J. Phycol. **49,** 61–68 (2013)

DOI: 10.1111/jpy.12009

THERMAL ACCLIMATION OF RESPIRATION AND PHOTOSYNTHESIS IN THE MARINE MACROALGA *GRACILARIA LEMANEIFORMIS* (GRACILARIALES, RHODOPHYTA)[1]

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The responses of respiration and photosynthesis to temperature fluctuations in marine macroalgae have the potential to significantly affect coastal carbon fluxes and sequestration. In this study, the marine red macroalga *Gracilaria lemaneiformis* was cultured at three different temperatures (12, 19, and 26°C) and at high- and low-nitrogen (N) availability, to investigate the acclimation potential of respiration and photosynthesis to temperature change. Measurements of respiratory and photosynthetic rates were made at five temperatures (7°C–33°C). An instantaneous change in temperature resulted in a change in the rates of respiration and photosynthesis, and the temperature sensitivities (i.e., the Q_{10} value) for both the metabolic processes were lower in 26°C-grown algae than 12°C- or 19°C-grown algae. Both respiration and photosynthesis acclimated to long-term changes in temperature, irrespective of the N availability under which the algae were grown; respiration displayed strong acclimation, whereas photosynthesis only exhibited a partial acclimation response to changing growth temperatures. The ratio of respiration to gross photosynthesis was higher in 12°C-grown algae, but displayed little difference between the algae grown at 19°C and 26°C. We propose that it is unlikely that respiration in *G. lemaneiformis* would increase significantly with global warming, although photosynthesis would increase at moderately elevated temperatures.

Key index words: **acclimation; global warming; *Gracilaria lemaneiformis*; marine macroalgae; N availability; photosynthesis; respiration; temperature**

***Abbreviations*: R_d, dark respiration; FW, fresh weight; P_g, gross photosynthesis; P_n, net photosynthesis**

Marine macroalgae are distributed in intertidal and subtidal zones of coastal areas and may contribute to the annual biological drawdown of CO_2 and the global carbon cycle (Gao and McKinley 1994, Chung et al. 2011). Respiration and photosynthesis are the primary physiological processes governing carbon flux and sequestration, and are affected by various environmental variables. The significant effect of temperature on biochemical reactions makes it a major environmental determinant of photosynthesis and respiration in marine macroalgae. Marine macroalgae often experience remarkable fluctuations of temperature, both on a daily and a seasonal scale (Raven and Geider 1988, Davison 1991, Davison and Pearson 1996). In addition, the ongoing increase of CO_2 in atmosphere is predicted to be accompanied by rising temperatures globally, and the mean global sea surface temperature is anticipated to increase by 1.0°C–4.4°C by the end of this century (Solomon et al. 2007). Changing water temperature will affect macroalgal metabolism, maintenance of a positive carbon balance, and productivity (Henkel and Hofmann 2008, Suzuki et al. 2008, Staehr and Wernberg 2009, Rothäusler et al. 2011).

Temperature fluctuations can result in an immediate change in photosynthesis and respiration rates, with the magnitude of change being determined by the short-term sensitivity of each process to temperature. In contrast to instantaneous responses, the effect of prolonged temperature change on respiration and photosynthesis rates relies on the extent to which these processes acclimate. Acclimation is defined as adjustments in these processes in such a way that plant performance (and potentially fitness) is improved at the new growth temperature (Lambers et al. 1998). Acclimation may eventually result in complete metabolic homeostasis (i.e., identical rates of photosynthesis and respiration in plants growing at contrasting temperatures; Atkin and Tjoelker 2003).

A large number of investigations on terrestrial plants have shown that the degree of respiratory and photosynthetic acclimation differs among species (e.g., Berry and Björkman 1980, Atkin et al. 2006), with some species exhibiting full acclimation whereas others appear incapable of even partial acclimation. It is well established that many species of marine macroalgae have a high, genetically fixed potential for

[1]Received 13 March 2012. Accepted 10 July 2012.
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photosynthetic acclimation, enabling them to adjust and optimize photosynthesis to the prevailing temperature conditions (e.g., Davison et al. 1991, Kübler et al. 1991, Terrados and Ros 1992, Kübler and Davison 1995, Stengel and Dring 1998, Zou and Gao 2005, Staehr and Wernberg 2009). The ability of macroalgae to maintain positive photosynthesis over a broad range of temperatures may play an important role to their wide distribution and success (Lüning 1990, Machalek et al. 1996).

