Comparative Mechanisms of Photosynthetic Carbon Acquisition in *Hizikia fusiforme* Under Submersed and Emersed Conditions

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**Abstract:** The economic seaweed *Hizikia fusiforme* (Harv.) Okamura (Sargassaceae, Phaeophyta) usually experiences periodical exposures to air at low tide. Photosynthetic carbon acquisition mechanisms were comparatively studied under submersed and emersed conditions in order to establish a general understanding of its photosynthetic characteristics associated with tidal cycles. When submersed in seawater, *H. fusiforme* was capable of acquiring HCO₃⁻ as a source of inorganic carbon (Ci) to drive photosynthesis, while emersed and exposed to air, it used atmospheric CO₂ for photosynthesis. The pH changes surrounding the *H. fusiforme* fronds had less influence on the photosynthetic rates under emersed condition than under submersed condition. When the pH was as high as 10.0, emersed *H. fusiforme* could photosynthesize efficiently, but the submersed alga exhibited very poor photosynthesis. Extracellular carbonic anhydrase (CA) played an important role in the photosynthetic acquisitions of exogenous Ci in water as well as in air. Both the concentrations of dissolved inorganic carbon in general seawater and CO₂ in air were demonstrated to limit the photosynthesis of *H. fusiforme*, which was sensitive to O₂. It appeared that the exogenous carbon acquisition system, being dependent of external CA activity, operates in a way not enough to raise intracellular CO₂ level to prevent photorespiration. The inability of *H. fusiforme* to achieve its maximum photosynthetic rate at the current ambient Ci levels under both submersed and emersed conditions suggested that the yield of aquaculture for this economic species would respond profitably to future increases in CO₂ concentration in the sea and air.

**Key words:** *Hizikia fusiforme*; photosynthesis; inorganic carbon; carbonic anhydrase; submersion; emersion; tide cycle

The intertidal seaweeds spend alternatively part of their time in atmosphere and part in seawater throughout the day with the fluctuation of tidal level. They therefore undergo two very distinct environmental conditions for photosynthesis and growth. It is of general interest to study the physiology of intertidal seaweeds when considering how to deal with the high frequency cycles of the aquatic and aerial conditions (Raven, 1999; Zou and Gao, 2002a). When the tide is high, the intertidal seaweeds are submersed in seawater and exposed to two potential sources of exogenous carbon for photosynthesis: dissolved CO₂ and bicarbonate (HCO₃⁻). In air-equilibrium natural seawater, at normal pH 8.2 and 20 °C, the bulk of total dissolved inorganic carbon (DIC) is HCO₃⁻ (ca. 2.0 mmol/L), and CO₂ (only 12 μmol/L) is less than 1% of the total DIC. It is reported that a large number of seaweeds have developed mechanisms that permit the acquisition of HCO₃⁻ pool in seawater during photosynthesis (Raven, 1997; Larsson and Axelsson, 1999; Zou and Gao, 2001). Paradoxically, intertidal seaweeds, which are exposed to atmospheric CO₂ periodically during emersion at low tide, seem to acquire HCO₃⁻ in seawater more efficiently than those growing in the subtidal zone (Maberly, 1990; Mercado et al., 1998). An immediate change in the “CO₂” supply for intertidal seaweeds will take place when they get out of the water at low tide, although a seawater film usually retains the algal thalli surface due to their viscosity and hydrophilia. The exact effect of this change in CO₂-supply on the photosynthetic rates of intertidal seaweeds is waiting for being fully established. It appears that CO₂ will become limiting for photosynthesis more often for the seaweeds under emersed condition than under submersed condition (Gao et al., 1999; Raven, 1999; Zou and Gao, 2002b; Zou and Gao, 2004).

