intensity fluctuations treatments (90±20 and 90±40 μmol m⁻² sec⁻¹) with a 12L:12D light-dark ratio. The light intensity fluctuations treatments were programmed to follow a circadian rhythm (Fig. 1) (Wang *et al.*, 2007a, b). The experiments were repeated under two initial NO₃-N concentrations (50 and 500 μmol L⁻¹) and PO₄-P concentration (3 and 30 μmol L⁻¹) with three replicates and grown under a temperature of 20°C. During the 15 days trial, the medium was renewed every 3 days.

**Photosynthesis experiment:** The yield Fv/Fm value of chlorophyll fluorescence can be used to measure the efficiency of PSII (Kang *et al.*, 2008). In our experiment, Gracilaria asiatica was cultured under different light intensity fluctuations and temperature 20°C throughout the experiment.

**Relative Growth Rate (RGR):** The relative growth rate (% day⁻¹) of the fresh weight was calculated based on the following equation (Chirapart and Lewmanomont, 2004):

\[
\text{Relative Growth Rate (RGR)} = \left[ \left( \frac{W_t}{W_0} \right)^{1/3} - 1 \right] \times 100
\]

Fig. 1: Light intensity fluctuation mode: White and black behalf of light (L) and dark (D), letters on behalf of A and Li is light intensity rhythms range and average light intensity.
Where:

\[ W_0 = \text{Initial fresh weight} \]
\[ W_t = \text{Fresh weight at the end} \]
\[ t = \text{Time (days)} \]

**Uptake rates (V):** The uptake rates \((\mu\text{mol g}^{-1} \text{ DW day}^{-1})\) were calculated from changes in substrate concentration \((S)\) during each sampling interval (Kang *et al.*, 2008):

\[
V = \frac{[(S_0 \times vol_0) - (S_t \times vol_t)]}{t \times B}
\]

**Removal efficiency (Re):** The removal efficiency (%) shows the average reduction (%) in concentration. The removal efficiency shows the amount of NO\(_3\)-N and PO\(_4\)-P removed per unit of time (Kang *et al.*, 2008):

\[
Re = \frac{S_0 - S_t}{S_0 \times t} \times 100
\]

Where:

\( S_0, vol_0 = \text{Nutrient concentration and the volume at the beginning of sampling interval} \)
\( S_t, vol_t = \text{Nutrient concentration and the volume at the end of sampling interval} \)
\( t = \text{Time between two samplings} \)
\( B = \text{Dry weight (DW) biomass} \)

**Photosynthetic efficiency:** The photosynthetic efficiency was measured as the change of the maximum quantum yield \((F_v/F_m)\) of photo system II (PSII) by Phyto-PAM Analyzer.

**Statistical analysis:** All results are presented as Mean±Standard Error of the mean. Data was analyzed by one-way analysis of variances (ANOVA) to test the effects of different temperatures and light intensities on growth and nutrient uptake of *Gracilaria asiatica*. Where significant differences were found \((p<0.05)\), Duncan multiple comparison analysis processing was used to rank the mean. Statistical analyses were made by SPSS for windows software (Version 19.0).

**RESULTS**

**Effects of light intensity fluctuation and initial nutrient concentration on growth of *Gracilaria asiatica*:** The growth rate of *Gracilaria asiatica* at different light intensity fluctuations under low and high nutrient concentrations are shown in Fig. 2, 3. After 15 days trial, growth rate was affected by different light intensities under both low and high nutrient concentrations. Under low nutrient concentrations, the highest RGR was observed at 90±20 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\) (10.56% day\(^{-1}\)) followed by 90±40 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\) (9.66% day\(^{-1}\)) with the lowest growth rate recorded at 90 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\) (9.44% day\(^{-1}\)). The same trend was observed under high nutrient concentration although the growth rate values recorded were slightly higher than those cultured under low nutrient concentration. The highest growth rate of 18.87% day\(^{-1}\) was recorded at 90±20 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\) which was closely followed by 11.68% day\(^{-1}\) recorded at 90±20 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\). The least growth rate of 10.54% day\(^{-1}\) was however recorded at 90 \(\mu\text{mol m}^{-2} \text{ sec}^{-1}\).
Effects of light intensity fluctuation and initial nutrient concentration on NO$_3$-N and PO$_4$-P uptake: Figure 4 shows the nutrient uptake rate by *Gracilaria asiatica* under low and high concentrations. The uptake rate of NO$_3$-N and PO$_4$-P by *Gracilaria asiatica* under low and high concentrations were affected by light intensity. At high light intensity of 90±40 µmol m$^{-2}$ sec$^{-1}$, the highest nutrient uptake of NO$_3$-N was recorded (423.39±16.50 µmol g$^{-1}$ DW day$^{-1}$). This was followed by 415.50 µmol g$^{-1}$ DW day$^{-1}$ which were recorded at 90 µmol m$^{-2}$ sec$^{-1}$ with the least uptake of 365.35 µmol g$^{-1}$ DW day$^{-1}$ recorded at 90±20 µmol m$^{-2}$ sec$^{-1}$. The same trend was observed at low light intensity with 55.26, 54.58 and 52.82 µmol g$^{-1}$ DW day$^{-1}$ being recorded at 90±40, 90 and 90±20 µmol m$^{-2}$ sec$^{-1}$, respectively. With respect to PO$_4$-P uptake rate, there was a trend of increasing uptake rates with increasing low light intensity. The uptake rates of 1.89, 1.90 and 1.93 µmol g$^{-1}$ DW day$^{-1}$ were recorded for 90, 90±20 and 90±40 µmol m$^{-2}$ sec$^{-1}$, respectively.
When the light intensities were high, the highest PO₄-P uptake rate was recorded at 90±40 μmol m⁻² sec⁻¹ (11.29 μmol g⁻¹ DW day⁻¹), followed by 90 μmol g⁻¹ DW day⁻¹ (10.70 μmol g⁻¹ DW day⁻¹) with the least being recorded at 90±20 μmol g⁻¹ DW day⁻¹ (9.27 μmol g⁻¹ DW day⁻¹).

