Botanica Marina 50 (2007): 275–279 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/BOT.2007.031

Daily timing of emersion and elevated atmospheric CO_2 concentration affect photosynthetic performance of the intertidal macroalga *Ulva lactuca* (Chlorophyta) in sunlight

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Abstract

The lunar day differs in length from the solar day so that times of low tide vary from day to day. Thus, aerial exposure of intertidal seaweeds may be during the day or during the night. We measured photosynthetic CO_2 assimilation rates of the intertidal green macroalga *Ulva lactuca* during exposures of varied daily timings during sunny days of summer to establish how photosynthetic performance responds to emersion timing under varied CO_2 levels [at ambient (360 ppmv) and 2× ambient (720 ppmv) atmospheric CO_2 concentrations]. There was an increase in net photosynthetic rates following some duration of exposure when the initial timing of exposure occurred during early morning (06.30 h) and late afternoon (17.15 h). In contrast, net rates exhibited a sharp decline with exposure duration when the initial timing of exposure occurred at 09.30 h, 15.30 h and especially at noon (12.30 h), implying the occurrence of a severe photoinhibition resulting from mid-day insolation. Doubled atmospheric CO_2 concentration significantly enhanced the emersed photosynthetic rates, indicating that the emersed photosynthesis is CO_2-limited at ambient CO_2 levels. However, increasing CO_2 barely stimulates the emersed photosynthetic rates during mid-day insolation.

Keywords: CO_2; emersion; photosynthesis; tidal rhythm; *Ulva lactuca*.

Introduction

Intertidal macroalgae experience continual alternation of living in air and in water, as the tidal level changes. Emersion will expose intertidal macroalgae to a variety of potentially adverse environmental conditions, such as photoinhibitory irradiance, extreme temperature and desiccation (Davison and Pearson 1996), which would significantly affect photosynthetic activities. Intertidal macroalgae can tolerate emersed conditions, and emersed photosynthesis contributes significantly to the total carbon fixation budget (Maberly and Madsen 1990, Matta and Chapman 1995, Peña et al. 1999). Some intertidal macroalgae even exhibit faster photosynthetic rates under emersed conditions than when submersed (Johnston and Raven 1986, Gao and Aruga 1987, Madsen and Maberly 1990).

Most studies on the emersed photosynthesis of intertidal macroalgae have been carried out in the laboratory under constant and moderate conditions of light and temperature (Johnston and Raven 1986, Madsen and Maberly 1990, Matta and Chapman 1995, Gao et al. 1999, Peña et al. 1999, Kawamistu et al. 2000, Ji and Tanake 2002, Zou and Gao 2002, 2004a,b), and comparatively little attention has been focused on field conditions (Beer and Eshel 1983, Hanelt et al. 1993, 1994, Sagert et al. 1997), although the effects of natural radiation on immersed photosynthesis during a daily cycle have been much documented (Hanelt et al. 1993, Figueroa et al. 1997, Sagert et al. 1997, Jiménez et al. 1998, Cabello-Pasini et al. 2000, Mercado et al. 2003). To our knowledge, no study has assessed the emersed photosynthetic performance of intertidal macroalgae in relation to changes in the daily timing of low tides. Because the lunar period differs from the solar period, high and low tides occur at varying times through the day. Intertidal macroalgae are exposed to considerable diurnal changes of solar irradiance as a result of tidal level and position of the sun. The harshest combination of irradiance and temperature occurs when low tide is around mid-day in summer when the sky is clear; these conditions may cause photoinhibition. In contrast, the irradiance to which the intertidal macroalgae are exposed is low or even zero when low tide occurs in early morning, late afternoon or night, and the emersed photosynthesis may be irradiance-limited or even shut down. Integrated diurnal photosynthetic performance may therefore vary with tidal phasing.

Intertidal macroalgae may be significantly sensitive to the atmospheric CO_2 rise, as atmospheric CO_2 is the main (or the only available) exogenous carbon source for their photosynthesis while exposed at low tide (Raven 1999, Mercado and Niell 2000, Zou and Gao 2004b). Laboratory studies have shown that the current ambient level of CO_2 in the atmosphere would become limiting for photosynthesis of intertidal macroalgae more often during emersion than during submersion (Surif and Raven 1990, Gao et al. 1999, Raven 1999, Zou and Gao 2002, 2004a,b). However, little is known about the impact of timing of emersion on the photosynthetic response to atmospheric CO_2 rise in intertidal macroalgae under natural solar radiation. We investigated how varied timings of low tide during daytime affect the photosynthesis of the intertidal green macroalga *Ulva lactuca* L. at ambient and elevated CO_2 levels. The following questions were examined: (i) does the timing of low tide affect the relationship between photosynthesis and desiccation? (ii) Does the ambient level of CO_2 in the atmosphere limit emersed photosynthesis of *U. lactuca*, and if so, is such limitation affected by the daily timing of emersion?