This study focused on the marine red macroalga *Gracilaria lemaneiformis* (Bory de Saint-Vincent) Greville (Gracilariales, Rhodophyta). This species of *Gracilaria* occurs in tropical and warm temperate waters of the world. Many *Gracilaria* species are economically important mainly due to the production of phycocolloids, accounting for more than half of all agar production worldwide (McHugh 1991). *G. lemaneiformis* is the principal species for seaweed cultivation in China, used as a high-quality raw material for agar industry and a good food source for intensive cultivation of abalone. Moreover, the cultivation of this species can be an effective bioremediation measure for eutrophication control in coastal waters (Fei 2004). This species is now being considered for further development of large-scale cultivation in China due to the strong market demand (Zou et al. 2004). *G. lemaneiformis* has been cultivated on large scales in both the southern and the northern parts of China, covering a large latitudinal gradient in ocean temperature. In addition, the mariculture of *G. lemaneiformis* at Nanao Island, Shantou, China (south coast of China), commences in January, and finishes in June. Therefore, *G. lemaneiformis* is subjected to strong variation in temperature (from 10°C to 28°C) throughout the cultivation period due to the seasonal change and/or latitudinal gradient.

We previously evaluated the photosynthetic acquisition of inorganic carbon and regulation by growth conditions, elevated CO_2 supply, and irradiance levels (Zou et al. 2004, Zou and Gao 2009). In this study, we examined the responses of respiration and photosynthesis to increasing temperature in *G. lemaneiformis*. Our objectives were to: (i) establish whether or not photosynthesis and respiration rates in *G. lemaneiformis* acclimate to increased temperature, (ii) address whether such rates acclimate similarly, (iii) establish whether the degree of acclimation of photosynthesis and respiration differs among different growth conditions such as N availability, and (iv) determine whether acclimation reestablishes the balance between thalli respiration and photosynthesis. We measured thallus respiratory and photosynthetic rates at five different temperatures on algae grown at three temperatures and at high- and low-N availabilities. In each case, respiration rates were measured in darkness, and photosynthesis was measured at saturating irradiances and at the same temperatures.

MATERIALS AND METHODS

Plant materials and experimental treatments. *Gracilaria lemaneiformis* was collected from a cultivation field at Shenao Bay, Nanao Island, Shantou, China (23°20′ N, 116°55′ E) in March 2007. The ambient surface seawater temperature at the site of collection over the sampling periods was around 20°C; *G. lemaneiformis* cultivated are subjected to a wide range of in situ temperature from 11°C–13°C in January to 25°C–28°C in June over the period of sea cultivation. The thalli were gently rinsed of any accumulated sediments and cleared of visible epiphytes, and then placed into a plastic barrel containing natural seawater, kept cool and dark during the transportation to the laboratory (about 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 prior to further treatment. The algae received an irradiance of 180 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (PAR) illuminated by a bank of cool-white fluorescent tubes with a 12:12 light:dark period. The seawater was changed (50%) daily and was continuously aerated by a filter pump to keep air equilibrium of the dissolved inorganic carbon.

For the experimental treatments, *G. lemaneiformis* thalli were cultured at three levels of temperature: 12, 19, or 26°C (i.e., culture temperature). They were arbitrarily assigned as low, intermediate, and high temperature, respectively. Experimental treatments were started when 2.5 g fresh weight (FW) algae were introduced into each of 18 Erlenmeyer flasks containing 5 L filtered seawater. The flasks were placed into three illumination chambers (GXZ-300D; Jiangnan Instrument Factory, Ningbo, China), the temperature conditions of which were controlled at 12, 19, or 26°C, respectively. The light conditions (light intensity and light period) for all the treatments were the same as indicated above. In each chamber, half of the flasks (3) contained N (200 μM NO_3^-)-supplemented filtered natural seawater (high-N availability, HN), and the remainder (3) contained non-N-supplemented filtered natural seawater (low-N availability, LN, total inorganic N concentration less than 10 μM). For all of the treatments, the culture seawater was supplemented with 40 μM $H_2PO_4^-$ to avoid phosphate limitation. Three replicate cultures were maintained at each growth treatment. The seawater media were completely renewed every 2 d, and were aerated vigorously and continuously with ambient atmospheric air by a filter pump to keep air equilibrium of the dissolved inorganic carbon. The concentration of inorganic N in the seawater media in low-N treatments after 2 d of cultivation was nearly undetectable, but was more than 150 μM in the high-N treatment (still maintaining high-N conditions). The algae were grown at the above conditions (three temperature levels and two N availabilities) for 3 weeks and then harvested in an effort to determine respiration and photosynthesis–temperature responses.

