

CARBON ISOTOPES IN ORGANIC MATTER FROM A BENTHIC ALGA *HALIMEDA INCRASSATA* (BERMUDA): EFFECTS OF LIGHT INTENSITY

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Abstract

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Carbon isotope composition of organic matter from the calcareous benthic alga *Halimeda incrassata* grown under controlled in situ conditions in Harrington Sound, Bermuda, suggests that isotope fractionation is not necessarily dependent upon temperature but appears to be related to light intensity. Light-related fractionation of carbon isotopes should be taken into account when making paleoclimatic interpretations from $\delta^{13}\text{C}$ -values of marine organic matter.

1. Introduction

Since Urey (1947) pioneered the use of stable isotopes in natural systems, there have been various reports on the effect of temperature on ($^{13}\text{C}/^{12}\text{C}$) fractionation in organic matter. For marine plankton a well-defined increase in $\delta^{13}\text{C}$ with temperature has been reported (Sackett et al., 1965; Fontugne and Duplessy, 1981). However, Rau et al. (1982) found that latitudinal trends in plankton $^{13}\text{C}/^{12}\text{C}$ differ between northern and southern oceans. Studies on various kinds of plants (Smith et al., 1973; Whelan et al., 1973; Troughton and Card, 1975; Christeller et al., 1976; Estep et al., 1978; Manzany et al., 1980) do not have shown a coherent general relationship between $\delta^{13}\text{C}$ and temperature. Experiments on the effect of light on plant

$\delta^{13}\text{C}$ have shown both positive and negative correlations (Park and Epstein, 1960; Smith et al., 1976; Farquhar, 1983).

We report here the first evidence that changes in the organic carbon isotopic fractionation of a marine macro-alga are not necessarily dependent upon temperature but appear to be related to light intensity. Our data are derived from the calcareous benthic alga *Halimeda incrassata* grown under controlled in situ conditions in Harrington Sound, Bermuda.

2. Material and methods

The alga we chose to analyse is a common representative of modern green algae (Chlorophyta). All material was collected by diving in Harrington Sound, Bermuda. Three specimens grew during September 1978, at 2-, 6- and 10-m

water depth, respectively. During their growth period the surface-water temperatures varied between 26° and 29°C at the pier of the Bermuda Biological Station (daily recordings). From long-term observations (Morris et al., 1977) it is known that Harrington Sound shows similar temperature variations and that vertical temperature differences are negligible within the uppermost 10 m of the water column. Samples were also obtained from three specimens growing at the same water depth (~2 m) but over three different time periods: September 1978, October/November 1979 and November/December 1979, respectively, when surface temperature varied between 19.8° and 29°C.

For the determination of the growth period Alizarin Red-S was used as a time marker. Algae were covered for one day by a clear plastic tent with a volume of ~3 m³, into which the Alizarin stain was injected as a concentrated seawater solution. The algae incorporated Alizarin into calcifying tissue while they were thus covered. The stain remained fixed as a visible red band in the skeleton afterwards, as growth continued following removal of the cover, thus defining a "time line" in the skeleton. All skeletal carbonate deposited after staining appeared above this line. We assumed a linear growth rate for the distance between staining and sampling, and also used this rate to define time of growth for the portions below the stained horizon. The "time line" in the skeleton allowed us to line up the isotopic data from bottom to top of algae with environmental data gathered during the time of growth. *Halimeda* can produce up to one segment per day per branch on actively growing tips (Wefer, 1980).

Specimens were subsampled using a scalpel to separate individual parts of the segments. Depending on their size, either the entire segment or as little as one-eighth of a segment (sliced lengthwise) was used for the analysis. To remove carbonate, each subsample was soaked in cold 1 M hydrochloric acid until evolution of CO₂ ceased. Subsequently, it was

washed five times with deionized water and dried at 60°C. About 1 mg of the carbonate-free material was mixed with 0.5 g of cupric oxide and placed, together with a small strip of silver sheet, into a quartz tube (12 cm long, 7 mm i.d.) which was then evacuated and sealed by fusion. The tube was heated in a furnace at 900°C for at least 2 hr. to oxidize all carbon material to CO₂.

