

# Morphological and photosynthetic studies on Gracilarian species from Trat Province, eastern Thailand

Sukanya PHOOPRONG\*, Hisao OGAWA and Nobuyoshi NANBA

School of Fisheries Science, Kitasato University, Iwate 022–0101 Japan

\*E-mail: spoonprong@yahoo.com

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**Abstract**—Morphological and photosynthetic characteristics of the three Thai species of *Gracilaria*, *G. changii*, *G. firma* and *G. salicornia* distributed in the same habitat at Cho Bay, Trat Province, eastern Thailand were studied. The three species can be identified morphologically, however, some specimens have intermediate morphologies among them. *G. firma* has terminal branches without or with some furcations and blunt apices, first-order of branches always constricted at the bases; *G. changii* has many branches, inflated at the middle and tapering toward apices, all branches abruptly constricted at the bases; *G. salicornia* has cylindrical to compressed branches with regular or irregular arrangement of branches, constricted or continuous and intermediate species has branches inflated, tapering to apices, with and without constriction at bases. To elucidate the species status of *Gracilaria* species, we examined their photosynthetic and respiratory responses to irradiance and temperature by Oxygen Analyzer Series 3600. Net photosynthetic rate in species of *Gracilaria* from Trat Province was dependent on temperature. It increased with an increase in temperature from 25–35°C, while the rate decreased at 40°C. Photosynthetic responses were similar for all species when plants were exposed from 30 to 35°C. The  $P_{\max}$  was significantly highest at 35°C ( $0.39 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ) for intermediate species, while the lowest was at 40°C in *G. firma* ( $0.09 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ). The photosynthetic rate responses to high irradiance and temperature by intermediate species are indicative of either adaptation to environmental variation or an outcome of hybridization between the three species.

**Key words:** *Gracilaria*, photosynthesis, morphology irradiance, temperature, Thailand

## Introduction

Species of *Gracilaria* are major sources of agar, and also used as ingredients in salad and soups (Oliveira et al. 2002). Due to their economical importance these algae have attracted of research, however, their taxonomic status remains unclear. Early concepts of species of *Gracilaria* were mainly based on morphological characters. However, the characteristic of reproductive organs have later been considered in the classification of species (Yamanoto 1978 and references there in). Since 1980, many studies using morphological, reproductive and molecular data have suggest that species of *Gracilaria* are closely related and their taxonomic relationships complex. Hence the family Gracilariaceae remains taxonomically challenging (Bird 1995).

More than 18 years ago, Abbott (1988) recognized eight species of *Gracilaria* in Thailand, of which two were described as new species. Lewmanomont (1994) has reported 5 species, one of which was report as new. In eastern Thailand, six species of *Gracilaria* occur in Trat Province (Lewmanomont 1994), three of them, *Gracilaria changii*, *G. firma* and *G. salicornia* are distributed in same habitat in Cho Bay. Interestingly, some specimens have intermediate morphologi-

cal characters among the three species and are dominant along coast of Trat province.

Photosynthesis has been commonly used to study on plant physiology as it is a useful indicator of plant growth (Lapoint et al. 1984). Many studies have reported that species of *Gracilaria* showed photosynthetic response to a broad range of environmental variations such as irradiance, salinity and temperature in accordance with the plants estuarine distribution (Dawes et al. 1978, Penniman and Mathieson 1985, Rosenberg and Ramus 1892). In this study, first we use morphological characters to delineate the species boundaries among *Gracilaria changii*, *G. firma*, *G. salicornia* and intermediate species. Second, we examine the photosynthetic response of these species to irradiance and temperature. Third, we discuss both of their taxonomic status and photosynthetic characteristics.

