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# Effect of pH on the carbon stable isotope fractionation in photosynthesis by the kelp *Undaria pinnatifida*

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**Abstract**—Mathematic models have suggested that the accumulation of anthropogenic $CO_2$ in the atmosphere has caused a decreased in oceanic pH, and that the decrease should continue for years to come. A lower pH should affect the photosynthesis of primary producers in the sea, including seaweeds. Carbon stable isotope fractionation in photosynthesis should provide information regarding this possible influence of pH on photosynthesis. Here we show that lower pH may lead to enhanced isotopic fractionation in carbon assimilation in photosynthesis by a brown alga *Undaria pinnatifida*. This may reflect a shift to $CO_2$ absorption instead of $HCO_3^-$ absorption, which is consistent with a higher proportion of $CO_2$ relative to $HCO_3^-$ at lower pH. Since in photosynthesis $CO_2$ should be absorbed with less energy costs than $HCO_3^-$, ocean acidification may be favorable to *U. pinnatifida* and species with similar photosynthetic mechanisms. However, other aspects of the phenomenon must be considered before predictions are made concerning the ecology of these organisms.

**Key words:** $CO_2$, oceanic acidification, seaweeds, laboratory experiment

## Introduction

Modeling studies have suggested that ocean acidification has been occurring due to the input of anthropogenic $CO_2$ into the atmosphere (Zeebe and Wolf-Gladrow 2001, Caldeira and Wickett 2003). As this acidification goes on, it should have varied consequences for he creatures living in the ocean, including the primary producers (Raven et al. 2005). The most negative impact of ocean acidification on the marine biota should be influence on calcifying organisms (Andersson et al. 2009, Arnold et al. 2009, Wood et al. 2009). However, ocean acidification should affect several aspects of ocean chemistry, and for most primary producers, a big effect should come from the shift in the relative proportions of $CO_2$ and $HCO_3^-$ in the dissolved inorganic carbon (DIC) pool in seawater (Raven et al. 2005). As pH decreases, the relative proportion of $CO_2$ increases, while that of $HCO_3^-$ decreases. Among the forms of DIC, only $CO_2$ and $HCO_3^-$ can be absorbed in photosynthesis (Raven 1997). However, $CO_2$ can be obained by diffusion, while $HCO_3^-$ needs to be taken up actively, with an energy cost (Raven 1997). An increased $CO_2$ concentration in seawater would be favorable to some primary producers in the sea, as it would imply in reduced energy costs in photosynthesis.

The use of $CO_2$ or $HCO_3^-$ in photosynthesis can be investigated with stable isotopes (Maberly et al. 1992, Carvalho et al. 2009a, Mercado et al. 2009). This is because $CO_2$ and $HCO_3^-$ have different carbon stable isotope compositions ($\delta^{13}C$) in seawater (Mook et al. 1974), and this difference can be reflected on the $\delta^{13}C$ of primary producers. However, for most of the species studied it has not been possible to use $\delta^{13}C$ with the purpose of ruling out $CO_2$ or $HCO_3^-$ uptake, because many other factors can influence seaweed $\delta^{13}C$ (Raven et al. 2002).

Instead of measuring seaweed $\delta^{13}C$, a more direct way to use stable isotopes to investigate $CO_2$ or $HCO_3^-$ use in seaweeds is to measure $\Delta$, the stable isotope fractionation in photosynthesis. This is because $\Delta$ can be measured in short-term experiments that allow the direct testing of the effects of several environmental conditions (Carvalho et al. 2009b), which are much more difficult to be done if the $\delta^{13}C$ of the organism is measured. Here we employ this approach to investigate the influence of pH on $\Delta$ in a brown alga *Undaria pinnatifida* (Harvey) Suringar.

