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1 (515063)

2 (430072)

,

(*Ulva lactuca*)

,

,   (P_{max})

,   ;   15 % , P_{max} , P_{max}

,   80 %   0 P_{max}

(   ) P_{max} 10   ,   30   10   ,

6 h   ,   20   ,   2. 2 h   ,

;   30   ,

,

,   ,   ,   ,   ,   ,

Photosynthetic Responses to Light and Temperature in
Ulva lactuca **Under Aquatic and Aerial States**

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Abstract   The varied environmental conditions imposed by high frequency cycles of aquatic and aerial states is a unique problem confronted by the photosynthesis of intertidal macroalgae. The photosynthetic responses to light and temperature were examined in *Ulva lactuca* collected along the Shantou coast of China , to establish the photosynthetic characteristics in relation with tidal cycles for this common intertidal green macroalga. Under aerial state , the relationship of light- saturated net photosynthetic rates (P_{max}) and exposure duration could be modeled by the third-order polynomial equation , and temperature affected the coefficients of the equation. An increase of photosynthetic activity was evident at 15 % water loss , and the rates decreased thereafter with further desiccation till to zero at 80 % water loss. P_{max} was much more affected by temperature under aquatic state than under aerial state. Aerial P_{max} (fully hydrated state) was significantly greater at 10   , but was considerably lower at 30   , compared with aquatic P_{max}. The net carbon fixation under aerial state was greater than that under aquatic state within 6 h of photosynthetic duration at 10   , whereas was

slightly higher than that under aquatic state within 2. 2 h at 20　. At 30　, the net carbon production was always lower under aerial state than under aquatic state. It was concluded that the photosynthesis and carbon budget of *Ulva lactuca* under aquatic state differed from that under aerial state , and such difference was associated with the conditions of ambient temperature and water statues of thalli.

Key words　　*Ulva ,* Photosynthesis , Light , Temperature , Aquatic , Aerial , Carbon budget

A considerable number of eukaryotic algal species spend some or all of their time in aerial environments , though they evolved in aqueous (mainly marine) environments (Raven , 1999). Among them , intertidal macroalgae have been paid much attention on their ecophysiology (Davison and Pearson , 1996). When the tide is high , the intertidal macroalgae are submerged (under aquatic state) and photosynthesize in a relative stable environment of seawater with about 200 times HCO_3^- relative to CO_2 (i. e. , 2. 0 $mmol \cdot L^{-1}$ vs. 0. 01 $mmol \cdot L^{-1}$). Many of them use the HCO_3^- pool in seawater as the principal source of inorganic carbon to drive photosynthesis (Zou and Gao , 2001). Intertidal macroalgae will be exposed to air (under aerial state) when the tide goes out. The large buffering reservoir of HCO_3^- is no longer available and atmospheric CO_2 is the only exogenous carbon source for photosynthesis. The driving force for the flux of atmospheric CO_2 into the algal cell may be the CO_2 concentration gradient (Portielje and Lijklema , 1995 ; Mercado and Niell , 2000). Consequently , intertidal macroalgae might have different inorganic carbon utilization mechanism in water compared to in air. As macroalgae possess no anatomical features such as stomata or waxy cuticules as that in terrestrial higher plants , they cannot avoid desiccation (water loss) but only tolerate it under aerial state. It appears that if a species can survive from desiccation it may benefit from the greater availability of CO_2 in air to contribute to the total photosynthetic production (Maberly and Madsen , 1990 ; Pena *et al* , 1999). Additionally , aerial state may expose intertidal macroalgae to changing environmental conditions such as light and temperature. These environmental changes associated with tidal cycle will have immediate effects on the photosynthesis. Therefore , intertidal macroalgae may exhibit photosynthetic adaptation to aquatic and aerial states.