Five samples and an organic standard (N.B.S. 22 oil) prepared in this way were loaded into a length of copper tubing (1 cm i.d.) sealed at one end. The copper tubing was attached to a vacuum line leading to the mass spectrometer (Micromass® 602C) and evacuated. The quartz tubes were sequentially broken by external squeezing of the copper tubing and the released CO₂ was collected in a liquid-nitrogen trap after passing through two dry-ice-isopropanol traps to remove H₂O. After pumping away non-condensibles the gas was transferred to the mass spectrometer inlet and its isotope composition measured by standard procedures (Berger and Killingley, 1977). Analytical precision was found to be ±0.1‰ (1σ deviation). Results are given as deviation from PDB in parts per mil:

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}} - 1] \times 1000$$

3. Results and discussions

The stable carbon isotope ratios of the organic matter are shown in Fig. 1, together with the oxygen and carbon isotopic compositions of the algae carbonate. The fractionation of oxygen isotopes within the algal carbonate is temperature-related (Wefer, 1985), while for carbon a three-step fractionation process is proposed (Wefer and Berger, 1981). With regard to the carbon isotopes in organic matter it can be seen that there is a relative depletion in ¹³C with water depth (Fig. 1a) and also, apparently, with the seasonal progression (Fig. 1b).

Different temperature levels, during three

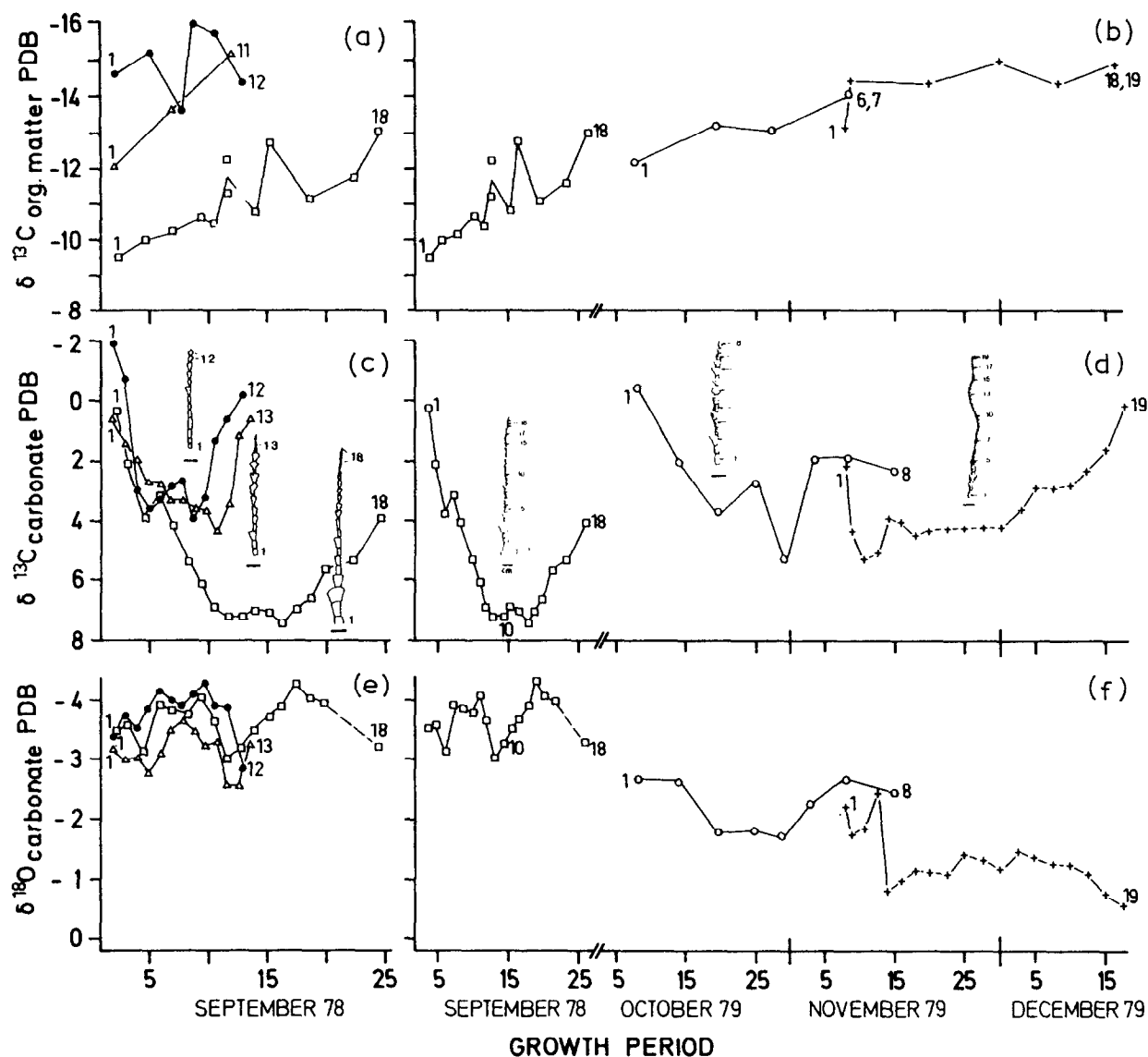


Fig. 1. Isotopic composition of subsamples from the benthic green alga *Halimeda incrassata* taken in Harrington Sound, Bermuda. The numbers on the curves refer to numbers on the drawings of the plants (scale is 1 cm). The life span was estimated using Alizarin Red-S as a stain for "time line" within the skeletons. Between staining and sampling, and for the skeletons growth before the staining, linear growth was assumed.

