

Photosynthetic Response of *Gracilaria fisheri* (Xia & Abbott) Abbott, Zhang & Xia to Irradiance, Temperature and Salinity Variation

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ABSTRACT

The red seaweed *Gracilaria fisheri* has been used for human food, for marine animal feeding, and for agar extraction. This algal species is extensively cultured, but its production is often unpredictable due to lack of basic biological information on this species. This work aims to determine the effects of irradiance, salinity and temperature on the photosynthesis of this species. Plants were subjected to different irradiance (0–1150 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), salinity (15–40‰), and temperature (12–40°C) levels in a growth chamber. Photosynthesis was determined using a dissolved oxygen sensor and pulse amplitude-modulated fluorometry. The results showed rapid light curves increasing with irradiance up to 1150 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The highest P_{net} was obtained at 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature 24°C and a salinity of 30‰, which also corresponded to the highest maximum quantum yield. Our study revealed that the P_{net} rates were highest from 24–28°C at an irradiance of 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and was lower at higher and lower temperatures.

Keywords: gracilaroid, photosynthesis, PAM, Thai seaweed

INTRODUCTION

The gracilaroid seaweeds are one of the mostly widely used taxa for commercial agar production throughout the world. This genus accounts for approximately 60% of all agar produced (Tseng, 2001). The species *Gracilaria fisheri* is widely distributed in the South China Sea (Lewmanomont, 1994; Ohno *et al.*, 1999; Phang *et al.*, 2016). In Thailand, this species is commonly found in sandy or muddy areas with turbid water along the coasts of the Gulf of Thailand and the Andaman Sea (Chirapart and Lewmanomont, 2004). In Thailand, *G. fisheri* is harvested by hand from natural populations, and its main uses are in food products for human consumption and feed additives for marine animals. In recent years, the demand for the algae has increased, and has led to commercial

mariculture of *G. fisheri* in Thailand. The annual production of *G. fisheri* is approximately 1–5 tonnes for each farm, with a selling price of ~US\$0.3–0.5 kg⁻¹ wet weight (Ruangchuay *et al.*, 2010). Mass cultivation of *G. fisheri* mostly occurs in shrimp pond effluent (Chirapart and Lewmanomont, 2004; Ruangchuay *et al.*, 2010), but production is not stable, which is compounded by a lack of basic biological information on this species. For more stable cultivation, the biology of *G. fisheri* needs to be clarified.

Environmental factors, such as irradiance, salinity and temperature, are the main contributors to the growth and distribution of the seaweed (Chirapart and Lewmanomont, 2004; Choi *et al.*, 2006; Araujo *et al.*, 2014). Additionally, these abiotic factors influence photosynthetic responses

and respiration (Lobban and Harrison, 1994), including in *Gracilaria* species (Phooprong *et al.*, 2008; Araujo *et al.*, 2014, Jayasankar, 2005).

There have been several studies measuring growth and agar production for species of *Gracilaria* (Chirapart and Lewmanomont, 2004; Chirapart *et al.*, 2006; Xu *et al.*, 2009; Ruangchuay *et al.*, 2010; Nguyen *et al.*, 2017), however, there are a few studies on physiological responses in tropical species of *Gracilaria* (Phooprong *et al.*, 2007) and none on *G. fisheri*.

In the present study, we investigated the physiological response of *G. fisheri* to controlled changes in irradiance, salinity and temperature. We focused the analysis on the effects of these factors on the photosynthetic activity of algal thalli. This study will provide information that can be used to improve the future mariculture of *G. fisheri* in Thailand.

MATERIALS AND METHODS

Samples of *G. fisheri* were collected from a shrimp culture pond in Phang Nga Province ($8^{\circ}24'0''\text{N}$, $98^{\circ}15'42''\text{E}$) in southern Thailand in 2015. The collected samples were allowed to adapt to the laboratory conditions at 25°C , 30‰ and a light intensity of $105 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for one month; then healthy thalli (1 g each) were selected for further experiments. Provasoli Enriched Seawater (PES) was used as the culture medium.