## Material and Method

### Collection area

Samples of *Gracilaria*, *G. changii*, *G. firma*, *G. salicornia* and intermediate species were collected in November 2003 in Cho Bay, Trat province, on the eastern part of the

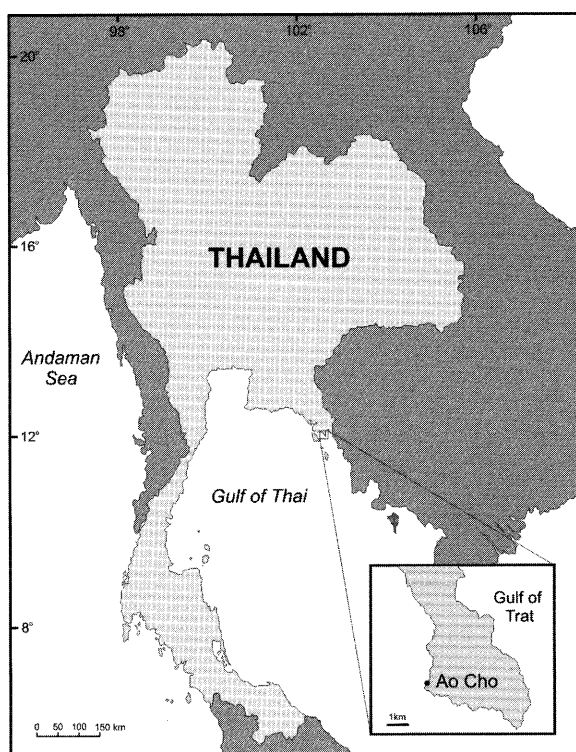


Fig. 1. Map of study sites of Cho Bay, Trat Province, Thailand.

Gulf of Thailand (Fig. 1). The study areas are sandy-muddy, alternating with rocky shores. The bottom was intermingled with gravels, shell, bivalves and hermit crabs. Seawater temperature along the coast ranged from 25°C in winter to 35°C in summer.

### Morphological studies

The plants were transported to the laboratory of primary productivity, Kitasato University in Iwate. Sections of the materials were prepared by hand with a razor blade, stained in 1% aqueous Aniline blue, washed with distilled water to remove excess stain, acidified with diluted solution of HCL and mounted in 1% Karo corn syrup.

### Photosynthetic measurement

Photosynthesis and respiration of *Gracilaria* from Thailand were measured by using Oxygen analyzer series 3600 instrument (Orbisphere Laboratories, Neuchatel-Geneva Switzerland). A xenon-discharge lamp (SERIC XC-100) was used as light source. Tip of branches were cut into 5–8 equal size of from which a square disc of 9 cm<sup>2</sup> was prepared. The disc was then set in the reaction vessel previously filled with 150 ml of sterile seawater (filtered by 0.45 µm).

In order to avoid the effects of dissolved oxygen will give photosynthetic inhibition resulting from high oxygen concentration. Seawater in reaction vessel was previously autoclaved to reduce oxygen concentration at lowest level (100°C, 15 minutes). The reaction vessel was hermetically closed connected with water bath (EL-8F COOL NIT BATH,

TATEC) for controlling temperature and the seawater was stirred by magnetic stirrer during the measurement.

Photosynthesis versus irradiance (P-I) curves were drawn for various photon flux densities in the range of 0 to 710.1 µmol photon m<sup>-2</sup>s<sup>-1</sup> by using filters between light source and reaction vessel. Irradiation was done for 30 minutes, but photosynthesis was measured only in the last 15 minutes. For carrying out dark respiration measurement, the samples were measured in complete darkness by switching off the light source. After completion of both measurements, the disc was photographed with a digital camera (OLYMPUS, CAMEDIA C-3030ZOOM) to calculate the area. To test the effect of temperature on photosynthesis, temperature conditions were set as 25, 30, 35 and 40°C. All of the measurements were carried out in four replicates.

Photosynthetic parameters were calculated by fitting P-I curve to the equation using modified non linear regression (Honda 1999);

$$v \approx \Phi I^2 / (\Psi_T + \Omega I + I^2)$$

where  $v$  is the gross photosynthetic rate,  $I$  is the irradiance,  $\Phi$  is light-saturated photosynthetic rate,  $\Omega$  is initial saturation irradiance, and constant values  $\Psi_T = 1/1000000$ . The compensation point was calculated by the equation,  $\Omega \times R_d / (\Phi - R_d)$  and photosynthetic efficiency by the equation,  $\Phi / \Omega$ .

