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Responses of dark respiration in the light to desiccation and temperature in the intertidal macroalga, *Ulva lactuca* (Chorophyta) during emersion

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Dark respiration (nonphotorespiratory mitochondrial CO_2 release) in the light (R_L) of the intertidal macroalga *Ulva lactuca* (Chorophyta) during emersion was investigated with respect to its response to variations in temperature and desiccation. R_L was estimated by CO_2 gas-exchange analysis using the Kok effect method, whereas dark respiration in darkness (R_D) was determined from CO_2 release at zero light. Rates of R_L were significantly and consistently lower than those of R_D in emersed *U. lactuca* across all the temperature and desiccation levels measured. This demonstrated that dark respiration was partially depressed in the light, with the percentage inhibition ranging from 32 to 62%. Desiccation exerted a negative effect on R_L and R_D at a high temperature, 33°C, whereas it had much less effect on respiration at low and moderate temperatures, 23 and 28°C. In general, R_L and R_D increased with increasing temperature in *U. lactuca* during all stages of emersion but responded less positively to temperature change with increasing desiccation. Additionally, the Q_{10} value (i.e. the proportional increase of respiration for each 10°C rise in temperature) for R_L calculated over the temperature range of 23 to 33°C was significantly higher than that for R_D in *U. lactuca* during the initial stages of emersion. Respiratory carbon loss as a percentage of gross photosynthetic carbon gain increased with increasing temperature and/or desiccation but was significantly reduced when estimated using R_L rather than R_D. It is suggested that measurements of R_L and how it changes in a variable environment are as important as estimates of R_D and photosynthesis in determining simultaneous balance between photosynthetic carbon uptake and respiratory carbon loss and in modeling the net daily carbon gain for an intertidal macroalga.

KEY WORDS: Dark respiration, Desiccation, Emersion, Intertidal macroalgae, Kok effect, Temperature, *Ulva*

INTRODUCTION

Dark respiration (nonphotorespiratory mitochondrial CO_2 release) is a pivotal metabolic pathway producing usable energy (ATP) and reductants [NAD(P)H], as well as carbon skeleton intermediates to support other metabolic processes during plant growth and maintenance (Krömer 1995; Amthor 2000; Atkin *et al.* 2005). It has been well documented that plants respire roughly half of their daily photosynthetic carbon gain (Ryan 1991; Amthor 2000). Therefore, dark respiration plays an essential role in determining the carbon budget of individual plants. Dark respiration occurs both in the light and in the darkness. Specifically, dark respiration that occurs in the light can modulate stromal redox balance during the photosynthetic process (Foyer & Noctor 2000). It may also aid in minimizing the production of potentially damaging reactive oxygen species through reoxidizing the excess cellular redox equivalents generated by chloroplasts under excess irradiance conditions (Saradadevi & Raghavendra 1992; Purvis 1997; Maxwell *et al.* 1999). Moreover, mitochondral respiratory activity during illumination provides ATP for the repair of photosynthetic proteins (in particular the D1 protein of PSII) degraded by photoinhibition, protecting against photoinhibitory damage of the photosynthetic apparatus (Raghavendra *et al.* 1994; Atkin *et al.* 2000b). Mitochondrial respiration therefore functions in light during photosynthesis, and it affects and is affected by the potential demands for this process to supply energy and carbon skeletons during hours of illumination (Hoefnagel *et al.* 1998; Shapiro *et al.* 2004).

Dark respiration in the light (R_L) has usually been assumed to be of the same magnitude as in darkness in most studies of the carbon balance of plants or plant organs (e.g. Graham 1980; Pooter *et al.* 1990; Collier *et al.* 1991; Zou & Gao 2002, 2003, 2005). However, there is growing evidence that R_L in terrestrial higher plants varies between 25 and 100% of dark respiration in darkness (R_D), indicating that light partially inhibits dark respiration in photosynthetic tissue (Sharp *et al.* 1984; Kirschbaum & Farquhar 1987; Brooks & Farquhar 1985; Villar *et al.* 1994, 1995; Krömer 1995; Atkin *et al.* 2000b; Wang *et al.* 2001; Shapiro *et al.* 2004). The inhibition of dark respiration in the light is even evident at irradiances as low as 3–50 μmol photons m^{-2} s^{-1} (Sharp *et al.* 1984; Brooks & Farquhar 1985; Atkin *et al.* 1998), regardless of the light quality (red, blue or white; Atkin *et al.* 1998). This inhibition appears to be a result of photosynthetic products acting as regulators of respiratory function (McCashin *et al.* 1988; Wang *et al.* 2001; Tovar-Mendez *et al.* 2003). It has been suggested that the mitochondrial pyruvate dehydrogenase complex is reversibly phosphorylated during illumination, which eventually influences the CO_2 efflux through the Krebs cycle (Budde & Randall 1990; Gemel & Randall 1992; Tovar-Mendez *et al.* 2003). Additionally, the mitochondrial isocitrate dehydrogenase was shown to be inhibited by the