Materials and methods

The experiments were carried out under field conditions near the Marine Biological Experimental Station of Shantou University located in Nanao Island, Shantou, China (23°20′ N, 116°40′ E) during 1–10 June, 2000. All of the days during our measurement period were clear and sunny. *Ulva lactuca* is one of the most common *Ulva* species found in the shoreline of Nanao Island. It thrives in the mid-intertidal zone. Thalli were collected at low tide from mid intertidal rocky shores near our experimental station, where tides were semi-diurnal, each approximately 50 min later than on the previous day (Figure 1). Based on the tidal amplitude (approximately 2.0 m) and the intertidal position of the samples, the duration of emersion at each low tide was estimated to be 2–6 h. The collected thalli were rinsed and cleared of any visible epiphytes and accumulated sediments, and only intact and healthy plants were selected as the experimental materials. The samples were then maintained outdoors in flowing sand-filtered natural seawater in a glass aquarium (4 m^3). The temperature was controlled at 26±0.5°C with through flowing seawater (similar to sea surface temperature at the collection site). The experiments were conducted within 2 days of sample collection.

To determine how emersed photosynthetic performances vary with the different timing of low tide, emersions were simulated to start at 06.30 h (early morning, local time), 09.30 h (morning), 12.30 h (noon), 15.30 h (afternoon) and 17.15 h (late afternoon), designated as 09.30 h, 12.30 h, 15.30 h and 17.15 h emersion phasings, respectively. Diurnal changes of solar radiation (PAR) were monitored with a quantum sensor (SKP200, ELE International, Leighton Buzzard, Bedfordshire, UK) each day during our experiments, and the aerial temperatures during the daily cycle were also recorded. The emersed net photosynthetic rates of *U. lactuca* were determined as CO_2 uptake by infrared gas analysis using a leaf chamber analyzer (LCA4, Analytical Development Company Ltd., Hoddersdon, UK) under sunlight at 360 (ambient) and 720 (enriched) ppmv CO_2. The enriched CO_2 level is double that in the atmosphere. Air bags were used to store the air to supply it at a stable CO_2 concentration. CO_2 (720 ppmv) was obtained by injecting pure CO_2 into the air bag before pumping in ambient air.

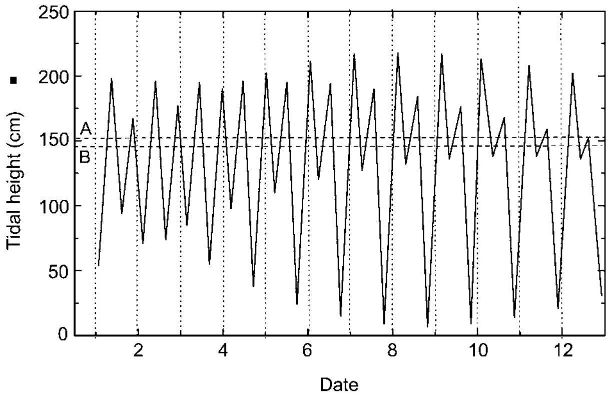

Figure 1 *Ulva lactuca*: diagram of predicted tidal height 1–13, June 2000 on the coast near the Marine Biology Experimental Station of Shantou University, Nanao Island.
Lines A/B illustrate the levels where thalli were collected.

Net photosynthesis (P_n) [μmol CO_2 g $(DW)^{-1}$ h^{-1}] was calculated as:

$$P_n = \Delta C \times F \times 60 \times 273/[(273+T) \times 22.4 \times B]$$

where ΔC is the difference in CO_2 concentration (ppmv) between the inlet and outlet air; F is the gas flow rate (l min^{-1}); T is temperature (°C) and B is the biomass of the samples (g dry weight, after drying at 80°C for 24 h).

Desiccation of the samples was estimated as percentage of water loss (D%):

$$D\% = (Wo-Wt)/(Wo-Wd) \times 100,$$

where Wo is the initial wet weight (i.e., fully hydrated weight) determined after removing surface water drops by lightly blotting with tissue paper; Wt is desiccated weight, indicating the instantaneous weight of the samples being desiccated and Wd is dry weight, determined after heating the sample at 80°C for 24 h and cooling in a desiccator.

