



Journal of  
**Fisheries and  
Aquatic Science**

ISSN 1816-4927



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## 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  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  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%  $\text{day}^{-1}$  and 10.56%  $\text{day}^{-1}$ , respectively were observed at the light intensity of 90±20  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ . Nutrient uptake was influenced by different salinities under both high and low concentrations. The removal efficiency of both  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  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  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  under high initial concentration while that of low concentration was 0.67 recorded at the light intensity of 90±40  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ . This study document that for a better growth rate, *Gracilaria asiatica* should be cultured at the light intensity of 90±20  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  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

Doty, 1989; Sousa-Pinto *et al.*, 1999). *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  $2500 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , the maximum photosynthesis of the sun algae is around  $500 \mu\text{mol m}^{-2} \text{sec}^{-1}$  and that of shade algae is around  $60\text{-}150 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . 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 ( $E_k$ ), between 60 and  $170 \mu\text{mol m}^{-2} \text{sec}^{-1}$ .

Xu *et al.* (2009) reported that, the highest specific growth rates of *Gracilaria lichenoides* ( $16.26\% \text{day}^{-1}$ ) and *Gracilaria tenuistipitata* ( $14.83\% \text{day}^{-1}$ ) were recorded at light intensity 287.23 and  $229.07 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , 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, *Dunaliella 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 agal thalli were selected, rinsed and the epiphytes removed using filtered seawater. Prior to the experiment,

*Gracilaria asiatica* were incubated at temperature below 20°C and intensity 90  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  with a 12L: 12D in filtered seawater. The culture medium, salinity 21‰ of 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 fluctuation on *Gracilaria asiatica*, three different light intensity fluctuations (90, 90±20 and 90±40  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) with two different initial concentrations of  $\text{NO}_3\text{-N}$  (50 and 500  $\mu\text{mol L}^{-1}$ ) and  $\text{PO}_4\text{-P}$  (3 and 30  $\mu\text{mol L}^{-1}$ ) were employed for the study.  $\text{NO}_3\text{-N}$  concentrations were enriched with  $\text{NaNO}_3$  while  $\text{PO}_4\text{-P}$  concentrations were enriched with  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .

The experiment lasted for 15 days. Seaweed biomass was measured at 3 days interval and  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  concentrations 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 a 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  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) and two light intensity fluctuations treatments (90±20 and 90±40  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) 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  $\text{NO}_3\text{-N}$  concentration (50 and 500  $\mu\text{mol L}^{-1}$ ) and  $\text{PO}_4\text{-P}$  concentration (3 and 30  $\mu\text{mol L}^{-1}$ ) 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  $F_v/F_m$  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 (%  $\text{day}^{-1}$ ) of the fresh weight was calculated based on the following equation (Chirapart and Lewmanomont, 2004):

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

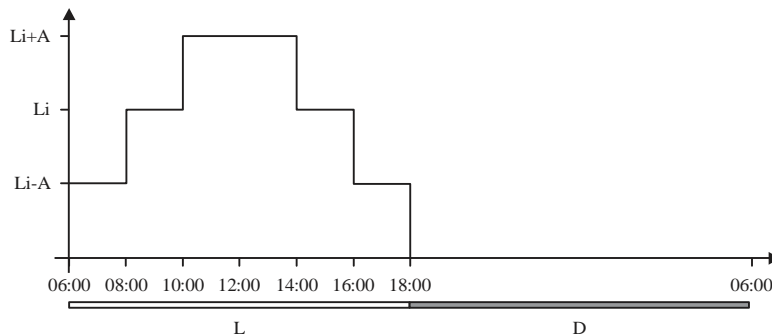


Fig. 1: Light intensity fluctuation mode: White and black behalf of light (L) and dark (D), letters on behalf of A and Li is light intensity rhythms range and average light intensity

Where:

$W_0$  = Initial fresh weight

$W_t$  = Fresh weight at the end

$t$  = 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):

$$V = \frac{[(S_0 \times \text{vol}_0) - (S_t \times \text{vol}_t)]}{t \times B}$$

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

$$\text{Re} = \left[ \frac{S_0 - S_t}{S_0 \times t} \right] \times 100$$

Where:

$S_0, \text{vol}_0$  = Nutrient concentration and the volume at the beginning of sampling interval

$S_t, \text{vol}_t$  = Nutrient concentration and the volume at the end of sampling interval