The marine green macroalgae of the genus *Ulva* are commonly found in intertidal and upper subtidal zone throughout the world. The mechanism of inorganic carbon utilization , during the periods of submersion have been studied extensively. They have developed two mechanisms to improve its utilization of HCO_3^- pool in seawater (Axelsson *et al* , 1999) , that is , the HCO_3^- dehydration mechanism through the catalysis of extracellular carbonic anhydrase activity and the HCO_3^- uptake mechanism via a transmembrane bicarbonate transporter. Adaptation to aerial state during low tide is clearly an important factor in the ecophysiology of these littoral *Ulva* plants. However , the photosynthetic performances with regarding to the changes of the ebb and flow of the tides have attracted much less attention for these ecologically important algae. We have reported the different photosynthetic responses to external inorganic carbon between aquatic and aerial states in *Ulva lactuca* L. along the coast of Shantou , China. *U. lactuca* exhibited ambient inorganic carbon-saturated photo-

synthesis under aquatic state, whereas ambient CO_2-limited photosynthesis under aerial state (Zou and Gao, 2002). In the present work, we further investigated the photosynthetic responses of *U. lactuca* to irradiation and temperature under aquatic and aerial states, in order to comprehend the question for this algae how to cope with the environmental conditions imposed by frequent changes of submergence and emersion. The time course of desiccation from this poikilohydric plant and the photosynthetic responses over a time course of exposure duration at different temperature conditions were evaluated. We also quantified the comparative net carbon production under aerial state and fully aquatic state at different temperatures.

1　Materials and Methods

1.1　Samples collection and maintenance

Thalli of *Ulva lactuca* L. were collected at low tide from the middle intertidal zone of Nanao Island of Shantou (23°20 N, 116°40 E). Collected thalli were gently rinsed and cleared to remove sediments and epiphytes, and the unwounded and healthy thalli were selected as experimental materials. Samples sealed in plastic bags with some seawater were transported to the laboratory in an insulated cooler with temperature of 1 - 4　to minimize metabolic activity within 4 h, and then were maintained in filtered natural seawater (salinity app. 32) in glass tanks under $100\mu mol\cdot m^{-2}\cdot s^{-1}$ (PAR, L D = 14 h 10 h) and room temperature (18 - 22). The seawater was aerated and half was renewed every day. Samples were used for the experiments within 5 days of laboratory maintenance, a period being demonstrated that the algae had not deteriorated (Zou and Gao, 2002).

1.2　Desiccation experiments

The samples of thalli were desiccated under standardized conditions in environmental chamber to determine the effects of desiccation on photosynthesis. The fluence rate of light exposed to thalli was $100\mu mol\cdot m^{-2}\cdot s^{-1}$ (PAR) supplied by fluorescent lamps, and the relative humidity was 75 % - 80 %. The temperature was set at 10 , 20 , or 30　respectively. Different degrees of desiccation were manipulated by the duration of the aerial exposure. The percent desiccation (D %) after a period of aerial exposure was calculated from the following equation: $D \% = [(W_o - W_t) / (W_o - DW)] \times 100$. Where W_0 (fresh weight) was determined after removing excess surface water drops by slightly blotting with paper tissue. W_t (desiccated weight) was determined after a certain period of desiccation. DW (dry weight) was determined after the samples had been heated at 80　for 24 h and cooled in a desiccator.

1.3　Measurements of photosynthetic carbon exchange in air

The net photosynthetic rates (P_n) of the thalli in air were determined as CO_2 uptake by infrared gas analysis using a leaf chamber analyzer (LCA4, Analytical Development Company LTD, UK) in open circuit systems. The irradiation was provided with a halogen lamp. The dark respiratory rate (R_d) was determined by covering the leaf chamber with an opaque cloth. Temperature in the leaf chamber was controlled by maintaining it in an incubator. P_n or R_d [μmol CO_2 g (DW)$^{-1}\cdot$ h^{-1}] was calculated as follows: P_n or $R_d = C \times F \times 60 \times 273 / ((273 + T) \times 22.4 \times DW)$.