### Statistical analysis

Photosynthetic rates among species of each light treatment were compared using one-way ANOVA. The light saturated photosynthetic rate ( $\Phi$ ), dark respiration ( $R_d$ ), photosynthetic efficiency ( $\alpha$ ), initial saturation irradiance ( $\Omega$ ) and compensation point ( $I_c$ ) were analysed by two-way ANOVA.

## Results

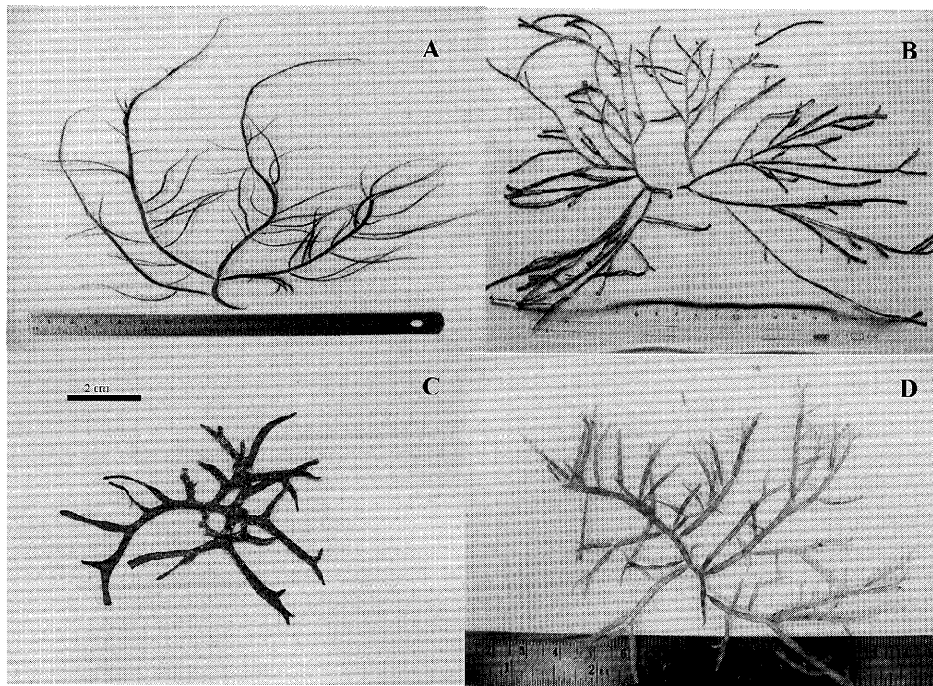
### Taxonomic observation

*G. changii* has all branches constricted at the bases, branches inflated, gradually tapering toward apices (Fig. 2-A). Transition of cells abrupt (Fig. 3-A), pericarp thick, consisting of two kinds of cells, the outer 5–6 rows rounded, the inner 6–9 rows compressed.