*Hizikia fusiforme*, belonging to Sargassaceae (Phaeophyta), is distributed uniquely in the west-northern parts of the coast of the Pacific. It has traditionally been used as a food delicacy in China, Japan and Korea (Zhang et al., 2002). Suzuki et al. (1996) showed that *Hizikia*...
contained higher soluble dietary fiber than other seaweeds. The extract from this seaweed has an immunomodulating activity on human, which might be useful for clinical application to treat diseases (Shan et al., 1999; Katayama et al., 2002). Additionally, *H. fusiforme* is an important raw material for alginates production. It now becomes one of the potential important species for seaweed cultivation, owing to its high commercial value and market demand (Zhang et al., 2002). A large number of studies have been carried out on its life history (Park et al., 1995; Ruan and Xu, 2001) and cultivation technique (Hwang et al., 1997; Li, 2001). However, the photosynthetic characteristics of *H. fusiforme* have been less studied (Zou et al., 2003). *H. fusiforme* is distributed at lower parts of the intertidal zone, frequently spending a part of tidal cycles in the emersed state. The aim of this study is to compare its photosynthetic strategies for exogenous inorganic carbon acquisition under submersed and emersed conditions, in order to establish a general knowledge about its physiological behavior associated with tidal cycles.

1 Materials and Methods

1.1 Algal materials

*Hizikia fusiforme* (Harv.) Okamura was collected from lower intertidal rocks along the coast of Nanao, Shantou, China when the tide went out. Samples sealed in a plastic bag with some seawater were transported to the laboratory in an insulated cooler (ca. 5 °C) within 4 h. The material was maintained in a glass aquarium tank containing filtered natural seawater (salinity ca. 33 ‰) under 100 μmol·m⁻²·s⁻¹ (PAR, 400-700 nm) illuminated by fluorescent tubes for 14 h out of each 24 h and at room temperature (18-22 °C). The seawater was aerated vigorously and was renewed daily. Experiments were conducted within a period of 5-d laboratory maintenance for each collection, during which the algal material showed stable photosynthetic activity. After this period, the remains were abandoned and fresh material was collected again.

1.2 Effects of pH and inhibitor on photosynthetic rates

Buffered natural seawater of varied pH values with or without the addition of acetazolamide (AZ, 100 μmol/L of final concentration) were prepared. Different pH values were obtained by adding a known amount of biological buffers (Sigma) to give final concentrations of 20 mmol/L. TRIS was used for buffering pH 8.2 (a pH value representative of that in natural seawater) and 9.0, and CAPS for pH 10.0. AZ stock solutions prepared with 40 mmol/L NaOH, were added into the buffers to the final concentrations of 100 μmol/L. AZ is known as a relatively membrane-impermeable inhibitor of extracellular carbonic anhydrase (CA) activity (Axelsson et al., 1999; Moroney et al., 2001).

Photosynthetic rates of submersed plants were measured as oxygen evolution using a Biological Oxygen Monitor (YSI Model 5300, USA) at 20 °C and at saturating photon flux density of 500 μmol·m⁻²·s⁻¹. The oxygen electrode was held in a temperature-controlled chamber. The fronds of *H. fusiforme* were cut into small segments (0.5-0.7 cm length) with a shape razor blade and incubated in seawater under 500 μmol·m⁻²·s⁻¹ and 20 °C for at least 2 h before the measurements. This pre-treatment aimed to minimize the possible effect of cutting damage (wound respiration). Segments of *H. fusiforme* of about 0.3 g FW were incubated in the reaction chamber with 10 mL of buffered seawater that was magnetically stirred, and the linear O₂ evolution versus time was recorded.

The emersed photosynthetic rates were determined as CO₂ uptake with an infrared gas analyzer (LCA-4, Analytical Development Company Ltd., UK) in an open circuit under the same light/temperature conditions as for the submersed samples. Before introducing into the photosynthetic leaf chamber, the samples were respectively immersed in above seawater buffers for 30 min, aiming to adjust the pH value within the surface water film surrounding the fronds when the algal samples were emersed. The buffered seawaters used were in equilibrium with atmosphere in terms of CO₂. Thus, in our photosynthesis-determining system, the difference of CO₂ concentration between the inlet and outlet of the assimilation chamber was due to CO₂ uptake by the algal photosynthesis. The rate of CO₂ uptake (Pₐ) (μmol CO₂·g⁻¹FW·h⁻¹) was calculated as follows: Pₐ = ΔC × FX × 60 × 273 / ((273+T) × 22.4 × FW), where ΔC is the difference in CO₂ concentration (μL/L) between the inlet and outlet air; F, the gas flow rate (L/min); T, temperature (°C); FW, fresh weight (g).