Where C is the difference in CO_2 concentration (μmol·mol^{-1}) between the inlet and outlet air from the leaf chamber; F, the gas flow rate (L·min^{-1}); T, the temperature in the chamber ( ); DW, dry weight (g, 80   , 24 h). The photosynthetic response to light (P-I curve) was determined by altering the distance between the light resource and the leaf chamber.

1.4   Measurements of photosynthetic carbon exchange in water

The rates of net photosynthesis and dark respiration of the thalli under seawater were measured as the changes of dissolved inorganic carbon (DIC) in seawater, using Shimadzu Total Organic Carbon Analyzer (TOC-5000A, Japan). The filtered and sterilized natural seawater was used as the reaction medium (DIC ca. 2.2 mmol·L^{-1}) and its pH value was buffered at 8.2 by using 20 mmol·L^{-1} HEPES (Sigma). Several pieces of the samples were placed in a sealed 25 mL Perspex chamber containing 20 mL magnetically stirred reaction medium, which was equipped with a thermostatted water bath. Varied levels of irradiation (0, 50, 100, 300, 600, 900μmol·m^{-2}·s^{-1}, PAR) were obtained by interposing neutral filters or altering the distance between the light source and chamber to measure the P-I curve. The rate of net aquatic photosynthesis (P_n) was calculated using the equation: $P_n = C \times V/ (T \times DW)$, where C was the difference in DIC concentration (mmol·L^{-1}) in medium before and after the incubation, which was determined by Total Organic Carbon Analyzer; V, the medium volume (L); T, the incubation duration (h); and DW, dry weight of the samples (g).

1.5   Calculation of the photosynthetic parameters and net carbon production

The apparent photosynthetic efficiency ( ) was estimated as the light-limited slope of the P-I curve. The light compensation point (I_c) and light saturation point (I_k) were calculated as $R_d/$ and $(P_{max} + R_d)/$ respectively (Henley, 1993). Here P_{max} was the light-saturated maximum P_n, which was calculated as the mean of three values in the asymptote region of the P-I curve; R_d was the dark respiration rate.

Provided that net rate of photosynthesis (y) is the function of incubation time (x), $y = f(x)$, the net total carbon production (Y) within incubation time (T) was calculated from the equation: $Y = \int_0^T f(x)\, dx$. In this study, the net photosynthetic rates of *Ulva lactuca* during aerial exposure changed with duration, their function relationship could be modeled elegantly by third-order polynomial equation.

1.6   Statistical analyses

The data were expressed as the means ± SD (standard deviation). ANOVA and t-test were employed to examine the significance of means at $p < 0.05$.

2   Results

2.1   Desiccation and its effect on photosynthesis

When exposed to air, the thalli of *Ulva lactuca* began to dry out. *U. lactuca* exhibited significant different rates of water loss from the thalli when subjected to different temperature conditions

（Fig. 1）. The time for the thalli losing 50 % water was 2. 2 , 1. 6 and 0. 9 h when the temperature was 10 , 20 and 30 , respectively. After 2. 5 h of desiccation , the water content of *U. lactuca* was 46 % at 10 , whereas that was 31 % at 20 , and 15 % at 30 .

The effect of desiccation on light-saturated net photosynthesis was shown similar changes patterns with time among the temperature levels（Fig. 2）. The photosynthesis increased with slight water loss to reach a maximum , then declined with further desiccation. When water loss was about

Fig. 1 Time course of desiccation expressed as a percentage of water loss in *Ulva lactuca* at different temperatures （10 , 20 , 30 ）

15 % or 80 % , the net photosynthetic rate reached maximum or zero , respectively. Higher temperature resulted in a faster photosynthetic fluctuation. Net photosynthesis reached its maximum in 1. 0 , 0. 5 and 0. 3 h , and approached to zero in 4. 4 , 3. 4 and 1. 8 h after the air exposure at 10 , 20 and 30 , respectively.