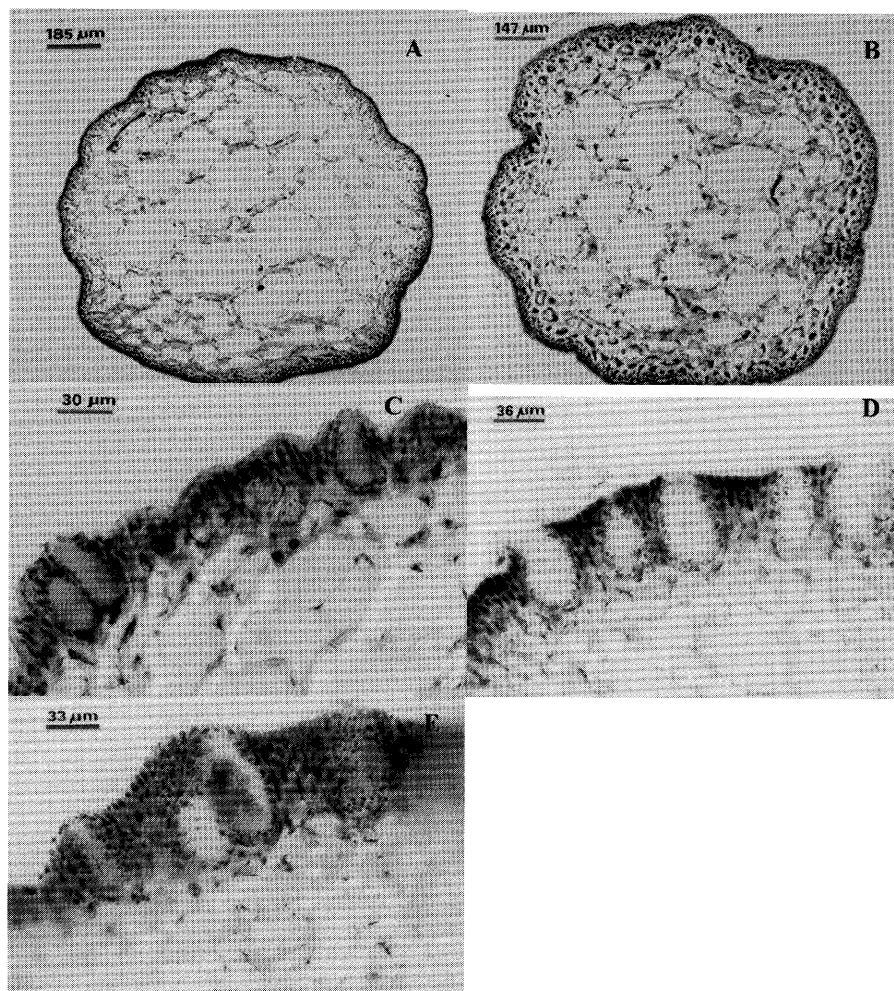
*G. firma* has first order of branches constricted at bases, other orders with or without constriction, branches cylindrical with blunt or acute apices (Fig. 2-B), transition from medulla to cortex gradual, pericarp thick with rounded to squared cells arranged in rows (Fig. 4-D).

*G. salicornia* has branches constricted or continuous, branches cylindrical to compressed (Fig. 2-C), transition from medulla to cortex gradual, pericarp thick, consisting of two kinds of cells, the outer 6–8 rows rounded, the inner 5–8 rows compressed.

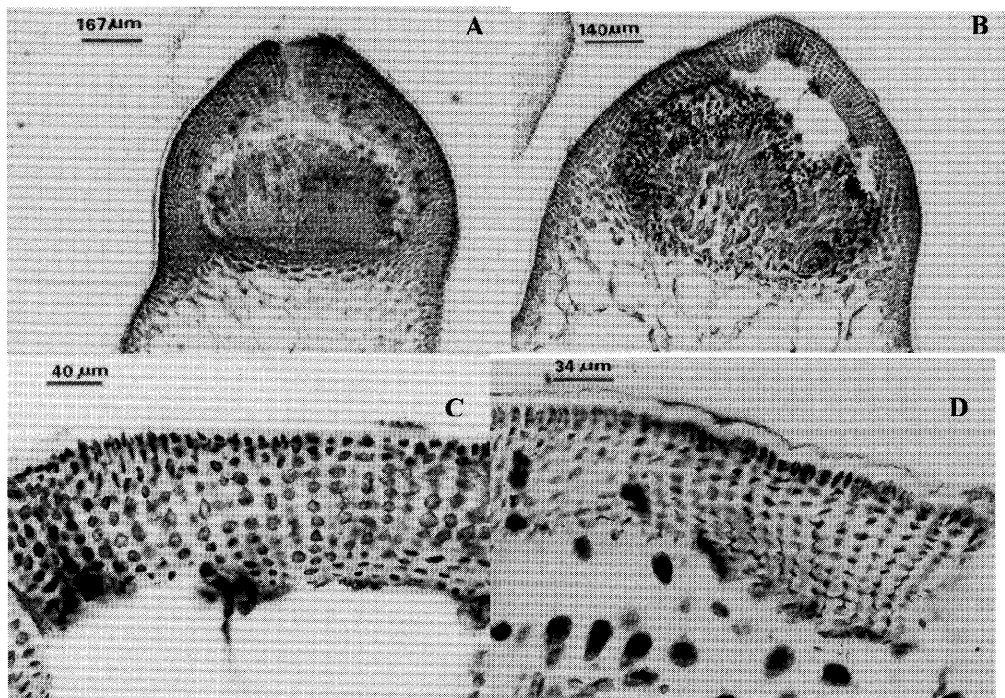
Intermediate species has branches with and without constricted at the bases (Fig. 2-D), transition of cells abrupt (Fig.