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high ratios of NADPH/NADP occurring during illumination (Igamberdiev & Gardeström 2003).

Intertidal macroalgae, being the important source of primary production in intertidal zone, are exposed to the atmosphere periodically resulting from tidally driven emersion–immersion cycles. This aerial exposure can last several hours at night as well as during the daytime. Emersed intertidal macroalgae suffer pronounced variations of potentially stressful environmental conditions (such as tissue water loss, increased irradiance and varied thallus temperature) as tissues dry with respect to submersion, which can bring about direct physiological effects on them (e.g. Dring & Brown 1982; Johnston & Raven 1986; Madsen & Maberly 1990; Bell 1993; Matta & Chapman 1995; Davison & Pearson 1996; Beach & Smith 1997; Peña *et al.* 1999).

There exists considerable information on the ability of intertidal macroalgae to photosynthesize during periods of exposure. It appears that not only intertidal algae tolerate the emersed conditions and recover their photosynthetic activity following resubmersion but also the carbon fixation during emersion contributes significantly to the daily carbon balance of the plants (Madsen & Maberly 1990; Bell 1993; Beach & Smith, 1997; Peña *et al.* 1999; Kawamistu *et al.* 2000). Although diffusion boundary layers are usually thicker in air relative to water, this constraint on inorganic carbon supply is more than offset by a diffusion coefficient for CO_2 in air that is 10,000-fold higher than in water (Madsen & Maberly 1990; Raven 1997, 1999). Therefore, emersion may facilitate inorganic carbon flux at the macroalgal surface and thereby elevate carbon fixation rates, even for species able to utilize HCO_3^- in photosynthesis (Beer & Eshel 1983; Holbrook *et al.* 1988; Madsen & Maberly 1990; Raven 1999). For example, some intertidal macroalgae have been demonstrated to exhibit higher photosynthetic rates under emersed conditions than under submerged conditions, and photosynthesis can be enhanced when partially desiccated (e.g. Johnson *et al.* 1974; Quadir *et al.* 1979; Johnston & Raven 1986; Gao & Aruga 1987; Einav & Beer 1993; Lipkin *et al.* 1993; Einav *et al.* 1995). However, the advantage in inorganic carbon acquisition by use of CO_2 in air rather than aqueous inorganic carbon is offset as water loss from the thallus. Decreased photosynthesis as a result of desiccation during emersion is a major reason why the time spent emersed is not more productive regarding net carbon assimilation (Raven 1997, 1999). Macroalgae possess no anatomical features analogous to the stomata or impermeable cuticules of terrestrial vascular plants that may effectively protect against water loss (exceptions are the saccate algae; Oates 1985, 1986), and thus cannot avoid desiccation during emersion at low tide (see review by Lüning 1990). However, photosynthesis is much more tolerant to desiccation in intertidal macroalgae than in terrestrial vascular plants because of differences in the molecular environment around the photosynthetic enzymes (Madsen & Maberly 1990; Kawamitsu *et al.* 2000) and the capacity of recovering photosynthesis within only 1–2 h following severe desiccation in intertidal macroalgae (Pearson *et al.* 2000). Tolerance to desiccation in air had been thought to be an important physiological requirement for macroalgae growing high up in the intertidal (e.g. Dring & Brown 1982; Beer & Kautsky 1992; Peña *et al.* 1999).

