

# Implications of $^{13}\text{C}$ natural abundance measurements for photosynthetic performance by marine macrophytes in their natural environment

John A. Raven<sup>1</sup>, Diana I. Walker<sup>2</sup>, Andrew M. Johnston<sup>1</sup>, Linda L. Handley<sup>3</sup>,  
Janet E. Kübler<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, United Kingdom

<sup>2</sup>Department of Botany, University of Western Australia, Nedlands, Western Australia 6907, Australia

<sup>3</sup>Soil-Plant Dynamics Group, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom

**ABSTRACT:** Marine macroalgae and seagrasses collected from Penguin Island, Western Australia, and red marine macroalgae from Australasia and Europe were analysed for the natural abundance of stable isotopes in their organic matter. These measurements revealed 1 species of green macroalgae and 9 species of red macroalgae with a  $\delta^{13}\text{C}$  below  $-30\text{‰}$ . These new observations bring the total of reports of wild-collected marine macrophytes with  $\delta^{13}\text{C}$  values below  $-30\text{‰}$  to 3 species of ulvophycean chlorophytes and 22 species of florideophycean rhodophytes. The 22 rhodophyte species are in 18 genera in 10 families of 4 orders. Marine algae with very negative  $\delta^{13}\text{C}$  values have been shown in other work to be unable to use  $\text{HCO}_3^-$  and rely on  $\text{CO}_2$  diffusion into the thallus for photosynthesis. The quantitative implications of the low  $^{13}\text{C}/^{12}\text{C}$  ratio and the inability to use  $\text{HCO}_3^-$  were analysed to predict the maximum *in situ* rate of photosynthesis. The relatively low predicted rates agree with measured rates (by other workers) of C assimilation in photosynthesis and growth. These low potential rates of C acquisition can be related to the low mean photon flux density required for growth in 17 of the 22 species of red algae and all 3 species of green algae. Low mean photon flux densities are characteristic of habitats in the subtidal (18 species of red algae, 3 species of green algae) and shaded microhabitats in the intertidal (1 species of red algae). Even the 5 other algae, littoral and infralittoral, seem from the literature to have low photosynthetic and relative growth rates, although it is not clear whether the low metabolic rates result from dependence on  $\text{CO}_2$  diffusion or whether diffusive  $\text{CO}_2$  entry as the sole means of inorganic C supply is permitted by a low metabolic rate imposed by some other cause.

**KEY WORDS:**  $\text{CO}_2$  ·  $\delta^{13}\text{C}$  · Chlorophyta ·  $\text{HCO}_3^-$  · Phaeophyta · Rhodophyta · Seagrasses

## INTRODUCTION

Measurements of the  $^{13}\text{C}/^{12}\text{C}$  ratio in the organic matter in plants relative to the  $^{13}\text{C}/^{12}\text{C}$  ratio in the inorganic C from which cell organic C was derived have been useful in the study of photosynthetic C assimilation by plants in controlled growth conditions and, especially, in agricultural and natural growth conditions outdoors (O'Leary 1981, Farquhar et al. 1989, O'Leary et al. 1992). The  $^{13}\text{C}/^{12}\text{C}$  ratio in plant organic C is generally lower than that in the inorganic C from which it was derived. This discrimination against  $^{13}\text{C}$  in producing plant organic C results

mainly from more rapid fixation of  $^{12}\text{C}$ -inorganic C by  $\text{CO}_2$ -using carboxylases: less discrimination is associated with transport processes and with the  $\text{HCO}_3^-$ -using carboxylases (Raven & Farquhar 1990, Arnelle & O'Leary 1992).

The simplest case to analyse is that of diffusive entry of  $\text{CO}_2$  followed by  $\text{C}_3$  metabolism.  $\text{C}_3$  metabolism means that  $\geq 95\%$  of the inorganic C is fixed by the  $\text{CO}_2$ -using RUBISCO (ribulose biphosphate carboxylase-oxygenase) while the remaining  $\leq 5\%$  of the C found in the accumulated organic C in the plant is fixed by anaplerotic carboxylases. The main anaplerotic carboxylase contributing to harvested organic C is one

which catalyses the production of a C<sub>4</sub> dicarboxylic acid by a C<sub>3</sub>+C<sub>1</sub> reaction. The enzymes involved are PEPC (phosphoenolpyruvate carboxylase) in the Chlorophyta and higher plants as well as the Rhodophyta, cyanobacteria and some Dinophyta and Haptophyta (formerly Prymnesiophyta; see Green & Leadbeater 1994) and phosphoenolpyruvate carboxykinase (PEPCK) which occurs as a supplement to PEPC in some green algae, and as the sole C<sub>3</sub>+C<sub>1</sub> carboxylase in most Prymnesiophyta, some Dinophyta and all Bacillariophyta and Phaeophyta (see Raven et al. 1989, 1990, Raven & Farquhar 1990, Descolas-Gros & Oriol 1992). Pyruvate carboxylase (PC) is used by some Dinophyta and may also occur in higher plants (Wurtelle & Nikolau 1990, Descolas-Gros & Oriol 1992). A smaller role in anaplerotic inorganic C fixation is played by carbamyl phosphate synthetase (CPS) (Raven & Farquhar 1990). Of these anaplerotic carboxylases only PEPCK uses CO<sub>2</sub>; PEPC, PC and CPS use HCO<sub>3</sub><sup>-</sup>.