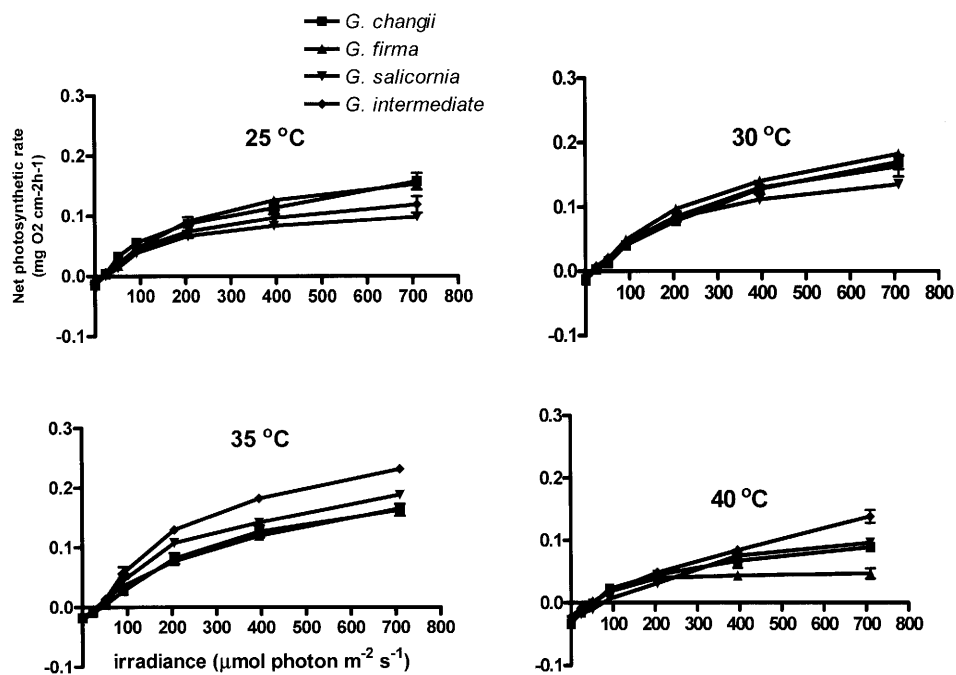
**Fig. 2.** Species of *Gracilaria* from Cho Bay, Trat Province. A: *G. changii*, B: *G. firma*, C: *G. salicornia*, intermediate species.



**Fig. 3.** A: Transverse section of *Gracilaria changii*, B: Intermediate species, C: Transverse section of spermetangial conceptacles (*verrucosa* type) of *G. changii*, D: *G. firma*, E: Intermediate species.



**Fig. 4.** A: Longitudinal section of cystocarp, of *G. changii*, B: Intermediate species, C: Longitudinal section of pericarp of Intermediate species, D: *G. firma*.



**Fig. 5.** Photosynthesis rate to vary photon flux density at variation of temperature in species of *Gracilaria* from Trat province. Bars represent standard deviations ( $n=4$ ).

3-B), spermatangial conceptacles oval (verrucosa type) (Fig. 3-E), pericarp with rounded to squared cells arranged in rows (Fig. 4-C).

#### Photosynthetic observation

Net photosynthetic rate in species of *Gracilaria* from Trat Province was dependent on temperature. It increased

with an increase in temperature from 25–35°C, while the rate decreased at 40°C, particularly for *G. firma*. Photosynthetic responses were similar for all species when plants were exposed from 30 to 35°C (Fig. 5). Furthermore, the responses of *G. changii* and *G. firma* from 25 to 30°C were higher than that of *G. salicornia* and intermediate species.

Parameters for P-I curve were calculated for the combi-

**Table 1.** Photosynthetic parameters for species of *Gracilaria* from Trat Province at temperature variation,  $\Phi$ : light-saturated photosynthetic rate,  $R_d$ : dark respiration ( $\text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ) and  $\alpha$ : Initial slope ( $\mu\text{mol photon m}^{-2} \text{ s}^{-1} / \text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ), values represent standard deviations ( $n=4$ ).