Although there is a large literature detailing the photosynthetic responses of intertidal macroalgae to emersion-related environmental factors (see review by Davison & Pearson 1996 and references therein; Beach & Smith 1997; Harker *et al.* 1999; Kawamitsu & Boyer 1999; Peña *et al.* 1999; Zou & Gao 2002, 2003), respiratory responses to changing environmental conditions are much less well understood (Madsen & Maberly 1990; Romaine *et al.* 1997; Zou & Gao 2002) in spite of the importance of dark respiration in determining the instantaneous daily carbon balance and algal metabolism. In particular, to our knowledge, there is no report focusing on the extent to which changes of temperature and cellular water content influence mitochondrial respiration that takes place during the hours of illumination in intertidal macroalgae when exposed to air during low tides. Achieving a better knowledge of the mechanistic response of R_L to the environmental variables associated with emersion has the potential to estimate more precisely the instantaneous or daily carbon balance in intertidal macroalgae. In the present study, we focused on the dark respiration in an intertidal macroalga during emersion. We have attempted to address two specific questions during emersion cycles using the intertidal green macroalga *Ulva lactuca* as the experimental material: (1) Is R_L lower than R_D in common with the light inhibition of dark respiration occurring in many species of terrestrial higher plants? (2) How are R_L and the proportion of R_L to gross photosynthesis at saturating light (A_{gross}) modified by temperature and desiccation in emersed *U. lactuca*? *Ulva* species (Chlorophyta) are commonly found along the intertidal and subtidal regions throughout the world. *Ulva lactuca* is one of the most common *Ulva* species found in the shoreline of Nanao Island, Shantou, China. It thrives in the mid- to low intertidal zone and therefore spends a significant fraction of its time out of the seawater during the tidal cycles.

MATERIAL AND METHODS

Plant materials and laboratory maintenance

Thalli of *U. lactuca* L. were collected at low tide from the middle intertidal zone of Nanao Island, Shantou, China, in May 2005. Collected algae were gently rinsed and cleaned of sediments and epiphytes. They were placed into a plastic barrel with some natural seawater and were transported to the laboratory within 3 h. The algae were then maintained in filtered natural seawater (salinity c. 32) in plexiglass aquaria under about 180 μmol photons m^{-2} s^{-1} (PAR, LD cycle 12 h : 12 h) and 28 ± 0.5°C (corresponding to the ambient surface seawater temperature at the site of collection). The seawater was continuously aerated and renewed every day. The algal samples were used for experiments within 3 days of collection. After this period, the algae remains were discarded and fresh samples recollected. The ambient surface seawater temperature at the site of collection over the sampling period in May 2005 was between 27.5 and 28.5°C. Such a small change in seawater temperature did not have a significant influence on the temperature–response curves of the algal samples.

CO_2 gas exchange measurement

Although it is easy to measure R_D, measurements of R_L are not straightforward as a result of the presence of other instan-

taneous processes of CO_2 exchanges in the light such as glycine decarboxylation and RuBP carboxylation (Villar *et al.* 1994; Atkin *et al.* 1998). There are two commonly used approaches to estimate R_L based on gas-exchange techniques: the Kok method (Kok 1948) and the Laisk method (Laisk 1977). The Laisk method, described by Laisk (1977) and extended by Brooks & Farquhar (1985), measures photosynthesis at low internal CO_2 concentrations and varying irradiances. Given that at such low CO_2 concentrations where CO_2 fixation and photorespiration are balanced, the rate of CO_2 release stand for R_L. As pointed out by Villar et al. (1994), the main shortcoming of this approach is that the analysis must be carried out at a very low CO_2 concentration that is far from the normal growth conditions. In addition, it is not known whether there is a direct short-term effect of CO_2 concentrations on R_L (Shapiro *et al.* 2004).

We therefore chose the Kok method, rather than the Laisk method, to estimate R_L in the present experiments. The Kok method analyzes the response of net rate of photosynthesis over low irradiances and can be measured at normal CO_2 conditions. At very low levels of irradiance, the response is linear, and the slope (i.e. the photosynthetic efficiency) is relatively steep, but near the light compensation point (the irradiance level at which net photosynthesis is zero), there is an abrupt break in the linear response, and the slope decreases. This change, termed the 'Kok effect', has been attributed to an increase in the respiration rate resulting from a gradual disappearance of the light-induced inhibition of dark respiration (Kok 1948; Sharp *et al.* 1984; Villar *et al.* 1994). The linear section of the light curve above the break extrapolates to an estimate of R_L (Villar *et al.* 1994; Krömer 1995; Wang *et al.* 2001), while the line at irradiance below the break stretches to R_D, which is determined at zero irradiance.