Results and discussion

Figure 2 shows the field conditions of the solar radiation (PAR) and aerial temperature on a representative day during the measurement period (6 June 2000) near the Marine Biology Experimental Station of Shantou University, Nanao Island. Emersed *Ulva lactuca* would have been subjected to photon irradiance as high as 2000 μmol m^{-2} s^{-1} in coincidence with a temperature of

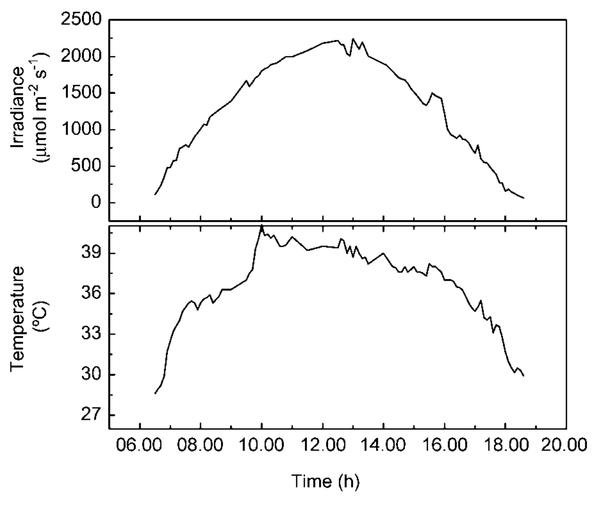

Figure 2 *Ulva lactuca*: diurnal changes in solar photon irradiance (PAR) and aerial temperature on 6 June, 2000, a representative day during the experimental period, near the Marine Biology Experimental Station of Shantou University, Nanao Island.
Irradiance is photon fluence rate.

approximately 40°C when low tide occurred at noontime. However, the irradiance and temperature that the emersed algae encountered would have been drastically reduced had the low tide occurred during early morning or late afternoon.

There was an increase of net photosynthetic rate in emersed thalli of *U. lactuca* following a period of exposure during the initial stage of emersion for the 06.30 h and 17.15 h emersion phasing (Figures 3, 4). It has been reported that many intertidal algae increase photosynthetic capacity when moderately desiccated (Johnston and Raven 1986, Lipkin et al. 1993, Peña et al. 1999, Ji and Tanaka 2002), presumably due to a reduction in the aqueous diffusion barrier for CO_2 on the surface of thalli. However, we found this photosynthetic phenomenon when making determinations under laboratory conditions, i.e., constant and moderate light-temperature environment. In the present study, we showed that field conditions must be taken into account. Thus, the initial increase of net photosynthesis for the 06.30 h emersion phasing resulted from two factors: (1) the increase in solar irradiance from photosynthesis limited level of 105 μmol photons m^{-2} s^{-1} to above saturation level within the initial emersion stage of 20 min; and (2) the generally recognized putative reduction in the diffusion barrier for CO_2 on the surface of thalli. In contrast, the initial increase of photosynthesis for the 17.15 h emersion phasing resulted from only one factor, i.e., the reduction of the diffusion barrier for atmospheric CO_2 on the surface of thalli. Although the solar photon irradiance decreased from 575 to 520 μmol m^{-2} s^{-1} during the initial exposure stage of 10 min, this did not affect the photosynthetic rate, because solar photon irradiance was above the saturation point of *U. lactuca* (~400 μmol m^{-2} s^{-1}). The changing temperature within the initial stage of emersion was so small that the effects on photosynthetic rates could be safely ignored.

The net photosynthetic rates for the 06.30 h emersion phasing maintained high values for rather long periods and decreased thereafter with progressive exposure duration, which was likely due to water loss from thalli. A decline of photosynthetic capacities with desiccation was reported by Oates and Murray (1983), Madsen and Maberly (1990) and Peña et al. (1999). However, in the present study, net photosynthesis for the 17.15 h emersion phasing was reduced sharply with exposure duration after reaching its maximum value; this may be attributable to the substantial photon irradiance decline with decreasing solar elevation.