1.3 Inorganic carbon-dependent photosynthetic rates

DIC-free seawater was prepared by removing inorganic carbon (Ci) from the natural seawater by lowering pH to less than 4.0 with 0.5 mol/L HCl and sparging with pure N₂ gas for 2 h at least. A known amount of TRIS (Sigma) was added to give a final concentration of 20 mmol/L, and the pH was then adjusted to 8.2 with freshly prepared 0.5 mol/ L NaOH and 0.5 mol/L HCl. All manipulations were carried out under N₂. Segments of *H. fusiforme* of about 0.3 g FW were incubated in the reaction chamber with 10 mL of buffered DIC-free seawater. The algae were left to photosynthesize to deplete the possible Ci present in the medium and in the algal cells till no further O₂ evolved, which took about 20 min. Aliquots of NaHCO₃ stock solution were then measured for photosynthetic activity.
injected into the chamber in order to create the appropriate final concentrations of Ci in the reaction medium. O₂ evolution was recorded after addition of NaHCO₃. Additionally, the Ci-dependent O₂ evolution (i.e. P-C response curve) was carried out with the presence of AZ (100 µmol/L).

The P-C response curve was also determined in air (under emersed condition). Samples were pretreated in buffered seawater (Tris 20 mmol/L, pH 8.2) with or without the addition of AZ (100 µmol/L of final concentration) for 30 min. Photosynthetic CO₂ uptake was then determined at different CO₂ concentrations (over the range of 2.6–62.4 µmol/L). CO₂ in the ambient air was removed to varied degrees by pumping it through a soda lime column to obtain lower concentrations of CO₂. Concentrations of CO₂ higher than ambient air were obtained by injecting pure CO₂ before pumping ambient air into an air bag (1 m³). The air bags were used to maintain constant CO₂ supply.

1.4 Oxygen sensitivity

Photosynthetic O₂ evolution rate of submersed samples was respectively measured at two levels of O₂, i.e. less than 30% of air-equilibrated level of O₂ (low O₂) which was achieved by bubbling N₂ gas into the reaction chamber, and 100% of air-equilibration concentration of O₂ (ambient O₂). Similarly, the emersed photosynthetic CO₂ uptake was respectively examined under normal atmosphere (ambient O₂ 21% of O₂ concentration) and under atmosphere with low O₂ concentration (< 6%) which was obtained by pumping air through a Na₂S₂O₃ solution. The O₂ concentration in air and in water were examined by the Infrared Gas Analyzer (CGT-7000, Shimadzu Corporation, Japan) and the Biological Oxygen Monitor (YSI Model 5300, USA) respectively.