Photosynthetic rates of the emersed *U. lactuca* thalli were measured as CO_2 exchange in an open-flow gas-exchange system, using an infrared gas analyzer (LCA-4, Analytical Development Co) at ambient atmospheric CO_2 concentrations (c. 360 ppm). The light source was a metal halide lamp (220/240 V, 150 W, Hikaric-J) suspended above the photosynthetic leaf chamber. Temperature was controlled by maintaining the chamber in a temperature-controlled cabinet. About 1.5 g fresh weight (initial wet weight) of algal material were spread out in the photosynthetic chamber, and the dry weight (DW) was determined after each experiment by oven drying (80°C for 24 h). Net photosynthesis (P_n) or dark respiration (R) [μmol CO_2 g(DW)$^{-1}$ h^{-1}] was calculated as follows: P_n (or R) = $\Delta C \cdot F \cdot 60 \cdot 273/[(273 + T) \cdot 22.4 \cdot DW]$, where ΔC is the difference in CO_2 concentration (ppm) between the inlet and outlet air; F is the gas flow rate (L/min), T is temperature (°C), and DW is the dry weight (g).

Eight irradiances between 0 and 120 μmol photons m^{-2} s^{-1} were used to analyze the photosynthetic responses at low irradiance. Irradiance was adjusted by altering the distance between the light source and the photosynthetic chamber. R_L was estimated by the method described above. A representative light–response curve for emersed *U. lactuca* at low irradiance is presented in Fig. 1. R_D was determined at zero irradiance by turning off the light source and covering the chamber with a black cloth. In addition, the light-saturated maximum net photosynthesis (A_{max}) for each algal sample case was measured at irradiance of 600 μmol photons m^{-2} s^{-1}, which saturated photosynthesis without causing photoinhibition.

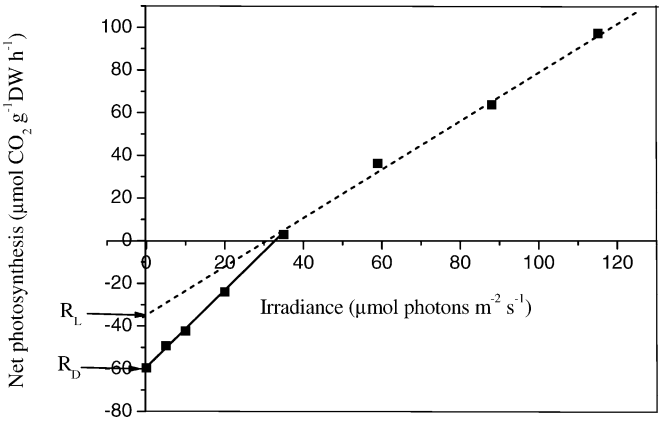

Fig. 1. A representative photosynthetic light–response curve of *Ulva lactuca* during emersion at low irradiance. Temperature was 28°C, and the percent desiccation of thalli was 0–8%. The linear regression section of light–response curve before the distinct break in the slope extrapolated back to the 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.

Photosynthetic response to irradiance was measured under three temperatures (23, 28 and 33°C) and three levels of desiccation (thallus water loss of 0–8%, 30–36% and 60–65%) in an effort to analyze the effects of those variables on R_L and R_D. Initially, thalli submersed in seawater were acclimated to a single measuring temperature for 30 min. Algae were then emersed and desiccated in an incubator at 160 μmol photons m^{-2} s^{-1}, 75 ± 5 % relative humidity and the same temperatures as used for initial acclimation and photosynthesis measurement. Samples were weighed at regular intervals through the various desiccation regimes in order to obtain the desired levels of desiccation. The percent desiccation (D %) was estimated as follows: D % = (Wo − Wt)/(Wo − Wd)·100, where Wo is the initial wet weight (i.e. fully hydrated weight) measured after removing superficial water from the thalli by gently blotting with tissue paper, Wt is the desiccated weight after a known time interval and Wd is the dry weight (80°C for 24 h). We sorted the samples in three classes of desiccation (desiccation 0–8%, 30–36% and 60–65%) in terms of the levels of water loss measured in the present study. These classes of desiccation were arbitrarily designated as low, medium and high, respectively. When the above measurements had been carried out at one temperature, the procedure was then repeated at a new measurement temperature, using similar algal thalli that had not previously been subjected to the measurement protocol. The order of temperature treatments (28, 23 and 33°C) was randomly assigned.

