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TEMPERATURE RESPONSE OF PHOTOSYNTHETIC LIGHT- AND CARBON-USE CHARACTERISTICS IN THE RED SEAWEED *GRACILARIOPSIS LEMANEIFORMIS* (GRACILARIALES, RHODOPHYTA)[1]

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The red seaweed *Gracilariopsis* is an important crop extensively cultivated in China for high-quality raw agar. In the cultivation site at Nanao Island, Shantou, China, *G. lemaneiformis* experiences high variability in environmental conditions like seawater temperature. In this study, *G. lemaneiformis* was cultured at 12, 19, or 26°C for 3 weeks, to examine its photosynthetic acclimation to changing temperature. Growth rates were highest in *G. lemaneiformis* thalli grown at 19°C, and were reduced with either decreased or increased temperature. The irradiance-saturated rate of photosynthesis (P_{max}) decreased with decreasing temperature, but increased significantly with prolonged cultivation at lower temperatures, indicating the potential for photosynthesis acclimation to lower temperature. Moreover, P_{max} increased with increasing temperature (~30 μmol $O_2 \cdot g^{-1}FW \cdot h^{-1}$ at 12°C to 70 μmol $O_2 \cdot g^{-1}FW \cdot h^{-1}$ at 26°C). The irradiance compensation point for photosynthesis (I_c) decreased significantly with increasing temperature (28 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at high temperature vs. 38 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at low temperature). Both the photosynthetic light- and carbon-use efficiencies increased with increasing growth or temperatures (from 12°C to 26°C). The results suggested that the thermal acclimation of photosynthetic performance of *G. lemaneiformis* would have important ecophysiological implications in sea cultivation for improving photosynthesis at low temperature and maintaining high standing biomass during summer. Ongoing climate change (increasing atmospheric CO_2 and global warming) may enhance biomass production in *G. lemaneiformis* mariculture through the improved photosynthetic performances in response to increasing temperature.

Key index words: **biomass production; global warming; *Gracilariopsis lemaneiformis*; light; photosynthesis; seaweeds cultivation; temperature; thermal acclimation**

***Abbreviations*: ACE, apparent carboxylating efficiency; CA, carbonic anhydrase; Ci, inorganic carbon; FW, fresh weight; I_c, irradiance compensation point for photosynthesis; I_k, irradiance saturation point for photosynthesis; $K_{0.5}$, half-saturation constant of photosynthesis for total Ci; *P-C*, photosynthesis versus Ci curve; P_g, gross photosynthesis; *P-I*, photosynthesis versus irradiance curve; P_{max}, irradiance-saturated rate of photosynthesis; P_n, net photosynthesis; R_d, dark respiration; α, initial slope of the P-I curve(the apparent photosynthetic efficiency)**

The significant effect of temperature on biochemical reactions makes it a major determinant among the marine environmental factors controlling the growth and metabolic rates in seaweeds. In addition to the differences in temperature among different habitats, seaweeds are subjected to substantial temporal variability in temperature on different timescales, including rapid shifts associated with tidal displacement of the thermocline or tidal immersion/emersion, diurnal and seasonal variability, and long-term inter-annual variability associated with natural climatic cycles and possibly, human influence (Raven and Geider 1988, Davison 1991). The ongoing increase in CO_2 in the atmosphere is expected to be accompanied by rising temperatures globally, and by more frequent and severe weather events (Christensen et al. 2007). Although global mean sea surface temperatures are rising at only half the rate as that of land (0.13 vs. 0.27°C per decade since 1979), increasing temperature is the most pervasive of present-day influences on marine systems (Halpern et al. 2008, Brierley and Kingsford 2009).

Plants and algae may respond to short-term temperature change (minutes to hours) in an

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unregulated way without active changes in resource utilization (e.g., Field et al. 1992). However, phenotype changes of acclimation in photosynthesis and other key aspects of metabolism (e.g., dark respiration) have been observed in response to long-term temperature change (days to weeks; Davison 1987, 1991, Madsen and Brix 1997, Sage and Kubien 2007). Seaweeds grown for several days or weeks at different temperatures generally exhibit a photosynthetic response of photosynthesis to the short-term temperature. Such responses may include increased photosynthetic capacity, increased resistance to low-temperature induced photoinhibition, lower optimum temperature for photosynthesis, reduced sensitivity to changes in temperature (lower Q_{10} value), and reduced tolerance to high temperature for low temperature-grown plants compared to high temperature-grown ones (e.g., Davison 1987, 1991, Kübler and Davison 1995, Davison and Pearson 1996, Major and Davison 1998). These changes would result in the net effect that realized growth rates are either less temperature-dependent than predicted from the short-term measurements (partial compensation), or independent of temperature.

Red seaweeds of the genus *Gracilariopsis*, which include more than 100 described species, are widely distributed in tropical and warm temperate waters (Bird and McLachlan 1984, Fei et al. 1998). In China, more than 30 species have been recorded so far, with cultivation of *Gracilariopsis* starting in the 1950s (Tseng 2001). Among them, *Gracilariopsis lemaneiformis* (Bory de Saint-Vincent) E.Y.Dawson, Acleto & Foldvik (Gracilariaceae, Rhodophyta) is regarded as the most important commercial species due to its rapid growth and the high-quality phycocolloid production compared to other species in the genus *Gracilariopsis* (Santelices and Doty 1989, Tseng 2001, Zou et al. 2004). *Gracilariopsis lemaneiformis* has been cultivated at large scales in both southern and northern China, for the agar industry and a good fodder for industrialized abalone cultivation in Shenao Bay, Nanao Island, Shantou, Guangdong Province (Zou et al. 2004). Moreover, the cultivation of this species can be an effective bioremediation measure for eutrophication control in coastal waters (Fei 2004). *G. lemaneiformis* is being considered for even further development of large-scale cultivation in Shenao Bay, Nanao Island due to the strong market demand (Zou et al. 2004).