Measurements of respiration and photosynthesis. Photosynthetic and respiratory rates were measured as oxygen exchange using a Clark-type oxygen electrode (YSI Model 5300; YSI, Yellow Springs, OH, USA) that was held in a circulating water bath (Cooling Circulator; Cole Parmer, Chicago, IL, USA) to keep the desired measurement temperature. To examine the effect temperature had on respiratory and photosynthetic flux for each culture, respiratory and photosynthetic rates were measured at different temperatures by adjusting the temperature in the O_2 electrode chamber. The thalli of *G. lemaneiformis* were cut into small segments with a razor blade and incubated in seawater at the identical culture treatment and identical light-temperature conditions for at least 2 h. Preliminary experiments showed that this pretreatment minimized the possible effect of damage due to cutting cell

segments (wound respiration) on the respiratory and/or photosynthetic determinations.

The algal samples were allowed to equilibrate for about 15 min at each of five temperatures (7, 12, 19, 26, and 33°C) before the respiratory and photosynthetic rates were taken. Temperature treatments were randomly assigned and respiration and photosynthetic rates at each temperature were measured with the same algal samples. Moreover, the O_2 electrode was recalibrated at each temperature.

Measurements of R_d were initiated after at least 2 h of photosynthesis in the growth chambers. Algal samples were left in darkness for 20–30 min following transfer to the electrode cuvette in an effort to avoid possible postillumination transients of gas exchange. About 0.2 g FW segments were transferred to the O_2 electrode cuvette containing 8 mL reaction medium, which was magnetically stirred. The algal samples were allowed to equilibrate for about 15 min at each temperature before the commencement of respiration measurements. Respiration was recorded when the O_2 uptake stabilized, usually within 4–8 min. Immediately following the respiration measurement, irradiance-saturated net photosynthetic rates were determined at the irradiance of 500 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} supplied from a halogen lamp. Preliminary experiments showed that photosynthesis was saturated at this irradiance level and that no photoinhibition occurred.

The temperature sensitivity of respiration or photosynthesis can be quantified by the Q_{10} value (i.e., the proportional change in metabolic rate per 10°C rise). Q_{10} values were calculated over the temperature intervals of 7°C to 26°C using the following equation according to Atkin and Tjoelker (2003): $Q_{10} = 10^{(\text{slope} \cdot 10)}$, where the slope is the regression slope of a $\log_{10}$-transformed respiratory and/or photosynthetic rates versus temperatures plot.

Biochemical components. Some biochemical components of the algal thalli were determined. To determine Chl *a*, about 0.2 g FW per sample were extracted in 100% acetone. The concentration of Chl *a* was calculated spectrophotometrically using the equation given by Jensen (1978). For phycobiliprotein (PB) determination, samples of about 0.2 g FW of algal biomass were placed in 5 mL of 0.1 M phosphate buffer (pH 6.8), homogenized at 4°C using a mortar and pestle (with a little acid washed sand), and rinsed with a further 5 mL of buffer. The extracts were then centrifuged at 5000*g* for 20 min. The supernatants were used for the spectrophotometrical measurement of PB (including phycoerythrin and phycocyanin) using the chromatic equations of Beer and Eshel (1985). The contents of soluble protein (SP) were determined from the same supernatants by the Coomassie Blue G-250 method (Bradford 1976). SP contents were calculated by comparison with a standard of bovine serum albumin. Soluble carbohydrates (SC) contents were measured colorimetrically according to Kochert (1978) using phenol sulfuric acid method for color development and glucose as standard.

Statistics. The data were expressed at the mean values ± SD ($n = 3$) for the three independent replicate cultures. Statistical significance of the data was analyzed with *t*-test and analysis of variance (ANOVA) followed by the Student–Newman–Keuls post hoc procedure of multiple comparisons if necessary using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$.

RESULTS

Respiration. Overall, the rates of dark respiration (R_d) in *G. lemaneiformis* increased strongly with temperature (Fig. 1), showing no clear optimum temperature (T_{opt}) for respiration over the tested temperature range (7°C–33°C). R_d was consistently higher ($P < 0.01$) in 12°C-grown algae compared with 19°C- and 26°C-grown algae for each temperature. There was no significant difference in values of Q_{10} (i.e., the sensitivity of instantaneous respiration to a 10°C increase in temperature) between the algae grown at 12°C and 19°C, irrespective of the N availability in culture (Table 1). However, Q_{10} value declined significantly at growth temperature of 26°C compared with 12°C or 19°C. Moreover, for 26°C-grown algae, Q_{10} value was higher in low-N (LN) than high-N (HN) growth condition.