a. $\delta^{13}\text{C}$ variations in organic matter in three *Halimeda* specimens from 2 m (squares), 6 m (triangles), and 10 m (circles) water depth grown during September 1978.

b. $\delta^{13}\text{C}$ variations in organic matter in three *Halimeda* specimens from ~2-m water depth grown during September 1978 (squares), October/November 1979 (open circles), and November/December 1979 (crosses).

c. $\delta^{13}\text{C}$ variations in carbonates [see (a)].

d. $\delta^{13}\text{C}$ variations in carbonates [see (b)].

e. $\delta^{18}\text{O}$ variations in carbonates [see (a)].

f. $\delta^{18}\text{O}$ variations in carbonates [see (b)].

Data on the stable-isotope composition of the carbonates are from Wefer and Berger (1981) and Wefer (1985).

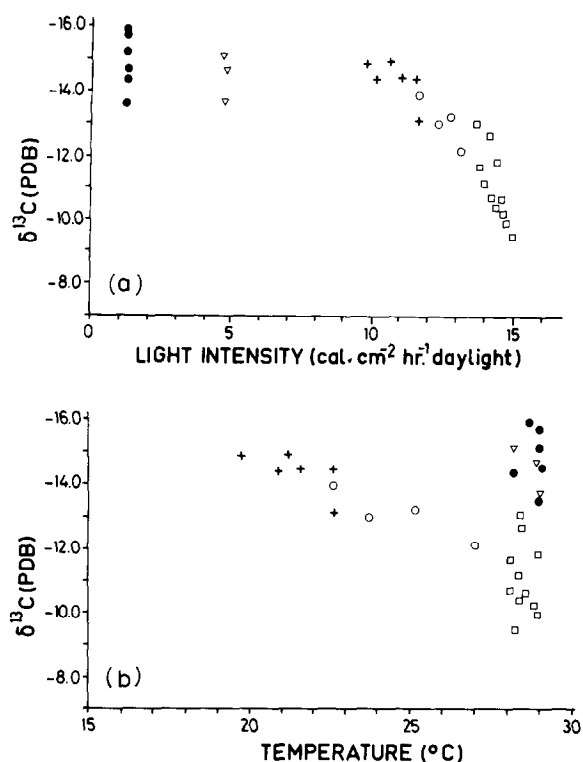


Fig. 2. Relationship between the $\delta^{13}\text{C}$ of organic matter (hypochloric acid-insoluble fraction) in the segments of the benthic calcareous alga *Halimeda incrassata* and light intensity (a) and temperature (b) during growth of the plants. For meaning of symbols see Fig. 1.

distinct portions of the years 1978 and 1979 are reflected in the $\delta^{18}\text{O}$ records in Fig. 1f. Likewise, one might expect a relationship between corresponding $\delta^{13}\text{C}$ (organic) -values and water temperature in Fig. 1b. However, the substantial differences in $\delta^{13}\text{C}$ -values between algae from different depths, shown in Fig. 1a, cannot be due to temperature differences. Weekly measurements at this site indicate that the maximum temperature difference between 2- and 10-m depth was less than 0.3°C . Also, there was no gradient in the nutrient concentration within the uppermost 10 m (B. von Bodungen, pers. commun., 1985).

The one important environmental factor which did show large differences at the three sample depths during the September 1978 period was light intensity. Average light intensities measured at noon were 130–160, 25–60

and 7–15 W m^{-2} at 2-, 6- and 10-m depth, respectively.

We plotted the $\delta^{13}\text{C}$ (organic) data against light intensity (Fig. 2a) and against temperature (Fig. 2b). Light intensities were estimated by reference to Bermuda data (Fairbanks and Dodge, 1979) corresponding to the growth periods for individual samples and appropriate corrections were made for attenuation with depth based on average values of measured light intensities. Clearly the $\delta^{13}\text{C}$ -values are directly related to light intensity. The apparent temperature effect on $\delta^{13}\text{C}$ -values on the left-hand side of Fig. 2b is readily accounted for considering that Bermuda water temperatures are linearly related to solar intensity from September through December (Fairbanks and Dodge, 1979).

The reason for the correlation between $\delta^{13}\text{C}$ -values and light intensity in these benthic algae is not known, but by analogy with a model developed for plants (Farquhar, 1983), there may be an increase in $\delta^{13}\text{C}$ with an increase in photosynthetic rate. The mechanism of carbon fixation by photosynthesis can vary with different types of plants (Craig, 1953; Park and Epstein, 1960; Hatch and Slack, 1970).

