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Dark respiration in the light and in darkness of three marine macroalgal species grown under ambient and elevated CO_2 concentrations

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Abstract
Dark respiration (non-photorespiratory mitochondrial respiration), which occurs both in the light and in darkness, is vital for growth and survival of algae and plays a critical role in modulating the carbon balance of them. In the present study, we have investigated dark respiration in the light (R_L) and in darkness (R_D) in three marine macroalgal species, *Hizikia fusiformis* (phaeophyta), *Gracilaria lemaneiformis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta), cultured at 20 °C using aeration with two CO_2 conditions: current ambient (CO_2 concentration about 380 μl/L) and elevated CO_2 (approximately 720 μl/L) air. R_L was estimated by using the Kok method, whereas R_D was determined as the rate of O_2 influx at zero light. The results showed that both R_L and R_D were unchanged for the elevated CO_2-grown algae relative to ambient CO_2 concentration for all the algal species tested. However, R_L was significantly lower than R_D across all the algal species and growth CO_2 treatments, demonstrating that daytime respiration was partly depressed by the light. The percentage of inhibition of respiration by light was similar between ambient and elevated CO_2-grown algae. The ratio of respiration to photosynthesis, which tended to decrease when estimated using R_L instead of R_D, was not altered for the elevated relative to ambient CO_2 concentration. The results suggest that R_L, rather than R_D, is a more accurate estimate of nonphotorespiratory carbon loss in marine macroalgae during the daytime. It would not be anticipated that elevated atmospheric CO_2 would exert a substantial influence on respiratory flux either in the light or in darkness in these particular marine macroalgal species.

Key words: marine macroalgae, respiration, CO_2, carbon balance

1 Introduction

Dark respiration (R, non-photorespiratory mitochondrial respiration) is vital for growth and survival of plants and plays a critical role in modulating the carbon balance of individual cells and whole-plants. Much of the usable energy (ATP), reducing power (e.g. NADPH and NADH) and carbon skeleton intermediates required for biosynthesis and cellular maintenance are generated by dark respiration in a regular manner (Atkin et al., 2005; Amthor, 2000). Specifically, dark respiration in the light (R_L) can even be regarded as the essential component of the photosynthetic process, because the oxidative phosphorylation is demanded to mediate the state of stromal redox and to sustain the cytosolic ATP pool during photosynthesis (Foyer and Noctor, 2000). It is often assumed that non-photorespiratory dark respiration continues at the same magnitude (at constant temperature) in the light as in darkness (e.g. Collier et al., 1991; Pooter et al., 1990). However, several investigations on terrestrial plants species using the Kok and/or the Laisk methods demonstrated that the rate of dark respiration occurring in the light is lower than that in darkness (R_D) (e.g. Atkin et al., 2000, 1997; Villar et al., 1994), indic-

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ating that respiration is inhibited in the light. The degree to which light inhibits respiration seems to be highly variable, with inhibition values of 16%–77%, being dependent on the species and/or environmental conditions such as irradiance and temperature (Atkin et al., 2000, 1997).

Marine macroalgae (including Chlorophyta, Rhodophyta and Phaeophyta) are distributed in the intertidal and subtidal zones of the coastal area that are most populated. They contribute significantly to the productivity of the coastal ecosystem and play an important role in the coastal carbon cycle (Gao and McKinley, 1994). The trend of the ongoing increase of CO_2 concentration in atmosphere suggests that atmospheric CO_2 concentration is to double from its current level by the end of this century. It is anticipated that increasing atmospheric CO_2 will result in a linear proportionality increase in the dissolved CO_2 concentrations and a concomitant reduction of pH in near-shore areas, as a consequence of the continuous gas exchange between the air and seawater (Takahashi et al., 1997). In order to predict how increasing atmospheric CO_2 will affect the individual marine macroalgal species and coastal ecosystem functioning and to estimate the coastal carbon budget more accurately, it is essential to achieve a better recognition of the mechanistic response of respiration as well as photosynthesis in marine macroalgae to the increase of atmospheric CO_2 concentration. In the present study, we cultured three marine macroalgal species, *Hizikia fusiformis* (a brown alga), *Gracilaria lemaneiformis* (a red alga) and *Ulva lactuca* (a green alga) under two CO_2 conditions: the current ambient (CO_2 concentration about 380 μl/L) and elevated CO_2 (approximately 720 μl/L CO_2). Among them, *H. fusiformis* and *G. lemaneiformis* are two economically important species maricultivated on large scale in southeastern China (Zou, 2005; Zou et al., 2004), while *U. lactuca* is a world widely-distributed species occurring from the mid-intertidal to upper subtidal zone. Two type of dark respiration (R_L and R_D) were examined. R_L was estimated by using the Kok method, whereas R_D was determined as the rate of O_2 influx at zero light. Our primary objective was to investigate the effects of elevated CO_2 on R_L and R_D in these three species. Additionally, as data from previous studies in terrestrial counterparts have demonstrated that R_L and R_D are not necessary equivalent (e.g. Shapiro et al., 2004; Atkin et al., 2000), a further objective of the present study was to explore whether R_L was significantly lower than R_D in these species across the growth CO_2 conditions.