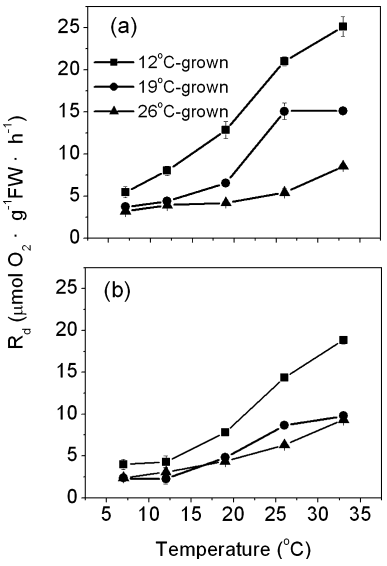

FIG. 1. Rates of dark respiration (R_d) as a function of culture temperatures in *Gracilaria lemaneiformis* grown at the three different temperatures (12, 19, and 26°C) and high- (a) and low-N (b) availability. Vertical bars represent ± SD of the means ($n = 3$).

The long-term response of R_d (determined by actual R_d at each respective growth temperature) was much less pronounced compared with the instantaneous temperature response. The rates of R_d in 19°C-grown algae were considerably lower ($P < 0.01$) than in 12°C-grown algae when measured at either 12°C or 19°C. Similarly, the rates of R_d in 26°C-grown algae were much lower ($P < 0.01$) than in 19°C-grown algae when measured at either 19°C or 26°C. Collectively, the long-term response of R_d was only slightly effective or even practically unchanged with growth temperature ('thermal homeostasis').

There was no significant difference ($P > 0.05$) in the rates of R_d between HN algae and LN algae at high growth temperature (26°C), but a significant increase ($P < 0.01$) in R_d was observed in HN algae relative to LN algae when growth temperatures were 12°C or 19°C.

Photosynthesis. Figure 2 presents the rates of net (P_n) and gross (P_g; i.e., P_n plus R_d) photosynthesis as a function of measuring temperatures in

TABLE 1. (A) The Q_{10} values for respiration and gross photosynthesis in *Gracilaria lemaneiformis* grown at the three different temperatures (12, 19, and 26°C) and high- and low-N availability. Data are means (±SD), $n = 4$–6. (B) The F and P values from ANOVA analysis assessing the single and interactive effects to Q_{10} of growth conditions, i.e., culture temperature (CT) and N availability (high vs. low).

Culture temperature	N availability	Q_{10} (R_d)	Q_{10} (P_g)
(A)			
12°C	HN	2.03 ± 0.10	3.13 ± 0.20
12°C	LN	2.04 ± 0.11	3.25 ± 0.01
19°C	HN	2.08 ± 0.11	3.37 ± 0.11
19°C	LN	2.12 ± 0.06	3.35 ± 0.05
26°C	HN	1.29 ± 0.04	2.32 ± 0.05
26°C	LN	1.67 ± 0.14	2.75 ± 0.13

Source	F	P
(B)		
Q_{10} (R_d)		
CT	96.801	<0.001
N	12.979	0.002
CT × N	8.983	0.002
Q_{10} (P_g)		
CT	122.784	<0.001
N	15.185	0.001
CT × N	8.423	0.003

Q_{10} values were calculated over the interval of 7°C–26°C using the equation: $Q_{10} = 10^{(\text{slope}\cdot 10)}$, where the slope was the regression slope of a $\log_{10}$ transformed rates versus temperatures plot (linear fittings $P < 0.01$).