Sea cultivation of seaweeds is the most effective means for obtaining large amount of seaweed biomass. An understanding of physiology (e.g., growth and photosynthetic performance) is important for the successful mariculture of *G. lemaneiformis*. *G. lemaneiformis* cultivation is usually by means of raft cultivation methods, using macroscopic algal fragments of tetrasporophytes of *G. lemaneiformis* attached to polyethylene ropes, which are then suspended at the sea surface. At Shenao bay, Nanao Island, Shantou, China, transplantation of *G. lemaneiformis* fragments to offshore rafts generally occurs in January (winter), when the mean seawater temperatures are ~11°C–13°C. During January and February, the standing stock of *G. lemaneiformis* is low and thalli lengths are generally less than 0.5 m. In April and May with increasing seawater temperatures, plants grow in length, branch number, and biomass. The maximum height of *G. lemaneiformis* can reach up to 1.5 m, and biomass up to 5 $kg \cdot m^{-2}$ in late May and early June when temperatures are over 26°C (personal observation). *G. lemaneiformis* thalli experience decreased irradiance as light was sharply attenuated by seawater and the sinking of thalli to deeper water, and self-shading due to high stocking density (Zou and Gao 2009). The deteriorating light conditions threaten the growth and photosynthesis of *G. lemaneiformis* thalli that must be exposed to irradiances that exceed the critical light demand for a positive carbon balance. Moreover, the pH value of the seawater surrounding the algae might rise due to the processes of inorganic carbon (Ci) and NO_3^- uptake by algae, especially at high standing stock severely reducing Ci acquisition by *G. lemaneiformis* (Zou et al. 2004). Overall, *G. lemaneiformis* thalli are subjected to variable environmental conditions such as seawater temperature and light level. Most recently, we showed that *G. lemaneiformis* respiration and photosynthesis may acclimate to long-term changes in temperature, with the extent of acclimation being much higher for respiration than photosynthesis (Zou and Gao 2013). Here, we hypothesized that photosynthetic adjustment due to thermal acclimation in *G. lemaneiformis* may not only increase photosynthetic rates at low temperatures, but also improve photosynthetic performance and allow high stocking biomass during the warmest period of the year (May to June). In this study, we investigated the effects of short-term and long-term temperatures change on photosynthetic light- and carbon-use properties, in an effort to evaluate the acclimation potential of photosynthetic performance due to changing temperature and specially to evaluate the ecophysiological implications of photosynthetic adjustment with regard to sea cultivation of *G. lemaneiformis*. We cultured *G. lemaneiformis* at three different temperature regimes for 3 weeks and then determined the photosynthetic light- and carbon-use characteristics at different temperature levels and tested for any interaction between the effects of short- and long-term temperature change.

MATERIALS AND METHODS

Sample collection. *G. lemaneiformis* was collected from a cultivation field at Shenao Bay, Nanao Island, Shantou, China (23°20′ N, 116°55′ E) in March 2007 (Fig. 1). The ambient surface seawater temperature at the site of collection over the sampling periods was 20°C. The algal thalli were gently rinsed and cleared of visible epiphytes and of any accumulated

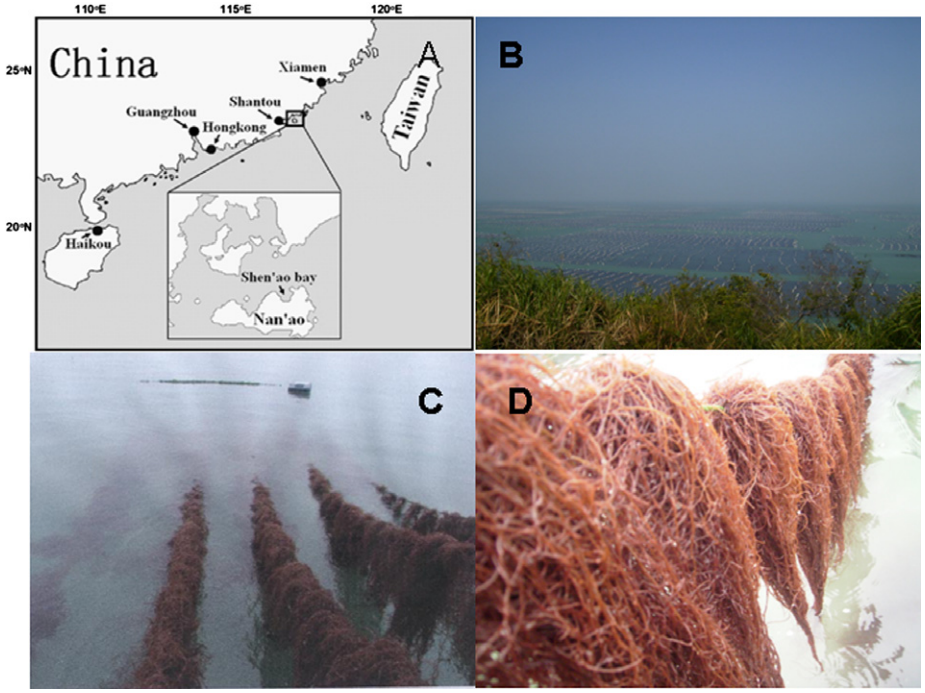

FIG. 1. Sea cultivation of *Gracilariopsis lemaneiformis* at Shenao Bay, Nanao Island, Shantou, China. (A) Schematic map showing the site of Shenao Bay, Nanao Island, Shantou, China; (B and C) Sea cultivation of *G. lemaneiformis*; (D) *G. lemaneiformis* thalli attached to the ropes.