$t$  = Time between two samplings

$B$  = 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 $\pm$ 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  $90 \pm 20 \mu\text{mol m}^{-2} \text{sec}^{-1}$  ( $10.56\% \text{day}^{-1}$ ) followed by  $90 \pm 40 \mu\text{mol m}^{-2} \text{sec}^{-1}$  ( $9.66\% \text{day}^{-1}$ ) with the lowest growth rate recorded at  $90 \mu\text{mol m}^{-2} \text{sec}^{-1}$  ( $9.44\% \text{day}^{-1}$ ). 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  $18.87\% \text{day}^{-1}$  was recorded at  $90 \pm 20 \mu\text{mol m}^{-2} \text{sec}^{-1}$  which was closely followed by  $11.68\% \text{day}^{-1}$  recorded at  $90 \pm 20 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . The least growth rate of  $10.54\% \text{day}^{-1}$  was however recorded at  $90 \mu\text{mol m}^{-2} \text{sec}^{-1}$ .

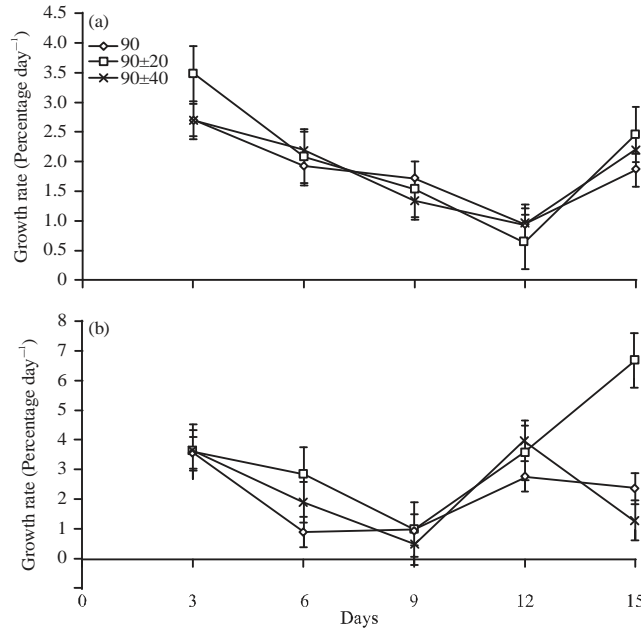


Fig. 2(a-b): Growth rate of *Gracilaria asiatica* at different light intensity fluctuations under (a) Low and (b) High nutrient concentration every 3 days, during 15 days

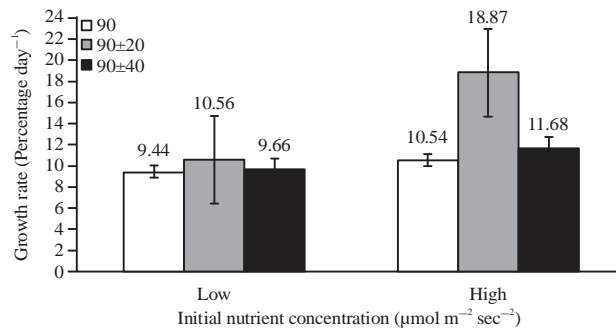


Fig. 3: Growth rate of *Gracilaria asiatica* at different light intensity fluctuations under low and high nutrient concentration at 15 days

**Effects of light intensity fluctuation and initial nutrient concentration on NO<sub>3</sub>-N and PO<sub>4</sub>-P uptake:** Figure 4 shows the nutrient uptake rate by *Gracilaria asiatica* under low and high concentrations. The uptake rate of NO<sub>3</sub>-N and PO<sub>4</sub>-P by *Gracilaria asiatica* under low and high concentrations were affected by light intensity. At high light intensity of 90±40 µmol m<sup>-2</sup> sec<sup>-1</sup>, the highest nutrient uptake of NO<sub>3</sub>-N was recorded (423.39±16.50 µmol g<sup>-1</sup> DW day<sup>-1</sup>). This was followed by 415.50 µmol g<sup>-1</sup> DW day<sup>-1</sup> which were recorded at 90 µmol m<sup>-2</sup> sec<sup>-1</sup> with the least uptake of 365.35 µmol g<sup>-1</sup> DW day<sup>-1</sup> recorded at 90±20 µmol m<sup>-2</sup> sec<sup>-1</sup>. The same trend was observed at low light intensity with 55.26, 54.58 and 52.82 µmol g<sup>-1</sup> DW day<sup>-1</sup> being recorded at 90±40, 90 and 90±20 µmol m<sup>-2</sup> sec<sup>-1</sup>, respectively. With respect to PO<sub>4</sub>-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<sup>-1</sup> DW day<sup>-1</sup> were recorded for 90, 90±20 and 90±40 µmol m<sup>-2</sup> sec<sup>-1</sup>, respectively.