## Materials and Methods

Mature *U. pinnatifida* sporophytic thalli were obtained from a culture rope in Okkirai Bay, Northeastern Japan in April 2008. They were kept in an outdoor tank for less than one week until the experiments were carried out. One day before each experiment, pieces were cut from the thalli (a sin-

gle piece measuring ca. 3 $cm^2$ from a single blade was taken). These pieces were tightened with a transparent nylon line to a thin transparent plastic sheet and kept overnight in the dark in aerated filtered seawater. This probably allowed time for healing the wounds in the seaweed pieces. After this adaptation process, the plastic sheet was tightened to a probe (Model 3600, Orbisphere Laboratories, Neuchatel, Switzerland) to measure dissolved oxygen concentration (DO) with care not to disturb DO measurements nor wound the seaweed pieces, and put in an incubation chamber specially designed to allow water sampling without air contamination, [details in Carvalho et al. (2009b)] containing a stir bar and the incubation medium, which was artificial seawater prepared by the addition of the following salts to distillated water (in mmol $L^{-1}$): NaCl, 423.0; KCl: 9.0; $CaCl_2$: 9.3; $MgCl_2$: 22.9; $MgSO_4$: 25.5 (Cavanaugh 1975). The chamber was placed over a magnetic stirrer so that the algal piece faced the light source ("Solax lamp", model SET-140F, Seric, Tokyo, Japan).

In the first experiment, incubations were done for a single algal piece in artificial seawater, at natural pH (around 8.1) or at low pH (around 5.9), which was obtained by adding small amounts of concentrated HCl solution to seawater and keeping this acidified seawater overnight in a closed bottle without air bubbles to avoid DIC striping. Irradiances of 20 and 80 $\mu$mol $m^{-2}$ $s^{-1}$ were employed to obtain different photosynthetic rates. Temperature was kept at around 15°C, and stirrer rotated at 500RPM.

In the second experiment, another algal piece was incubated at low pH (around 6.5) under irradiances from 10 to 160 $\mu$mol $m^{-2}$ $s^{-1}$. Temperature was kept at around 15°C, and stirrer rotated at 400RPM.

A third experiment was done by incubating a single algal piece in artificial seawater at low (~6.5) and high (~7.8) pH under 80 $\mu$mol $m^{-2}$ $s^{-1}$, 15°C and stirrer rotation of 400RPM. Water was sampled 5 times in each incubation, so that discrimination was calculated for 4 incubation periods in each case. Respiration flux was measured only at the end of the fourth incubation, and was assumed to be valid for each entire incubation period. Measurements of respiration flux were not done for the other incubations in this experiment because this would lead to changes in [DIC] and DIC $\delta^{13}C$, and this would make calculations of $\Delta$ complicated.

Incubations were finished after approximately 5% of total DIC was consumed (estimated from DO concentration and assuming a photosynthetic $O_2$:$CO_2$ quotient of 1; this approximation was done only to decide the time to end the incubation, and for calculation of $\Delta$, fluctuation in this ratio was allowed, details ahead). Water was sampled at the beginning and at the end of each incubation, and each water sample was divided in aliquots: one for $\delta^{13}C$ measurement (using a Gas Bench II coupled to a mass spectrometer, $Delta^{XP}_{plus}$, Thermo Fisher, Yokohama, Japan) and another for DIC quantification (based on pH and alkalinity, measured by Gran titration with a pH meter, model IQ240, IQ Scientific Instruments, Carlsbad, USA). DIC measurements were used only to determine the initial [DIC], and DIC consumption was calculated based on $O_2$ evolution assuming a photosynthetic quotient (PQ) between 1.0 and 1.2, which can be expected when nitrate is not being assimilated (Falkowski and Raven 2007), and which was very probably the case here, because no nitrate was added to the artificial seawater. This was done because DIC measurements based on alkalinity in water used to incubate seaweeds can be inaccurate (Pokorny et al. 1989). Rates of respiration in the light were estimated by turning off the light just after the incubation (Pringault et al. 2007), followed by the measurement of the change in DO concentration in water, and assuming a respiratory quotient (RQ) between 0.8 and 1.0, a commonly employed range (Wissel et al. 2008). After incubations, algal samples were dried at 60°C, weighed and kept in a desiccator until stable isotope and elemental composition analysis (precision of respectively 0.1‰ and 0.5%), which was done using an elemental analyzer (Flash EA, Thermo Fisher), coupled to the mass spectrometer. Photosynthetic flux was calculated by the change in [DIC] divided by the carbon content of the algal piece and incubation time in hours. The stable isotope composition of respired carbon was considered to be approximately equal to the stable isotope composition in the plant, which is a reasonable assumption (Carvalho et al. 2009b). Calculations of $\Delta$ and photosynthetic fluxes were performed as in Carvalho et al. (2009b).