2. 2 Comparison of photosynthesis in water and in air

The photosynthetic responses to light for the thalli of *U. lactuca* in water and in air were measured at three temperatures （10 , 20 , 30 ）, and that in air were also determined at two statuses for the exposed thalli , i. e. , the fully hydrated and desiccated （50 % water loss）. The light-saturated net photosynthetic rates （P_{max}）of *U. lactuca* in water increased significantly with the increase of temperature within the tested range of 10 - 30 （Fig. 3）, indicating that temperature optimum for aquatic photosynthesis was 30 or higher. However , P_{max} in air was less affected by temperature. Aerial P_{max} （fully hydrated state）was greater by 89. 0 % than that of P_{max} in water at 10 , whereas the former was considerably lower than the latter at 30 . Under aerial condition , P_{max} declined substantially with the thalli desiccated 50 % of their water. Thus , P_{max} of desiccated thalli was considerably lower than
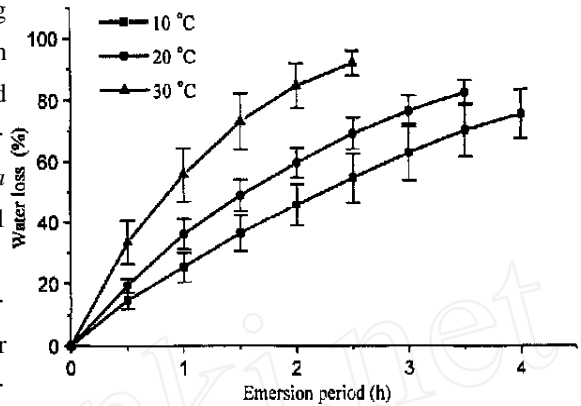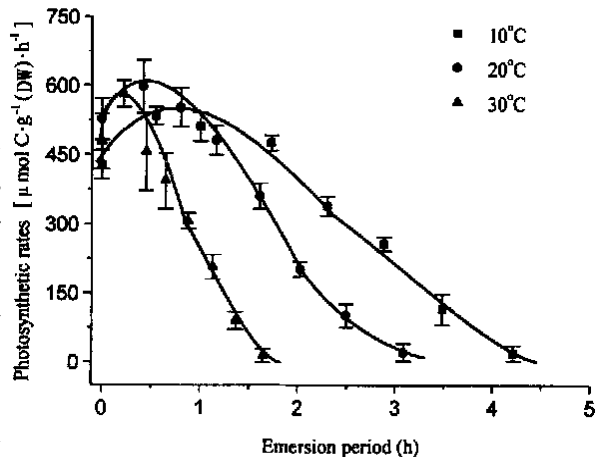

Fig. 2 Time course of photosynthesis of *Ulva lactuca* at different temperatures （10 , 20 , 30 ）
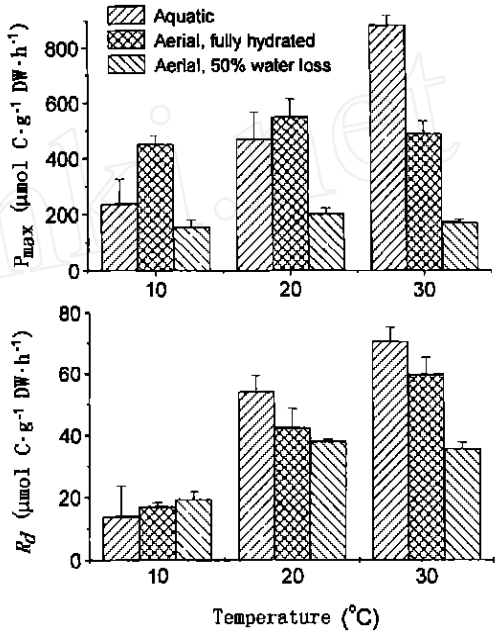The courses could be modeled by third-order polynomial equations : $y = 432. 7 + 238. 5 x - 163. 0 x^2 + 19. 7 x^3$ （10 ）, $y = 531. 5 + 264. 1 x - 338. 1 x^2 + 64. 5 x^3$ （20 ）, $y = 502. 3 + 250. 2 x - 768. 0 x^2 + 264. 5 x^3$ （30 ）

that of submersed thalli at 20   and 30   . Dark respiratory rates（R_d）in both water and air（fully hydrated state）increased markedly as temperature increased（Fig. 3）. R_d exhibited insignificant difference between in and out of water at each temperature. Under aerial state , R_d maintained unchanged for dehydrated thalli（50 % of water loss）compared to fully hydrated thalli at 10   and 20   , while R_d of dehydrated thalli was lower than that of fully hydrated thalli at 30   .