The C3 mechanism involves the carboxylation of ribulose biphosphate with CO_2 , whereas the C4 mechanism involves an additional step using the carboxylation of phosphoenol pyruvate from a HCO_3^- substrate. Various estimates have been made of the degree of discrimination against ^{13}C in these reactions.

O'Leary (1981) estimates the isotopic fractionation ($\Delta\delta^{13}\text{C}$) for the C3 carboxylation of ribulose biphosphate, relative to CO_2 , to be $-30 \pm 10\text{‰}$, and the fractionation during carboxylation in the C4 case (carboxylated phosphoenol pyruvate relative to HCO_3^-) to be in the range -2 to -2.5‰ . Throughton et al. (1974) estimate the isotopic values ($\delta^{13}\text{C}$) for C3 plants to be ~ -25 to -30.5‰ and values for C4 plants to lie within the range -11.9 to -15.2‰ . Considering a typical value of $\delta^{13}\text{C}$ for CO_2 to be -7‰ and for HCO_3^- to be $+1\text{‰}$ then the estimated values for C3 and C4 plants,

when compared with their respective carboxylation fractionations, indicate that for C4 plants the carboxylation step alone cannot account for the overall ^{13}C depletion.

Our $\delta^{13}\text{C}$ results are in the range of -9.5 to -16‰ for *Halimeda* and therefore include the C4 range but are heavier than the C3 range suggested by Troughton et al. (1974). Measured $\delta^{13}\text{C}$ -values on *Halimeda opuntia* (Fry et al., 1983) are within the range -9 to -21‰ (also outside of the C3 range) but other data on a variety of macro-algae (Black and Bender, 1976; Doohan and Newcomb, 1976) extend the range to -25.8‰ which is just within the C3 range. There is then a possibility that the photosynthetic pathway of *Halimeda* is of the C4 type but experimental work is necessary to confirm this.

It may be that the mechanism of fractionation is related to isotopic discrimination during the enzyme-catalysed carboxylation step or to changes in membrane characteristics affecting the physical transport of inorganic carbon species to the carboxylation site. Studies on aquatic plants (O'Leary, 1981) have shown that the C3 pathway is operating but the relatively small isotope fractionation observed are presumed to be due to the slow diffusion of CO_2 or due to slow permeability rates across the membrane. Widespread among the algae, however, is the ability to use HCO_3^- for photosynthesis (Benedict, 1978).

Conceivably, changes in the spectral composition of the light could affect algal $\delta^{13}\text{C}$ -values. Light attenuation between 2- and 10-m depth is greater at the red end of the visible spectrum, while green-blue light penetrates water most readily. Possibly, there could be an effect on the rate and/or mechanisms of photosynthesis, with a corresponding effect on $\delta^{13}\text{C}$. A relationship between $\delta^{13}\text{C}$ and rates of photosynthesis was found by Erez (1978). Lighter $\delta^{13}\text{C}$ in the carbonate corresponds with higher rates of photosynthesis in the symbiotic algae.

Part of the trend of increasing $\delta^{13}\text{C}$ with increasing age of the *Halimeda* segments may

be due to an "aging" effect on the organic material. During growth *Halimeda* plants add not only new segments but also deposit carbonate and organic material into already existing parts. It has been shown (Liebezeit and Dawson, 1982) that a decrease with age in the relative content of structural polysaccharides in Bermudan *H. incrassata* is paralleled by an increase in reserve polymers. Therefore the "aging" effect could be due to differences in the composition of the organic material between younger and older segments.

The $\delta^{13}\text{C}$ of the aragonitic skeleton, $\delta^{13}\text{C}_{(\text{A})}$, of the specimens show an interesting trend from low to high and back to low values (Fig. 1c and d). These results have been discussed (Wefer and Berger, 1981) with the suggestion that changes may be related to: (1) admixture of carbon from metabolic pathways; and (2) variable fractionation between the various stages of calcification. In the typical case, three stages of calcification can be distinguished (Wilbur et al., 1969; Flajs, 1977). Comparison of the $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_{(\text{A})}$ curves in Fig. 1 suggests that fractionation of carbon isotopes during the formation of organic matter and fractionation associated with precipitation of the various types of carbonate crystals are not related. Because of the small size the different carbonate minerals precipitated during distinct stages of calcification cannot be analyzed. The cause for the observed $\delta^{13}\text{C}_{(\text{A})}$ trend in *Halimeda* plants remains to be discovered.

Our results imply that some caution should be exercised when making paleoclimatic interpretation from $\delta^{13}\text{C}$ -values of marine organic matter. It is doubtful that only temperature-related fractionation of carbon isotopes is important during photosynthesis. Effects of light intensity on $\delta^{13}\text{C}$ may complicate matters and should be excluded before temperature is accepted as controlling factor.

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