2 Materials and methods

2.1 *Algal materials and laboratory maintenance*

Gracilaria lemaneiformis (Bory) Weber-Van Boss (Rhodophyta) was collected from the Shengao bay, Nanao Island, Shantou, where it was artificially cultivated by means of the pole-system. *Ulva lactuca* L. (Chlorophyta) and *Hizikia fusiformis* (Harv.) Okamura (Phaeophyta) were collected from the intertidal rocky shore, along the coast of Nanao Island, Shantou, China. Healthy thalli free from visible epiphytes and any accumulated sediments were selected, and transported to the laboratory in an insulated cooler (6–10 °C) within 4 h. The algae were maintained in a controlled environment chamber (Conviron EF7, Canada) for 3 d in glass aquaria containing filtered natural seawater (salinity ca. 33), enriched with 400 μmol/L $NaNO_3$ and 50 μmol/L NaH_2PO_4 (final concentration). The seawater was vigorously aerated with ambient air and changed by a half of the amount daily. The algae received an irradiance of about 180 μmol photons m^{-2} s^{-1} supplied with fluorescent illumination for 12 h a day, and the temperature was controlled at 20 °C.

2.2 *Experimental treatments*

Algae were cultured under two CO_2 conditions (current ambient and elevated CO_2 concentrations) at the same temperature, light and nutrient conditions as mentioned above for maintenance. There are three replicates performed for each growth treatment. For each algal specie in experimentation, about 3 g fresh weight of algae was introduced in each Erlenmeyer flask contained 5 L of seawater media. Six flasks were placed into each of two CO_2 chambers (Conviron EF7, Canada). The one of the CO_2 chambers was programmed to supply 380 μl/L CO_2 (current ambient CO_2 treatment) in aeration for culture, and the other was programmed to supply a CO_2 concentration of 720 μl/L (elevated CO_2 treatment). The seawater motion generated by the aeration allowed the algal thalli to move gently without tumbling. The seawater in each culture was renewed every 2 d in all treatments with their respective ambient or elevated CO_2 air-equilibrated seawater supplemented the above mentioned nutrients. The pH values of the sea-

water were monitored in regulated interval to inspect the CO_2 conditions in the culture. They fluctuated with the day time for the cultures aerated with either current ambient or CO_2-enriched air, with increased values occurring during the light period and decreasing values in the darkness. The pH values in culture aerated with CO_2-enriched air were generally 0.3-0.9 units lower than those in culture with current ambient air, reflecting higher dissolved CO_2 concentrations in seawater in culture treated with elevated CO_2 relative to ambient CO_2 air. The algae were harvested and used for the experimental purposes after two weeks of cultures, a period which could be enough for acclimation in marine macroalgae (Zou, 2005; Zou et al., 2003; Andra et al., 2001; Mercado et al., 1999).