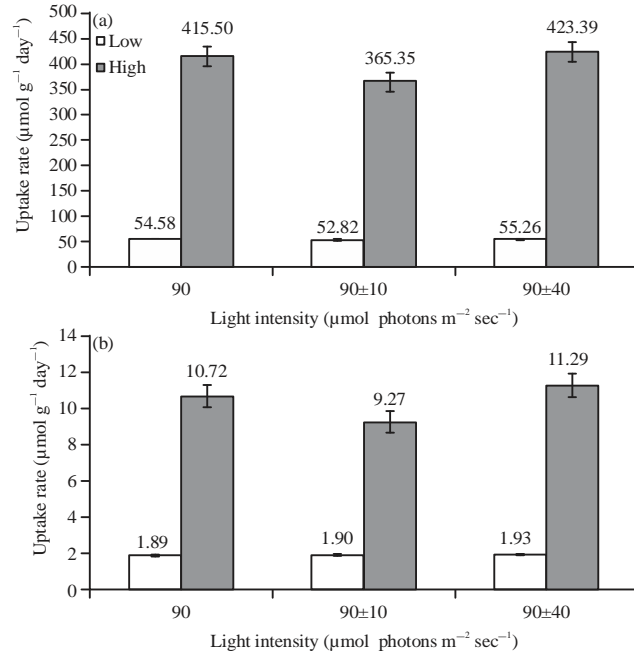


Fig. 4(a-b): (a) Nitrate (NO<sub>3</sub>-N) and (b) Phosphate(PO<sub>4</sub>-P) uptake rate at different light intensity fluctuation under low and high concentrations

When the light intensities were high, the highest PO<sub>4</sub>-P uptake rate was recorded at 90±40 µmol m<sup>-2</sup> sec<sup>-1</sup> (11.29 µmol g<sup>-1</sup> DW day<sup>-1</sup>), followed by 90 µmol g<sup>-1</sup> DW day<sup>-1</sup> (10.70 µmol g<sup>-1</sup> DW day<sup>-1</sup>) with the least being recorded at 90±20 µmol g<sup>-1</sup> DW day<sup>-1</sup> (9.27 µmol g<sup>-1</sup> DW day<sup>-1</sup>).

**NO<sub>3</sub>-N and PO<sub>4</sub>-P removal efficiency at different light intensity fluctuation under low and high concentrations:** The NO<sub>3</sub>-N and PO<sub>4</sub>-P removal efficiency at different light intensities with different nutrient concentration are shown in Fig. 5. The NO<sub>3</sub>-N and PO<sub>4</sub>-P removal efficiency at low initial concentration were higher than high initial concentration under the various light intensity fluctuations. The values of NO<sub>3</sub>-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<sup>-2</sup> sec<sup>-1</sup> was significantly lower than the two other treatments (90 and 90±20 µmol m<sup>-2</sup> sec<sup>-1</sup>). The NO<sub>3</sub>-N removal efficiency at high initial concentration under every different light intensity fluctuations increased from beginning until the end of experiment. The least PO<sub>4</sub>-P removal efficiency recorded under different low concentration was recorded on the day 3 when the light intensity was 90±40 µmol m<sup>-2</sup> sec<sup>-1</sup>. 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 PO<sub>4</sub>-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