For the 09.30 h, 12.30 h and 15.30 h emersion phasings, net photosynthetic rates displayed a stable reduction with duration of exposure (Figure 3) and with water loss (Figure 4). The rates declined to half of their initial values at exposure durations of 15, 8 and 16 min and approached zero at approximately 30, 20 and 44 min, respectively. It appeared that the declining rate of photosynthesis was greatest for the 12.30 h emersion phasing. In this latter case, the relative water content of the thalli was still 70% when net photosynthetic rate reached zero. Thalli of *U. lactuca* had relatively high photosynthetic activity with 70% water content under laboratory conditions, i.e., constant and moderate light and temperature

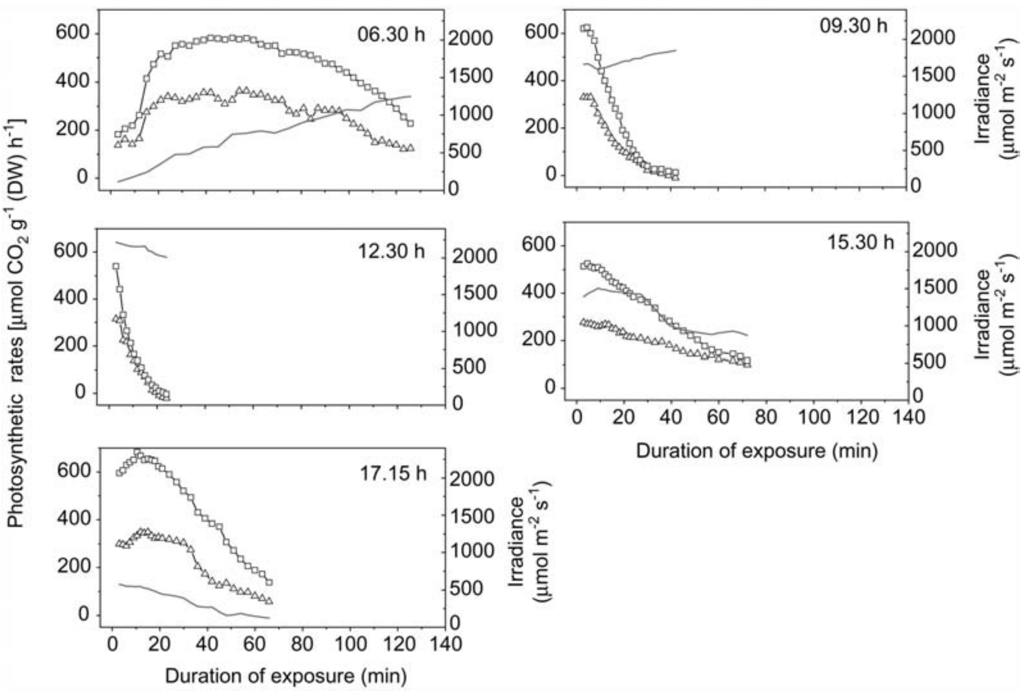

Figure 3 *Ulva lactuca*: the course of net photosynthetic rates with the exposure duration under field conditions with initial exposure occurring at different times of day: 06.30 h, 09.30 h, 12.30 h, 15.30 h and 17.15 h (local time).
Photosynthesis was measured under ambient atmospheric CO_2 concentration (360 ppmv, △) and double atmospheric CO_2 concentrations (720 ppmv, □). Data are the means of duplicate measurements. The solar photon irradiances (—) to which the emersed thalli were subjected are also indicated for each emersion phasing. Irradiance is photon fluence rate.

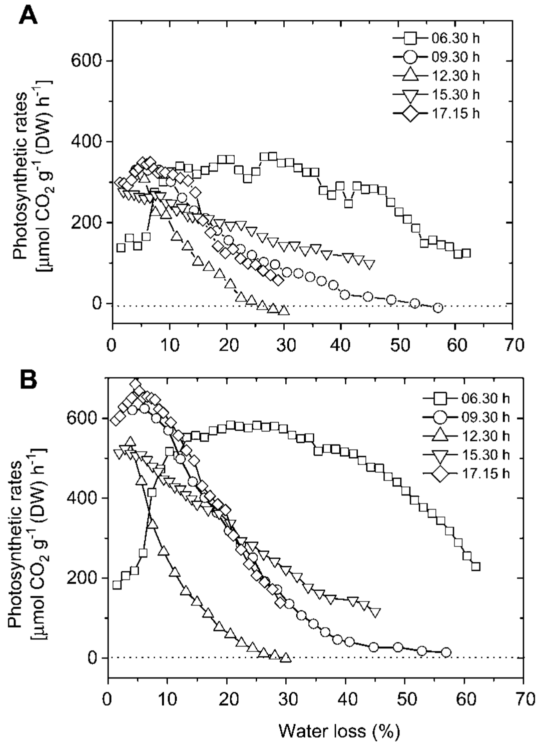

Figure 4 *Ulva lactuca*: relationship between emersed net photosynthetic rates and percentage desiccation (water loss) of thalli under field conditions with the initial exposure occurring at different times of day: 06.30 h, 09.30 h, 12.30 h, 15.30 h and 17.15 h (local time).
Photosynthesis was measured under ambient atmospheric CO_2 concentration [360 ppmv, (A)] and double atmospheric CO_2 concentrations [720 ppmv, (B)]. Data are the means of duplicate measurements.