Rapid light curves (relative electron transport rate)

Rapid light curves (RLCs) were generated by running the standard algorithm of a Pulse Amplitude Modulated (PAM) fluorometer (Junior-PAM, Walz/Germany) using an incremental sequence of actinic illumination periods, with photosynthetic active radiation (PAR) intensity increasing in nine steps from 0 to $1,150 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Relative electron transport rate (rETR) was calculated using the following equation:

$$\text{rETR} = 0.5 * Y * \text{PAR} * \text{AF},$$

where Y is the effective quantum yield of PSII, the factor 0.5 assumes that half of the photons are absorbed by PSII and AF is the fraction of incident light assumed to be absorbed by the sample (i.e., 0.84).

Effect of irradiance on photosynthesis

Photosynthetic rates were determined at different levels of light intensity (0, 30, 60, 100, 150, 200, 250 and $500 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 25°C (three replicates per level). The net photosynthetic rates (P_{net}) and the dark respiration were determined by measuring the dissolved oxygen concentration (mg L^{-1}) every 5 min for 30 min. After these measurements, a 30-min pre-incubation period was used to allow the samples to acclimate to each set of experimental conditions. The slope of the linear regression was determined from the data from 30-min measurements of the estimated rates. Dissolved oxygen (DO) was measured using a DO meter (YSI 5000). The light saturated point was considered to be the optimum density and was used to determine the effects of salinity and temperature on photosynthesis.

Effect of salinity on photosynthesis

Photosynthetic rates were determined at different levels of salinity (15, 20, 25, 30, 35 and 40‰), at 25°C (three replicates per level). Samples were incubated under the saturating light intensity (from data described above); then the P_{net} were determined as described above.

Effect of salinity on maximum quantum yield of charge separation

The maximum quantum yields of charge separation at $0 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were determined at different salinities of 15, 20, 25, 30, 35 and 40‰ after periods of 0, 3, and 7 days. Each increment in salinity occurred over a 30-min period with an additional 30 min allowed for dark and salinity acclimatization. The maximum quantum yields were measured using a Junior-PAM (pulse-amplitude-modulation) chlorophyll fluorometer.

Effect of temperature on photosynthesis and dark respiration

Photosynthetic rates were determined at different levels of temperature (12, 16, 20, 24, 28, 32, 36 and 40°C) at salinity of 30‰ (three replicates per level). Samples were incubated under the light saturation point (from the data described above), and the P_{net} and dark respiration were determined as described above.

Effect of temperature on maximum quantum yield of charge separation

The maximum quantum yield of charge separation at 0 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was determined at temperatures of 12, 16, 20, 24, 28, 32, 36 and 40°C. The maximum quantum yield was measured using the Junior-PAM.

Measurement of chlorophyll-a

Samples were extracted under refrigeration using 10 mL of N,N-dimethylformamide (DMF) for 24 h. The absorbance (Abs) was determined using a spectrophotometer at 750, 646.8, and 663.8 nm. Chlorophyll-a (chl-a) levels were calculated using the equation (Porra *et al.*, 1989):

$$\text{Chl-a } (\mu\text{g}\cdot\text{mL}^{-1}) = 12.00 (\text{Abs}_{663.8} - \text{Abs}_{750}) - 3.11 (\text{Abs}_{646.8} - \text{Abs}_{750}),$$

where Abs 663.8, Abs 646.8, and Abs 750 represent the absorbance values at these wavelengths (in nm).

Statistical analysis

The data are presented as means \pm standard deviation (SD). Statistical analyses were performed using following by a one-way analysis of variance (ANOVA) at the confidence level of 95%.