sediments, placed into a plastic barrel containing natural seawater, and kept cool and dark, during the transportation to the laboratory (~3 h). The samples were maintained in filtered natural seawater (salinity 32) enriched with 100 μM $NaNO_3$ and 20 μM NaH_2PO_4 (final concentration) in a 30 L plexiglass aquarium at 20 ± 1°C for 7 d. The algae received an irradiance of about 200 μmol photons · m^{-2} · s^{-1} (PAR) illuminated by a bank of cool-white fluorescent tubes with a 12:12 h light:dark period (set from 9 a.m. to 9 p.m.). Seawater was renewed by 50% daily and was continuously aerated by a filter pump in order to keep air equilibrium of the dissolved Ci.

Experimental treatments. *Gracilariopsis lemaneiformis* thalli were cultured at three temperature levels: 12, 19, or 26°C, arbitrarily assigned as low, intermediate, and high temperature, respectively. Experimental cultures began when 2.5 g fresh weight (FW) algae were introduced into each of nine Erlenmeyer flasks containing 5 L filtered seawater. The flasks were placed into three illumination incubators (GXZ-300D, Jiangnan Instrument Factory, Ningbo, China), whose temperature conditions were controlled at 12, 19, or 26°C, respectively; three replicate cultures were maintained at each temperature treatment. Nutrient and irradiance conditions were the same as indicated above. Water motion resulting from the aeration by ambient atmospheric air allowed the algae to move gently without tumbling, and the seawater media were changed every 2 d. Total dissolved Ci concentration (using a Total Organic Carbon Analyzer, TOC-5000A; Shimadzu, Kyoto, Japan) was 2.05 mM, pH value was 8.21, and measured total alkalinity was 2250 μeq · L^{-1}. The algae were grown at these different temperature regimes for 3 weeks, and then were harvested to determine photosynthetic characteristics in response to additional experimental temperature variability.

Growth rates. Biomass was measured in each experimental culture to estimate growth. The relative growth rate (RGR), expressed as percentage of change in FW biomass per day (% · d^{-1}), was estimated assuming exponential growth during the culture period according to the formula: $RGR = [(\ln W_t - \ln W_o)/t] \times 100$, where W_o represents the initial and W_t the final FW, and t is the culture time in days. Algal samples were softly blotted on filter paper before FW weighing.

The response of photosynthesis to irradiance. Photosynthetic rates were measured as net O_2 evolution by using a Clark-type oxygen electrode (YSI Model 5300, Yellow Spring, OH, USA), which was held in a circulating water bath (Cooling Circulator; Cole Parmer, Chicago, IL, USA) to keep the desired temperature. The illumination was provided by a halogen lamp. It had been shown that proton buffers (such as TRIS) might disturb photosynthetic utilization of Ci in some brown macroalgal species (e.g., Axelsson et al. 2000, Mercado et al. 2006) and other macrophytes (e.g., Beer et al. 2002, Uku et al. 2005), because these buffers eliminate proton extrusion forming low pH (acid zones) in the external HCO_3^- dehydration on the thalli surface, and thereby could disrupt the functioning of Ci-acquisition mechanisms. However, in the case of *G. lemaneiformis*, we have previously shown that TRIS had no inhibitory impacts on photosynthetic carbon acquisition (Zou et al. 2004, Zou and Gao 2009). Therefore, the response of photosynthetic rates to irradiance and Ci, concentrations, were determined using TRIS buffer (20 mM final concentration).

For the measurement of net photosynthetic rates (Pn) as a function of photon irradiance (P-I curve), ~0.15 g FW of algal segments were introduced into the electrode chamber with 8 mL of filtered TRIS (20 mM)-buffered (pH 8.1) natural seawater, which was magnetically stirred. The algal samples were allowed to equilibrate in the darkness until the rate of oxygen consumption was constant (~4–6 min) and respiratory rate (R_d) was monitored. The samples were exposed to a series of increasing irradiances from 0 to 600 μmol photons · m^{-2} · s^{-1}, which was adjusted by changing the distance between the light source and the assimilation chamber. The irradiance levels were measured with a PAR quantum sensor (SKP 200; ELE International, Leighton Buzzard, UK). The algal samples were allowed to equilibrate for 3–6 min at each light level during which period a linear change in oxygen concentration was obtained, and then the reading was

recorded. Preliminary experiments showed that slight changes of pH and dissolved Ci within the seawater did not affect photosynthesis over the period during the P-I curve measurement.

In order to estimate the temperature effect, P-I curves for each temperature treatment (12, 19, and 26°C) were manipulated by adjusting the temperature in the O_2 electrode chamber. The algal samples were allowed to equilibrate for about 15 min at each temperature change before the photosynthetic rates were taken. Moreover, the O_2 electrode was recalibrated for each temperature change.