1.5 Calculations of the photosynthetic parameters and theoretical photosynthetic rates

Ci-saturated maximal rate of photosynthesis (Vₘₚₖ) and half-saturation constant (Kᵣₚₖ, the inorganic carbon concentration required to give half of Vₘₚₖ) were estimated by fitting the P-C curve to the Michaelis-Menten equation. The maximum rates of CO₂ supply derived from spontaneous (uncatalysed) dehydration of HCO₃⁻ in seawater were calculated according to Matsuda et al. (2001) as the theoretical rates of CO₂ supply for photosynthesis. The assumption was adopted that the algal samples consumed CO₂ at a rate causing the CO₂ concentration in seawater surrounding the algae to approach zero. This gave a theoretical maximal rate of uncatalysed conversion of CO₂ from HCO₃⁻ in seawater. The flux of CO₂ across the surface seawater film under emersed condition was not considered. The volume of the surface water film surrounding the algal samples was estimated as a mass difference before and after blotting off the superficial water. The value obtained from this procedure was (0.6 ± 0.2) mL per gram fresh weight of alga sample. The theoretical rate of CO₂ supply (d(CO₂)/dt) was calculated by the following equations: d(CO₂)/dt = Kᵣₚₖ[A] + Kᵣₚₖ[DIC][H⁺]/K₃[HCO₃⁻], and A = 1 + [H⁺]/K₃ + [H⁺]/K₄, where [DIC] is the concentration of dissolved inorganic carbon in seawater. K₁ and K₃ are the rate constants of reactions HCO₃⁻ → CO₂ + OH⁻ and H₂CO₃ → CO₂ + H₂O, respectively. K₃[HCO₃⁻] and K₄ represent respectively the dissociation constants of the reactions H⁺ + HCO₃⁻ ↔ H₂CO₃ and H⁺ + CO₃²⁻ ↔ HCO₃⁻. The values of K₁, K₃, K₃[HCO₃⁻] and K₄ are according to Johnson (1982) and Stumm and Morgan (1996). Photosynthetic rates based on O₂ evolution or CO₂ uptake were compared by assuming the photosynthetic quotient of 1.0.

1.6 Statistics

The data were expressed as the mean values ± SE (n ≥ 3). Statistical significance of the data was tested with ANOVA or t-test at P<0.05.

2 Results

2.1 Effects of pH and AZ on the photosynthetic rates under submersed and emersed conditions

Figure 1 shows the effects of pH and AZ on photosynthetic rates of H. fusiforme fronds under submersed and emersed conditions.
emersed conditions. Photosynthetic O_2 evolution of submersed plants was reduced drastically as the pH in the seawater increased from 8.2 to 10.0. O_2 evolution at pH 9.0 and 10.0 was reduced by 54.4% and 90.5%, respectively, compared to that at pH 8.2. By contrast, there was only a slight decrease in photosynthetic CO_2 uptake of emersed plants with the increasing pH in the surface water film surrounding _H. fusiforme_ fronds. There was no significant difference (P > 0.1) in CO_2 uptake between pH at 8.2 and 9.0. CO_2 uptake at 10.0 was reduced by 44.5% compared to that at pH 8.2. It was shown that the photosynthetic rate was significantly (P < 0.01) greater at pH 8.2, but was conspicuously (P < 0.01) lower at pH 10.0, in submersed plants than in emersed plants. AZ remarkably (P < 0.01) inhibited the photosynthetic rate at all the pH values tested for both submersed plants and emersed plants. The inhibitory effect of AZ ranged from 76% to 82% in water, but from 87% to 68% in air, when the pH value raised from 8.2 to 10.0.

In order to test whether or not the CO_2 supplies derived from uncatalysed spontaneous dehydration of HCO_3^- in seawater medium surrounding the algal fronds were enough to support the measured photosynthetic rates of O_2 evolution by emersed plants or CO_2 uptake by emersed plants, respectively, the observed rates of photosynthesis were compared with the theoretical rates (Table 1). The observed rates at all the pHs tested for the submersed plants exceeded (P < 0.01) those supported solely by the spontaneous CO_2 formation. This indicated that submersed _H. fusiforme_ frond was capable of using external HCO_3^- as a source of Ci to drive photosynthetic O_2 evolution. However, the theoretical rate was fast enough to account for the observed rate of photosynthetic O_2 evolution at pH 8.2 with the presence of AZ. Under emersed condition, the ratios of observed to theoretical rates were much higher than 1.0, and those increased by two orders of magnitude with increasing pH from 8.2 to 10.0. The ratios in case of AZ were also much greater than 1.0 and dramatically increased with increasing pH, though the values of these ratios were reduced compared to the controls. As the CO_2 fluxes from the atmosphere to the fronds were not taken into account in calculating the theoretical values, it could be inferred that the atmospheric CO_2 was the predominant source of Ci driving the photosynthesis of _H. fusiforme_ fronds under emersed condition.

