PRELIMINARY EVIDENCE OF GROWTH INFLUENCE ON CARBON STABLE ISOTOPE COMPOSITION OF *UNDARIA PINNATIFIDA*

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ABSTRACT

Cultured sporophytic thalli of *Undaria pinnatifida* were collected at different periods of the year from Okkirai Bay, northeastern Japan. ′^{13}C of the thalli collected in January was much higher than that of the thalli collected in March, and young thalli collected in March showed a tendency towards lower ′^{13}C values comparing with adult ones. *U. pinnatifida* thalli grew fast in January, while young thalli in March showed slow growth due to the light limitation caused by shading adult thalli. Therefore, the growth rate of thallus may relate to ′^{13}C. Ongoing studies aim to quantitatively describe this relation and to search similar results for other species.

Keywords: ′^{13}C, Undaria pinnatifida, Gross primary production.

INTRODUCTION

It is known that carbon stable isotope ratios of primary producers usually vary in a predictable way that allows their use to trace the source of organic matter in ecosystems and food webs (Peterson and Fry, 1987; Wada *et al.*, 1987). However, this possibility is not made clear in seaweeds. Their carbon stable isotope composition shows wide variation (Raven *et al.*, 2002) and this variability makes it too difficult to use the carbon stable isotope ratio as a parameter to identify the organic matter source in ecosystems where seaweeds play an important role as primary producers (Stephenson *et al.*, 1984; Fenton and Ritz, 1989).

Some attempts to understand this variation in seaweeds suggest that there are correlations between carbon stable isotope composition of seaweeds and the following parameters: water velocity (Raven *et al.*, 1982), carbon assimilation mechanism (Maberly *et al.*, 1992), air exposure (Surif and Raven, 1990), taxonomy (Maberly *et al.*, 1992; Raven *et al.*, 2002) and growth (Wiencke and Fischer, 1990; Kubler and Raven, 1994; 1995). However, the last observations by Wiencke and Fischer and Kubler and Raven were mainly based on data from laboratory experiments and no evidence of growth influence on carbon stable isotope ratios of seaweeds in nature has been presented to date. On the other hand, the case of seagrasses is different from that of seaweeds, because it has been found that the growth of the thallus in field gives an influence to their carbon stable isotope composition (Cooper and DeNiro, 1989; Durako and Hall, 1992). This influence is attributed to the higher carbon assimilation rate necessary for faster growth. Faster carbon assimilation should deplete more rapidly the carbon pool in water, affecting the isotopic equilibrium of that pool, and leading to a change in the stable isotope composition of assimilated carbon (Peterson and Fry, 1987; Wiencke and Fischer, 1990). If the relation between the carbon stable isotope composition and the growth rate of seaweeds in nature is significant, it might be possible to use this parameter to estimate the primary production of seaweeds in the same way as already suggested for phytoplankton (Laws *et al.*, 1995).

In this report, we present preliminary evidence that there is a relation between the growth rate and the carbon stable isotope composition of *Undaria pinnatifida* thalli cultured in the natural environment.

MATERIALS AND METHODS

Sporophytic thalli of *Undaria pinnatifida* grown on culture rope were collected from Okirai Bay, Iwate Prefecture in northeastern Japan in January and March 2006. The collected materials were brought to the laboratory where their thallus were measured. Individuals were classified as adult or young according to the presence or not of sporophyl, respectively. A piece of ca. 2 cm^2 was cut at the midrib near the meristem of each collected thallus and they were washed in distilled water and dried under 60°C over night. Then they were powdered with pestle and mortar, and stored in a desiccator for posterior stable isotope analysis.

Stable isotope measurement was conducted in a stable isotope mass spectrometer (Delta^{plus}XP, ThermoFinnigan) connected to an elemental analyzer (Flash EA, ThermoFinnigan) by a flow controller (Conflo, ThermoFinnigan). Aliquots (about 1.5mg) of each powdered sample were folded with tin capsules and were put in the elemental analyzer where they are burned. The combustion gases (including CO_2) flowed through the machines until the mass spectrometer, where the stable isotope ratios of carbon and nitrogen were measured. The stable isotope ratio of Alanin as working standard was measured in several times during a single run to evaluate the quality of the analyses. The standard deviation of replicated samples was kept 0.15‰ or less than it.

Carbon stable isotope ratios are mentioned following the standard notation:

$$\delta^{13}C = (R/R_{ref} - 1)10^3$$

Where R is the ratio of $^{13}C/^{12}C$, and ref indicates the substance of reference, in the case, the Pee Dee belemnite.

RESULTS AND DISCUSSION

′^{13}C of thalli collected in January was much higher than ′^{13}C of thalli collected in March (Fig. 1). In March, values were higher for adult thalli. A Student *t* test for the average of carbon stable isotope ratio showed a significant difference between the thalli collected in January and March (P<0.05). The difference in the average of carbon stable isotope ratio between young and adult thalli collected in March was also statistically signifficant (P<0.05). The following discussion analyses the possible reasons for the observed results.