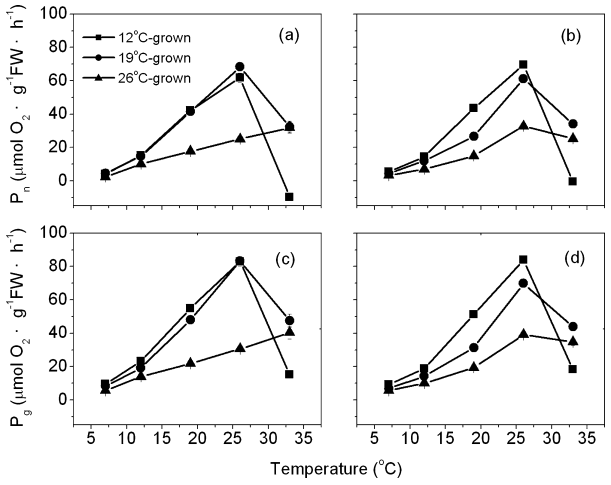

FIG. 2. Net (P_n; a, b) and gross (P_g, i.e., P_n plus R_d; c, d) photosynthetic rates as a function of culture temperatures in *Gracilaria lemaneiformis* grown at the three different temperatures (12, 19, and 26°C) and high- (a, c) and low-N (b, d) availability. Vertical bars represent ± SD of the means ($n = 3$).

G. lemaneiformis grown at the three different temperatures and high- and low-N availabilities. The instantaneous response of photosynthesis to change in temperature showed that light-saturated rates of photosynthesis (either P_n or P_g) increased with increasing measurement temperature up to 26°C (T_{op}), then decreased drastically at supraoptimal temperature (33°C) in 12°C- and 19°C-grown algae. This contrasted with 26°C-grown algae, which exhibited enhanced stability of photosynthetic rates at 33°C.

The Q_{10} for photosynthesis (gross) was similar ($P > 0.05$) between the 12°C- and 19°C-grown algae, with the value being around 3.2 (Table 1). The Q_{10} value declined significantly ($P < 0.01$) at high growth temperature (26°C), and this was accentuated in HN algae with respect to LN algae.

The acclimated photosynthetic response to temperature, as determined by actual values of Pg at their respective culture temperature, was less pronounced than the instantaneous responses. The rate of P_g measured at 12°C for 19°C-grown algae was higher than that of P_g measured at 12°C for 12°C-grown algae; reduction percentages of P_g rate by low temperature was lowered with acclimation (60.3% vs. 52.0% for HN algae, or 54.8% vs. 40.2% for LN algae). On the other hand, the rate of P_g measured at 19°C for 12°C-grown algae was lower than that of P_g measured at 19°C for 19°C-grown algae. This percentage increase in P_g by exposure of higher temperature was lowered with acclimation (138.0% vs. 108.4% for HN algae, or 173.5% vs. 67.3% for LN algae).

Nitrogen addition in culture strongly enhanced the photosynthetic capacity for the algae grown at 19°C, however, this photosynthetic enhancement by N addition was much less pronounced when algae were grown at low (12°C) or high (26°C) temperature.

The ratio of respiration to photosynthesis. Dark respiration was generally between 0.12 and 0.35 of P_g in *G. lemaneiformis*. A tendency for gradual decrease with increasing temperature could be seen for the values of R_d/P_g ratios, until the ratios leveled off over the temperatures of 19°C–26°C (Fig. 3). The values of R_d/P_g increased dramatically at a temperature of 33°C, with the value being more that 1.0 for 12°C-grown algae. This was the result of increasing R_d and simultaneously decreasing P_n (or P_g). As for the effects of culture temperature, 12°C-grown algae exhibited the highest value of R_d/P_g ratio among the different growth temperatures. However, there was little difference in the R_d/P_g ratio between the algae grown at 19°C and 26°C.

At a culture temperature of 12°C, N addition significantly increased ($P < 0.01$) the value of R_d/P_g ratio, whereas the values were similar ($P > 0.05$) between HN and LN algae when the culture temperature was 19°C or 26°C.

Biochemical components. Both culture temperature and N availability exerted a significant effect on the biochemical components in *G. lemaneiformis* (Table 2). It appeared that the contents of Chl *a*, SP, and SC were significantly higher, but the contents of PB were lower, in low temperature-grown algae than in high temperature-grown algae. High-N availability strongly increased the contents of Chl *a*,

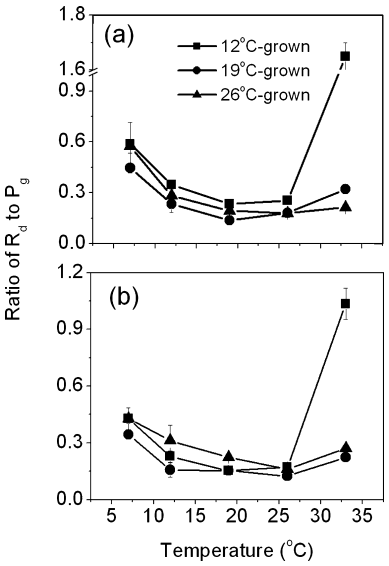

FIG. 3. The dark respiration (R_d) to gross photosynthesis (P_g) ratios as a function of culture temperatures in *Gracilaria lemaneiformis* grown at the three different temperatures (12, 19, and 26°C) and high- (a) and low-N (b) availability. Vertical bars represent ± SD of the means ($n = 3$).