### 2.2 The dependence to exogenous inorganic carbon for photosynthetic rates under submersed and emersed conditions

Effects of external inorganic carbon concentrations on net photosynthetic rate with or without AZ are shown in Fig.2 for the submersed _H. fusiforme_ fronds and in Fig.3 for the emersed fronds. The photosynthesis was far from saturated with ambient Ci levels under both submersed and emersed conditions. This was in accordance with the high _Kc_ values in water as well as in air (Table 2). Saturating Ci level seemed to be reached at about 8.8 mmol/L for the submersed plants. It appeared that the emersed photosynthetic rate was not to be saturated over the range of CO_2 in air (2.6-62.4 µmol/L) used in the experiments. Though the

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**Table 1** Ratios of measured to theoretically calculated photosynthetic rates at different pH values for _Hizikia fusiforme_ under submersed and emersed conditions

<table>
<thead>
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<th>pH 8.2</th>
<th>pH 9.0</th>
<th>pH 10.0</th>
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<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.5 ± 0.2</td>
<td>10.0 ± 1.5</td>
<td>55.3 ± 6.2</td>
</tr>
<tr>
<td>+AZ</td>
<td>0.4 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>9.8 ± 12.8</td>
</tr>
<tr>
<td>Air</td>
<td>36.3 ± 2.4</td>
<td>424.0 ± 60.6</td>
<td>7 699.0 ± 2 005.6</td>
</tr>
<tr>
<td>+AZ</td>
<td>4.9 ± 0.2</td>
<td>75.3 ± 8.0</td>
<td>2 450.0 ± 506.2</td>
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AZ, acetazolamide.

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**Table 2** The inorganic carbon-saturated maximum photosynthetic rates (V<sub>max</sub>) and the apparent half-saturation constant (K<sub>c</sub>(µmol/L)) in _Hizikia fusiforme_ under submersed and emersed conditions

<table>
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<th>V&lt;sub&gt;max&lt;/sub&gt; (µmol O₂ or CO₂·g⁻¹ FW·h⁻¹)</th>
<th>K&lt;sub&gt;c&lt;/sub&gt; (DIC) (µmol/L)</th>
<th>K&lt;sub&gt;c&lt;/sub&gt; (CO₂) (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>44.7 ± 13.1 2.72 ± 0.91  14.8 ± 5.0</td>
<td></td>
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<tr>
<td>+AZ</td>
<td>11.2 ± 1.3  3.88 ± 1.76  21.2 ± 9.6</td>
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</tr>
<tr>
<td>Air</td>
<td></td>
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<tr>
<td>Control</td>
<td>49.7 ± 15.5 -  51.0 ± 14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+AZ</td>
<td>13.6 ± 5.3 -  41.2 ± 21.4</td>
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AZ, acetazolamide; DIC, dissolved inorganic carbon.
net photosynthetic rates at ambient Ci were significantly higher in submersed plants than in emersed plants, the Ci-saturated maximum photosynthetic rates (V_max) were similar (P > 0.1) in both submersed and emersed plants (Table 2). AZ strongly depressed photosynthetic activities of H. fusiforme fronds at all Ci levels of the measurements under submersed condition as well as emersed condition (Figs.2, 3). However, the K_0.5 values showed no significantly (P > 0.05) difference between with and without the presence of AZ (Table 2).

2.3 Sensitivity to O_2 concentration for photosynthetic rates under submersed and emersed conditions

Effect of O_2 concentration on photosynthetic rates under submersed and emersed conditions are shown in Fig. 4. Photosynthetic rates is significantly inhibited (P < 0.05) by O_2 at ambient O_2 concentration in comparison with low O_2 concentration for H. fusiforme fronds under both submersed and emersed conditions, indicating a C_3-like photosynthetic gas exchange physiology.