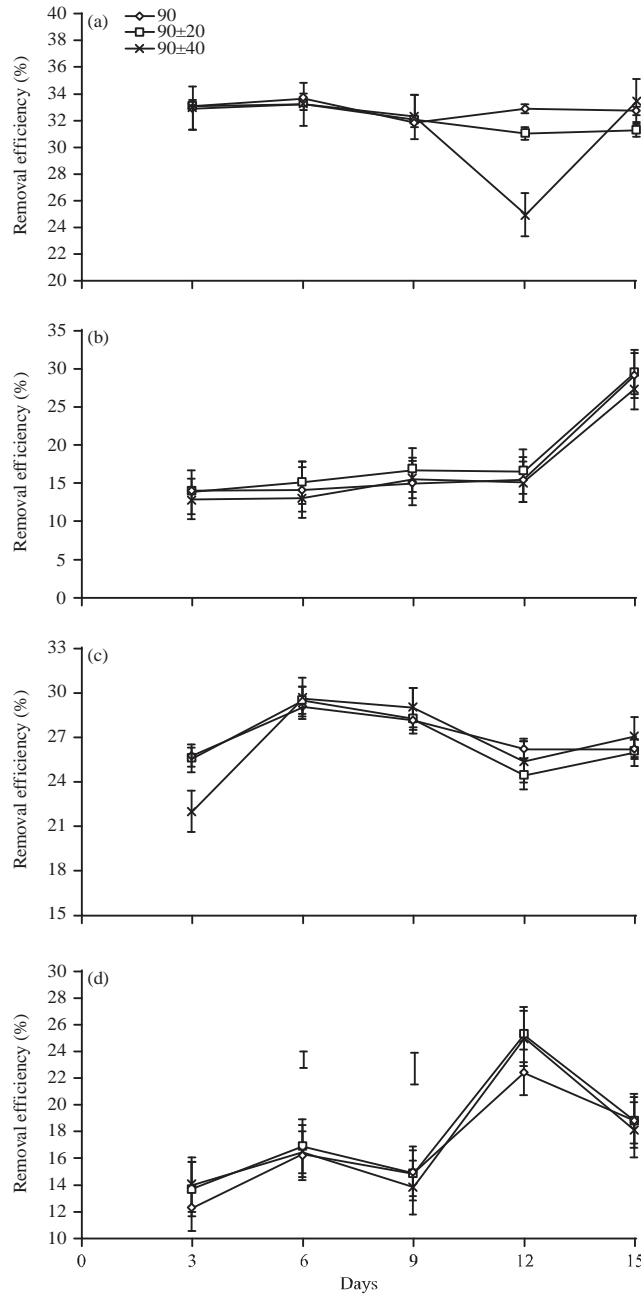


Fig. 5(a-d): Removal efficiency at different light intensity fluctuation under low and high concentrations every 3 days, during 15 days, (a) Low NO<sub>3</sub>-N concentration, (b) High NO<sub>3</sub>-N concentration, (c) Low PO<sub>4</sub>-P concentration and (d) High PO<sub>4</sub>-P concentration

highest yield of 0.67 was recorded when the intensity was 90±40 μmol m<sup>-2</sup> sec<sup>-1</sup>. At intensities of 90 and 90±20 μmol m<sup>-2</sup> sec<sup>-1</sup>, 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<sup>-2</sup> sec<sup>-1</sup> with yield of 0.61 and 0.58, respectively.



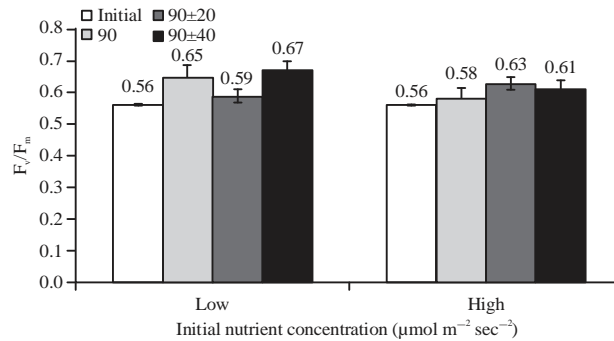


Fig. 6: Yield ( $F_v/F_m$ ) at different light intensity fluctuation under low and high concentrations,  $F_v/F_m$ : photosynthetic efficiency

## DISCUSSIONS

Light is one of the most important environmental factors influencing seaweed growth (Yang *et al.*, 2015). In this study, when light intensity was  $90 \pm 20 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$  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  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  uptake existed at  $90 \pm 40 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$  under high initial concentration ( $423.39 \pm 16.50$  and  $11.29 \pm 2.10 \mu\text{mol g}^{-1} \text{DW day}^{-1}$ ), respectively. The higher value of  $\text{NO}_3\text{-N}$  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  $\text{NO}_3$ -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  $\text{PO}_4\text{-P}$  remain too high. In contrast, the  $\text{NO}_3\text{-N}$  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  $90 \pm 20 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . 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  $90 \pm 20 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . Also more studies should be conducted on other factors that affects growth and performance of cultured *Gracilaria asiatica*.

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