## Results and Discussion

Experimental conditions and measurement results for all incubations in this study are shown in Tables 1 and 2, each one correspondent to figures with the same numeration. Incubation time varied between a little more than 10 min to more than 2 h, depending on the experimental conditions. The incubations did not reach extreme temperatures or irradiance, and thus the influence of measured experimental factors on photosynthetic and respiratory rates in general followed the expected patterns: higher photosynthetic rates for higher water velocity, irradiance and temperature; higher respiratory rates for higher temperature (Falkowski and Raven 2007). Net photosynthetic rates were higher than respiratory rates.

DIC $\delta^{13}C$ increased in all incubations (Tables 1 and 2), which could be expected to happen because of $\Delta$ effect (faster removal of $^{12}C$ than of $^{13}C$ from water in photosynthesis). Similar results were observed in a previous study (Carvalho et al. 2009b). Another phenomenon observed in all incubations was an increase in pH of the incubation water. The increase in pH in these short-term incubations was a consequence of the carbonate equilibrium in aqueous solution, by

**Table 1.** Details of incubation conditions for Fig. 1. DW: dry weight; $\delta^{13}C_p$: carbon stable isotope composition of the incubated algal piece; %C: organic carbon content in a dry weight basis of the incubated algal piece; Treat.: experimental treatments; *E*: irradiance; T: time in minutes; Vol: chamber volume during incubation in mL; c: [DIC]; J: carbon flux in mmol C $h^{-1}$ seaweed organic $C^{-1}$ for net photosynthetic flux (subscript l) and respiration (subscript r); o: beginning of the incubation; w: end of the incubation. co was measured directly, but cw was estimated from the change in dissolved oxygen concentration assuming $O_2$:$CO_2$=1. $J_l$ and $J_r$ were calculated based on these assumptions as well. Units: DW: g; *E*: $\mu$mol $m^{-2}$ $s^{-1}$; $\delta^{13}C$: ‰; c: mM; $J_l$ and $J_r$: mmol C $h^{-1}$ mol $C^{-1}$.

| | Treat. 1 | Treat. 2 | T | Vol | $c_o$ | $c_w$ | $pH_o$ | $pH_w$ | $J_l$ | $J_r$ | $\delta^{13}C_o$ | $\delta^{13}C_w$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fig. 1A | PH: 8.1 | *E*: 20 | 70 | 27.3 | 2.06 | 1.95 | 8.09 | 8.32 | 3.41 | 1.7 | −9.9 | −8.1 |
| DW=0.023 | | 80 | 20 | 30.8 | 2.09 | 1.98 | 8.07 | 8.33 | 13.4 | 1.4 | −9.9 | −9.3 |
| $\delta^{13}C_p$=−14.5 | 5.9 | 20 | 104 | 28.7 | 2.06 | 1.96 | 5.89 | 5.98 | 2.21 | 0.9 | −9.9 | −8.4 |
| %C=39.6 | | 80 | 16 | 34.4 | 1.99 | 1.89 | 5.98 | 6.11 | 18.5 | 1.4 | −10.0 | −8.5 |
| Fig. 1B | 6.5 | 20 | 125 | 33.1 | 1.91 | 1.69 | 6.39 | 6.63 | 6.28 | 1.9 | −5.6 | −2.0 |
| DW=0.019 | | 40 | 50 | 35.9 | 1.81 | 1.67 | 6.45 | 6.53 | 10.9 | 1.4 | −5.3 | −3.3 |
| $\delta^{13}C_p$=−16.5 | | 80 | 17 | 36.6 | 1.84 | 1.74 | 6.44 | 6.52 | 24.1 | 1.2 | −5.7 | −4.6 |
| %C=34.9 | | 160 | 13 | 35.2 | 1.80 | 1.69 | 6.52 | 6.61 | 32.2 | 1.9 | −5.6 | −4.5 |
| | | 10 | 114 | 33.1 | 1.79 | 1.68 | 6.39 | 6.52 | 3.38 | 1.4 | −5.6 | −3.8 |