RESULTS

The rapid light curves of *Gracilaria fisheri* increased until reaching an asymptote, photoinhibition (measured as relative electron transport (rETR)), which was not apparent until PAR reached 1150 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1a). Net photosynthetic rates (P_{net}) also increased from 0 to 500 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1b). P_{net} steadily increased from 100 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with the highest P_{net} (2.9 $\mu\text{g O}_2\cdot\text{g}^{-1}\text{ww}\cdot\text{min}^{-1}$) at the light-saturation point of 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1b).

The experiment on the effect of salinity on the P_{net} rate, at 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, showed that after seven days of culturing, P_{net} increased with salinity from 15 to 20‰ (i.e., -0.54, -0.34 $\mu\text{g O}_2\cdot\text{g}^{-1}\text{ww}\cdot\text{min}^{-1}$) and decreased from 35 to 40‰ (-0.21, -4.53 $\mu\text{g O}_2\cdot\text{g}^{-1}\text{ww}\cdot\text{min}^{-1}$). The optimum salinity level for photosynthesis was between 25 and 30‰. The highest P_{net} was obtained at a salinity of 30‰ ($3.32 \pm 0.62 \mu\text{g O}_2\cdot\text{g}^{-1}\text{ww}\cdot\text{min}^{-1}$) on the third day of incubation (Fig. 2a). In addition, the highest maximum quantum yield of 0.54 ± 0.003 was also obtained at 30‰. Lower yields were obtained at the other salinities of 15, 20, 25, 35 and 40‰ (0.51 ± 0.003 , 0.52 ± 0.003 , 0.53 ± 0.003 , 0.50 ± 0.004 and 0.50 ± 0.003 , respectively) (Fig. 2b). On the first day of incubation, the maximum quantum yield was significantly different ($p < 0.05$) from that on the third and the seventh days of incubation. However, the maximum quantum yield was somewhat stable, within the range of 0.50 and 0.54. Different chlorophyll-a content was obtained at salinities of 15, 20, 25, 35 and 40‰ (9.6, 10.9, 11.5, 8.4 and 6.6 $\mu\text{g mL}^{-1}$, respectively) (Fig. 2c), with the highest content at 30‰ (13.1 $\mu\text{g mL}^{-1}$). Therefore highest rates of net photosynthesis, maximum quantum yield and chlorophyll-a content were all at 30‰.