Q_{10} values (i.e. the proportional change in dark respiration per 10°C rise) were calculated for R_L and R_D over the measured temperature interval of 23 to 33°C using the following equation according to Atkin et al. (2000a): $Q_{10} = 10^{(\text{slope} \cdot 10)}$, where the slope is the regression slope of a $\log_{10}$-transformed respiration rate (either R_L or R_D) vs temperature plot. As pointed out by Atkin et al. (2000a), this would reduce the chances of erroneous Q_{10} values coming from unusually high or low respiration values at each of the low and high measuring tem-

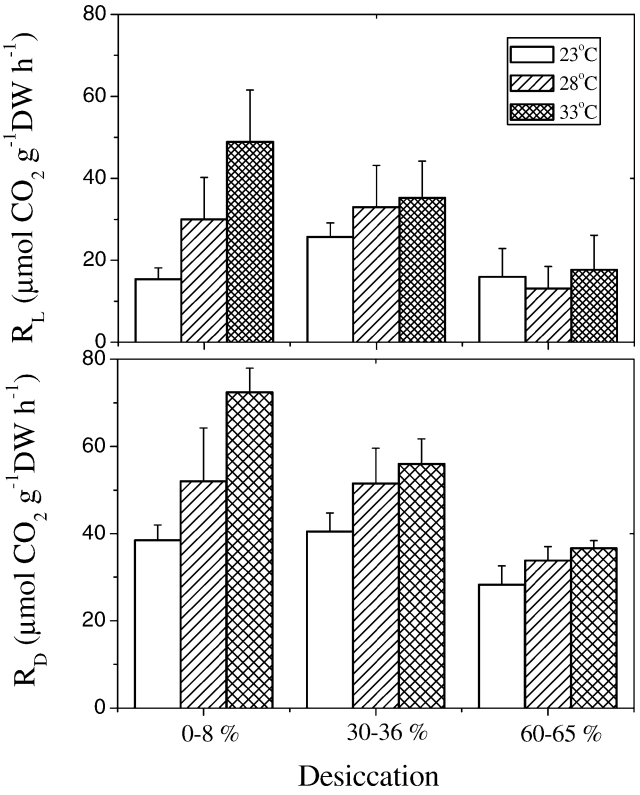

Fig. 2. R_L and R_D of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent ± standard deviation of the means (n = 3–6).

peratures used in the study. We calculated a single Q_{10} value using mean rates of respiration of all replicates.

The data plotted on graphs were mean values with standard deviations (s) and were analyzed with one- and two-way analysis of variance and Student's t test by using SPSS for Windows software package version 10. Differences were considered to be significant if $P < 0.05$.

RESULTS

The values of R_L and R_D of *U. lactuca* during emersion under different temperature and thalli desiccation conditions are shown in Fig. 2. It was evident that R_L values were significantly and consistently lower ($P < 0.01$) than R_D across all the temperature and desiccation levels measured, demonstrating that dark respiration was partially inhibited in the light. The ratio of R_L to R_D, which reflected the magnitude of inhibition of dark respiration by light, ranged from a low of 38% to a maximum of 68% (Fig. 3). However, no consistent trend in the R_L/R_D ratio was clear under the various combinations of temperature and desiccation conditions.

There was a decreasing trend in the rates of both R_L and R_D with increasing desiccation at 33°C (Fig. 2). However, for algae incubated at 23 and 28°C, mild desiccation (30–36%) did not effect ($P > 0.1$) either R_L or R_D. A positive effect of the mild desiccation was even observed for R_L at 23°C.

In general, both R_L and R_D increased with increasing measurement temperature from 23 to 33°C during all the stages of emersion, with the exception that R_L remained unchanged

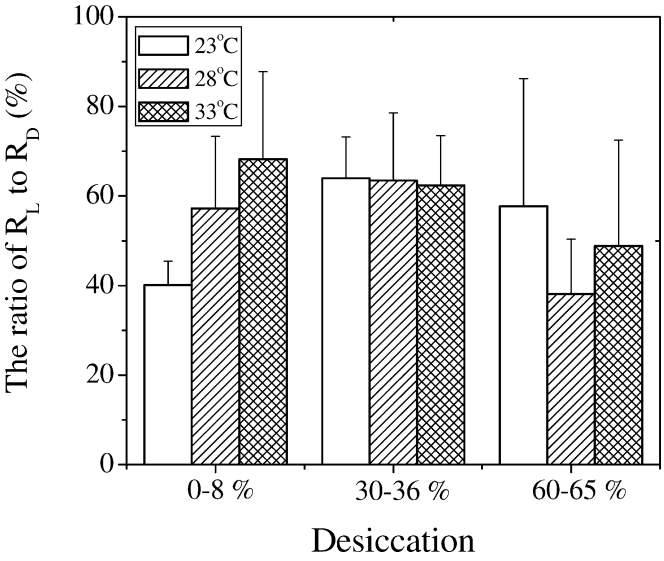

Fig. 3. The ratio of R_L to R_D of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent ± standard deviation of the means (n = 3–6).