The response of photosynthesis to Ci. Pn as a function of external Ci concentration in seawater (P-C curve) was measured at 400 μmol photons $\cdot m^{-2} \cdot s^{-1}$ using the oxygen electrode described above. Preliminary experiments showed that photosynthesis was fully saturated at this irradiance level, and no photoinhibition occurred. The Ci-free seawater was prepared by acidifying the filtered seawater to pH less than 4.0 with 0.5 M HCl, and sparging for at least 2 h with high purity N_2 gas to remove the Ci in seawater. TRIS buffer was added to give a final concentration of 20 mM and the pH was adjusted to 8.1 with freshly prepared 0.5 M NaOH and HCl under N_2 aeration. About 0.2 g of *G. lemaneiformis* segments were transferred to the electrode chamber containing 8 mL of Ci-free seawater. Samples were left to photosynthesize and deplete any remaining Ci in the seawater medium and the intracellular and intercellular pools of Ci, until zero net oxygen evolution was reached. Different aliquots of 200 mM $NaHCO_3$ stock solution were then injected into the chamber, to create different Ci concentrations as desired, and photosynthesis was measured over a range of Ci concentrations up to 4.4 mM. P-C curves for each growth treatment were respectively determined at three assay temperatures: 12, 19, and 26°C, the same method described for the P-I curves.

Calculations and statistics. Daily carbon budget was estimated by multiplying gross photosynthesis (P_g; net photosynthesis plus dark respiration) by daily light-saturated photosynthesis (over 12 h) and then subtracting the daily (24 h) dark respiration. Parameters for P-I curve were analyzed. The light-saturated photosynthetic rate (P_{max}) was calculated from the mean values in the asymptote region of the P-I curve. Apparent photosynthetic efficiency (α) was estimated as the ascending slope at limiting irradiance levels. The irradiance saturation (I_k) and compensation points (I_c) were calculated as $(R_d+P_{max})/\alpha$ and R_d/α, respectively, according to Henley (1993). For the P-C parameters, the apparent carboxylating efficiency (ACE; i.e., the initial slope of P-C curve) was calculated by linear regression over the range of 0-0.55 mM Ci. This value was used to indicate how effectively algae use low concentration of Ci, as discussed by Johnston et al. (1992). The half-saturation constant of photosynthesis for total Ci ($K_{0.5}$) was estimated using double reciprocal plots of the rates of O_2 evolution and the Ci concentration.

The data were expressed as the means ± SD. Statistical significance of the data was analyzed with one- and two-way ANOVA followed by the S-N-K post hoc procedure of multiple comparisons by using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). A significant effect of long-term temperature would indicate an acclimation to the growth conditions, a significant effect of short-term temperature would that the parameter was influenced by instantaneous thermal fluctuation (i.e., on the timescale of minutes). A significant interaction between short- and long-term temperatures would indicate that there was a synergism between thermal acclimation to the growth temperature and the effect of thermal variation on the timescale of minutes. The significance level was set at $P < 0.05$.

RESULTS

The growth of *G. lemaneiformis* thalli remained positive in each temperature treatment. The RGR was highest in *G. lemaneiformis* grown at intermediate temperature (19°C) among the different growth treatments, and the rate was reduced ($P < 0.05$) with a change in temperature (Fig. 2).

The photosynthetic response to irradiance (P-I curves) of *G. lemaneiformis* over different short- and long-term temperatures are illustrated in Figure 3, and the parameters of P-I curves are given in Table 1. No photoinhibition was observed over the irradiance range tested (0–600 μmol photons $\cdot m^{-2} \cdot s^{-1}$). It appeared that the differences among the curves were more pronounced for lower than higher short-term temperatures. The irradiance-saturated rates of photosynthesis (P_{max}) were significantly affected by both short- and long-term temperatures (Table 1). It was evident that *G. lemaneiformis* exhibited acclimation potential of photosynthesis to lower temperature. Firstly, when measured at 12°C, rates of P_{max} in 12°C-grown algae were much higher than those in 19°C or 26°C-grown algae ($P < 0.01$). Secondly, when measured at 19°C, rates of P_{max} in 19°C-grown algae were much higher than those in 26°C-grown algae ($P < 0.01$). On the other hand, P_{max} increased with the increasing short-term temperature (the rates ranging from 30 μmol $O_2 \cdot g^{-1}$ FW $\cdot h^{-1}$ at 12°C to 70 μmol $O_2 \cdot g^{-1}$ FW $\cdot h^{-1}$ at 26°C), and this stimulation persisted. The evidence was as follows: (1) P_{max} measured at 19°C was similar between in 12°C- and 19°C-grown algae; (2) P_{max} measured at 26°C were similar among the algae grown at all the three temperatures (12, 19, and 26°C).

According to the measured values of photosynthetic and respiratory rates, the daily carbon

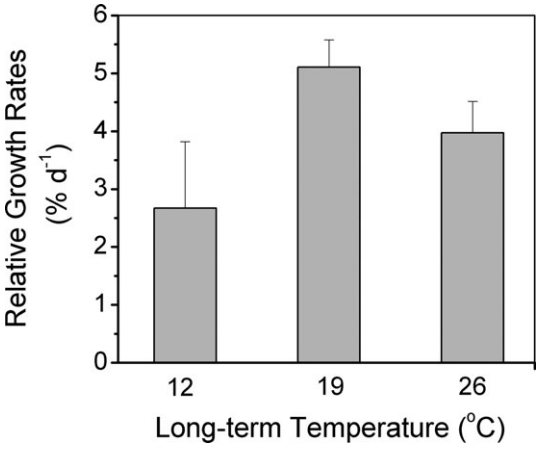

FIG. 2. Average relative growth rate (RGR) of *Gracilariopsis lemaneiformis* grown at different temperatures (12, 19, or 26°C). Vertical bars represent ±SD ($n = 3$).