PB, and SP, but decreased the contents of SC, for the algae grown at high temperature. However, N availability had only a slight effect on the biochemical components in algae grown at low temperature.

DISCUSSION

Our present results showed that change in temperature resulted in an immediate alternation in the rate of R_d of *G. lemaneiformis*. The extent of such alternation is usually determined by the short-term temperature coefficient (Q_{10}), i.e., the proportional increase in respiratory rate per 10°C rise in temperature. For *G. lemaneiformis* thalli grown at low and intermediate temperatures, the calculated Q_{10} value for R_d was around 2.0, the value often assumed for R_d in plants (Tjoelker et al. 2001). However, a high culture temperature resulted in a reduced temperature sensitivity for R_d, which was reflected in the decreased value of Q_{10}. This result was consistent with many terrestrial species in which Q_{10} of respiration decreases with increasing temperature (Tjoelker et al. 2001). Our results also contrasted the findings in the kelp *Saccharina latissima*, which exhibited similar Q_{10} values of respiration between two different culture temperatures (12°C and 22°C).

Given the high sensitivity of respiration to changing short-term temperatures for *G. lemaneiformis* thalli across all culture temperatures, it is interesting to establish whether and to what extent the acclimation of respiration to a new culture temperature will occur in this algal species. It has been reported that rates of R_d in many terrestrial species acclimate to changes in ambient temperature, often capable of maintaining constant rates of R_d (measured at their respective growth temperature) when grown at contrasting temperatures, although they may exhibit large variation in respiratory acclimation potential to thermal environment (Atkin and Tjoelker 2003). To our knowledge, very little information is available on the thermal acclimation of respiration in marine macroalgae. The present study demonstrated that the respiration of *G. lemaneiformis* exhibited strong acclimation to prolonged exposure to thermal fluctuation. Our results showed that the rates of R_d (measured at their respective culture temperatures) in warmer temperature-grown *G. lemaneiformis* were much lower than would be predicted from the instantaneous temperature responses exhibited by cooler temperature-grown algae. Conversely, the rates of R_d in cooler temperature-grown algae were much higher than would be predicted from the instantaneous temperature responses exhibited by their warmer temperature-grown counterparts. Such adjustment in the rate of R_d following prolonged exposure to a new temperature strongly compensated for the initial change in temperature, irrespective of the N level under which the algae were grown. Collectively, the acclimated response of R_d at the actual growth temperature was much less pronounced than the instantaneous response with nearly identical rates of respiration at contrasting temperatures, indicating nearly respiratory homeostasis over the tested temperature range (12°C–26°C).

This study showed that under low and intermediate growth temperatures, the rate of R_d in *G. lemaneiformis* was higher in high-N-grown algae than low-N-grown algae and these results were in agreement with our previous findings on the brown macroalga, *Hizikia fusiformis* (Harvey) Okamura (Zou et al. 2011). High-N-grown macroalgae were reported to have higher contents of N, Rubisco and higher uptake rate of nutrient compared with low-N-grown algae (Gordillo et al. 2001). Therefore, increased respiration in high-N-grown *G. lemaneiformis* might be necessary to support increased costs associated with higher maintenance demands (e.g., increased Rubisco contents) and greater uptake and assimilation of extra N in seawater, as suggested in our previous study on *H. fusiformis* (Zou et al. 2011).

Gracilaria lemaneiformis photosynthetic rates were significantly affected by the instantaneous change in temperature. Like respiration, the temperature sensitivity of photosynthesis could be described by the Q_{10} value. Our results showed that while the value of Q_{10} for photosynthesis (calculated over the range 7°C–26°C) did not differ between the 12°C- and 19°C-grown algae, the value was reduced for 33°C-grown algae. In terrestrial species, some studies showed that exposure to a high culture temperature decreased the value of Q_{10} for photosynthesis (Atkin and Tjoelker 2003); whereas other studies showed that culture temperature had no effect on

TABLE 2. (A) Chl *a*, phycobiliprotein (PB), soluble protein (SP), and soluble carbohydrates (SC) contents in *Gracilaria lemaneiformis* grown at the three different temperatures (12, 19, and 26°C) and high- and low-N availability. Data are means (±SD), $n = 4–6$. (B) The *F* and *P* values from ANOVA analysis assessing the single and interactive effects to Chl *a*, PB, SP and SC contents of growth conditions, i.e., culture temperature (CT) and N availability (high vs. low).