**Table 2.** Details of incubation conditions for Fig. 2. Abbreviations and units as in Table 1.

| | Treat. 1 | T | Vol | $c_o$ | $c_w$ | $pH_o$ | $pH_w$ | $J_l$ | $J_r$ | $\delta^{13}C_o$ | $\delta^{13}C_w$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DW=0.019 | pH: 6.3–6.6 | 29 | 37.3 | 1.61 | 1.52 | 6.32 | 6.42 | 12.39 | 1.38 | −5.5 | −4.3 |
| $\delta^{13}C_p$=−16.5 | | 18 | 31.6 | 1.52 | 1.39 | 6.42 | 6.49 | 25.41 | 1.38 | −4.3 | −2.5 |
| %C=34.9 | | 13 | 27.4 | 1.39 | 1.29 | 6.49 | 6.57 | 21.52 | 1.38 | −2.5 | −1.2 |
| | | 12 | 23.9 | 1.29 | 1.20 | 6.57 | 6.62 | 20.71 | 1.38 | −1.2 | −0.2 |
| | pH: 7.6–8.4 | 19 | 37.3 | 2.35 | 2.27 | 7.56 | 7.71 | 17.45 | 3.25 | −6.5 | −6.2 |
| | | 19 | 33.8 | 2.27 | 2.17 | 7.71 | 7.95 | 18.92 | 3.25 | −6.2 | −5.5 |
| | | 21 | 30.9 | 2.17 | 2.06 | 7.95 | 8.21 | 17.89 | 3.25 | −5.5 | −5.0 |
| | | 18 | 26.0 | 2.06 | 1.96 | 8.21 | 8.35 | 14.79 | 3.25 | −5.0 | −4.5 |

which a decrease in [DIC] leads to an increase in pH (Stumm and Morgan 1981, Zeebe and Wolf-Gladrow 2001). The differences observed in this increase among the incubations done here were probably a result of the different buffer intensity of the carbonate system under the different incubation conditions. Buffer intensity is pH dependent (Stumm and Morgan 1981), and, in the case of the carbonate system, its lowest value is near pH=8 (the initial condition of most incubations here), with higher values near pH=6. Hence, the lower increase in pH in these incubations can be explained by a higher buffer capacity of the carbonate system in those incubations.

In Fig. 1, a comparison between incubations under low (5.9 or 6.5) and high (8.1) pH is shown. A trend of lower Δ for higher pH becomes clearer for higher photosynthetic rates. However, due to the error in the measurements, this trend cannot be evaluated with much confidence. Subsequent experiments (Fig. 2A) confirmed the trend of higher Δ for lower pH at relatively high photosynthetic rates, encouraging us to suggest that this trend existed in the incubations done in Fig. 1 as well. Differently from previous results (Carvalho et al. 2009b), photosynthetic rates seemed not to affect Δ much in Fig. 2A. This is not surprising, since incubations in this experiment were done all under the same irradiance and water velocity, and consequently photosynthetic rates did not vary as much as in those previous experiments.