**NO₃-N and PO₄-P removal efficiency at different light intensity fluctuation under low and high concentrations:** The NO₃-N and PO₄-P removal efficiency at different light intensities with different nutrient concentration are shown in Fig. 5. The NO₃-N and PO₄-P removal efficiency at low initial concentration were higher than high initial concentration under the various light intensity fluctuations. The values of NO₃-N removal efficiency at low concentration for all light intensities were not significantly different at days 3, 6 and 9. However, the removal efficiency recorded for 90±40 μmol m⁻² sec⁻¹ was significantly lower than the two other treatments (90 and 90±20 μmol m⁻² sec⁻¹). The NO₃-N removal efficiency at high initial concentration under every different light intensity fluctuations increased from beginning until the end of experiment. The least PO₄-P removal efficiency recorded under different low concentration was recorded on the day 3 when the light intensity was 90±40 μmol m⁻² sec⁻¹. The values however increased on the 6th day but there was a general trend of decreasing removal efficiency on days 9, 12 and 15. The highest removal efficiency of PO₄-P at high concentration during the 15 days trial occurred on he 12th day.

**Photosynthetic efficiency:** The yields (Fv/Fm) at three different light intensities as well as the initial are shown in Fig. 6. The yield recorded for the three different light intensities under both low and high concentrations were higher than the initial yield. Under low concentrations, the
Fig. 5(a-d): Removal efficiency at different light intensity fluctuation under low and high concentrations every 3 days, during 15 days, (a) Low NO$_3$-N concentration, (b) High NO$_3$-N concentration, (c) Low PO$_4$-P concentration and (d) High PO$_4$-P concentration. The highest yield of 0.67 was recorded when the intensity was 90±40 µmol m$^{-2}$ sec$^{-1}$. At intensities of 90 and 90±20 µmol m$^{-2}$ sec$^{-1}$, the yield recorded was 0.65 and 0.59, respectively. The highest yield under high concentration however occurred at 90±20 (0.63) which was followed by 90±40 and 90 µmol m$^{-2}$ sec$^{-1}$ with yield of 0.61 and 0.58, respectively.
Fig. 6: Yield ($F_v/F_m$) at different light intensity fluctuation under low and high concentrations, $F_v/F_m$: photosynthetic efficiency

DISCUSSIONS

Light is one of the most important environmental factors influencing seaweed growth (Yang et al., 2015). In this study, when light intensity was 90±20 μmol photons m$^{-2}$ sec$^{-1}$ under low and high concentration, the highest growth rate were observed and were significantly different, respectively. Under all light intensities, *Gracilaria asiatica* recorded the highest growth rate when nutrient concentrations were higher. This is because cultivated seaweeds grow very fast under high nutrient concentrations especially when N is high (Fei, 2004; Sode et al., 2013; Yang et al., 2015).

Factors such as light, temperature, desiccation and water motion influence uptake kinetics (Harrison and Hurd, 2001). The highest rate of NO$\text{}_3$-N and PO$\text{}_4$-P uptake existed at 90±40 μmol photons m$^{-2}$ sec$^{-1}$ under high initial concentration (423.39±16.50 and 11.29±2.10 μmol g$^{-1}$ DW day$^{-1}$), respectively. The higher value of NO$\text{}_3$-N could be explained that nitrogen was important more than phosphorus because Nitrogen compounds are important for amino acid, amines and protein synthesis (Lobban and Harrison, 1994). Contrary to this study is however the results of Wheeler (1982) which documented that NO3-uptake by macrocystis decreased with increasing irradiance. The difference could be attributed to the difference in culture material (Seaweed).

At high initial concentration under every condition PO$\text{}_4$-P remain too high. In contrast, the NO$\text{}_3$-N removal efficiency under high initial concentration increased day by day nearly exhausted from the medium. This could be reconciled with the fact that light is source energy of photosynthesis in seaweed and used for absorb nutrient into cell, especially nitrogen (Lobban and Harrison, 1994).

CONCLUSION

In conclusion, we documented that different light intensity fluctuations affected the growth rate of *Gracilaria asiatica*. The maximum growth rate was obtained when light intensity was 90±20 μmol m$^{-2}$ sec$^{-1}$. Also nutrient uptake, removal efficiency as well as photosynthetic efficiency recorded for *Gracilaria asiatica* were affected by different light intensities and nutrient concentrations. We recommend that for maximum growth rate *Gracilaria asiatica* should be cultured at 90±20 μmol m$^{-2}$ sec$^{-1}$. Also more studies should be conducted on other factors that affects growth and performance of cultured *Gracilaria asiatica*.

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