Analysis of the <sup>13</sup>C/<sup>12</sup>C ratio in the final harvested plant organic C in terms of carboxylation and transport (diffusion) reactions requires weighting of the discrimination factors for the contribution to the total carboxylation of RUBISCO and the various anaplerotic carboxylases which operate in parallel, and the fractional contribution to controlling the steady-state rate of CO<sub>2</sub> fixation, of CO<sub>2</sub> diffusion, and of the sum of the (parallel) carboxylations operating in series. We make the usual assumption (Farquhar et al. 1989) that post-carboxylation losses of C from plants (e.g. by respiration) show negligible discrimination between C isotopes.

The equation which describes the <sup>13</sup>C/<sup>12</sup>C of organic C in the plant relative to that of bulk phase CO<sub>2</sub> is (Raven & Farquhar 1990):

$$\Delta = 1 - \left( \alpha_{\text{diff}} \frac{C_b - C_c}{C_b} \right) + \left( \alpha_{\text{carb}} \frac{C_c}{C_b} \right) \quad (1)$$

where C<sub>b</sub> is the CO<sub>2</sub> concentration in the bulk phase in mol m<sup>-3</sup>; C<sub>c</sub> is the CO<sub>2</sub> concentration in the chloroplast during steady-state photosynthesis in mol m<sup>-3</sup>; α<sub>diff</sub> is the ratio of the rate constant for diffusion of <sup>12</sup>CO<sub>2</sub> through water to that for <sup>13</sup>CO<sub>2</sub> diffusion through water; α<sub>carb</sub> is the weighted mean ratio of the rate constants for carboxylation by the carboxylases acting in parallel when using <sup>12</sup>CO<sub>2</sub> to the rate constants when using <sup>13</sup>CO<sub>2</sub> (referring the α values for the HCO<sub>3</sub><sup>-</sup>-using carboxylases to CO<sub>2</sub> as notional substrate via the equilibrium <sup>13</sup>C/<sup>12</sup>C of dissolved CO<sub>2</sub> relative to that for HCO<sub>3</sub><sup>-</sup>); and Δ is as defined here (Raven & Farquhar 1990):

$$\Delta = \frac{\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{plant}}}{1 + \delta^{13}\text{C}_{\text{plant}}} \quad (2)$$

where δ<sup>13</sup>CO<sub>2</sub> is the δ<sup>13</sup>C value of source CO<sub>2</sub> in the bulk phase, and δ<sup>13</sup>C<sub>plant</sub> is the δ<sup>13</sup>C of the organic C in the plant, i.e. with all inorganic C (e.g. CaCO<sub>3</sub> deposits) removed. The δ<sup>13</sup>C values used here are decimal fractions, not the ‰ (parts per thousand) usually employed. δ<sup>13</sup>C is defined as:

$$\delta^{13}\text{C} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} - 1 \quad (3)$$

where 'sample' refers to the material whose δ<sup>13</sup>C is to be determined (in this case source inorganic C or plant organic C), and 'PDB' refers to the C in the CaCO<sub>3</sub> of Pee-Dee Belemnite. Conversion of the decimal fraction produced by Eq. (3) into the commonly used parts per thousand (‰) involves multiplication by 1000.

Previous work (Osmond et al. 1980, Raven et al. 1982, 1987, 1992b, 1994, Maberly 1990, MacFarlane & Raven 1990, Johnston et al. 1992, Keeley & Sandquist 1992, Maberly et al. 1992) strongly suggests that Δ values (Eq. 1) in excess of 20‰ for freshwater and marine macrophytes are associated with diffusive CO<sub>2</sub> entry and (with the exception of *Udotea*; Dauby 1989, Raven 1993) the C<sub>3</sub> pathway of CO<sub>2</sub> fixation. Values of Δ below 20‰ do not rule out diffusive CO<sub>2</sub> entry (with a large fractional limitation due to diffusion) but are also consistent with HCO<sub>3</sub><sup>-</sup> use and CO<sub>2</sub> uptake by a non-diffusive mechanism (see Raven & Farquhar 1990, Maberly et al. 1992, Raven & Johnston 1992, Raven et al. 1992b).

One objective of this paper is to use this criterion of a Δ value greater than 20‰ to indicate which of the marine macrophytes for which we have measured δ<sup>13</sup>C values have diffusive CO<sub>2</sub> entry with a relatively small diffusive limitation on the CO<sub>2</sub> fixation which has contributed to the measured δ<sup>13</sup>C of the plant.

A further objective is to use Eq. (1) to determine the extent to which the photosynthesis contributing to plant δ<sup>13</sup>C for plants with Δ in excess of 25‰ is limited by CO<sub>2</sub> diffusion [the (C<sub>b</sub> - C<sub>c</sub>)/C<sub>b</sub> term in Eq. (1)] relative to CO<sub>2</sub> fixation and downstream biochemical reactions (the C<sub>c</sub>/C<sub>b</sub> term in Eq. 1). To this end Eq. (1) can be transformed to give an explicit expression for C<sub>c</sub>/C<sub>b</sub>:

$$\frac{C_c}{C_b} = \frac{1 + \Delta - \alpha_{\text{diff}}}{\alpha_{\text{carb}} - \alpha_{\text{diff}}} \quad (4)$$

Furthermore, if C<sub>b</sub> is known, the value of (C<sub>b</sub> - C<sub>c</sub>), which can be derived from C<sub>c</sub>/C<sub>b</sub>, can be used, with an estimate of the diffusion boundary layer plus internal CO<sub>2</sub> diffusion pathway thickness, to give a value for the mean rate of photosynthesis during accumulation of the organic C for which δ<sup>13</sup>C was measured (see Maberly et al. 1992). This involves a version of Fick's law of diffusion.

$$J = \frac{D_{\text{CO}_2}(C_b - C_c)}{l} \quad (5)$$

where  $J$  is the net  $\text{CO}_2$  fixation rate in photosynthesis (in  $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $l$  is the diffusion boundary layer thickness ( $\delta$ ) plus length of the internal diffusion pathway (in m),  $D_{\text{CO}_2}$  is the diffusion coefficient for  $\text{CO}_2$  in water (in  $\text{m}^2 \text{ s}^{-1}$ ), and  $C_b$  and  $C_c$  are as previously defined for Eq. (1).