At ordinary seawater pH (8.1, in Okkirai Bay), $CO_2$ comprises a negligible portion of total DIC, the biggest portion (more than 95%) being $HCO_3^-$ (Zeebe and Wolf-Gladrow 2001). The situation is completely different at pH=5.9: at this condition, about one half of total DIC is $CO_2$, and the other half is $HCO_3^-$ (Zeebe and Wolf-Gladrow 2001). Therefore, at low pH $CO_2$ availability is much higher than at normal seawater pH, and it is quite probable that the possible pH effect on Δ in Fig. 1 was related to [$CO_2$], since the latter has often been suggested as an important factor determining Δ in phytoplankton (Laws et al. 2002). Thus, [$CO_2$] were calculated from pH, temperature and [DIC] [for procedures see Zeebe and Wolf-Gladrow (2001)] for incubations in Fig. 2A, and Δ was plotted as a function of [$CO_2$] in Fig. 2B. In this figure it is observed that Δ showed a strong positive correlation with [$CO_2$], which suggests that this factor was possibly the main determinant of Δ in these incubations. Therefore, it is probable that results in Fig. 1 can also be explained by [$CO_2$] effect on Δ.

Aquatic plants may rely on $CO_2$ or $HCO_3^-$ for their pho-

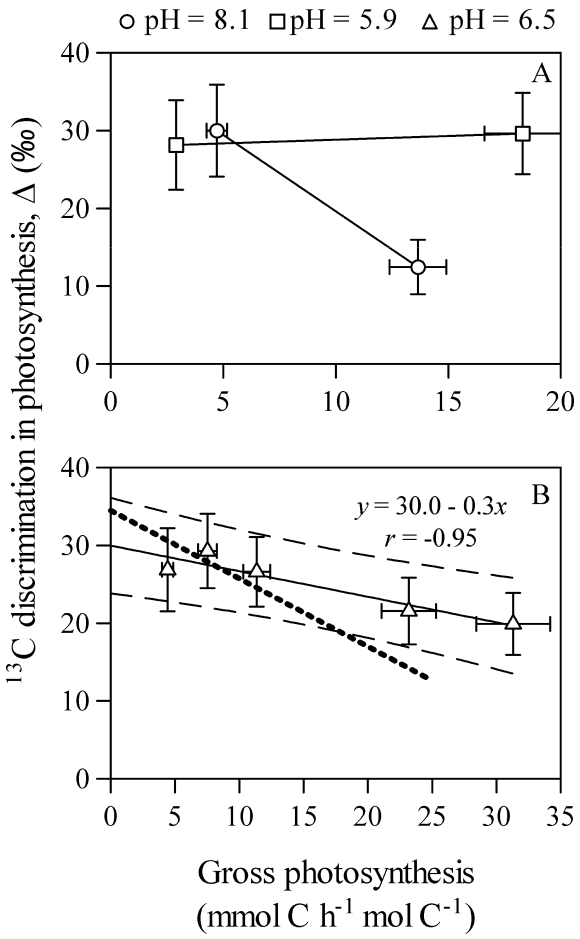


**Fig. 1.** Test of pH influence on carbon stable isotope discrimination in photosynthesis (Δ) by *U. pinnatifida*. Symbols: midpoints of the estimations for Δ and photosynthetic rates. Error bars: calculated ranges for Δ and photosynthetic rates. A: direct comparison between low and high pH at low and high photosynthetic rates. B: incubations done at low pH and several different irradiances. Continuous line in Fig. 3B: linear regression fit, with the respective describing equation and correlation coefficient. Dashed lines in Fig. 1B: prediction bands (C.I.=95%) for the linear regression. Dotted line: regression fit for *U. pinnatifida* incubations done at normal seawater pH in a previous study (Carvalho et al. 2009b).