No significant differences were observed for the photosynthetic efficiency（ ）between in water and air（fully hydrated state）at each temperature （Fig. 4）. However ,   of desiccated thalli were lower than that of submersed thalli at all temperatures. Temperature had positive effects on light compensation points（I_c）under both aquatic and aerial states（Fig. 4）. Additionally , the values of aquatic I_c were similar to aerial values（fully hydrated state）. Since 50 % water loss resulted in a significant increase in I_c under aerial state , the vaules of I_c of desiccated thalli were significant higher than that of submersed thalli at each tested temperature. The values of aquatic I_k were lower than the aerial values（fully hydrated state）at 10   , but the former were greater than the latter at 30   . Moreover , the aquatic I_k at 30   was significantly higher than that at 10 or 20   , whereas the temperature or desiccation（50 % water loss） showed little effects on the aerial I_k（Fig. 4）.

Fig. 3   The light-saturated net photosynthetic rate （P_{max}）and the dark respiratory rate（R_d）of *Ulva lactuca* under aquatic and aerial（fully hydrated or 50 % desiccation）states at different temperatures （10 , 20 , 30   ）

2.3   Comparison of carbon budget between in water and in air

Net carbon gain is the result of net photosynthesis and time. At saturating light , the net carbon production for *U. lactuca* under aquatic state could be expressed by equations : $Y = 238.4 \times T$ （10   ）, $Y = 469.9 \times T$（20   ）and $Y = 884.6 \times T$（30   ）. Taking account into the effects of desiccation on net photosynthesis of *U. lactuca ,* the net carbon production under aerial state could be expressed by following equations : $Y = 432.7 \times T + 119.2 \times T^2 - 54.3 \times T^3 + 4.9 \times T^4$ （10   ）, $Y = 531.5 \times T + 132.1 \times T^2 - 112.7 \times T^3 + 16.1 \times T^4$（20   ）, $Y = 502.3 \times T + 125.1 \times T^2 - 256 \times T^3 + 66.1 \times T^4$（30   ）. Where *Y* was the net carbon production ［μmol C· g^{-1}（DW）］, and *T* was the time（h）. It could be seen from Fig. 5 that the net carbon gain in thalli under aerial state was greater than that under aquatic state within 6 h of photosynthesizing time at 10   , whereas was slightly greater than that under aquatic state within 2.2 h at 20   . When the temperature was 30   , the net carbon production in thalli was always lower under aerial state than

under aquatic state. Under aerial state , the net carbon production reached the highest point after 4
h（10　）, 3 h（20　）, or 1. 5 h（30　）of desiccation , owing to the net photosynthetic rates de-
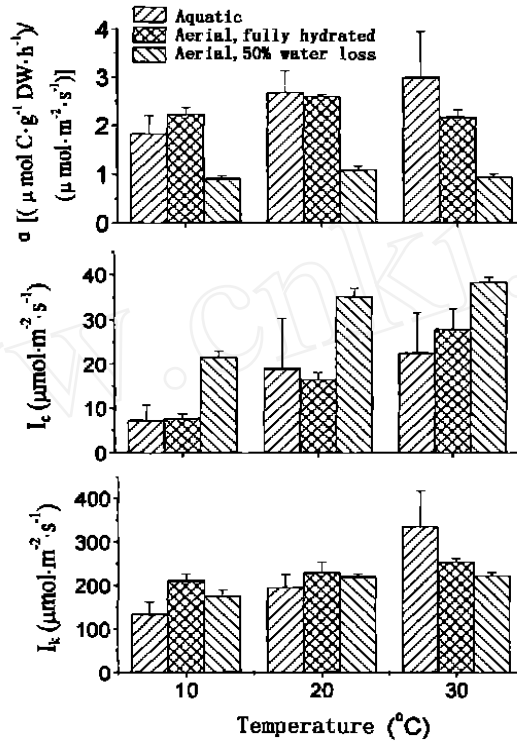clining to zero after this period.