Experimental conditions	$\Phi$	$R_d$	$\alpha$	$\Omega$	$I_c$
25°C					
<i>G. changii</i>	0.23±0.05	0.02±0.001	0.0010±0.0002	216±31	15±2
<i>G. firma</i>	0.24±0.02	0.01±0.002	0.0009±0.0001	276±37	16±2
<i>G. salicornia</i>	0.14±0.01	0.01±0.002	0.0008±0.0000	171±21	18±3
Intermediate species	0.17±0.03	0.01±0.001	0.0008±0.0002	226±62	12±5
30°C					
<i>G. changii</i>	0.31±0.04	0.01±0.002	0.0007±0.0000	471±23	23±5
<i>G. firma</i>	0.30±0.03	0.01±0.002	0.0008±0.0000	362±21	16±2
<i>G. salicornia</i>	0.21±0.02	0.01±0.003	0.0008±0.0001	262±59	17±3
Intermediate species	0.27±0.05	0.01±0.001	0.0007±0.0001	390±68	10±3
35°C					
<i>G. changii</i>	0.28±0.03	0.02±0.002	0.0006±0.0001	491±121	32±5
<i>G. firma</i>	0.30±0.04	0.02±0.004	0.0007±0.0002	437±60	30±4
<i>G. salicornia</i>	0.32±0.03	0.02±0.004	0.0010±0.0001	347±80	25±7
Intermediate species	0.39±0.02	0.02±0.003	0.0010±0.0002	375±73	19±4
40°C					
<i>G. changii</i>	0.15±0.03	0.03±0.009	0.0009±0.0003	195±94	52±15
<i>G. firma</i>	0.09±0.02	0.03±0.005	0.0010±0.0001	90±8	45±15
<i>G. salicornia</i>	0.15±0.02	0.03±0.006	0.0008±0.0000	182±23	42±7
Intermediate species	0.21±0.01	0.02±0.006	0.0009±0.0001	241±26	29±7

nation of species and temperature. The light-saturated photosynthetic rate ( $\Phi$ ) was significant highest ( $p<0.05$ ) at 35°C ( $0.39 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ) for intermediate species, while the lowest was 40°C in *G. firma* ( $0.09 \text{ mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ ) (Table 1). No significant ( $p>0.05$ ) differences in dark respiration ( $R_d$ ) were detected among the four species for each temperature investigated. However, at 40°C, intermediate species showed lower  $R_d$  which was not significantly ( $p>0.05$ ) (Table 1). Photosynthetic efficiency (initial slope) as express by  $\alpha$ , was similar in species of *Gracilaria* from 25 to 40°C, ranging from 0.0007–0.001  $\mu\text{mol photon m}^{-2} \text{ s}^{-1} / \text{mg O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ , with the acceptance at 35°C for *G. salicornia* and intermediate species showed higher significance ( $p<0.05$ ) than that of *G. changii* and *G. firma* (Table 1). Compensation ( $I_c$ ) and saturation irradiance increased with the temperature increase from 25 to 35°C in all species and ranged from 10 to 45 and 90 to 491  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$  respectively (Table 1). No photoinhibition of photosynthesis was observed within the light intensity range investigated, whatever the water temperature (max, 720  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ).

## Discussion

The taxonomic identity of the three species of *Gracilaria*, *G. firma*, *G. changii* and *G. salicornia* collected

from natural population was confirmed based on morphology. However, some specimens showed intermediate morphological characters among these species. Our photosynthetic observation showed that temperature controlled net photosynthesis of Thai *Gracilaria* significantly, as indicated by the variation of both light-saturated photosynthetic rate and dark respiration. Temperature has fundamental effects on chemical reaction rate (Lobban & Harrison 1994), usually the rates of photosynthesis double for every 10°C rise in temperature. The photosynthetic responses of *G. salicornia* and intermediate species to temperature showed their remarkable tolerance in this factor as their photosynthetic response increased by two-fold, while *G. changii* and *G. firma* increased only one-fold per 10°C rise in temperature from 25 to 35°C.