2.3 *Gas-exchange measurements*

There are two main methods used to estimate R_L by analysis of gas exchange: the Laisk method and the Kok method (Atkin et al., 2005, 1997; Shapiro et al., 2004; Wang et al., 2001; Villar et al., 1994). We choose the Kok method in the present experiments to estimate R_L. This method analyses the response of net photosynthesis to light (P-I curve) at low irradiances (Shapiro et al., 2004; Villar et al., 1994). The linear response of photosynthesis to light at low irradiances changes abruptly at the vicinity of the light compensation points (the Kok effect) so that the slope (i.e. the quantum yield of photosynthesis) appears to decrease as irradiance increases. This has been attributed to an increase in the respiration rate due to a progressive disappearance of the light-induced inhibition of dark respiration. The line at irradiance above the break extrapolates to an estimate of R_L under growth CO_2 conditions, and the line at irradiance below the break extends to R_D, where it is determined at zero irradiance. Figure 1 illustrates a representative example P-I curve for *H. fusiformis* at relatively low irradiances, from which R_L could be estimated. Similar curves were obtained for the other two algal species (data not shown).

Gas exchanges (respiration and photosynthesis) were carried out by measuring the O_2 uptake or evolution using a Clark-type oxygen electrode (YSI Moedel 5300, USA). The irradiance was provided by a slide projector, and the temperature was maintained at desired levels by using a cooling circulator (Cole Parmer, USA). The harvested algae were cut into small segments (0.7–1.0 cm in length) with a shape razor blade and were incubated under the identical conditions of

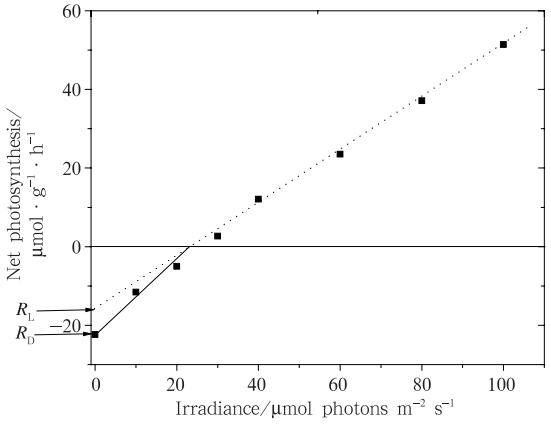

Fig.1. A representative example of photosynthetic light-response curve at low irradiance. The data are from *Hizikia fusiformis* grown at current ambient CO_2. The linear regression section of light-response curve before the distinct break in the slope extrapolated back to the Y axis, and the intercept was given an estimate of dark respiration in the light (R_L), according to the Kok method. The value for the dark respiration in darkness (R_D) was taken at zero irradiance. The inflection in the slope of the curves occurs at the vicinity of the light compensation point.

growth cultures (i.e. 180 mmol photons m^{-2} s^{-1}, 20 °C, and the same CO_2 levels in seawater as growth treatment) for at least 2 h. This pre-treatment was served to avoid the possible effect of cutting damage (wound respiration) on the O_2 exchange measurements. 0.1–0.3 g of algal samples was transferred to the O_2 electrode chamber containing 8 ml magnetically stirred seawater medium with their respective growth CO_2 concentrations, i.e. either ambient or elevated CO_2 air-equilibrated seawater. The rate of O_2 evolution was expressed as per unit of algal dry weight. The dry weight was determined after heating the algal sample at 80 °C for 24 h and cooling in a desiccator.

P-I curves were generated at growth temperature (20 °C) with a series of step-up lower light levels (0, 5, 10, 20, 30, 40, 60, 80, 100 μmol photons m^{-2} s^{-1}). The irradiance levels were measured with a PAR quantum sensor (SKP 200, ELE International, UK). The algal samples were allowed to equilibrate for 4–6 min at each light level before any reading was recorded. The light-saturated maximum photosynthetic rates (A_{max}) were also determined to estimate the ratio of respiration (R_D or R_L) to photosynthesis.

2.4 *Statistics*

The data plotted on graphs were mean values with standard deviations (SD). Statistical significance of the data was performed with SPSS for Window version

10 using analysis of variance (ANOVA) and student's t-test, with the significance level being set at $p < 0.05$.

3 Results and discussion

3.1 *Effects of elevated CO_2 on R_L and R_D*

There were no significant differences ($p > 0.05$) in R_D or R_L between elevated CO_2-grown algae and ambient CO_2-grown algae for each the three species tested (Fig. 2), demonstrating that elevated atmospheric CO_2 had no significant effects on R_L and R_D in these three macroalgal species.