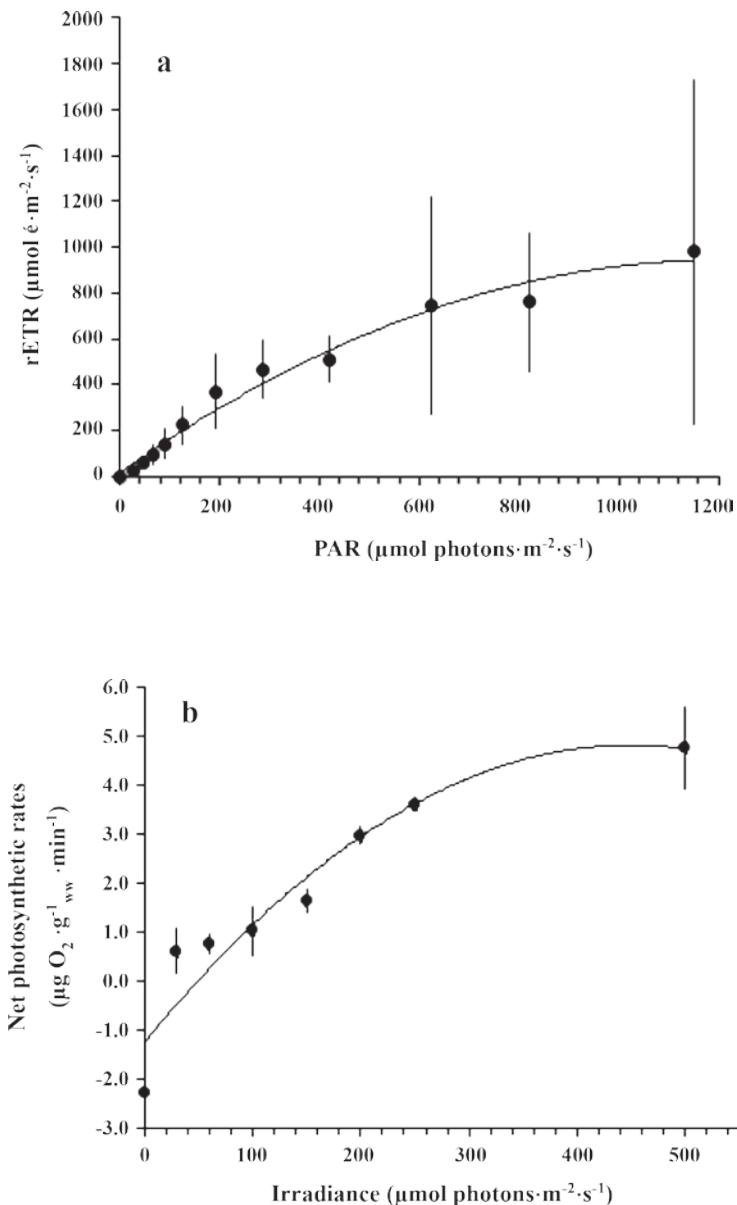


Figure 1. Influence of exposure to irradiance between 0–1150 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the relative electron transport rate (rETR) of Chl *a* fluorescence of *Gracilaria fisheri* (a) and influence of exposure to irradiance between 0–500 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the net photosynthetic rates (P_{net}) and respiration of Chl *a* fluorescence of *G. fisheri* (b). Data are mean \pm SD. PAR = photosynthetic active radiation.