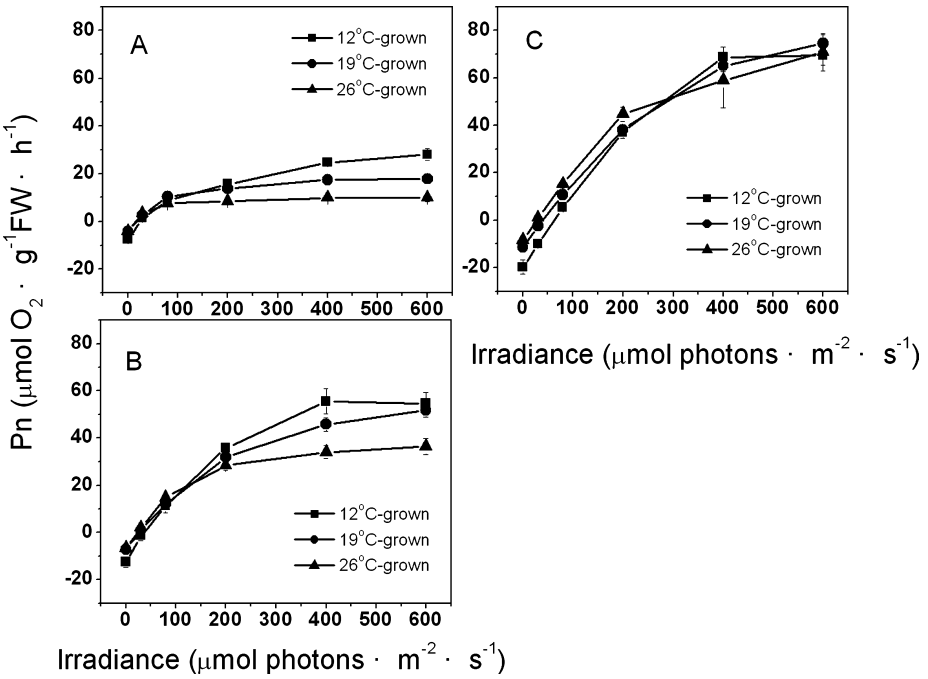

FIG. 3. Net photosynthesis versus irradiance curves (P-I curves) of *Gracilariopsis lemaneiformis* grown at different temperatures (12, 19, or 26°C). Curves were measured at (A) 12°C, (B) 19°C, and (C) 26°C, respectively. Vertical bars represent ±SD ($n = 3$).

budgets were calculated, assuming the photosynthetic quotient of 1.0. The calculated values of daily net carbon gains were 396.9 ± 32.4, 677.8 ± 35.5, and 914.8 ± 96.6 μmol $CO_2 \cdot g^{-1}$ FW $\cdot d^{-1}$, respectively, for 12-, 19-, and 26°C-grown algae. It was clear that the daily net carbon gains increased significantly ($P < 0.01$) with increasing long-term temperature.

Temperature effects on the apparent photosynthetic efficiency (α) followed a similar pattern observed for P_{max} (i.e., the value of α increased with increasing short- and long-term temperature changes). The irradiance compensation point for photosynthesis (I_c) significantly increased with increasing temperature in 12°C- and 19°C-grown algae. However, the short-term effects of temperature on I_c was not significant for 26°C-grown algae ($P > 0.1$). Another salient result was that, when assayed at their respective long-term temperatures, I_c was much higher ($P < 0.01$) in 12°C-grown algae (38 μmol photons $\cdot m^{-2} \cdot s^{-1}$) than 19°C- and 26°C-grown algae (28 μmol photons $\cdot m^{-2} \cdot s^{-1}$). As for the irradiance saturation point (I_k), it exhibited the general trend that the value was increased with increasing short- and long-term temperature.

The photosynthetic responses to Ci concentration (P-C curves) are shown in Figure 4, and the parameters of P-C curves are presented in Table 2. It appeared that the difference among the curves of the algae grown at different temperatures was more pronounced in low with regard to high short-term temperatures. Photosynthetic rate was fully or nearly saturated at Ci of 2.2 mM, the concentration representative of that in normal natural seawater. This was in accordance with the relative low values of $K_{0.5}$(Ci) (the half-saturation constant for photosynthesis), which being less that 1.1 mM Ci over all temperatures. No consistent tendency for the values of $K_{0.5}$(Ci) with regard to temperature was seen due to the large standard deviation of the values. The ACE, the initial slope of the P-C curve, displayed a trend of increase ($P < 0.01$) with increasing short- and long-term temperatures (Table 2).

DISCUSSION

The short- and long-term temperature tested in this study were within the range of temperatures to which *G. lemaneiformis* are normally encountered in their natural conditions during the cultivation season in Nanao Island, Shantou, China (i.e., from 11°C–13°C in January to 25°C–28°C in June). *G. lemaneiformis* sea cultivation in January, and are harvested in late May or early June. Our results showed that *G. lemaneiformis* grew fastest in intermediate temperature (19°C), which roughly corresponded to the seawater temperature in March and April at the cultivation location. The growth rate was reduced with a change in temperature.

It was noted that, unlike growth, photosynthesis of *G. lemaneiformis* increased remarkably with increasing temperature (both short- and long-term) within the tested range (12°C–26°C). In this study we only adopted three temperature regimes for exploring photosynthesis and growth, and thereby the accurate values of the optimum temperature for growth and photosynthesis could not be given. Nevertheless, a rough assessment could be made. The temperature-growth pattern reflected the temperature optimum of growth being around 19°C, while the temperature optimum for photosynthesis in

TABLE 1. (A) Photosynthetic parameters of the P-I curves in *Gracilariopsis lemaneiformis* cultured at different temperatures (12, 19, or 26°C). (B) Two-way ANOVA analyses assessing the main and interactive effects in response to additional experimental temperature variability on the parameters of the P-I curves.