Culture temperature	N availability	Chl *a* ($g \cdot g^{-1}$ FW)	PB ($\mu g \cdot g^{-1}$ FW)	SP ($mg \cdot g^{-1}$ FW)	SC ($mg \cdot g^{-1}$ FW)
(A)					
12°C	HN	101.1 ± 2.8	342.4 ± 40.7	7.34 ± 0.97	42.59 ± 4.76
12°C	LN	95.2 ± 6.6	346.2 ± 31.2	6.37 ± 0.30	42.35 ± 3.18
19°C	HN	78.1 ± 11.2	345.4 ± 26.7	6.58 ± 0.23	34.11 ± 3.46
19°C	LN	68.4 ± 9.7	207.0 ± 12.4	4.33 ± 0.32	39.02 ± 0.96
26°C	HN	76.2 ± 5.4	445.1 ± 9.3	5.91 ± 0.03	32.71 ± 2.31
26°C	LN	65.9 ± 5.7	286.7 ± 17.7	4.37 ± 0.22	39.45 ± 3.37

Source	*F*	*P*
(B)		
Chl *a*		
CT	32.638	<0.001
N	8.131	0.011
CT × N	0.204	0.818
PB		
CT	26.938	<0.001
N	87.969	<0.001
CT × N	24.024	<0.001
SP		
CT	24.191	<0.001
N	57.199	<0.001
CT × N	4.306	0.030
SC		
CT	14.615	<0.001
N	12.543	0.001
CT × N	3.782	0.034

the Q_{10} in some species (Tjoelker et al. 2001). In the kelp *S. latissima* (Gerard 1997), the Q_{10} of gross photosynthetic capacity was stable among the two different culture temperatures (12°C and 22°C). Suggesting that the response of the Q_{10} of photosynthesis to culture temperature is highly variable among species. In addition, our results showed that Q_{10} value was higher in photosynthesis than in respiration. This suggests that photosynthetic processes (such as the activities of enzymes in Calvin cycle, photosynthetic electron transport chain) in *G. lemaneiformis* had a higher degree of temperature dependence than respiratory processes (such as the activities of enzymes in glycolysis and Krebs cycle, and respiratory electron transport chain), as had been generally shown by Davison (1991) and Atkin and Tjoelker (2003).

Rates of photosynthesis have been well known to acclimate to prevailing temperature, and ecotypic differentiation in thermal traits of photosynthesis occurs in marine macroalgae (Berry and Björkman 1980, Gerard and Du Bois 1988, Davison 1991, Eggert et al. 2003, 2006). The present results showed that, for the seaweed *G. lemaneiformis*, 19°C-grown algae displayed a decline in P_g following short-term exposure to 12°C, but with subsequent acclimation, photosynthetic rates increased. 12°C-grown algae exhibited an increase in P_g rate with instantaneous exposure to 19°C, but with the subsequent acclimation, photosynthetic rates decreased. Therefore, the response of photosynthesis in *G. lemaneiformis* to short-term temperature change was followed by partial readjustment with the prolonged exposure to increased temperature.

Thermal acclimation of photosynthesis and/or respiration was associated with temperature-mediated changes in cellular biochemical compositions (Kübler and Davison 1995, Machalek et al. 1996, Staehr and Wernberg 2009). This study showed higher Chl *a*, soluble proteins, and soluble carbohydrates in low temperature-grown algae compared with high temperature-grown algae. These changes were associated with reduced constraints of low temperatures on metabolic rates (Atkin and Tjoelker 2003, Sage and Kubien 2007, Staehr and Wernberg 2009). It has been recognized that increased cellular level of enzymes (e.g., Rubiso and other Calvin-cycle enzymes) is an acclimation response to low temperature, compensating for reduced specific activity at lower temperatures (Davison 1987, Davison and Davison 1987, Machalek et al. 1996). At low temperatures, an increase in Chl (in the reaction centers) and a decrease in PB (in the antennae) were consistent with a decrease in the ratio of antenna capacity to reaction centers, and a re-allocation from light capture toward electron conversion/transport capacity. This made sense physiologically as lower temperatures might slow the electron transport, so increased ratio of reaction centers to antennae could partly compensate. In addition, photosynthetic light

reactions are usually far less affected by temperature than enzymatic reactions (Davison 1991), resulting in a different response to temperature between photosynthesis and respiration. Further research is needed to establish the biochemical underpinnings of photosynthetic and respiratory acclimation in *G. lemaneiformis*, such as qualitative changes in the photosynthetic apparatus and quantitative change in resource investment.

