Effect of different temperature fluctuations and different initial concentrations of NO\textsubscript{3}-N and PO\textsubscript{4}-P on growth, nutrient uptake and photosynthetic efficiency of \textit{Gracilaria asiatica}

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The effects of three temperature fluctuations combined with different initial NO\textsubscript{3}-N and PO\textsubscript{4}-P concentrations on growth rate, nutrient uptake as well as photosynthetic efficiency were assessed. \textit{Gracilaria asiatica} were collected from the coast of Fujian, China for this present study. Study revealed that after 15 days, the highest growth rate of \textit{G. asiatica} was recorded at 20ºC under low initial nutrient concentration whiles the lowest was recorded at 25ºC under high initial nutrient concentration. The NO\textsubscript{3}-N uptake rate of \textit{G. asiatica} under low and high initial concentration at temperatures 20ºC and 20±2ºC were higher than other conditions after 15 days. On the other hand the PO\textsubscript{4}-P uptake rate under high initial concentration at temperatures 20ºC and 20±2ºC were lower than other conditions after 15 days. After the third day, NO\textsubscript{3}-N and PO\textsubscript{4}-P were exhausted from the medium when the initial concentration were low under different temperature fluctuations. Photosynthetic efficiency observed at temperatures 15ºC and 15±2ºC decreased from beginning whiles the photosynthetic efficiency at 25ºC and 25±2ºC increased from beginning. We concluded that temperature fluctuations integrated with different initial NO\textsubscript{3}-N and PO\textsubscript{4}-P concentrations affects growth rate, nutrient uptake as well as photosynthetic efficiency of \textit{G. asiatica}.

[Keywords: \textit{Gracilaria}, temperature fluctuations, nutrient uptake rate, nutrient concentrations, photosynthetic efficiency]

Introduction

\textit{Gracilaria} is one of the genera, belonging to the Rhodophyta family\textsuperscript{1}. \textit{Gracilaria} is worldwide distributed, but grows mostly in tropic and subtropic waters\textsuperscript{2,5,8&7}. Nasmia et al. documented that the production of seaweed, and \textit{Gracilaria} is estimated to increase concurrent with the increasing demand of seaweed for the food industry, pharmacy and cosmetics\textsuperscript{6}. Due to its high yields and commercial value, products such as agar-agar are used for human consumption and fodder for marine animals\textsuperscript{5,7}. There are more than 100 species of the genus \textit{Gracilaria}\textsuperscript{8}. \textit{Gracilaria} grows well in the tropic and sub-tropic sea waters\textsuperscript{2,5,8}. Some species have characteristics such as strong adaptability, rapid growth rate, high yield, asexual reproduction and high uptake of the nitrogen (N) and phosphorus (P) hence making them suitable candidates for cultivation\textsuperscript{5,8,9&10}. Environmental factors not only affect the growth of seaweeds, but also have a significant influence on other biochemical components, such as pigmentation, protein synthesis and the antioxidantive defense system of the algae\textsuperscript{10,11&12}. Environmental factors play important roles in the growth, reproduction and distribution of seaweed which have to adapt to the several conditions in their natural habitat\textsuperscript{13&14}. This is because most of the seaweeds are sessile organisms and are unable to migrate themselves during environmental stress, such as, changes in temperature, salinity, solar radiation and nutrients.

Temperature has an important influence on the seasonal and geographic distribution, and the growth rate of \textit{Gracilaria}\textsuperscript{15}. Most enzymes are effective at optimal temperatures and if the temperature is higher than optimal range, the
enzyme activity will be deteriorated and cell-molecular activities will be interrupted. Similarly, the nutrient absorption is also affected by temperature, especially nitrogen. Moreover, the chemical reaction, respiratory metabolism, enzyme activity and growth of organisms in the tidal zone are also affected by temperature. The fluidity in the cell membranes is decreased when temperature levels fall very low. This leads to cell compensation by increasing the unsaturated fatty acid to increase fluidity thereby making the membranes more susceptible to damage by free radicals. Mitsu documented that when temperature increases there is a degradation of the starch produced. Temperature also has impacted on the starch content in the algal cell. Increasing temperature beyond the optimum reduces protein synthesis and consequently results in decrease of growth rates.