Parameters	Long-term temperature	Short-term temperature 12°C	19°C	26°C
(A)				
P_{max}	12°C	**27.93 ± 2.50^{a}**	54.76 ± 4.96^{d}	69.34 ± 4.06^{f}
	19°C	12.88 ± 1.40^{b}	**51.74 ± 2.84^{d}**	74.42 ± 3.82^{f}
	26°C	9.80 ± 1.48^{c}	36.46 ± 3.38^{e}	**70.70 ± 7.97^{f}**
R_d	12°C	**7.71 ± 0.69^{ab}**	12.45 ± 2.14^{d}	19.68 ± 2.88^{e}
	19°C	4.15 ± 0.72^{c}	**7.11 ± 0.35^{a}**	11.43 ± 0.77^{d}
	26°C	4.21 ± 0.55^{c}	6.58 ± 0.75^{a}	**8.30 ± 0.44^{b}**
α	12°C	**0.202 ± 0.009^{a}**	0.292 ± 0.019^{d}	0.312 ± 0.030^{d}
	19°C	0.178 ± 0.008^{b}	**0.238 ± 0.018^{e}**	0.275 ± 0.008^{d}
	26°C	0.138 ± 0.010^{c}	0.265 ± 0.019^{de}	**0.293 ± 0.022^{d}**
I_c	12°C	**38.2 ± 2.4^{ab}**	42.9 ± 9.1^{b}	62.9 ± 4.6^{f}
	19°C	23.3 ± 4.0^{c}	**29.9 ± 1.6^{d}**	41.6 ± 3.0^{b}
	26°C	30.7 ± 5.8^{cd}	24.9 ± 2.4 ^{cd}	**28.4 ± 2.0 ^{cd}**
I_k	12°C	**177.0 ± 16.0^{a}**	230.9 ± 29.6^{d}	286.8 ± 19.5^{e}
	19°C	124.1 ± 8.6^{b}	**247.7 ± 7.0^{d}**	312.3 ± 20.8^{e}
	26°C	101.5 ± 6.7^{c}	163.1 ± 20.5^{a}	**271.9 ± 44.7^{de}**

Source	Degrees of freedom	*F*-value	*P*-value
(B)			
P_{max}			
ST	2	516.120	<0.001
LT	2	27.559	<0.001
ST×LT	4	8.939	<0.001
R_d			
ST	2	105.039	<0.001
LT	2	94.247	<0.001
ST×LT	4	9.504	<0.001
α			
ST	2	157.749	<0.001
LT	2	18.431	<0.001
ST×LT	4	4.649	0.006
I_c			
ST	2	32.353	<0.001
LT	2	67.966	<0.001
ST×LT	4	10.367	<0.001
I_k			
ST	2	145.270	<0.001
LT	2	20.721	<0.001
ST×LT	4	5.236	0.003
Error	27		

Values were derived from Figure 3. Values are means ± SD (n = 3). Bolded values are those measured at the respective long-term culture temperature. For each parameter, values associated with different letters were significantly different ($P < 0.05$). Units of P_{max} and R_d were μmol $O_2 \cdot g^{-1}$ FW $\cdot h^{-1}$, α were (μmol $O_2 \cdot g^{-1}$ FW $\cdot h^{-1}$). (μmol photons $\cdot m^{-2} \cdot s^{-1})^{-1}$, and those of I_C and I_K were μmol photons $\cdot m^{-2} \cdot s^{-1}$. ST, short-term temperature; LT, long-term temperature.

G. lemaneiformis was 26°C or greater according to the photosynthesis-temperature pattern. It was clear that the temperature optimum for photosynthesis was higher than that for growth in *G. lemaneiformis*. This is a common phenomena in seaweeds (e.g., Davison 1991, Kübler et al. 1991, Eggert and Wiencke 2000, Zou and Gao 2005) and plants (Berry and Björkman 1980). In addition, like photosynthesis-temperature responses, the daily net carbon gains in *G. lemaneiformis* were increased with increasing long-term temperatures. Therefore, the temperature-growth pattern did not correspond to the temperature-photosynthesis pattern and temperature-daily carbon budget pattern in *G. lemaneiformis*. The physiological reason was that growth is an integration of the effect of temperature on the total metabolism, rather than a specific physiological process (e.g., photosynthesis and/or daily carbon gain). One possible physiological process might involve the efflux of dissolved organic matter increased with temperature due to reduced longevity of thalli and a concomitant enhanced rate of cell lysis at high temperature (Gordillo et al. 2001, Barron et al. 2012). In addition, the extrapolation of the daily net carbon gains calculated from the experiments to the natural habitat should be cautious, since the photo-period in the field is variable through the annual cycle.