The upper limit on the achieved rate of photosynthesis estimated from Eqs. (4) & (5) can then be used, with other environmental data, to put limits on the productivity of the macrophytes.

## MATERIALS AND METHODS

The bulk of the organisms whose  $^{13}\text{C}/^{12}\text{C}$  values are presented in this paper came mostly from attached plants collected on 5 October 1993 from the littoral and shallow sublittoral, or as drift, at the southeast bay of Penguin Island, Warnbro Sound, Western Australia ( $32^\circ \text{S}$ ,  $115^\circ \text{E}$ ). Collection sites for the other algae examined are cited where data on  $^{13}\text{C}/^{12}\text{C}$  are given.

After collection, the algae and seagrasses were identified (nomenclature as in Huisman & Walker 1990, Walker 1991), freed of extraneous organisms, and dried at  $80^\circ\text{C}$ . For calcified specimens, a 1 h treatment with  $100 \text{ mol HCl m}^{-3}$  followed by a rinse in distilled water was used to remove the  $\text{CaCO}_3$ . The  $\delta^{13}\text{C}$  value of the organic C in the specimens was determined as described by Maberly et al. (1992) and Raven et al. (1994). In brief, dried material was ground, and 1 mg samples were analysed in a VG ISOGAS SIRA Series II isotope ratio mass spectrometer with a Carlo-Erba CHN analyser employed as a combustion unit. The overall precision of the combustion and mass spectrometric analysis (1 SD) was  $0.08\text{‰}$  ( $n = 6$ ). Values separated by semicolons in tables and text refer to replicates.

The concentration and  $\delta^{13}\text{C}$  value of the  $\text{CO}_2$  in seawater around Penguin Island was computed as in the appendix to Maberly et al. (1992). A mean seawater temperature of  $20^\circ\text{C}$  was used based on the summer maximum of  $23^\circ\text{C}$  and the winter minimum of  $14^\circ\text{C}$  for the southeast bay of Penguin Island (T. Carruthers, M.Sc. thesis, University of Western Australia). The use of a yearly mean rather than the temperature on the day of collection or the mean for a few months previously relates to the perennial habit of most of the organisms examined. Using the solubility of  $\text{CO}_2$  at  $20^\circ\text{C}$  in water at  $35 \text{ kg m}^{-3}$  salinity of  $33.22 \text{ mol m}^{-3} \text{ atm}^{-1}$  (Skirrow 1975) and a water vapour pressure of  $2.337 \text{ kPa}$  (Benson & Krause 1984) in a total (standard) atmospheric pressure of  $101.3 \text{ kPa}$ , the dissolved  $\text{CO}_2$

will be  $11.36 \text{ mmol m}^{-3}$  in equilibrium with atmospheric  $\text{CO}_2$  of  $350 \text{ } \mu\text{mol mol}^{-1}$ . With a carbonate alkalinity of  $2.3 \text{ mol equivalents m}^{-3}$ , the seawater pH can be computed as pH 8.11, using values for the first and second dissociation constants of the inorganic carbon system from Goyet & Poisson (1989). At this pH,  $\text{CO}_2$  comprises  $0.55\text{‰}$  of the total inorganic carbon ( $\text{CO}_2 = 11.36 \text{ mmol m}^{-3}$ ,  $\text{CO}_3^{2-} + \text{HCO}_3^- = 2068.69 \text{ mmol m}^{-3}$ , total inorganic C =  $2080.08 \text{ mmol m}^{-3}$ ). The measurements of Mook et al. (1974) show that dissolved  $\text{CO}_2$  has a  $\delta^{13}\text{C}$   $9.54\text{‰}$  more negative than that of  $\text{HCO}_3^-$  at  $20^\circ\text{C}$ . No fractionation occurs between  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . Thus the  $\delta^{13}\text{C}$  of dissolved  $\text{CO}_2$  is  $-9.49\text{‰}$  and that of  $\text{HCO}_3^-$  is  $+0.05\text{‰}$  based on a  $\delta^{13}\text{C}$  for total inorganic C in seawater of  $0\text{‰}$  (Maberly et al. 1992, Simenstad et al. 1993). It is assumed throughout that the inorganic C system is in equilibrium with atmospheric  $\text{CO}_2$ . Furthermore, it is assumed that there is no lowering of the  $\delta^{13}\text{C}$  of inorganic C in the bulk phase by respiration of  $^{13}\text{C}$ -depleted organic matter (Durako & Sackett 1991, Lin et al. 1991) or increase in  $\delta^{13}\text{C}$  of bulk phase inorganic C by photosynthetic consumption (Durako & Sackett 1991, Simenstad et al. 1993). While the use of a mean annual temperature may be more appropriate for the perennial than the annual species, the procedure used here is analogous to that employed by Maberly et al. (1992), i.e. the use of the mean annual temperature with algal collection just after the spring solstice. In view of the assumptions made in computing the  $\delta^{13}\text{C}$  of  $\text{CO}_2$  dissolved in seawater, the  $\Delta$  values calculated from Eq. (2) using the computed values of  $\delta^{13}\text{C}_{\text{CO}_2}$  have been rounded to 1 ( $\Delta < 10\text{‰}$ ) or 2 ( $\Delta \geq 10\text{‰}$ ) significant digits.