3 Discussion

H. fusiforme, which normally grows in low intertidal zone, will be exposed to air when the tide goes out, especially during a spring tidal cycle. However, field desiccation seldom occurs due to the continuous waves, sea spray and extensive shingle-overlapping. The morphology of coarsely-branched fronds of H. fusiforme further reduces the probability of desiccation. Thus, H. fusiforme could often maintain lengthy hydrated statue while exposed. The present work showed that H. fusiforme exhibited different photosynthetic activity under emersed condition compared to submersed condition, which might result from different availability of exogenous inorganic carbon for photosynthesis, and/or the mechanism of carbon acquisition between in and out of water. For example, the concentration of DIC in seawater is about 140 times greater in water than in air (2.2 mmol/L vs 15.6 µmol/L), but the diffusion rate of CO_2 in air is 10 000 times higher than in water. Oates (1985) and Romaine et al. (1997) guessed that the difficulty in absorbing CO_2 in its molecular form or blockage of its movement into the cells might result in the lower photosynthetic rate in air. On the other hand, the carbon-saturated maximum photosynthetic rate of H. fusiforme was similar between in water and in air (Table 2), implying the comparable carboxylatory capacity of Rubisco in both the environmental conditions.

Rates of theoretical CO_2 supply lower than the observed rates of photosynthetic O_2 evolution could be considered as evidence for the capacity of submersed H. fusiforme to acquire external HCO_3^- in seawater to drive photosynthesis, as reported in some other algae for their abilities of using HCO_3^- (Johnston et al., 1992; Gao and Zou, 2001). The inhibition of photosynthetic O_2 evolution by AZ addition indicated that extracellular CA activity acted as an essential part of HCO_3^- acquisition by submersed H. fusiforme. It has been previously shown that extracellular CA evidently occurred in H. fusiforme (Zou et al., 2003). In some intertidal seaweeds, there seems a poor correlation between external CA activity and the capacity of HCO_3^- acquisition, and the main cause is that those seaweeds possessed the mechanism of direct HCO_3^- uptake (Mercado et al., 1998). However, the experiment of culturing H. fusiforme under different CO_2 concentrations was in support that the external CA activity was closely linked to the ability of acquiring HCO_3^- in seawater (Zou et al., 2003). Therefore, when Hizikia was submersed in seawater, external CA catalyzed
the dehydration of HCO$_3^-$ externally, and the CO$_2$ formed was the species of Ci that crossed the plasma membrane. However, although the rate of conversion between HCO$_3^-$ and CO$_2$ catalyzed by extracellular CA activity is almost instantaneous, the resulting equilibrium CO$_2$ concentration is rather low at high pH (Raven, 1997; Axelson et al., 1999; Zou and Gao, 2001). As a consequence of that, the extracellular CA-mediated conversion of HCO$_3^-$ to CO$_2$ is much less efficient at high pH values. This gave the physiological explanation for the results that submerged photosynthetic rate of _H. fusiforme_ was conspicuously reduced with increasing pH (Fig. 1). On the other hand, the relationship between the photosynthetic rate and pH under emersed condition differed from that under submerged condition, in which the emersed photosynthetic rate did not significantly decrease as pH rose. Such discrepancy of photosynthetic performances in _H. fusiforme_ between in water and in air could be ascribed to the different source of Ci for photosynthesis, as suggested by Mercado and Niell (2000). Though submerged _H. fusiforme_ mainly used HCO$_3^-$ pool in seawater for photosynthesis, emersed _H. fusiforme_ acquired CO$_2$ in air as a principal source for photosynthesis. The CO$_2$ uptake rate substantially exceeded the theoretical CO$_2$ flux derived from the spontaneous conversion of HCO$_3^-$ in the surface seawater film surrounding the fronds of _H. fusiforme_, supplying further evidence that the main source of Ci for emersed photosynthesis came from atmospheric CO$_2$.