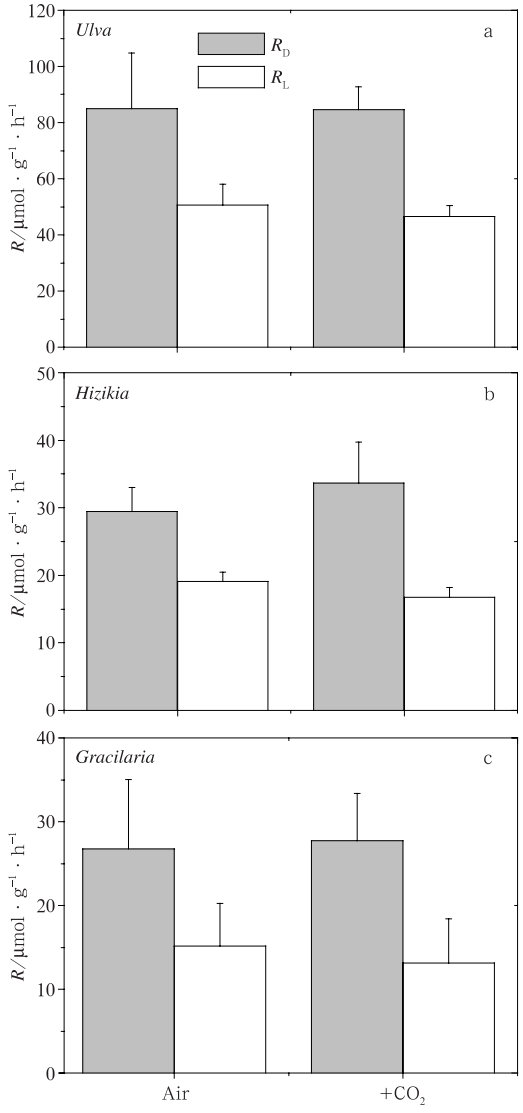

Fig.2. R_L and R_D of *Ulva lactuca* (a), *Hizikia fusiformis* (b) and *Gracilaria lemaneiformis* (c) cultured in seawater with aeration of ambient (Air) or CO_2-enriched air (+CO_2). R_L and R_D are measured at their respective growth CO_2 conditions. Vertical bars represent ±SD of the means (n=3).

There are much attentions on the effects of increasing CO_2 on marine macroalgae, with regard to the growth and biology such as photosynthesis, nutrient assimilation and elemental ratios (e.g. Zou, 2005; Zou et al., 2003; Israel and Hophy, 2002; Gordillo et al., 2001). However, to our knowledge, little is known how algal respiration responds to the increased CO_2. There are two possible concurrent processes which can contribute to adjustment of respiration in marine macroalgae grown at elevated CO_2. On the one hand, growth at elevated CO_2 might increase soluble carbohydrates content in macroalgal species (Zou and Gao, 2002; Andría et al., 1999), which is expected to increase respiration rate by providing more respiratory substrates. On the other hand, exposure and acclimation to high CO_2 would cause a decrease in tissue nitrogen content (such as contents of soluble protein and chlorophyll) in marine macroalgae (Zou and Gao, 2002; Andría et al., 1999; Mercado et al., 1999), which potentially affect the maintenance respiration. Therefore, the stimulation of respiration due to the accumulation of soluble carbohydrates would be offset by the reduction in maintenance respiration resulting from lower tissue nitrogen. The present experiments show that R_L and R_D of marine macroalgae grown in CO_2-enriched seawater were similar to those of algae grown in CO_2-nonenriched seawater, suggesting that significant changes in the energy and carbon skeleton output in these macroalgal species would not be expected when facing the ongoing increase of atmospheric CO_2 concentrations. These results contrast the findings in the terrestrial higher plant, *Xanthium strumarium* (Shapiro et al., 2004; Wang et al., 2001), in which leaf R_L and R_D were significantly higher in elevated CO_2 compared with ambient CO_2-grown plants. It is now generally expected that terrestrial plants grown at elevated relative to current ambient CO_2 concentrations exhibit an overall trend of a moderate increase in respiration rates of leaves (Davey et al., 2004; Gonzalez-Meler et al., 2004). It seems that the responses of R_L and R_D to increasing atmospheric CO_2 concentrations in marine marcoalgae were smaller compared with the terrestrial counterparts.