with the rise of temperature in highly desiccated algae (Fig. 2). Temperature had a smaller effect on R_L and R_D with increasing water loss from algal thalli (Fig. 2), which was further evident from the change of the Q_{10} values (Table 1). The Q_{10} values, calculated over the measured temperature interval of 23 to 33°C, were reduced from 3.17 to 1.11 or from 1.88 to 1.29, respectively, for R_L and R_D with the increasing degree of desiccation. It was evident that R_L and R_D differed significantly in their relative response to temperature during the initial stages of emersion. The higher Q_{10} value of R_L (3.17) relative to that of R_D (1.88) in low desiccated algae may have been a consequence of the small absolute value of R_L determined at low temperature (23°C), which thereby amplified the relative difference in R_L over the measured temperature range.

A large variability was found in respiratory carbon loss (R_L or R_D) as a percentage of gross photosynthetic carbon gain (A_{gross}, i.e. $A_{max} + R_L$) in *U. lactuca* when emersed and subjected to various combinations of temperature and desiccation (Fig. 4). R_L and R_D were 4.6–30.4% and 11.4–77.1% of light-saturating gross photosynthetic carbon gain, respectively. A consistent trend was clear that both $R_L : A_{gross}$ and $R_D : A_{gross}$ ratios increased with increasing temperature and/or increasing degree of desiccation. Additionally, the ratios of $R_L : A_{gross}$ were substantially and consistently lower than those of $R_D : A_{gross}$ across all the combinations of temperature and desiccation measured.

Table 1. Q_{10} values for R_L and R_D of emersed *Ulva lacutuca* subjected to varying degree of desiccation.[1]

	Desiccation		
	0–8%	30–36%	60–65%
Q_{10} for R_L	3.17	1.37	1.11
Q_{10} for R_D	1.88	1.38	1.29

[1] Values are based on Fig. 2.

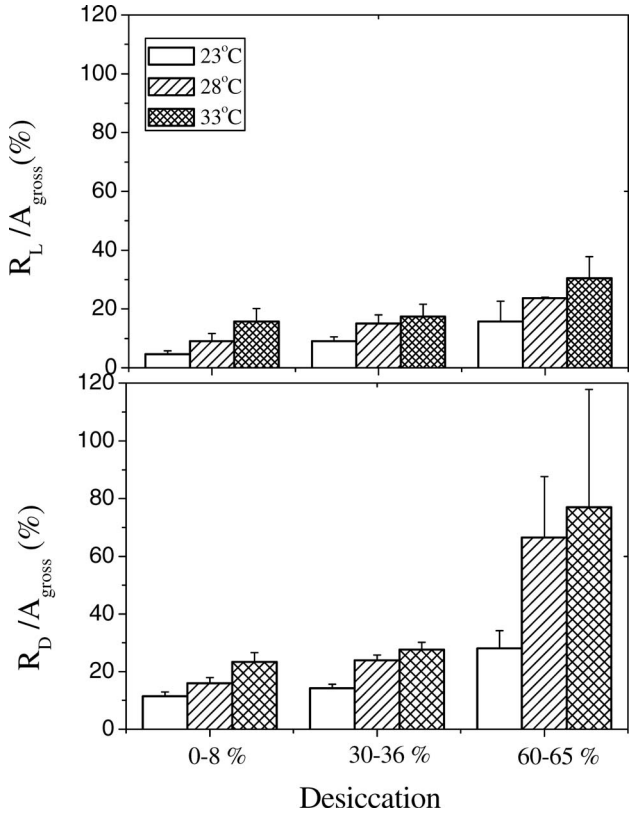

Fig. 4. Percentage of R_L and R_D to gross photosynthesis at saturating light (A_{gross}, i.e. A_{max} + R_L) of emersed *Ulva lactuca* subjected to different conditions of temperature and thalli water loss. Vertical bars represent ± standard deviation of the means (n = 3–6).