Fig. 4　The photosynthetic efficiency（ ）, light compensation point（I_c）and light saturation point（I_k）of *Ulva lactu-*
ca under aquatic and aerial（fully hydrated or 50 % desiccation）states at different temperatures（10 , 20 , 30　）
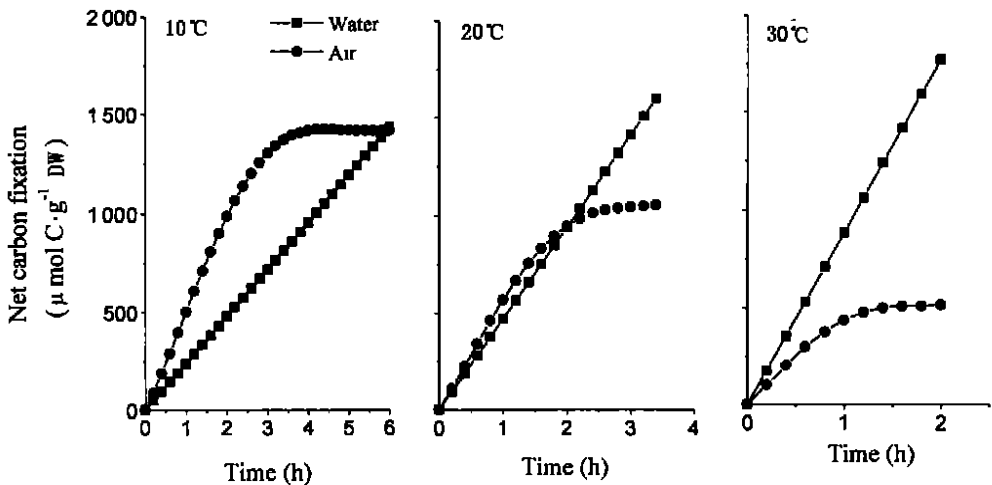

Fig. 5　Comparison of net carbon fixation of *Ulva lactuca* between aquatic and aerial states at different temperatures
（10 , 20 , 30　）

3　Discussion

　　Intertidal algae（nonsaccate algae）begin to dry out following a short period of exposure to air , and the ratio of surface area to volume of the algae is the key factor controlling the rate of water loss （Dromgoole , 1980）. The rate of water loss in *U. lactuca* during aerial exposure could be rapid due to its membranous form in thallus. This study showed that water loss of *U. lactuca* thalli proceeded substantially faster as temperature increased from 10　to 30　. During the initial stages of exposure , *U. lactuca* exhibited an increase of net photosynthesis and reach a peak after the thalli lost about 15 % of its water content. That slight desiccation stimulating the photosynthesis had been reported in some other intertidal macroalgae（Gao and Aruga , 1987 ; Pena *et al* , 1999 ; Ji and Tanaka , 2002）, which was presumably attributed to the reduction in the aqueous diffusion barrier for CO_2（Davison and Pearson , 1996）. The net photosynthesis of *U. lactuca* in air reached the maximum earlier and thereafter declined rapidly as temperature became higher , because the rate of water loss in thalli proceeded substantially faster as increasing temperature. It was interesting to note that , compared with photosynthesis , aquatic/ aerial state or desiccation had much less effects on dark respiration rates in *U. lactuca*. This implied that the respiratory processes such as the activities of respiratory enzymes were less water-sensitive compared to such photosynthetic processes as electron transfer and enzymatic reactions of the Calvin cycle. Kawamitsu *et al*（2000）suggested that the capacity of intertidal macroalgae tolerating desiccation during aerial exposure was correlated with molecular environment around the photosynthetic enzymes , which was mainly featured that prevented membrane breakage but promoted the retention of small amounts of water that were critical for viability. Desiccation might negatively affected the enzymes of photophosphorylation and electron transport and plastoquinone diffusion. Consequently , the photosynthetic efficiencies （reflecting the light-harvesting efficiencies and photosynthetic energy conversion efficiencies）decreased with desiccation. In contrast , dark respiration remained stable relative to desiccation. These factors had the consequence of increasing of light compensation points with desiccation.