3.2 *Comparison between R_L and R_D*

Within each species, it was evident that R_D was significantly and consistently higher ($p < 0.01$) than R_L in ambient as well as elevated CO_2-grown plants (Fig. 2). This indicated that dark respiration was

partially depressed in the light. The percentage of inhibition of R_L relative to R_D ranged from 34.1% to 53.7% across the algal species and growth treatments (Fig. 3).

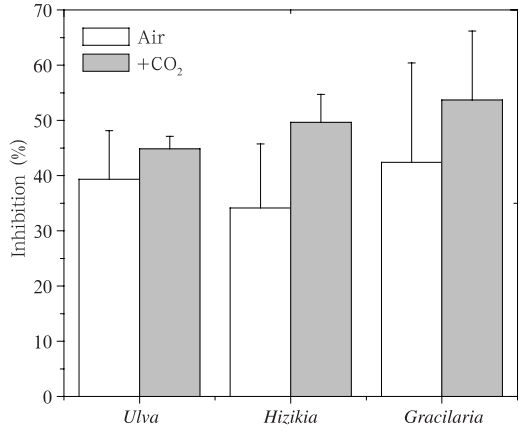

Fig.3. Percentage of inhibition of R_L relative to R_D in *Ulva lactuca*, *Hizikia fusiformis* and *Gracilaria lemaneiformis* cultured in seawater with aeration of ambient (Air) or CO_2-enriched air ($+CO_2$). The values of percentage inhibition was estimated as: Percentage inhibition (%)=$(1–R_L/R_D)\times100$. Vertical bars represent ±SD of the means (n=3).

The results in present study that dark respiration of the three marine macroalgae, *H. fusiformis, G. lemaneiformis* and *U. lactuca* being partially inhibited in the light are in accordance with the findings previously reported in several species of terrestrial counterparts (e.g. Shapiro et al., 2004; Wang et al., 2001; Atkin et al., 2000, 1997; Villar et al., 1994). The percentage of inhibition of respiration in the light at the growth temperature observed in marine macroalgae in this study was similar to the results (28%–53%) found in the terrestrial C_3 herbaceous plant, *Xanthium strumarium* (Shapiro et al., 2004). Our results suggest that compared to R_D, R_L is a more accurate estimate of nonphotorespiratory carbon loss in marine macroalgae during the light periods, irrespective of the growth CO_2 conditions. It is therefore essential that modeling the carbon budgets of these particular aquatic species during daytime incorporate corrected values of respiration by using R_L in place of R_D, which would bring increasing total amount of carbon gained. The physiological interpretation of the inhibition of respiration in the light has been attributed to the metabolites from photosynthetic process during light hours, such as ATP and NADPH, acting on respiratory enzymes as respiratory regulators and decreasing the demand for respiratory energy (Tovar-Méndez et al., 2003).

No significant differences were observed in the percentage of inhibition between the growth CO_2 treatments (Fig. 3), indicating that light affected respiration to the same extent in both ambient and elevated CO_2-grown algae. This also implies that elevated CO_2-grown algae have a similar demand for respiratory products such as energy and carbon skeletons during the light hours. Wang et al. (2001), however, found that in the terrestrial herbaceous *X. strumarium* light inhibited leaf dark respiration to a lesser degree for elevated than for ambient CO_2-grown plants, which was presumably attributed to a higher demand for energy and carbon skeletons in elevated CO_2-grown plants than in ambient CO_2-grown plants in light.

Obtaining an understanding of the ratio of respiration to photosynthesis regarding its response to increasing CO_2 concentration would be essential in determining if expected environmental changes alter algal carbon balance. Figure 4 showed that algal R_D and R_L at the growth temperature ranged from 15.4% to 25.0%, and from 9.2% to 14.0%, of net maximum photosynthesis (A_{max}), respectively, across the algal species. Each of the tested macroalgal species exhibited similar values for R_D: A_{max} and R_L: A_{max} (%) between the ambient and elevated CO_2 growth conditions (Fig. 4). However, a consistent trend was observed that the values of R_L: A_{max} (%) were lower than those of R_D: A_{max} (%) within each algal species, irrespective of the CO_2 levels in cultures.