## RESULTS

Tables 1 to 4 show values of  $\delta^{13}\text{C}$  (Eq. 3) for the 7 species of Chlorophyta, 7 species of Phaeophyta, 15 species of Rhodophyta and 5 species of seagrass collected at Penguin Island. The  $\delta^{13}\text{C}$  values for the seagrasses vary over a small range, are on average higher (i.e. less negative) than those of the other 3 taxa, and exhibit little overlap with the Phaeophyta and Chlorophyta  $\delta^{13}\text{C}$  values and no overlap with the Rhodophyta  $\delta^{13}\text{C}$  values. The Phaeophyta have a higher mean, a higher minimum, and a smaller range of  $\delta^{13}\text{C}$  values than for the Chlorophyta or Rhodophyta. The Chlorophyta have the largest range of  $\delta^{13}\text{C}$  values and the second-lowest minimum, while the Rhodophyta have the lowest maximum and minimum  $\delta^{13}\text{C}$  values.

Tables 1 to 4 also show the  $\Delta$  values (Eq. 2) for the 4 major taxa based on the bulk phase  $\text{CO}_2$   $\delta^{13}\text{C}$  value for  $\delta^{13}\text{C}_{\text{CO}_2}$ , i.e. making the assumption (discussed

Table 1  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for Chlorophyta collected from Penguin Island, Western Australia, 5 October 1993

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
Order Ulvales			
<i>Ulva rigida</i> C. Agardh	-16.91; -16.99	8	On leaf of <i>Posidonia australis</i>
Order Derbesiales			
<i>Bryopsis foliosa</i> Sonder	-18.48; -18.71	9	Drift
Order Codiales			
<i>Codium duthieae</i> Silva in Silva & Womersley	-12.09; -12.00	3	Upper sublittoral
Order Caulerpales			
<i>Caulerpa flexilis</i> var. <i>muelleri</i> (Sonder) Womersley	-28.20; -25.73; -26.89; -28.21	18	Drift
<i>Caulerpa longifolia</i> C. Agardh f. <i>crispata</i> (Harvey) Womersley	-30.29; -30.49 -32.58; -32.67	22 24	} Drift
<i>Caulerpa racemosa</i> (Forskall) J. Agardh var. <i>laetivirens</i> (Montagne) Weber-van Bosse f. <i>cylindracea</i> (Sonder) Weber-van Bosse	-16.19; -14.95; -15.79	6	

below) that  $\text{CO}_2$  is the exogenous inorganic C source. The ranges of  $\Delta$  values for the 4 major taxa are indicated in Fig. 1. On the basis of the conclusion from Maberly et al. (1992) that  $\Delta$  values in excess of 20‰ indicate the absence of  $\text{HCO}_3^-$  use (or active  $\text{CO}_2$  influx) and thus dependence solely on diffusive  $\text{CO}_2$  influx, it appears that 1 species (*Caulerpa flexilis* var. *muelleri*) in the Chlorophyta and 6 species (4 from the Gigartinales and 2 from the Ceramiales) of the Rhodophyta rely on diffusive  $\text{CO}_2$  supply. No members of the Phaeophyta come within 7‰ of a  $\Delta$  value of 20‰; for the seagrasses none come within 16‰ of the 'CO<sub>2</sub> diffusion only'  $\Delta$  value. One additional member of the Chlorophyta and one of the Rhodophyta have  $\Delta$  values of 18 and 19‰ respectively.

Since Maberly et al. (1992) was written, additional  $\delta^{13}\text{C}$  values for marine macroalgae from St. Andrews Bay and the Coast of Angus in eastern Scotland have been measured (Table 5). The  $\delta^{13}\text{C}$  value for the rhodophyte *Hildenbrandia rubra* is well within the range for the red algae examined by Maberly et al. (1992). However, both *Scytosiphon lomentaria* and *Desmarestia aculeata* have lower  $\delta^{13}\text{C}$  values than those for any of the 15 phaeophytes examined by Maberly et al. (1992); the  $\Delta$  values (Table 5) are still at least 5‰ lower than the 20‰ minimum value for unequivocal demonstration of diffusive  $\text{CO}_2$  entry in marine macroalgae. Very similar  $\delta^{13}\text{C}$  values to those for the Scottish *S. lomentaria* (Table 5) were found for *Scytosiphon* sp. (-25.59‰, -25.64‰) collected on 16 February 1991 from the intertidal of Gran Canaria

Table 2.  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for Phaeophyta collected from Penguin Island, Western Australia, 5 October 1993

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
Order Chordariales			
<i>Polycerea zostericola</i> (Harvey ex Kützing) Kylin	-14.91; -15.04	5	On leaf of <i>Posidonia australis</i>
Order Scytosiphonales			
<i>Colpomenia peregrina</i> (Sauvageau) Hamel	-12.01; -12.09	3	On leaf of <i>Posidonia australis</i>
Order Laminariales			
<i>Ecklonia radiata</i> (C. Agardh) J. Agardh	-20.71; -20.73	11	Drift
Order Fucales			
<i>Platythalia quercetifolia</i> (R. Brown ex Turner) Sonder	-16.90; -16.94	8	Drift
<i>Sargassum</i> sp. I 'leaf'	-20.24; -20.26; -20.15; -20.16	11	} Drift
<i>Sargassum</i> sp. I 'float'	-20.24; -20.26	11	
<i>Sargassum</i> sp. I 'stem'	-21.08; -21.09	12	
<i>Sargassum</i> sp. II 'leaf' + 'stem'	-13.62; -13.50	4	Intertidal
<i>Scytothalia dorycarpa</i> (Turner) Greville	-18.33; -18.49	9	Drift