The optimum temperature of photosynthesis for several *Gracilaria* species have been reported in the range of 25 to 35°C (Dawes et al. 1999, Penniman and mathieson 1985 and Mizusawa et al. 1978). Similarly, in the present study, the optimum temperature was received from 30 to 35°C of all species. Furthermore, the photosynthetic rates of *G. salicornia* and intermediate species increased with an increase temperature from 30 to 35°C while, the rates of *G. firma* and *G. changii* were stable within this temperature range (Table 1). However, Penniman and Mathieson (1985) reported that photosynthesis decreased dramatically at 37.5°C in *G. tikvahiae*. Similarly in our study, the photosynthetic rates decreased at

40°C in all species, most probably indicating that the high-temperature inhibition of photosynthesis is associated with a disruption of energy transfer phycobilisomes and PSII and affected by the photorespiration (Davison 1991 and references there in). Moreover, the present study highlights that Thai *Gracilaria* species may be capable of carrying out photosynthesis in high temperature. It is interesting to note that seasonal growth of *Gracilaria* species in Cho Bay occur from March to May with water temperatures from 30 to 35°C.

At subsaturating light levels, the effect of temperature on photosynthesis plays an important role in ecology (Davison 1991). Plants from Cho Bay are frequently subjected to subsaturating light level due to the sandy-muddy bottoms in intertidal habitats. Interestingly, these plants in Cho Bay, in particular intermediate species showed high photosynthetic efficiency ( $\alpha$ ) indicating adaptation to low light condition in high temperature (35 to 40°C). In contrast, *G. firma* and *G. changii* showed higher  $\alpha$  in low temperature (25°C).

A wide variation in initial light saturation irradiance ( $\Omega$ ) and photosynthetic response to high irradiance in many species of *Gracilaria* have been reported in the range 60 to 600  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Bird et al. 1979 and Orduna-Rojas et al. 2002), those are similar to the present study. Furthermore, *G. tikvahiae* from New Hampshire was observed by Penniman and Mathieson (1985) and *G. verrocosa* and *G. tikvahiae* from Florida respectively was observed by Dawes and Koch (1990). They reported that saturation irradiance occurred from 200 to 600  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  with no photoinhibition, although, the irradiance over 1200  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Similarly, the present study showed no photoinhibition up to 710  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Rosenberg and Ramus (1982) and Orduna-Rojas et al. (2002) reported that light compensation point for *G. tikvahiae* and *G. cornea* were from 20 to 37  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  respectively. However, the wide variation in compensation point and saturation irradiance (10 to 45 and 90 to 491  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  respectively) reported for Thai *Gracilaria* here suggest that these plants are sun plants (intertidal plants) adapted to shade plant with high level of efficiency for light gathering in turbid water.

Our study is the first to deal with the photosynthesis of Thai *Gracilaria* species in response to temperature and irradiance variation. The results show that intermediate species have broader photosynthetic responses to irradiance and temperature. These results help to explain the dominance of this species at Cho Bay. Furthermore, the existence of intermediate species with different morphological and photosynthetic characteristics among the three species, is indicative of either adaptation to environmental variation or of an outcome of hybridization between the three species.

All three species of *Gracilaria* have different distribution. *G. salicornia* is common in Thai water and is found in almost every province along the sea coast. *G. changii* is com-

mon along the east coast of the Gulf of Thailand and along Andaman Sea, but it is not common along the west coast of the Gulf of Thailand. *G. firma* occurs only along the coast of Trat Province (Lewmanomont 1994). Hence, *G. salicornia* show similar photosynthetic responses to intermediate species with broader tolerance to temperature and irradiance and this could explain why this alga is widely distribution.

The low temperatures tested (25 and 30°C) were tolerant by *G. changii* and *G. firma* and the high temperatures tested (35 and 40°C) were tolerance by *G. salicornia* and intermediate species. The responses suggest that any mariculture efforts using these species will require stable, low temperature and low irradiance for *G. changii* and *G. firma* and high temperature and low irradiance for *G. salicornia* and intermediate form.

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