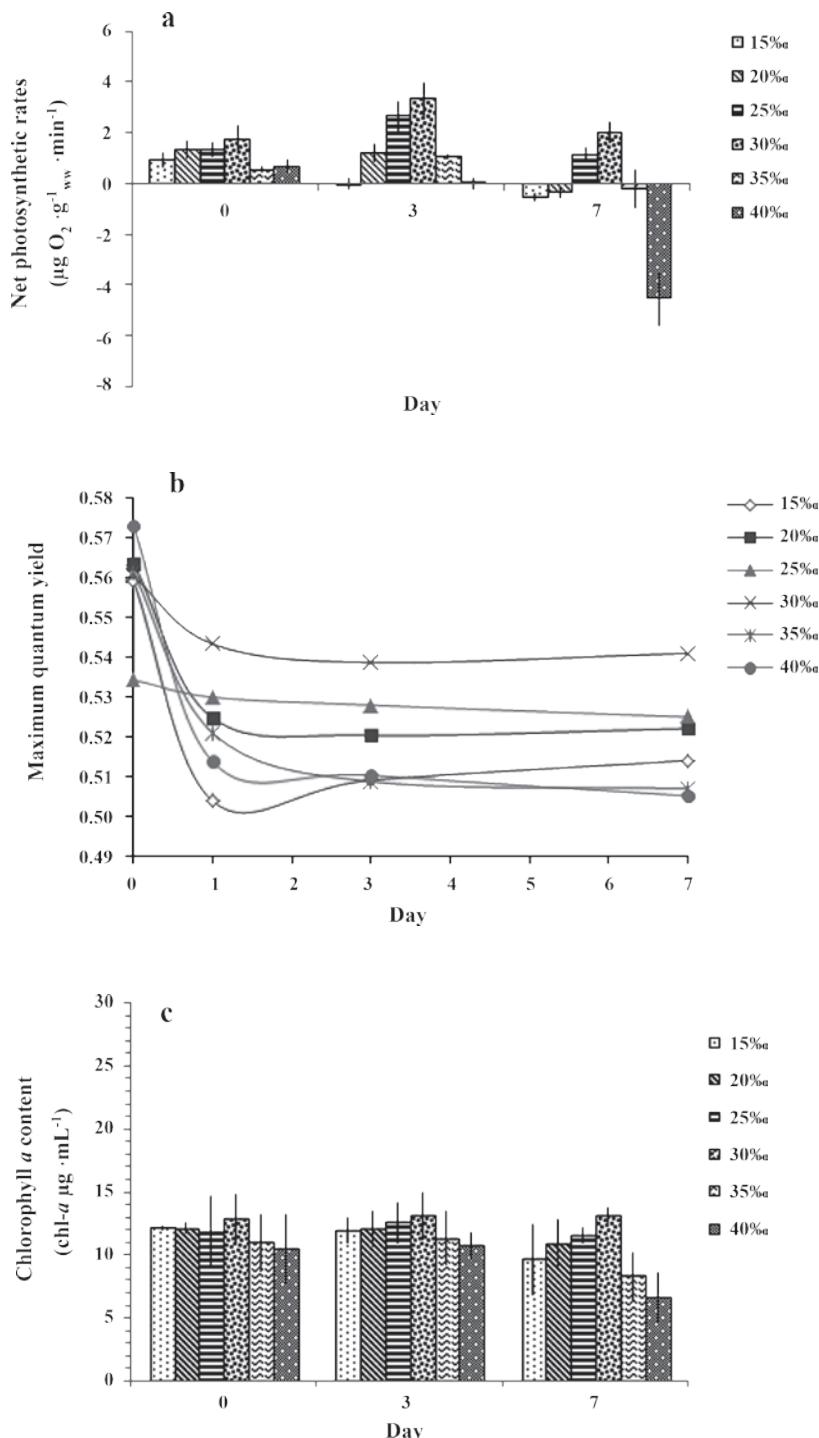


Figure 2. The response of net photosynthetic rates (a), maximum quantum yield (b) and Chlorophyll a content of *Gracilaria fisheri* under different levels of salinity (15, 20, 25, 30, 35 and 40‰) (c). Data are mean \pm SD.

The response of the photosynthetic rates of *G. fisheri* to different temperatures was measured at the saturating light intensity of 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 30% (Fig. 3). The P_{net} was highest at 24°C and 28°C and decreased with temperatures above and below this range (Fig. 3a). Similar significant differences were seen in gross photosynthesis ($p < 0.05$). The gross photosynthetic rates gradually increased with

increasing temperature and attained its maximum at 24°C ($5.89 \mu\text{g O}_2\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) and 28°C ($5.91 \mu\text{g O}_2\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) before sharply decreasing (Fig. 3b). Dark respiration rates decreased from a high at 12°C ($-0.61 \mu\text{g O}_2\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) to a low at 28°C ($-2.06 \mu\text{g O}_2\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) and then increased again at higher temperatures (Fig. 3c). Maximum quantum yield was highest at 24°C (0.46 ± 0.01) (Fig. 3d).