The highest specific growth rates of Gracilaria lichenoides (16.26% d⁻¹) and Gracilaria tenuistipitata (14.83% d⁻¹) (% increase in wet weight) occurred at temperatures of 31.30°C and 25.38°C, respectively. Lapointe et al. reported that, Gracilaria tikvahiae grew well at 25°C and the NO₃-N uptake increased when temperature increased. Although researchers have studied on the effects of environmental factors on seaweed, there is limited information with reference to Gracilaria asiatica. The objective of the present study was therefore to assess the effects of different temperatures and initial concentrations on growth, nutrient uptake and photosynthetic efficiency of G. asiatica. This study will serve as a fundamental information for the cultivation of G. asiatica.

Materials and Methods

G. asiatica were collected at the coast of Fujian province, China. 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, the algal were incubated at temperatures 18°C to 19°C and 90 µmol m⁻² s⁻¹ with a 12L:12D in salinity 20 filtered seawater. Culture medium, 21 of seawater salinity; Von Stoch's modified enriched seawater (VSE) was added at every 3 days and seawater was changed every 6 days.

To investigate the effects of different temperature fluctuations, G. asiatica was cultured under three different temperature fluctuations treatments (15±2°C, 20±2°C and 25±2°C), two different initial concentration of NO₃-N (50 and 500 µmol L⁻¹) and PO₄-P (3 and 30 µmol L⁻¹). The NO₃-N concentration were enriched with (NaNO₃) and PO₄-P concentration were enriched with (Na₂HPO₄·12H₂O) under light intensity of 90 µmol m⁻² s⁻¹ with a 12L:12D light–dark cycle. The experimental treatments were in triplicates. The seaweed biomass was measured every 3 days. NO₃-N and PO₄-P concentrations were determined by SKALAR Analyzer on every third day. Photosynthetic efficiency was measured at the beginning and end of the experiment using Hansatech Instrument (Oxy-Lab). The culture medium was changed every 3 days throughout the 15 days of experiment.

The mean initial weight of the seaweed was 0.3123±0.0049 g (Mean ± SD). Seaweed were put in 250 ml flasks containing 200 ml of seawater and was added into the culture medium (VSE). Control experiments were three constant temperature treatments (15°C, 20°C and 25°C). Temperature fluctuations treatments were programmed to follow a circadian rhythm (Fig. 1).

![Fig. 1—Temperature fluctuation mode: White and black behalf of light (L) and dark (D), A is temperature the rhythms range and T is average temperature.](image)
quantum yield \((F_v/F_m)\) of photo system II (PSII) by Phyto-PAM Analyzer, WAL-Z.

All results are presented as mean ± standard error of the mean (SEM). Data were analyzed by one-way Analysis of Variances (ANOVA) to test the effects of different temperatures and light intensities on growth and nutrient uptake of \(G. asiatica\). 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 temperature fluctuations and initial nutrient concentrations on growth of \(G. asiatica\)

Growth rate of \(G. asiatica\) at different temperature fluctuations under low and high nutrient concentrations are shown in Fig. 2 and Fig. 3. In all instances there was a general trend of decreasing growth rate. From Fig. 2A, growth rate after the 15th day was lower compared to the 3rd day. When temperature was fluctuated around 20 ºC with either low or high nutrient concentration, growth rate increased until the 6th day even though that of high nutrient concentration had decreased. Growth rate however decreased thereafter until the 15th day. With respect to growth rate under low nutrient concentration, 15 ºC recorded the highest (18.00% \(d^{-1}\) and 16.95% \(d^{-1}\)), followed by 25 ºC (13.09% \(d^{-1}\) and 12.53% \(d^{-1}\)) with the least being recorded at 20 ºC (12.04% \(d^{-1}\) and 11.57% \(d^{-1}\)) for constant and fluctuating temperatures respectively. When the initial concentrations were low, the highest growth rate for both constant and fluctuating temperatures were recorded at 20 ºC (18.93% \(d^{-1}\) and 14.74% \(d^{-1}\)) respectively. This was followed by 15 ºC (17.03% \(d^{-1}\) and 14.51% \(d^{-1}\)) and 25 ºC (16.25% \(d^{-1}\) and 14.64% \(d^{-1}\)) for constant and fluctuating temperatures respectively.

From Fig. 2C, it is evident there was a decrease in growth rate under low and high nutrient concentrations when the temperature was 25 ºC or 25±2 ºC until the 9th day, however growth rate increased until the 15th day although there was a slight decrease at low 25±2 ºC and low 25 ºC.

Fig. 2—Growth rate of \(G. asiatica\) at different temperature fluctuations under low and high nutrient concentration every 3 days, during 15 days; 15ºC (A), 20ºC (B) and 25ºC (C).