Table 3.  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for Rhodophyta collected from Penguin Island, Western Australia, 5 October 1993. The Cryptonemiales are subsumed into the Gigartinales (Kraft & Robins 1985)

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
Order Gigartinales			
<i>Callophycus oppositifolius</i> (C. Agardh) Silva			
Fronde	-32.46; -33.22	25	} Drift
Stipe	-33.49; -33.56	25	
<i>Hypnea musciformis</i> (Wulfen) Lamouroux	-21.31; -21.51	12	Drift
<i>Kallymenia cribrosa</i> Harvey	-33.32; -33.34	25	Drift
<i>Plocamium angustum</i> (J. Agardh) Hooker & Harvey	-31.31; -31.46	23	Drift
Order Corallinales			
<i>Metagoniolithon stelliferum</i> (Lamarck) Weber-van Bosse			
(After HCl treatment)	-19.75; -19.90	11	On leaf, stem of <i>Amphibolis antarctica</i>
(Before HCl treatment)	-10.31; -10.46	na	
Order Rhodymeniales			
<i>Champia viridis</i> C. Agardh	-22.78; -22.82	14	Drift
<i>Champia zostericola</i> (Harvey) Reedman & Womersley	-20.33; -20.48	11	On leaf, stem of <i>Amphibolis antarctica</i>
<i>Gloioderma halymenioides</i> (Sonder) De Toni	-19.10; -19.25	10	Drift
Order Ceramiales			
<i>Antithamnion hanowii</i> (Sonder) De Toni	-27.77; -27.81	19	Drift
<i>Spyridia filamentosa</i> (Wulfen) Harvey	-14.66; -14.81	5	on <i>Codium duthieae</i>
<i>Wrangelia plumosa</i> Harvey	-31.67; -31.91	23	Drift
<i>Jeannerettia pedicillata</i> (Harvey) Papenfuss	-30.85; -30.94	22	Drift
<i>Laurencia brongiartii</i> J. Agardh	-17.25; -17.56	8	Drift
<i>Laurencia majuscula</i> (Harvey) Lucas	-20.20; -20.29; -20.66; -21.09	} 11	Drift
<i>Lenormandia marginata</i> Hooker & Harvey in Harvey	-26.02; -26.15		

(27° 55' N, 15° 24' W; Raven & Johnston unpubl.). None of the other 16 species of intertidal and upper sublittoral macroalgae (8 green, 5 brown, 3 red) from Gran Canaria had  $\delta^{13}\text{C}$  values for organic C lower than -21.75‰ (Raven & Johnston unpubl.). Similarly, none of the 7 brown algae, 2 red algae and 1 seagrass collected on 28 June 1992 from the intertidal and sublittoral of Point Lobos (36° 30' N, 121° 56' W) and Point Sur (36° 17' N, 121° 55' W), California, USA, had  $\delta^{13}\text{C}$

values for organic matter lower than -19.0‰ (Raven & Johnston unpubl.). Further, none of the 4 brown algae, 3 red algae and 1 seagrass collected on 17 July 1992 from the intertidal of Cobbler's Brook (48° 26' N, 54° 8' W) and Arnold's Cove (47° 45' N, 54° 2' W) Newfoundland, Canada, had  $\delta^{13}\text{C}$  values for organic matter lower than -21.4‰ (Raven & Johnston unpubl.). Finally, none of the 2 brown algae (including *S. lomentaria*; see above), 3 red algae, 3 green algae and 1 sea-

Table 4.  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for seagrasses collected from Penguin Island, Western Australia, 5 October 1993

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
<i>Amphibolis antarctica</i> (Labillardiere) Sonder & Ascherson ex Ascherson	-11.93; -12.22	3	Subtidal
<i>Posidonia angustifolia</i> Cambridge & Kuo	-9.81; -9.75	0	Subtidal
<i>Posidonia australis</i> Hooker	-9.51; -9.53	0	} Subtidal
	-8.95; -10.18	0	
	-6.31; -8.71	-2	
<i>Posidonia sinuosa</i> Cambridge & Kuo	-9.83; -9.98	0	Subtidal
<i>Heterozostera tasmanica</i> (Martens ex Ascherson) den Hartog	-10.76; -10.86	1	Subtidal