CO$_2$ in air must first be dissolved into and across through the surface water film surrounding the fronds of _H. fusiforme_ before it reached the plasmolemma and was available for photosynthesis. The present results showed that AZ had a considerable inhibitory effect on photosynthetic CO$_2$ uptake rate of _H. fusiforme_ under emersed condition, indicating that the external CA facilitated the atmospheric CO$_2$ acquisition. Firstly, external CA catalysed the conversion of dissolved CO$_2$ into HCO$_3^-$ in the water film, which allowed a CO$_2$ gradient and produced a driving force facilitating the dissolving of gaseous CO$_2$ into water film (Portielje and Lijklema, 1995). Secondly, as CO$_2$ was the species of Ci that entering into cells, extracellular HCO$_3^-$ must be dehydrated to form CO$_2$ before acquired by the algal cells. This process was also mediated by external CA. Therefore, external CA simultaneously catalyzed hydration and dehydration reactions in the surface water film. However, those two adverse reactions must be spatially separated from each other. It might be proposed that the role of extracellular CA in _H. fusiforme_ could be regarded as a facilitating under emersion condition, whereas that as a qualitatively essential mechanism under submersion condition, as described in some other intertidal seaweeds (Raven, 1997; Mercado and Niell, 2000; Zou and Gao, 2004).

It was noted that at high pH value (10.0), the photosynthetic rate of _H. fusiforme_ under submersed condition was very low, indicating the acquisition of HCO$_3^-$ mediated by external CA activity was not function well in seawater under such high pH. By contrast, when exposed to air, _H. fusiforme_ could still photosynthesize efficiently when the pH value of the surface water film covering the fronds was as high as 10.0. Such high pH in the water film could accelerate the conversion of CO$_2$ into HCO$_3^-$ and then raise the CO$_2$ flux across the air-water interface (Portielje and Lijklema, 1995). In case of high standing stock or low seawater motion, the pH of seawater close to _H. fusiforme_ may rise due to the photosynthetic acquisition of HCO$_3^-$, and consequently the photosynthesis could be depressed. However, when the tide goes out, the fronds of _H. fusiforme_ retain surface seawater film with high pH and they could still photosynthesize efficiently. Such emersed photosynthetic performance could confer _H. fusiforme_ with ecological significance of increasing the daily carbon gain.

Marine seaweeds usually assimilate CO$_2$ via the C$_3$ biochemical pathway with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) as a carboxylating enzyme (Kerby and Raven, 1985; Raven, 1997). The carboxylase function of Rubisco can be competitively inhibited by O$_2$, and a high intracellular O$_2$:CO$_2$ ratio is favorable for oxygenase activity and the photorespiration pathway. However, O$_2$ tension had hardly effect on photosynthetic rates for many seaweeds (Kerby and Raven, 1985; Raven, 1997), i.e. they exhibited C$_3$-like photosynthetic gas exchange physiology. The common explanation is that they possessed a CO$_2$-concentrating mechanism (CCM) maintaining elevated CO$_2$ level intracellularly, which was based on the active HCO$_3^-$ utilization system (Beer, 1994), as the well-established CCM in microalgae (Kaplan and Reinhold, 1999). The O$_2$ sensitivity obtained in _H. fusiforme_ was consistent with a C$_3$-like photosynthetic gas exchange physiology, albeit this species had the ability of HCO$_3^-$ use. It was proposed that the external CA activity and the associated exogenous carbon acquisition mechanism in _H. fusiforme_ were not enough to maintain elevated CO$_2$ intracellularly and to prevent photorespiration. The present results showed that the photosynthesis of _H. fusiforme_ was not saturated with the current ambient Ci levels under submersed condition as well as under emersed condition, and substantially increased rates of photosynthesis could be gained by addition of DIC in seawater or CO$_2$ in air. It is generally believed that
the atmospheric CO₂ rises mainly due to anthropogenic effect (combustion of fossil fuels; deforestation), and consequently near-shore marine dissolved CO₂ levels may also increase (Bowes, 1993; Stumm and Morgan, 1996). Such an increase of CO₂ in air and/or in seawater would no doubt enhance the photosynthetic rate of *H. fusiforme* under both submersed and emersed conditions, which thereby would enhance the growth and production of aquaculture for this cultivated crop.

References:


(Managing editor: HE Ping)