Photosynthetic and respiratory metabolisms are tightly coupled (Atkin et al. 2006). Obtaining an understanding of the ratio of respiration to photosynthesis in response to short- and long-term temperature change is essential for determining if algal carbon balance and carbon flux throughout coastal ecosystems are altered by environmental changes. The temperature sensitivity of photosynthesis differed from that of respiration (Q_{10} value was higher for photosynthesis than respiration), resulting in a gradual decrease in R_d/P_g following an instantaneous change in temperature in *G. lemaneiformis* for all the culture treatments. However, the ratio of R_d/P_g jumped up sharply at high temperatures (33°C), with a more pronounced increase for low temperature-grown algae. This was because R_d increased, but P_n (or P_g) decreased simultaneously at 33°C.

Acclimation at moderate temperatures, especially from 15°C to 25°C, may result in near homeostasis of the ratio of R_d/P_g in plants (Atkin et al. 2006). However, our results showed that the ratio of R_d/P_g was drastically reduced at culture temperatures of 19°C relative to 12°C, although there was a slight increase under high-N conditions (or no change at low-N condition) in the ratio with increasing culture temperature over the range 19°C–26°C. The decreased R_d/P_g ratio might suggest that the algae used a lower quota of photosynthate as a respiratory substrate, thereby supplying more carbon for biosynthetic activities and ultimately growth. Moreover, the response in R_d/P_g implied that temperature increases over a moderate range may favor photosynthetic carbon fixation in *G. lemaneiformis* rather than respiratory carbon loss to the ocean or atmosphere, which may potentially affect the carbon cycle along macroalgal-dominated coasts.

Climatic changes predicted for the next century include a continued warming of near-surface air temperatures on the order of 2°C–7°C (Christensen et al. 2007). There is increasing concern over the potential consequences of anthropogenic climate change on marine systems and organisms (e.g., Brierley and Kingsford 2009). It is clear that temperature-mediated changes in photosynthesis and respiration are important components for the functioning of not only macroalgal primary production but also of the role of macroalgae in carbon sequestration and amelioration of atmospheric CO_2. Our results suggest that as temperatures increase, photosynthesis in *G. lemaneiformis* will be favored over respiration, as R_d is nearly unchanged, yet photosynthesis increases at a higher culture temperatures. Therefore, increasing temperature would enhance productivity in this red macroalgal species. It is worth noting, however, that the balance between respiration and photosynthesis is not enough to predict the ability of *G. lemaneiformis* to sequester atmospheric carbon. Many other processes might act as factors changing the overall increase in atmospheric CO_2 uptake by this species under warming conditions. For example, as thalli of *G. lemaneiformis* are generally subjected to large fluctuations in irradiance (Zou and Gao 2009), temperature effects as a function of light conditions should be taken into account. Staehr and colleagues recently suggested that, although temperature acclimation is significant, minimum light requirements still increase exponentially with increasing temperatures (Staehr and Wernberg 2009, Staehr and Borum 2011). More research is needed on the thermal acclimation potential of metabolism processes and the dependence of environmental conditions.

In summary, our results showed that both photosynthesis and respiration in *G. lemaneiformis* were strongly temperature sensitive in the short term. However, both metabolic processes acclimated to long-term changes in temperature, irrespective of the N availability under which the algae were grown. The results indicated that respiration exhibited significant levels of acclimation to changing culture temperatures, whereas photosynthesis only displayed a partial acclimation response to temperature. Therefore, the instantaneous response would be a poor predictor of long-term acclimated responses of carbon fluxes to changes in temperature in this species, especially for the respiratory carbon releases. There is little likelihood that respiration in *G. lemaneiformis* would increase significantly with moderate increases in temperature, whereas photosynthesis would be enhanced at moderately higher temperatures.

The authors would like to thank the three anonymous reviewers for helpful comments on the manuscript. This study was supported by the Chinese 973 Project (2009CB421207), the National Natural Science Foundation of China (41076094, 41276148, and 30970450), and the Fundamental Research Funds for the Central Universities, SCUT (2011ZG0013).

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