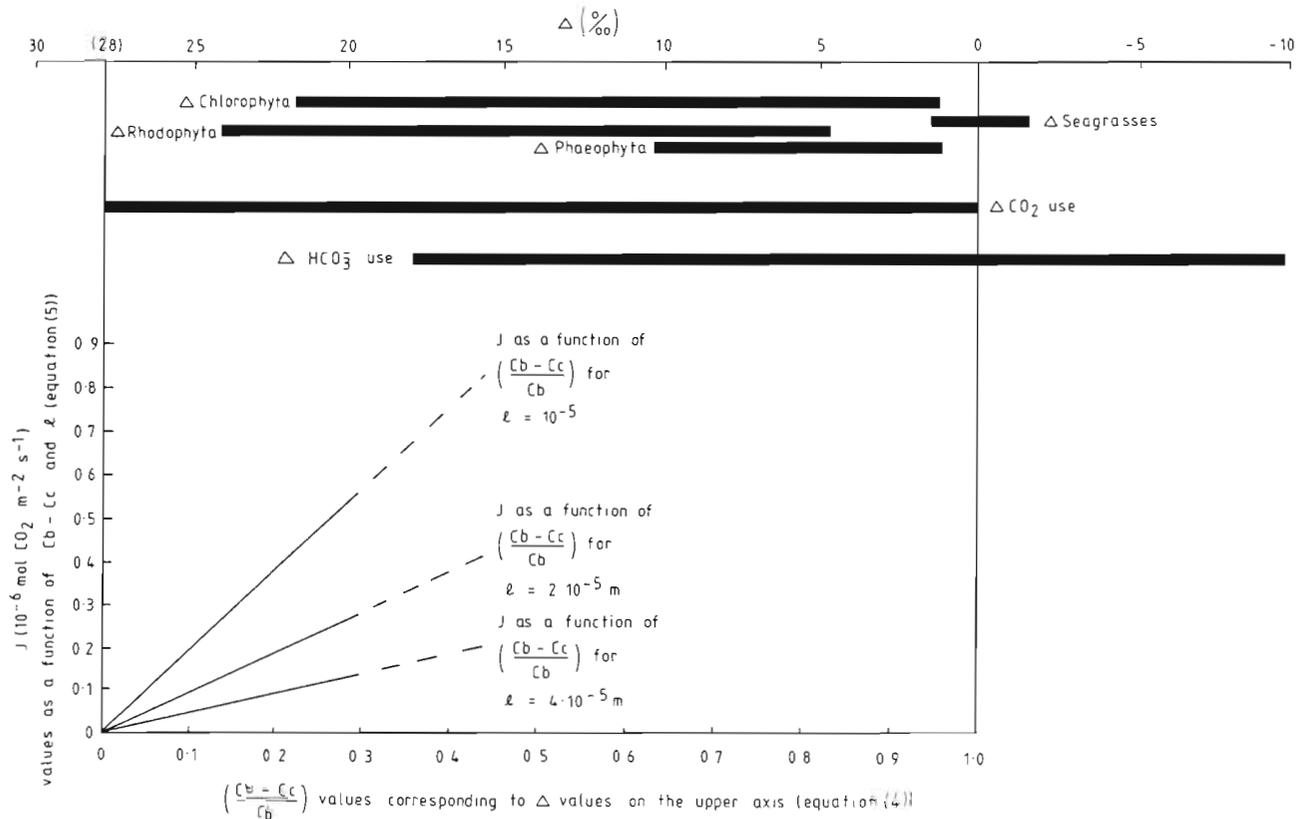


Fig. 1. The horizontal bar-charts and the upper ordinate show the ranges of  $\Delta$  values (relative to dissolved  $\text{CO}_2$ ) for green, brown and red algae and seagrasses from Penguin Island, and the range of  $\Delta$  values possible for  $\text{CO}_2$  use and  $\text{HCO}_3^-$  use in photosynthesis. The distribution of  $\Delta$  values for the 4 higher taxa investigated from Penguin Island are as follows:

- $\Delta < 10\%$ : 4/6 (4 out of 6) species of Chlorophyta, 4/7 species of Phaeophyta, 3/15 species of Rhodophyta and 5/5 species of seagrass;
- $\Delta 10\text{--}20\%$ : 1/6 species of Chlorophyta, 3/7 species of Phaeophyta, 7/15 species of Rhodophyta and 0/5 species of seagrass;
- $\Delta > 20\%$ : 1/6 species of Chlorophyta, 0/7 species of Phaeophyta, 5/15 species of Rhodophyta and 0/5 species of seagrass;

The lower ordinate shows the values of  $(C_b - C_c)/C_b$  corresponding to given values of  $\Delta$  (upper ordinate) using Eq. (4), with  $(C_b - C_c)/C_b = 1 - (C_c/C_b)$ . The abscissa shows the  $\text{CO}_2$  fixation rate ( $J$ ) computed from Eq. (5) as a function of  $(C_b - C_c)/C_b$  and  $l$ . The solid lines on the graph indicate this relationship for 3 values of  $l$  within the range of  $\Delta$ , and hence  $(C_b - C_c)/C_b$  values, for which inorganic C entry is solely by  $\text{CO}_2$  diffusion. Note that a value of  $J$  of  $1 \mu\text{mol m}^{-2} \text{s}^{-1}$  on a total area basis is equivalent to a photon flux density incident normal to 1 side of a planar flux density of  $42 \mu\text{mol m}^{-2} \text{s}^{-1}$  (400 to 700 nm), assuming a thallus absorbance of 0.8 and that  $0.06 \text{ mol CO}_2$  (net) is fixed per mol photons absorbed, taking into account mitochondrial respiration and photorespiration effects for an organism relying on diffusive  $\text{CO}_2$  entry (Raven 1984, Frost-Christensen & Sand-Jensen 1992, Markager & Sand-Jensen 1992)

Table 5.  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for 1 Rhodophyta and 2 species of Phaeophyta from eastern Scotland.  $\Delta$  values are computed relative to the  $\delta^{13}\text{C}$  of  $-10.64\%$  computed by Maberly et al. (1992) for seawater off eastern Scotland