Effects of temperature fluctuations and initial nutrient concentrations on \(NO_3-N\) and \(PO_4-P\) uptake

The uptake rate of \(NO_3-N\) as well as \(PO_4-P\) by \(G. asiatica\) was affected under low and high concentration with different temperatures (Fig. 4). Under low \(NO_3-N\) concentration, the highest uptake rate was recorded at 20 ºC which was significantly higher than those of 15 ºC and 25 ºC. Similarly, when the \(NO_3-N\) were high, the highest uptake rate was recorded at 20 ºC followed by 15 ºC and 25 ºC in that order. It is worth noting that this trend was observed under both constant and fluctuating temperatures. With respect to low and

Fig. 3—Growth rate of \(G. asiatica\) at different temperature fluctuations under low and high nutrient concentration at 15 days. Low nutrient concentration (A) and High nutrient concentration (B).
high PO$_4$-P concentrations, the highest nutrient uptake rate recorded occurred at 25 °C (14.64 µmol g$^{-1}$ DW d$^{-1}$) and 15 °C (14.97 µmol g$^{-1}$ DW d$^{-1}$) for fluctuating and constant temperatures, respectively. The least nutrient uptakes however occurred at 25 °C (1.79 µmol g$^{-1}$ DW d$^{-1}$) and 20 °C (1.71 µmol g$^{-1}$ DW d$^{-1}$) for constant and fluctuating temperatures respectively. Under high PO$_4$-P concentration, the least nutrient uptake of 6.74 µmol g$^{-1}$ DW d$^{-1}$ and 9.31 µmol g$^{-1}$ DW d$^{-1}$ for constant and fluctuating temperatures, respectively, were recorded at 20 °C. These values were significantly lower than those of 15 °C and 25 °C.

**Fig. 4**—NO$_3$-N and PO$_4$-P uptake rate at different temperature fluctuation under low and high concentrations every 3 days, during 15 days; Low NO$_3$-N concentration (A), High NO$_3$-N concentration (B), Low PO$_4$-P concentration (C) and High PO$_4$-P concentration (D).

**NO$_3$-N and PO$_4$-P removal efficiency at different temperature fluctuations under low and high concentrations**

The NO$_3$-N and PO$_4$-P removal efficiency reported for this study is presented in Fig. 5. The NO$_3$-N and PO$_4$-P removal efficiency recorded when the initial concentration were low happened to be higher than when initial concentration was high under every different temperature. After the third day, NO$_3$-N and PO$_4$-P were exhausted from the medium when the initial concentration were low under different temperature fluctuations (Fig. 5). However, at high NO$_3$-N concentration, the nutrient in the medium remained high (Fig 5A-C). The PO$_4$-P removal efficiency at high initial concentration under every different temperature fluctuations increased (Fig. 5D-F).
Photosynthetic efficiency

Under the temperatures 15°C and 15±2°C, the $F_v/F_m$ decreased from an initial value of 0.53 to 0.32-0.52 and were significant different after 15 days (Fig. 6A). The $F_v/F_m$ under temperatures 20°C, 20±2°C and 25°C, 25±2°C however increased from an initial value of 0.56 to 0.58-0.65 and 0.41 to 0.56-0.66, respectively, after 15 days although it was not significant different (Fig. 6B-C). The $F_v/F_m$ value recorded at 20°C and 20±2°C under low and high initial concentration were almost the same with value of 0.56 at beginning and 0.58-0.65 after 15 days (Fig. 6B).

Discussion

Gracilaria is one of the most important and the most attractive candidate for culture because of its high yields and commercial value products\(^7\). McLachlan and Bird reported that water temperature has been an important factor for production of Gracilaria\(^21\). It grows well when the temperature is 20°C or higher. Friedlander et al. reported that low and high temperatures were limiting factors of Gracilaria\(^22\). In nature, the G. lemaneiformis grows along the coast of Shandong Province, China. It grows well with high growth rate at eutrophic seawater areas in spring and autumn when temperature is between 12 and 23 °C\(^22\&23\). The results of this present study showed that the maximum growth rate of G. asiatica was observed at a constant temperature of 20°C under high nutrient concentration (18.93±4.06% d\(^-1\)). The result is similar to that of Zhou et al. who documented that the maximum growth rate of G. lemaneiformis (11.03% d\(^-1\)), was recorded when the seawater temperature ranged from 12.8 to 22.2 °C\(^7\). The reason for the highest growth rate occurring at 20°C but not 25°C could be attributed to the assertion that increasing temperature beyond its optimum reduces protein synthesis and consequently leads to a decrease in growth rate\(^19\). In Gracilaria, most species grow well when the temperature is 20 °C or higher. At
higher temperature, production may be reduced or even ceased\textsuperscript{15}.