DISCUSSION

To our knowledge, it was often assumed in previous studies of carbon balance in macroalgae that dark respiration during illumination continued at the same magnitude as that in darkness. However, the present results showed that R_L was consistently and significantly lower than R_D in the common intertidal macroalga *U. lactuca* during emersion over all measured temperatures and levels of desiccation. This demonstrated that dark respiration was repressed in the light, with the percentage inhibition of respiration in the light ranging from 32 to 62%. This finding was consistent with reports for many species of terrestrial higher plants (e.g. Villar *et al.* 1994, 1995; Atkin *et al.* 1997, 1998, 2000, 2006; Wang *et al.* 2001; Shapiro *et al.* 2004). The present results underline the importance of using R_L rather than R_D to more accurately estimate the proportion of photosynthetically fixed carbon that is respired and to model the total amount of carbon gained during the hours of illumination in such intertidal macroalga as *U. lactuca.*

The mechanism of light inhibition of dark respiration remains debatable and appears to be complex. The physiological explanation for the inhibition of dark respiration by light had been ascribed to the inhibiting effect of the light on the respiratory enzymes regulated by the accumulation of photosynthetic metabolites during the hours of illumination, such as NADPH and ATP (McCashin *et al.* 1988; Krömer 1995; Tovar-Mendez *et al.* 2003), which is suggested to down-regulate the respiratory pathway in the light regarding both glycolysis and the Krebs cycle. Recently, Tcherkez et al. (2005) reported the metabolic basis of inhibition by light of leaf respiration *in vivo* study by feeding experiments using ^{13}C-enriched substrates and followed the ^{13}C atoms with isotope ratio mass spectrometry and nuclear magnetic resonance. Their results indicated that metabolic down-regulation (glycolysis, Krebs cycle) accompanies the light/dark transition and emphasized the decrease of Krebs cycle decarboxylations as a metabolic basis of the light-dependent inhibition of mitochondrial respiration. Our results of the inhibition of respiration in the light might suggest that emersed *U. lactuca* had a lower demand for respiratory products such as energy and carbon skeletons during the illumination hours than during darkness periods.

Temperature has fundamental influences on chemical reaction rates. It is viewed as one of the most important environmental parameters affecting the rates of respiration in plants, with the temperature sensitivity of respiration reflecting the effects of temperature on enzyme activity, adenylate control (i.e. ADP/ATP ratios) and/or substrate supply (Atkin & Tjoelker 2003; Atkin *et al.* 2005). During emersion at low tide, intertidal macroalgae usually experience changes in temperature. The present experiments showed that, although both R_L and R_D responded positively to the increasing measurement temperature in emersed *U. lactuca,* increasing water loss from thalli resulted in a reduced temperature sensitivity for both R_L and R_D, which was reflected in the decreased value of Q_{10} (the proportional increase in respiratory rate for each 10°C rise) for both R_L and R_D with increasing desiccation. For algae subjected to low desiccation, the calculated Q_{10} value for R_D was 1.9, being similar to the value (2.0) often assumed for dark respiration in plants (Ryan 1991; Tjoelker *et al.* 2001). However, the Q_{10} value for R_L (3.2) was considerably higher than that for R_D in algae with low desiccation. This indicated that R_L responded much more positively to increasing temperature than R_D did in algae during the initial stages of emersion. Q_{10} values more than 3.0 have also been reported in many species of terrestrial higher plants (e.g. Atkin & Tjoelker 2003; Shapiro *et al.* 2004).

Ulva lactuca is highly susceptible to desiccation during emersion owing to its thin sheet-like thallus morphology. Desiccation might affect reactions catalyzed by enzymes located at the water/membrane interface such as ATPase or soluble enzymes (Kaiser 1987). Excessive water loss could also lead to increased surface pH, resulting in reduction or cessation of photosynthesis (Bidwell & McLachlan 1985). While the photosynthetic responses to emersion-related desiccation in intertidal macroalgae have been an area of intensive investigation (see review by Davison & Pearson 1996 and references therein; Beach & Smith 1997; Harker *et al.* 1999; Kawamitsu & Boyer 1999; Peña *et al.* 1999; Zou & Gao 2002, 2003), the responses of dark respiration to desiccation have received much less attention. Information available shows that, although increasing desiccation would decrease the rate of dark respiration, the rate of decline is usually lower than that of photosynthesis (Madsen & Maberly 1990; Zou & Gao 2002). The results in the present study also agree with this finding (the photosynthetic data not shown) since intense desiccation exerted a less deleterious influence on respiration (either R_L or R_D) than on photosynthesis. It was also suggested that the