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
<b>Phaeophyta</b>			
Order Scytosiphonales			
<i>Scytosiphon lomentaria</i> (Lyngb.) Link.	-24.76; -25.21; -26.10; -26.44	15	Rock pool, St. Andrews
Order Desmarestiales			
<i>Desmarestia aculeata</i> (L.) Lamour	-23.78; -24.00	14	Drift
	-23.26; -23.32	13	Monifieth
<b>Rhodophyta</b>			
Order Hildenbrandiales <sup>a</sup>			
<i>Hildenbrandia rubra</i> (Sommerf.) Menegh <sup>b</sup>	-17.66; -17.51; -18.56	7	Intertidal rock pool, Broughty Ferry

<sup>a</sup> Following Garbary & Gabrielson (1990); <sup>b</sup> Data from Raven et al. (1994)

Table 6.  $\delta^{13}\text{C}$  values and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for 5 species of red algae.  $\Delta$  values are computed relative to the  $-10.64\text{‰}$  for  $\delta^{13}\text{C}$  computed by Maberly et al. (1992) for seawater at  $10^\circ\text{C}$  for northwest European isolates, and relative to the  $-9.54\text{‰}$  for  $\text{CO}_2$   $\delta^{13}\text{C}$  computed in 'Materials and methods' for seawater at  $20^\circ\text{C}$  for the other isolates

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)	Site details
Order Bonnemaisoniales			
<i>Asparagopsis armata</i> Harvey	-29.61	21	Intertidal in Spanish estuary in W Mediterranean
Order Ceramiales			
<i>Bostrychia scorpioides</i> (Hudson) Montague	-31.20	22	Intertidal/littoral fringe; Spanish estuary in W Mediterranean
<i>Bostrychia scorpioides</i> (Hudson) Montague	-24.13 to -33.33	14 to 23	High intertidal/littoral fringe; France, The Netherlands
<i>Bostrychia mortiziana</i> (Sonder ex Keutzing) J. Agardh	-30.01	21	High intertidal/littoral fringe; Australia
<i>Stictosiphonia arbuscula</i> (J. D. Hooker et Harvey) R. J. King et Puttock	-29.77	21	High intertidal/littoral fringe; New Zealand
<i>Stictosiphonia hookeri</i> (Harvey) J. D. Hooker et Harvey	-27.87	19	High intertidal/littoral fringe; Australia

grass species collected at Hampton Bays, Long Island ( $40^\circ 53' \text{N}$ ,  $72^\circ 32' \text{W}$ ), USA, had  $\delta^{13}\text{C}$  values for organic matter lower than  $-20.75\text{‰}$  (Raven & Johnston unpubl.).

Table 6 shows the  $\delta^{13}\text{C}$  and  $\Delta$  (relative to  $\text{CO}_2$  dissolved in seawater) for 5 species of the Rhodophyta. Only the first 2 entries resulted from 'undirected' sampling; *Asparagopsis armata* (an introduction from Australia?) and *Bostrychia scorpioides* were the only 2 algae at the site examined by L.L.H. Following analysis showing relatively negative  $\delta^{13}\text{C}$  values, further specimens of *B. scorpioides* and specimens of other species of the Bostrychioidae (comprising the 2 genera *Bostrychia* and *Stictosiphonia*) were obtained by the good offices of Drs U. Karsten and R. J. King, and Prof. G. O. Kirst (see Karsten et al. 1990). One collection of northwest European *B. scorpioides*, and *Stictosiphonia hookeri*, have  $\Delta$  values lower than  $20\text{‰}$  (14 and  $19\text{‰}$  respectively); the other 5 specimens all have  $\Delta$  values greater than  $20\text{‰}$ , strongly indicating dependence on  $\text{CO}_2$  diffusion for their C supply.

Table 7 shows the  $\delta^{13}\text{C}$  and  $\Delta$  (relative to  $\text{CO}_2$  dissolved in seawater) for a further 3 species of the Rhodophyta collected from pneumatophores of the mangrove *Avicennia marina* (Forssk) Vierh. var. *australasica* (Walp.) Moldenke at Lytton on the southern side of the mouth of the Brisbane River, Queensland, Australia ( $27^\circ 32' \text{S}$ ,  $135^\circ 12' \text{E}$ ; mean salinity  $33.8\text{‰}$ ; site 1 of Mosisch 1993). The 3 algae include a species of *Bostrychia* and 2 other members of the Bostrychietum, *Caloglossa* (Ceramiales: Delesseriaceae) and *Catanella* (Gigartinales: Caulacanthaceae) (King & Puttock 1994). These 3 species had  $\delta^{13}\text{C}$  values just above  $-30\text{‰}$ , just short of the arbitrary cut-off value of  $\delta^{13}\text{C}$  more negative than  $-30\text{‰}$ , and  $\Delta$  values of

19 to  $20\text{‰}$ . Interpretation of these data is complicated by the density of the algal community on the pneumatophores which might restrict  $\text{CO}_2$  diffusion from the medium thus tending to decrease  $\Delta$ , and by the production of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  from pneumatophore respiration which might be expected to increase  $\Delta$  ( $\delta^{13}\text{C}$  of pneumatophores were  $-26.54$  and  $-26.63\text{‰}$ , and of leaves  $-29.38$  and  $-29.32\text{‰}$ ). Very similar values ( $-29.71\text{‰}$ ) were reported by Rao et al. (1994), for leaves of *Avicennia marina* from an ecologically similar 'fringing mangrove' site in Kenya. A further complication is that the water in the mangrove environment is usually very turbid, exacerbating the light limitation on submersed photosynthesis which might be expected from shading by the mangrove canopy. This could mean that photosynthesis mainly occurred during emersion, at the expense of atmospheric  $\text{CO}_2$  ( $\delta^{13}\text{C}$   $-8\text{‰}$ ) to an even greater extent than that suggested by Maberly et al. (1992) for *Lomentaria*.