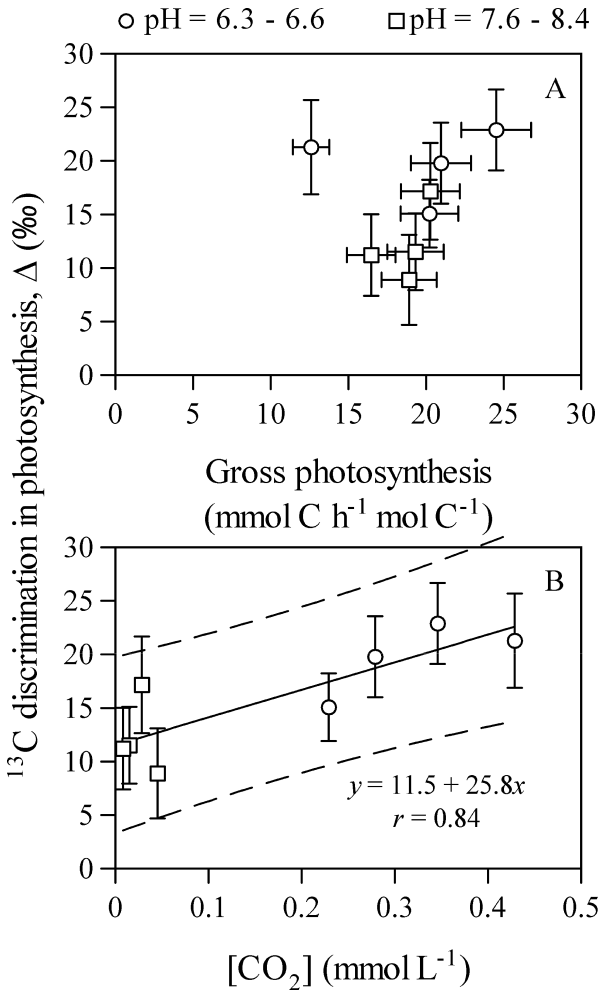


**Fig. 2.** Test of $[CO_2]$ influence on carbon stable isotope discrimination in photosynthesis (Δ) by *U. pinnatifida*. A: results shown as a function of photosynthetic intensity. B: results shown as a function of $[CO_2]$. Symbols and error bars as in Fig. 1. Lines in Fig. 2B as in Fig. 1B.

tosynthesis, as these are the forms of DIC that can cross the plasma membrane ($CO_3^=$ cannot) (Raven 1997). It is energetically advantageous for aquatic plants to use $CO_2$ instead of $HCO_3^-$, because $CO_2$ can be absorbed by diffusion, while $HCO_3^-$ can only be absorbed actively (Raven 1997). Therefore, in the incubations under low pH (and consequently higher $[CO_2]$), it is possible that a larger portion of the absorbed DIC was $CO_2$ and not $HCO_3^-$ than in the incubations at higher pH. In face of the probable advantage of using $CO_2$ and hot $HCO_3^-$ in photosynthesis (Raven 1997), $[CO_2]$ influence on Δ (Fig. 2B) suggests that $CO_2$ supply was also an important factor determining Δ in *U. pinnatifida*. This also suggests that *U. pinnatifida* prefers $CO_2$ compared to $HCO_3^-$ as a source of carbon.

The way of changing the DIC condition in incubation water in the present study consisted in changing total alkalinity and keeping DIC constant. However, the phenomenon of ocean acidification is more similar to a change in [DIC] under almost constant total alkalinity (Schulz et al. 2009). Despite these differences, the effects for organism physiology should be similar (Schulz et al. 2009), and thus the results obtained here should give a general idea of what we could expect in the case of ocean acidification. In a future ocean with lower pH, it is expected that carbon availability can be increased for some primary producers in face of the large $CO_2$ proportion in DIC in seawater. The results obtained here support this expectation for *U. pinnatifida*. Despite projections of future surface ocean pH point to a minimum pH around 7.5 (Raven et al. 2005), which is much higher than the low pH values tested here (Tables 1 and 2), the trends observed here should probably be valid for this reduction as well, but in a lesser degree. Thus, it seems that *U. pinnatifida* may benefit from ocean acidification. Among the

organisms expected to be more negatively affected by ocean acidification are calcareous algae. In ecosystems where these negatively affected species coexist with positively affected species, there may be a significant change in the structure of the ecosystem. *U. pinnatifida* is known as a species that easily invades new ecosystems (Hay and Luckens 1987, Casas et al. 2004). In the future, it may become an even stronger invasive species, if it benefits more of the ocean acidification than the others. *U. pinnatifida* is also an important aquacultured alga (Ogawa 2004), and increased $CO_2$ availability might have a positive impact on the activity. However, the possibilities considered here must be yet tested, and studies addressing them must be done at the community level in order to really understand the impacts of ocean acidification in coastal and aquacultural ecosystems.

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