Acclimation to long-term temperature change was evident in *G. lemaneiformis*, because cultivation at low temperature resulted in increased photosynthetic rates at low temperature relative to the

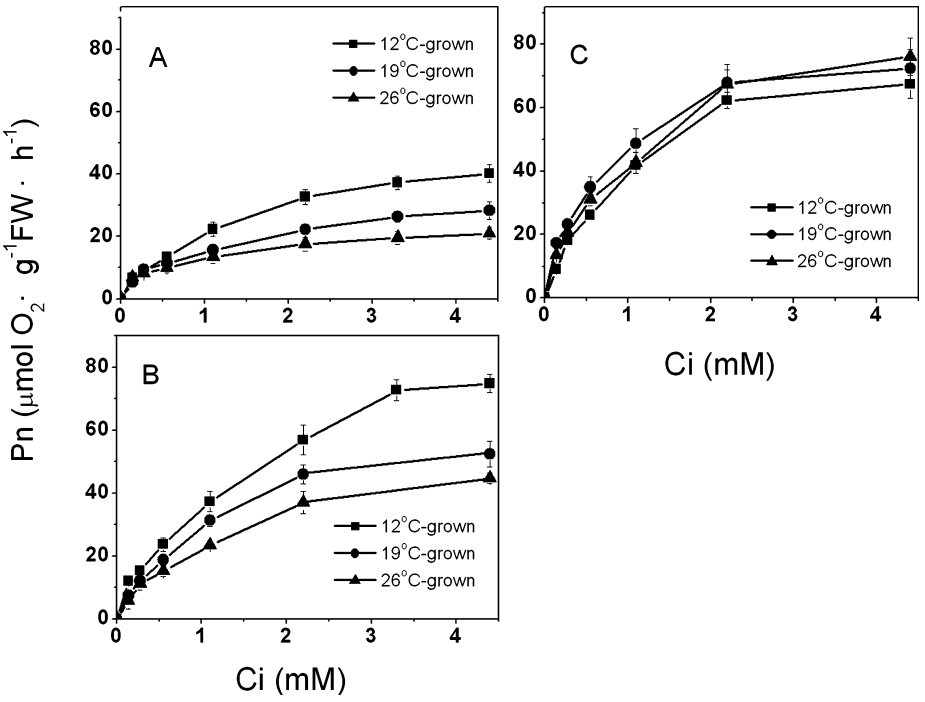

FIG. 4. Net photosynthesis versus inorganic carbon (Ci) concentration curves (P-C curves) of *Gracilariopsis lemaneiformis* grown at different temperatures (12, 19, or 26°C). Curves were measured at (A) 12°C, (B) 19°C, and (C) 26°C, respectively. Vertical bars represent ±SD ($n = 3$).

TABLE 2. (A) Parameters for photosynthetic responses to inorganic carbon (P-C curves) in *Gracilariopsis lemaneiformis* cultured under at different temperatures (12, 19, or 26°C). (B) Two-way ANOVA analyses assessing the main and interactive effects in response to additional experimental temperature variability on the parameters of the P-C curves.

Parameters	Long-term temperature	Short-term temperature 12°C	19°C	26°C
(A)				
ACE	12°C	**22.9 ± 2.9^{ab}**	40.0 ± 4.6^{d}	47.1 ± 1.1^{f}
	19°C	19.8 ± 1.2^{a}	**33.0 ± 1.2^{e}**	59.2 ± 7.5^{g}
	26°C	16.0 ± 2.5^{c}	27.1 ± 2.3^{be}	**53.9 ± 4.0^{g}**
$K_{0.5}$	12°C	**0.93 ± 0.45^{abc}**	0.82 ± 0.26^{bd}	1.06 ± 0.10^{ad}
	19°C	0.55 ± 0.05^{bc}	**1.03 ± 0.27^{ad}**	0.56 ± 0.15^{bc}
	26°C	0.42 ± 0.09^{c}	1.13 ± 0.66^{ad}	**0.67 ± 0.07^{b}**

Source	Degrees of freedom	*F*-value	*P*-value
(B)			
ACE			
ST	2	201.093	<0.001
LT	2	5.174	0.013
ST×LT	4	7.975	<0.001
$K_{0.5}$			
ST	2	3.730	0.037
LT	2	1.792	0.186
ST×LT	4	1.958	0.130
Error	27		

Values were derived from Figure 4. Values are means ± SD (n = 3). Bolded values are those measured at the respective long-term culture temperature. For each parameter, values associated with different letters are significantly different ($P < 0.05$). Units of ACE are (μmol $O_2 \cdot g^{-1}$ FW $\cdot h^{-1}$) $\cdot$ $(mM)^{-1}$, and that of $K_{0.5}$(Ci) is mM.

expected rates from the photosynthesis–temperature relationship. It was shown that prolonged exposure to low temperature might involve an improved capacity of temperature-limited enzymatic steps in the photosynthetic process, for example, an increase in the amount and activation state of key enzymes (e.g., Rubisco) and light reaction components, an alteration of enzyme characteristics and ratios of key photosynthetic components (Hikosaka et al. 2006, Sage and Kubien 2007). Those changes tended to compensate for the short-term effect of lower temperature on *G. lemaneiformis*. The photosynthetic acclimation to temperature was also reported in many other seaweeds species (e.g., Davison et al. 1991, Kübler et al. 1991, Eggert and Wiencke 2000) and in their terrestrial counterparts (e.g., Berry and

Björkman 1980, Atkin et al. 2006). During the earlier period of cultivation (i.e., January to February) when the seawater temperature is low (11°C–15°C), the capability of physiological adjustment to low temperature favored *G. lemaneiformis* with improved photosynthetic productivity, and thereby ameliorated the temperature limitation of growth.

Thermal acclimation of photosynthesis might be characterized by variations in the concentration of light-harvesting pigments (Kübler and Davison 1995) or chlorophyll *a* contents associated with the photosystem II reaction center densities (Machalek et al. 1996). Our data showed that photosynthetic efficiency (α) increased with increasing long-term temperature. Other investigations also reported the higher values of α in higher temperature-grown seaweeds or seagrasses (e.g., Davison et al. 1991, Olesen and Sand-Jensen 1993, Perez-Llorens and Niell 1993, Masini and Manning 1997). We observed that the increases of α with increasing long-term temperature were paralleled with an increase in phycobilin content, but a decline in chlorophyll *a* content, in higher long-term temperature (Zou and Gao 2013). We therefore suspected that light harvesting was closely coupled with the phycobilins contents in *G. lemaneiformis*.