In this present study, the highest NO\textsubscript{3}-N uptake rate occurred at 20\textdegree C and 20±2 \textdegree C under high initial concentration (152.64±23.49 and 132.65±59.91 \mu mol g\textsuperscript{-1} DW d\textsuperscript{-1} respectively), while the highest rate of PO\textsubscript{4}-P uptake rate existed at 25 \textdegree C and 15±2 \textdegree C under high initial concentration (11.56±0.69 and 14.97±0.90 \mu mol g\textsuperscript{-1} DW d\textsuperscript{-1} respectively). In contrast, at temperatures 20 and 20±2\textdegree C the PO\textsubscript{4}-P uptake rate was lowest. This could be reconciled with the fact that nitrogen is important more than phosphorus because nitrogenous compounds are important for amino acid, amines and protein synthesis and the nutrient absorption especially nitrogen is affected by temperature\textsuperscript{16}. The uptake rate increased when the initial concentration levels increased. The results of this study is in agreement to the results of Zhou et al. who documented that Nitrogen and Phosphorus uptake of \textit{G. asiatica} were 10.64 and 0.38 \mu mol g\textsuperscript{-1} DW h\textsuperscript{-1} respectively\textsuperscript{17}.

NO\textsubscript{3}-N and PO\textsubscript{4}-P removal efficiency at low initial concentrations under all conditions were exhausted from the medium before 3 days. This could be due to the fact that under low NO\textsubscript{3}-N and PO\textsubscript{4}-P initial concentration (50 and 3 \mu mol L\textsuperscript{-1}) there was starvation. At higher initial concentration under all conditions, NO\textsubscript{3}-N remained too high. In contrast, The PO\textsubscript{4}-P removal efficiency under high initial concentration increased day by day, especially at temperatures 25 and 25±2\textdegree C where the PO\textsubscript{4}-P was nearly exhausted from the medium. This could be due to the possibility that in this experiment growth occurred as epiphytes when at high initial (500 and 30 \mu mol L\textsuperscript{-1}) concentration (nutrient enrichment). Epiphytism on seaweed thallus is competition for nutrient, light, substrate and gases dissolved in the seawater, and decreased growth rate of seaweed.

Photosynthetic efficiency at the end of experiment under temperatures 20 and 20±2\textdegree C did not differ significantly from that of the initial. At temperatures under 25\textdegree C and 25±2\textdegree C, the F\textsubscript{v}/F\textsubscript{m} increased from an initial value due to the growth of epiphytes (Green and Blue-green algae). Thus, F\textsubscript{v}/F\textsubscript{m} under temperatures 25\textdegree C and 25±2\textdegree C were high. Similar to this study, Friedlander et al. documented the growth of epiphytes when \textit{G. conferta} were cultured in earthen ponds\textsuperscript{24}. Such as \textit{Ulva lactuca} and \textit{Ulva compressa} competed nutrient and light on thallus of \textit{G. conferta}, caused the growth rate of \textit{G. conferta} was decreased. However, the F\textsubscript{v}/F\textsubscript{m} of \textit{G. asiatica} at low and high concentrations were not affected by water temperature although that at low and high concentrations were affected by epiphytes.

**Conclusion**

It can be concluded that \textit{G. asiatica} grows well at the temperature of 20\textdegree C under high nutrient concentration. Also the highest nutrient uptake rate was recorded at 20\textdegree C under high initial concentrations which indicated that growth rate and nutrient uptake are affected by temperature and nutrient concentration especially nitrogen. Nutrient removal efficiency at low initial concentrations under all conditions caused nutrient starvation. High initial concentration (500 and 30 \mu mol L\textsuperscript{-1}) caused nutrient enrichment and grew as epiphytes (Green and Blue-green algae). The F\textsubscript{v}/F\textsubscript{m} at the end of the experiment under temperature 20 and 20±2\textdegree C almost were the same as beginning. In contrast, the F\textsubscript{v}/F\textsubscript{m} increased from the initial value under temperature 25\textdegree C and 25±2\textdegree C, resulting the growth of epiphytes (Green and Blue-green algae).

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**References**