of 400 μmol photons m^{-2} s^{-1} and 30°C (data not shown). Thus, the sharp reduction of photosynthetic rates with exposure duration is likely attributable to the mid-day insolation, which exposed the alga to an irradiance of more than 2000 μmol photons m^{-2} s^{-1} and a temperature of 40°C (Figure 3), but not to desiccation. Such an extremely high irradiance and probably the synergism with high temperature apparently caused a remarkable photoinhibition in the thalli, as has been demonstrated in non-desiccated thalli of *U. rotundata* Bliding during emersion (Henley et al. 1992). It was interesting to note that the initial net photosynthetic rates were almost the same for the 09.30 h, 12.30 h, 15.30 h and 17.15 h emersion phasings, indicating that the instantaneous mid-day insolation did not result in depression of photosynthesis, and it was the cumulative effect of harsh mid-day insolation that brought about severe photoinhibition. Bose et al. (1988) and Franklin et al. (1992) have also shown that photoinhibition is a function of duration of exposure to high irradiance. The presence of natural ultraviolet radiation might also amplify the photoinhibition effects of radiation (Sagert et al. 1997). Additionally, desiccation might reduce resistance to photoinhibition in *U. lactuca*, as suggested by Beer and Eshel (1983) and Henley et al. (1992). For the 09.30 h and 15.30 h emersion phasings (rather benign light-temperature conditions in comparison to the12.30 h emersion), the degrees of photoinhibition were somewhat less than for the 12.30 h emersion phasing.

Ulva lactuca has an enhanced capability for extracting inorganic carbon from seawater, and immersed thalli are able to uptake HCO_3^- directly from the dissolved external pool (Drechsler and Beer 1991, Axelsson et al. 1999) for incorporation into an internal carbon-concentrating mechanism (Beer 1994). Thus, the ambient inorganic carbon composition in seawater can fully saturate photosynthesis of *U. lactuca* when submersed (Beer and Koch 1996). However, the present study showed that photosynthesis of *U. lactuca* while exposed to air was significantly enhanced by doubled atmospheric CO_2 concentration (Figures 3, 4), indicating the occurrence of CO_2-limited photosynthesis of emersed *U. lactuca* at ambient CO_2 levels in air. For the 06.30 h, 09.30 h, 12.30 h, 15.30 h and 17.15 h emersion phasings, doubled atmospheric CO_2 level stimulated the initial net photosynthetic rates by 32%, 87%, 70%, 86% and 99%, respectively. The initial photosynthesis relative enhancement (32%) by elevated CO_2 during the 06.30 h emersion phasing was smaller than that for other emersion phasings. This discrepancy might result from light limitation of photosynthesis at 06.30 h. However, photon irradiance increased sharply with the increase in solar elevation, and photosynthetic enhancement with increased CO_2 also rose to its maximum. For the 12.30 h emersion phasing, the elevated CO_2 did not stimulate photosynthetic rate following very short exposure duration, because the capability for CO_2-fixation was severely depressed by mid-day insolation. After some exposure duration for 09.30 h and 15.30 h emersion phasings, elevated CO_2 also barely stimulated photosynthetic rates, again likely due to the photoinhibition. Enhancement of net carbon production during emersion resulting from atmospheric CO_2 (i.e., the area between photosynthetic curves at ambient atmospheric CO_2 and at double atmospheric CO_2) was greatest for the 06.30 h emersion phasing and smallest for the 12.30 h emersion phasing (Figure 3).

In conclusion, emersed photosynthetic performances under field conditions varied significantly by daily initial timing of exposure during sunny summer days. When low tide occurred during morning hours, *U. lactuca* was able to prolong a relatively high capacity of emersed photosynthesis and exhibited significant enhancement of photosynthesis from increased atmospheric CO_2. However, mid-day insolation considerably and rapidly depressed emersed photosynthetic capacity, in spite of the relatively high water content in thalli. In addition, elevation of CO_2 concentration in the atmosphere barely stimulated photosynthesis when low tide occurred at noon time.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (nos. 30670396, 30470343 and 90411018), the Key Project of the Chinese Ministry of Education (no. 207080) and the Guangdong Natural Science Foundation (nos. 2006B20601005 and 04010990).

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Received 22 December, 2006; accepted 2 August, 2007