decline of respiration in emersed *U. lactuca* was not simply due to the reduced photosynthesis (which thereby might reduce the supply of respiratory substrate) but to other effects of desiccation *per se* because the reduced photosynthesis was not necessary accompanied with the reduced respiration. However, the biochemical mechanism underpinning the reduced respiration in intense desiccated algae is waiting to be investigated. Additionally, it is important to note that the relation of dark respiration and desiccation in emersed *U. lactuca* was temperature dependent. The rate of decline of either R_L or R_D resulting from more intense desiccation was reduced remarkably with decreasing temperature. Moreover, R_L and R_D exhibited different response patterns to desiccation. At low temperature (23°C), high desiccation exerted no negative effect on R_L, whereas it reduced the rate of R_D by 26.3%. In contrast, the percentage decrease of respiration caused by greater desiccation was greater in R_L (64 %) than in R_D (50%) when the thalli were incubated at high temperature (33°C).

A large variability in percentage dark respiration relative to light-saturating rates of gross photosynthesis observed in this experiment in emersed *U. lactuca* might reflect the varying physical conditions of desiccation and temperature, indicating that variations in the physical conditions affected the carbon balance. Two important trends could be seen when evaluating the instantaneous photosynthetic carbon uptakes and respiratory carbon losses during daytime emersion. On the one hand, substituting R_L for R_D substantially increased estimates of the percentages of carbon gained to carbon lost in *U. lactuca* across all the combinations of temperature and desiccation. It is therefore proposed that estimates of net carbon balance during illumination for an emersed macroalga based on R_L/A_{gross} ratios might be more appropriate than estimates based on R_D/A_{gross} ratios. On the other hand, the percentages of carbon respired via R_L relative to gross carbon gained at A_{max} increased appreciably with the increasing temperature and/or desiccation, implying a decreasing balance between net carbon gain and desiccation or temperature.

The response pattern to temperature in dark respiration usually differs from that in photosynthesis (Davison 1991; Atkin & Tjoelker 2003). For example, unlike photosynthesis, which displays the optimum temperature of up to approximately 5–10°C higher than the ambient seawater temperature, respiratory rates in marine macroalgae can continue to increase with increasing temperature of seawater in excess of 40°C (Zou & Gao 2005). In the present experiments, a decreasing trend of light-saturated net photosynthesis in emersed *U. lactuca* was observed with increasing temperature over the tested range of 23–33°C (data not shown), suggesting the optimum temperature of photosynthesis by emersed *U. lactuca* to be 23°C or lower. By contrast, both R_L and R_D showed an increasing trend with increasing temperature. On the other hand, either R_L or R_D was much less sensitive to water loss than was photosynthesis, which can presumably be attributed to respiratory processes (such as the activities of respiratory enzymes in glycolysis and the Krebs cycle and respiratory electron transport chain) being less water status sensitive than the photosynthetic processes (such as photosynthetic electron transfer and the enzymatic reactions of the Calvin cycle). Collectively, our data that dark respiration (either R_L or R_D) exhibited a different temperature and/or desiccation sensitivity compared with light-saturated photosynthesis may supply the physiological interpretation for the observed increasing percentage of respiratory carbon loss relative to photosynthetic carbon fixation. Our results have important implications for accounting for the simultaneous budget between photosynthetic carbon fixation and respiratory carbon loss.

In conclusion, the results obtained in the present experiments indicated that R_L values in emersed *U. lactuca,* when measuring CO_2 gas exchange using the Kok method, were significantly and consistently lower than R_D values. This demonstrated that dark respiration is partially inhibited in the light in emersed *U. lactuca,* which is consistent with the findings obtained from many species of terrestrial higher plants. The difference in R_L and R_D emphasized the necessity of substituting R_L for R_D when trying to accurately estimate mitochondrial respiration sustained during illumination. We therefore proposed that measurements of R_L and how it changes in a variable environment are as important as estimates of R_D and photosynthesis in determining the simultaneous balance between photosynthetic carbon uptake and respiratory carbon loss and in modeling the net daily carbon gain for a macroalga.

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