One known factor to influence ′^{13}C of seaweeds is water velocity. Water velocity affects ′^{13}C through the boundary layer: faster water implies a thinner boundary layer (Mann and Lazier, 1996), and a thinner boundary layer implies lower ′^{13}C (Raven *et al.*, 1982; France, 1995). If this were the reason behind the results obtained here, it would mean that water movement was faster in March than in January. Observed data for Otsuchi Bay, not far from Okkirai Bay, does not support this (Otobe *et al.*, 2006). Then, it is not reasonable

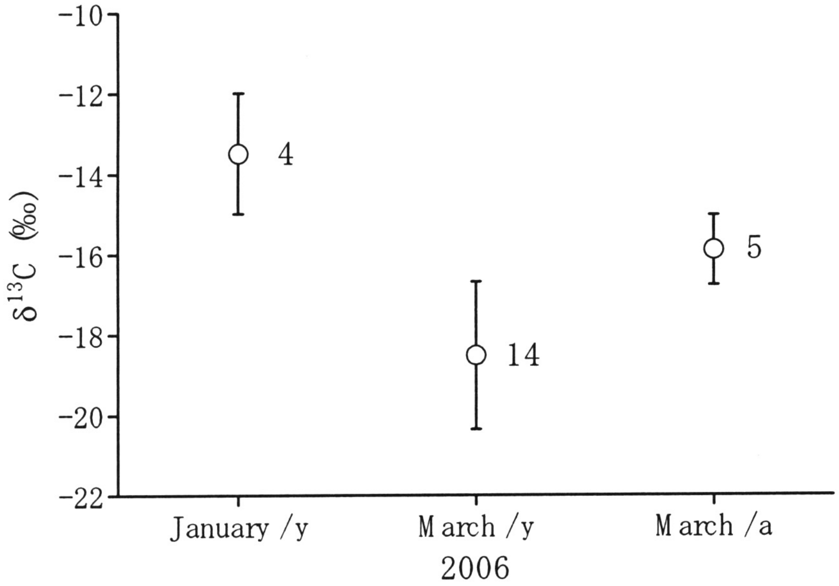

Figure 1. ′^{13}C (mean±SD) of the thalli of *Undaria pinnatifida* collected at different dates. Indicated numbers are the number of thalli measured. y: young thalli without sporophyl; a: adult thalli with sporophyl. The sporophyl was used as an indication of the probable age of the thallus.

to propose that water moment led the differences in ′^{13}C of the thalli collected in January and March. Moreover, there is another problem with the water velocity hypothesis to explain the ′^{13}C of *U. pinnatifida*: it is necessary that the predominant means of carbon acquisition of this species is diffusive carbon uptake (Maberly *et al.*, 1992). This is very unlikely to be the case, since this species grows very fast from January to March and sole diffusive carbon uptake would not supply enough amount of carbon for the growth of this species.

For algae that do not rely on sole diffusive carbon uptake, it is proposed the factor that leads to differences in ′^{13}C is the leakage of carbon assimilation (Sharkey and Berry, 1985). The ′^{13}C value could have another possibility to estimate the proportional amount of carbon that leaks through carbon assimilation. In this sense, higher value of ′^{13}C would mean both lower leakage and more efficient photosynthesis. The observation made here would suggest that the photosynthetic activity of *U. pinnatifida* was very efficient in January and young thalli in March were less efficient than older ones. "Efficiency" in the present case may be a reflex of the growth intensity. Cultured thalli of *U. pinnatifida* in the bay showed very fast growth in January, but slow growth in March. On the other hand, very young thalli germinated in March face severe light acquisition competition with adult thalli and most of them cannot grow like elders. Then, growth differences between the groups could explain the ′^{13}C observed.

Another factor implied in different ′^{13}C among seaweeds might be the difference of exposure time to the air among seaweeds growing in tidal zone (Surif and Raven, 1990). However, it is difficult to apply the factor in the thalli studied here, because they were kept in submerged condition all the time.

Finally, it could be argued that the ′^{13}C of carbon source for the thalli changed and this could have been caused the difference among the groups. The ′^{13}C difference in thalli collected in January and March allows us to imagine that the ′^{13}C of dissolved inorganic carbon (DIC) may have changed in both months and this change could cause such difference. The main factor to lead a change in the ′^{13}C of DIC would be a change in salinity, as the ′^{13}C of DIC in freshwater is usually much lower than that of DIC in seawater (Fry, 2002). It has been observed that the salinity in Okkirai Bay in January 2006 was higher than that in March, and it is conceivable that lower ′^{13}C of the thalli in March than that in January suggest a consequence of this difference. Then, the ′^{13}C of young thalli in January would be highest because of the highest ′^{13}C of the sources. This could also explain the difference between ′^{13}C of adult and young thalli in March. While adult thalli would have used a mixture of inorganic carbon with high and low ′^{13}C, young thalli would have used the carbon with low ′^{13}C only. However, it must be kept in mind that the difference in salinity was quite small (33.9psu in February against 33.3psu in March). This may have a negligible effect on DIC ′^{13}C (Fry, 2002), so that the differences observed among thalli collected in January and March may not have been due to salinity solely.

From the above discussion, it is possible to select two possible factors to explain the ′^{13}C of the sporophytic thallus of *U. pinnatifida*: growth rate or salinity, but the present evidence suggests that growth rate is possibly the most probable one. Subsequent studies will be focused on the elucidation of this question. If it is found that growth rates are the factor that explains ′^{13}C of *U. pinnatifida*, it may be possible to use ′^{13}C to estimate primary production of this species in nature. The determination of seaweed gross primary production is important for many purposes, but it is very difficult to propose reasonable estimations of this parameter (Lobban and Harrison, 1994). Eventually, ′^{13}C could be the tool to accomplish this task.

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