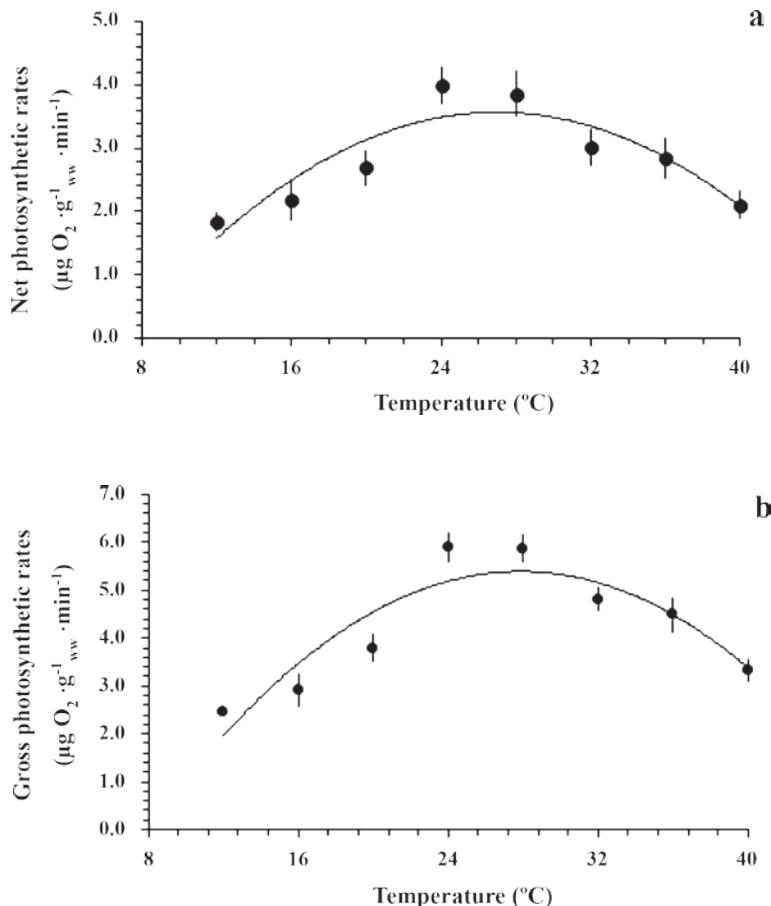


Figure 3. The response of photosynthetic rates (a), gross photosynthetic rates (b), respiration rates (c) and maximum quantum yield (d) of *Gracilaria fisheri* at different temperatures of 12, 16, 20, 24, 28, 32, 36 and 40°C. Data are mean \pm SD.

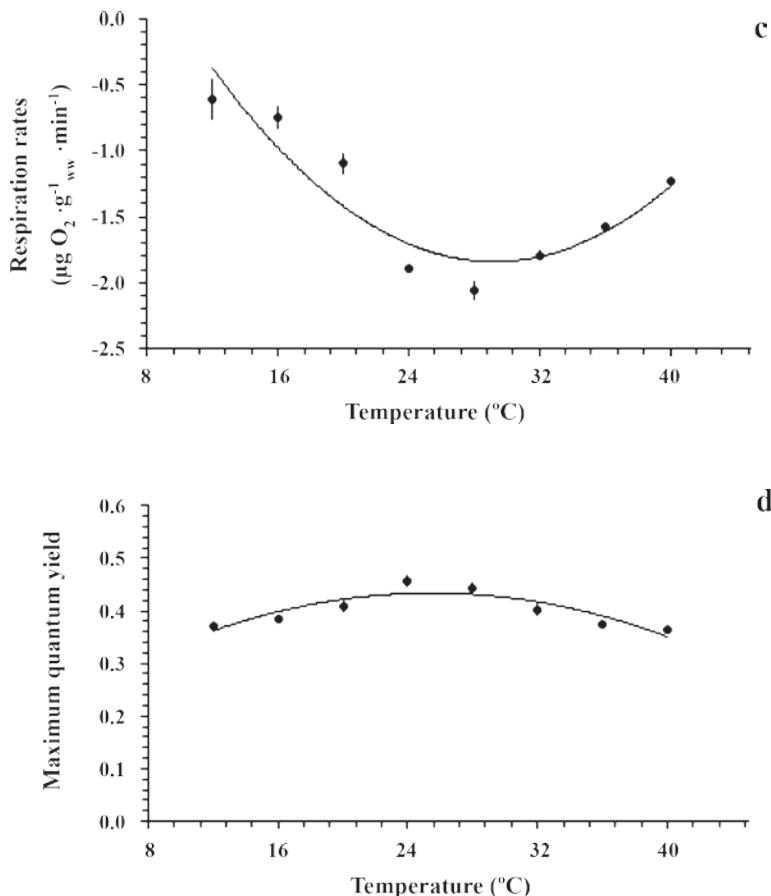


Figure 3. (Cont.) The response of photosynthetic rates (a), gross photosynthetic rates (b), respiration rates (c) and maximum quantum yield (d) of *Gracilaria fisheri* at different temperatures of 12, 16, 20, 24, 28, 32, 36 and 40°C. Data are mean \pm SD.