There was a significant effect on the compensation irradiance for photosynthesis (I_c) of interaction between growth and assay temperatures. While I_c was strongly influenced by assay temperature in algae grown at lower temperatures (12°C and 19°C), it was largely independent of short-term temperature change in 26°C-grown algae. Therefore, our results showed that increasing long-term temperatures significantly decreased the light level required for P_g to compensate the respiratory carbon loss. Moreover, the temperature sensitivity of the light compensation point was much weakened with increasing long-term temperature. Similar results had also been reported in the kelp *Ecklonia radiata* (Staehr and Wernberg 2009). On the other hand, our results showed that the ACE (i.e., the initial slope of the P-C curve) was increased in high temperature-grown *G. lemaneiformis* thalli compared to the low temperature-grown thalli. This concurred with higher photosynthetic rates in higher temperature than lower temperature. The increase in the photosynthetic carbon-use efficiency (photosynthetic carboxylating efficiency) was suggested to be related to the activation of the Ci-acquisition mechanism (Kübler and Raven 1995, Raven 1997, Mercado et al. 2000, Zou and Gao 2009). Our previous investigations demonstrated that photosynthesis of *G. lemaneiformis* depended on the external carbonic anhydrase (CA) activity that mediated the dehydration of HCO_3^- to CO_2 extracellularly, and that formed CO_2 is then taken up into the cells (Zou et al. 2004). There were two major steps involved in the supply of CO_2 to Rubisco from the external pool of HCO_3^- (Raven 1997, Mercado et al. 2002): first, the external conversion of HCO_3^- into CO_2 by external CA and second, the transport of Ci from the plasmalemma to the carboxylating site of Rubisco for final photosynthetic CO_2 fixation. It had been shown that internal CA is involved in Ci transport processes inside the algal cell (Sültemeyer 1998). Therefore, the higher photosynthetic capacity and ACE in *G. lemaneiformis* grown at higher temperatures implied higher capacity of Ci acquisition (including the capacity to use the external HCO_3^-, and the transport of Ci toward Rubisco within the cell), and/or higher capacity of electron transport and Rubisco carboxylating. Direct physiological determinations are waiting to be conducted to examine whether or not the activities of CA (including extracellular and internal activities), Rubisco carboxylating capacity and/or the electron transport capacity were enhanced in high temperature-grown *G. lemaneiformis* thalli relative to low temperature-grown thalli.

In the farming field at Nanao Island, Shantou, China, the environmental conditions of *G. lemaneiformis* changed greatly during the late cultivation period compared to the earlier cultivation period. First, *G. lemaneiformis* experienced a wide range of temperatures (from 11°C–13°C in January to 25°C–28°C in June) due to the seasonal change. Second, the thalli of *G. lemaneiformis* were subjected to sharp attenuation of light level mainly due to severe self-shading of the dense algal beds (Zou and Gao 2009). Carbon limitation might also occur within the algal beds as a result of impeded currents and increased pH (Zou et al. 2004). Under this scenario, the growth, photosynthesis, and especially the persistence of dense stocking biomass could be threatened. Therefore, thermal acclimation of photosynthetic performances in *G. lemaneiformis* (i.e., the lowered light compensation point, but increased light- and carbon-use efficiencies in response to high growth temperature) would have important ecophysiological implications during the late cultivation period (summer with high temperature conditions) when *G. lemaneiformis* forms dense beds.

Mean global sea surface temperatures are predicted to increase by 1.0°C–4.4°C by the end of this century, mainly due to the increasing atmospheric CO_2 concentrations (Solomon et al. 2007). To be able to predict the effects of climate change, and to adapt mariculture systems of seaweeds to a warmer ocean, it is imperative to understand how temperature changes affect photosynthesis of seaweeds during the period of sea cultivations. In this study, given the ability of *G. lemaneiformis* to acclimatize its photosynthetic performance to temperature, increasing seawater temperature might increase stocking densities and the critical depth limit for growth, as long as the seawater temperature does not surpass the upper thermal limit. At the same time, the increasing atmospheric CO_2 concentra-

tions and associated ocean acidification may benefit the photosynthetic carbon metabolism (Zou et al. 2004, Zou and Gao 2009). Therefore, we suggested that the ongoing climate change (increasing atmospheric CO_2 and global warming) might have a favorable influence on the mariculture of *G. lemaneiformis* through the improved photosynthetic performance. However, much more research effort is needed to highlight the interactive effects of increasing temperature and associated environmental changes (such as increasing atmospheric CO_2 and the ongoing coastal eutrophication) on growth and biochemical processes to fully understand the possible constrains of future environmental changes on *G. lemaneiformis* cultivation.

In summary, it is essential to investigate the thermal acclimation of *G. lemaneiformis*, when evaluating the possible consequences of seawater temperature of natural and/or anthropogentic temperature fluctuations. Our results suggested that *G. lemaneiformis* can optimize metabolic balance by adjustment of the light- and carbon-use in harmony with the changes in the prevailing temperature conditions, which would have important ecophysiological implications for sea cultivation of this species. We proposed that ongoing climate change (increasing atmospheric CO_2 and global warming) might exert a favorable influence on the mariculture of *G. lemaneiformis* through the improved photosynthetic performances.

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