It is noted that R_D and R_L differed significantly among species, with the rates of respiration (either R_D or R_L) in *U. lactuca* being three-time as high as those in the other two species. However, *U. lactuca* exhibited relative lower values of ratio of respiration to photosynthesis (either R_L:A_{max} or R_D:A_{max}), concurring with a much higher values of A_{max} and relative growth rates in *U. lactuca* (data not shown), compared with the other two species, *H. fusiformis* and *G. lemaneiformis*. It therefore appears that R_L and R_D are closely associated with growth in those algal species. Growth requires a supply of reducing equivalents and carbon skeletons for the biosynthetic reactions, which are produced only by respiration. As a consequence, the fast-growing species needs a higher rate of respiration that occurs both in the light and in darkness.

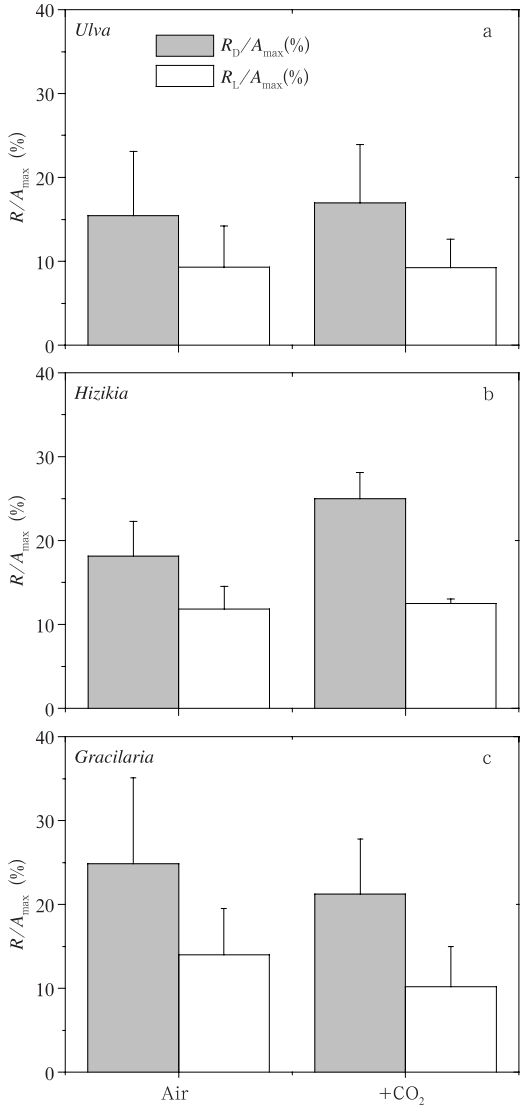

Fig.4. Percentage of R_L and R_D relative to light-saturating maximum net photosynthesis (A_{max}) in *Ulva lactuca* (a), *Hizikia fusiformis* (b) and *Gracilaria lemaneiformis* (c) cultured in seawater with aeration of ambient (Air) or CO_2-enriched air (+CO_2). Vertical bars represent ±SD of the means (n=3).

4 Conclusions

Our results would have important implications that R_L, rather than R_D, is a more accurate estimate of nonphotorespiratory carbon loss in marine macroalgae *Hizikia fusiformis*, *Gracilaria lemaneiformis* and *Ulva lactuca* during the daytime. It would not be anticipated that elevated atmospheric CO_2 would exert a substantial influence on respiratory flux either in the light or in darkness in these particular aquatic algal species. However, more species must be examined on this topic to gain a more general conclusion. In addition, more information is needed on the interactive effect of CO_2 and other important factors such as temperature and nutrients on respiration of marine macroalgae. Such investigations will improve our understanding of how marine macroalgal species may respond to natural and anthropogenic CO_2 variations in the atmosphere and/or ocean, and of how macroalgal species play a role in the carbon cycle budget in the ocean and/or atmosphere with respect to the forthcoming increase of global environmental changes.

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