DISCUSSION

Gracilaria fisheri showed a limited photosynthetic tolerance to light intensity (100–203 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Photosynthesis and respiration are important physiological processes in photosynthetic organisms and are related to an organism's overall growth rate and competitive advantage (Kumar *et al.*, 2014). We measured in *G. fisheri* a P_{net} initial saturation light intensity (E_k) of 203 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and compensation

point (E_c) of 45.7 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In contrast, *G. salicornia* has a broader E_k of between 172–557 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and no photo-inhibition was found even at 720 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Phooprong *et al.*, 2007). Our result was similar to the intertidal red algae *G. vermiculophylla* (Phooprong *et al.*, 2008).

In this study, the prolonged period of exposure to salinity affected the photosynthetic rate of *G. fisheri*. Highest P_{net} occurred at salinity

of 30‰, with higher (>30‰) or lower (<25‰) levels of salinity resulting in lower P_{net} , maximum quantum yield and chlorophyll-*a* content. The reduction of maximum quantum yield on the first day in all treatments may be due to salt stress, which is an important environmental factor that limits the growth and productivity of seaweeds (Lobban and Harrison, 1994). In combination with light stress, salt stress could affect PSII (Murata *et al.*, 2007). It has been suggested that salt stress might enhance photodamage to PSII in the cyanobacterium *Spirulina platensis* (Lu and Zhang, 1999). Kumar *et al.* (2014) stated that relative insensitivity of photosynthesis and respiration to salt and desiccation seems to be a prerequisite for the successful occupation of the eulittoral habitat, where there are large amplitudes of salinity and desiccation stress, in combination with other environmental stressors. The lower P_{net} of *G. fisheri* at 25‰ compared to that of 30‰, was similar to photosynthetic responses in *G. edulis* and *G. crassa* (Jayasankar, 2005).

Our study showed significant adverse effects of low and high temperature on *G. fisheri*. The temperature responses of algal species are often correlated with local thermal environments (Eggert, 2012). It has been stated that temperature limitations of growth and photosynthesis directly affect temperature-sensitive cellular components. Astrid *et al.* (2012) suggested that tropical macroalgae are the most stenothermal macroalgae, with their growth generally reaching a peak between 25 and 30°C. In this study, the response of oxygen evaluation (net and gross photosynthetic rates) showed a characteristic dome-like shape, with the highest P_{net} obtained at 24°C and highest gross photosynthetic rate obtained at 28°C. These results are similar to those for *G. salicornia* (Phooprong *et al.*, 2007) and *Gelidiella acerosa*, with highest gross photosynthetic rate at 28°C (Fujimoto *et al.*, 2015), but unlike the red alga *Kappaphycus alvarezii*, for which the optimal gross photosynthetic rates ranged from 30.3–32.0°C (Terada *et al.*, 2016). The maximum quantum yield of *G. fisheri* appeared to be temperature dependent and gradually increased with increasing temperatures

up to 24°C, but decreased thereafter. In this study, the net and the gross photosynthetic rates and the maximum quantum yield curves were similar.

In conclusion, *G. fisheri* expressed not a broad range of tolerance to irradiance, salinity, and temperature. This is the first study of the physiological response in *G. fisheri* to irradiance, salinity, and temperature. However, the results of this study are based on short-term laboratory measurements, and as such, the characteristics of *G. fisheri* have not yet been fully elucidated. In addition, we still do not know the responses of photosynthesis and respiration to environmental acclimation. Therefore, a longer time-scale is needed to better determine the growth of *G. fisheri* in mass cultivation systems.

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