　　This study showed that the rate of light-saturated net photosynthesis of *U. lactuca* in air（fully hydrated state）was significantly greater than that in water at lower temperature（10　）, but considerably lower at higher temperature（30　）. Madsen and Maberly（1990）reported that the light-saturated rate of photosynthesis of *Fucus spiralis* under aerial state was greater than that in water at higher temperature（15　and 20　）, but lower at lower temperature（10　）. Photosynthetic efficiencies and light compensation points of *U. lactuca* under aquatic and aerial （fully hydrated） states were similar at each experiment temperature , while these two parameters showed differences between desiccated（50 % water loss）state and aquatic state. Additionally , light saturation points exhibited temperature-dependent differences between aquatic and aerial states. Those findings indicated that *U. lactuca* had different light utilization capacities in aquatic and aerial states.

　　The results in this study showed that temperature had a much higher stimulating effect on light-saturated rates of net photosynthesis of *U. lactuca* under aquatic state than aerial state. *U. lactuca*

had different mechanism of external inorganic carbon acquisition between aquatic and aerial states. It could uptake directly HCO_3^- pool in seawater or indirect use HCO_3^- via extracellular carbonic anhydrase catalyzed dehydration for photosynthesis when submersed (Axelsson *et al*, 1999). Whereas *U. lactuca* acquired the external inorganic carbon only by diffusion from the atmospheric CO_2 under aerial state (Zou and Gao, 2002). Temperature-sensitive steps that could potentially limit the overall rate of photosynthesis include diffusion, carbonic anhydrase activity, and the active transport of CO_2 or HCO_3^- across the plasmalemma and / or chloroplast membranes (Davison, 1991). The degrees of effects of temperature on these physiological and physical processes under aquatic state would differ from that under aerial state. Thus, the different mechanisms of inorganic carbon utilization between in water and in air result in the different photosynthetic response to temperature in and out of water. On the other hand, temperature had a similar stimulating effect on rates of dark respiration of *U. lactuca* in water and in air, indicating that temperature had a similar way to affect the respiratory enzymes under submersed and aerial state.

Since intertidal macroalgae can perform photosynthesis effectively in air, aerial photosynthesis is an important component of the total net daily carbon production (Maberly and Madsen, 1990; Pena *et al*, 1999). However, it appeared that the growth rates of algal thalli with periodic aerial exposure was much slower than that of thalli without aerial exposure (Tajiri and Aruga, 1984; Gao and Aruga, 1987). Thus, whether the emersed state imposes a benefit or constraint on the overall carbon budget compared to fully submersed state is to be understood. Based on a mathematical model that incorporated the effect of desiccation on net photosynthesis, this study indicated that whether aerial exposure to air would actually increase the net carbon production in *U. lactuca* compared with a fully submersed situation depended on the duration of aerial exposure and the ambient temperature conditions. At lower temperature (10), aerial photosynthesis could brought about a greater benefit on the overall carbon production. Whereas at higher temperature (30), aerial state imposed *U. lactuca* a constraint on the overall carbon budget compared with fully aquatic state. However, this study did not take into account the photosynthetic recovery during resubmergence following desiccation. Further investigations are needed, therefore, to comprehend fully the effects of aerial state on overall carbon budget.

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