Table 7.  $\delta^{13}\text{C}$  and  $\Delta$  values relative to dissolved  $\text{CO}_2$  for 3 species of red algae from the Bostrychietum forming 'caps' over *Avicennia* pneumatophores collected on 22 September 1994 from Lytton, Queensland, Australia.  $\Delta$  values are computed relative to the  $-9.54\text{‰}$  for  $\text{CO}_2$   $\delta^{13}\text{C}$  computed in 'Materials and methods' for seawater at  $20^\circ\text{C}$

	$\delta^{13}\text{C}$ (‰)	$\Delta$ (‰)
Order Gigartinales		
<i>Catanella nipae</i> Zanard	-29.21; -29.40	20
Order Ceramiales		
<i>Bostrychia flagellifera</i> Post	-27.97; -27.06	19
<i>Caloglossa leprieurii</i> (Mont.) J. Ag. var. <i>leprieurii</i>	-28.84	20

## DISCUSSION

The data presented in Tables 1, 3 & 6 considerably extend the number of marine macrophytes with  $\Delta$  values in excess of 20‰ when referred to dissolved  $\text{CO}_2$  as their putative exogenous inorganic C source, and which indicates diffusive  $\text{CO}_2$  entry with no  $\text{HCO}_3^-$  use or active inorganic C influx (Maberly et al. 1992). The catalysed (by carbonic anhydrase) conversion of  $\text{HCO}_3^-$  to  $\text{CO}_2$  outside the cells is common, but not ubiquitous, in marine algae (Giordano & Maberly 1989). Such a conversion, followed by diffusive entry of  $\text{CO}_2$ , would yield a range of  $\Delta$  values closely similar to those based on diffusive  $\text{CO}_2$  entry from a bulk phase containing an equilibrium mixture of  $\text{CO}_2$  and  $\text{HCO}_3^-$ . This is because the kinetic fractionation of the carbonic-anhydrase-catalysed conversion of  $\text{HCO}_3^-$  to  $\text{CO}_2$  ( $\alpha = 1.0101$ ; O'Leary et al. 1992) is closely similar to the equilibrium fractionation of  $\text{HCO}_3^-$  and  $\text{CO}_2$  ( $\alpha^1 = 1.00954$  at 20°C, with the depletion of  $^{13}\text{C}$  in  $\text{CO}_2$ ; Mook et al. 1974).

Before considering the possible taxonomic and ecological significance of these data and of those available from earlier papers it is necessary to evaluate the validity of such 'snapshot' measurements of  $\delta^{13}\text{C}$  (and thus  $\Delta$ ). It is clear that significant variations (several ‰) are found for specimens of the same species of marine macrophytes as a function of the part of the thallus measured for differentiated macrophytes (Fenton & Ritz 1989, Simenstad et al. 1993), the time of year at which samples were taken (Maberly et al. 1992, Simenstad et al. 1993), the zone of the littoral or sublittoral from which the sample was taken (Wefer & Killingley 1986, Cooper & De Niro 1989, Durako & Hall 1992, Maberly et al. 1992) and the part of the geographical range of organism from which the sample came (Dauby 1989 and Maberly et al. 1992 for *Ceramium rubrum*).

The very large difference (17‰) between the  $\delta^{13}\text{C}$  of *Ceramium rubrum* collected from Corsica by Dauby (1989; -30‰) and that from a rockpool in eastern Scotland (Maberly et al. 1992; -13.09‰) might be related to genetic differences between the specimens attributed to the single taxon *Ceramium rubrum* (Maggs & Hommersand 1993, Ward & Maggs 1994) although phenotypic effects (see above) are perhaps more likely. Intraspecific genetic differentiation within *Plocamium cartilagineum* is also likely at least as far as thermal tolerance is concerned; Antarctic specimens are killed at temperatures at which North Sea specimens can grow, although acclimation has not been rigorously excluded (Vayda & Yuan 1994). For some of the within-taxon differences found by Maberly et al. (1992) between specimens from rockpools and those from the intertidal or subtidal (Table 5 of Maberly et al.

1992), a reasonable explanation is the incidence of boundary layer limitation of photosynthesis and especially of inorganic C depletion in the different habitats (Maberly et al. 1992). Variation in  $\delta^{13}\text{C}$  with zonation in the sublittoral has been plausibly related to the gradient in mean photon flux density, with more negative  $\delta^{13}\text{C}$  values for deeper-growing specimens with a lower mean photon flux density in accord with data for cultured specimens (e.g. Wefer & Killingley 1986, Cooper & De Niro 1989, Wiencke & Fischer 1990, Durako & Hall 1992, Kübler & Raven 1994a, b).

The considerations in the last 2 paragraphs show that intraspecific variations among specimens as a function of spatial (within the plant or between plants) and temporal differences among specimens can be rationalised by appealing to mechanistically plausible factors. Furthermore, while intraspecific variability can be large, the range for organisms known to only use  $\text{CO}_2$  on the basis of 'external' evidence either in wild-collected material (Maberly et al. 1992, Johnston & Raven unpubl.; data in Tables 1, 3 & 6, with the exception of *Bostrychia scorpioides*) and cultured material (Kübler & Raven 1994a, b) is relatively small ( $\leq 4$ ‰) and, most importantly, still yields  $\Delta$  values larger than 20‰. We note that these  $\Delta$  values are not subject to errors due to depletion of  $^{13}\text{C}$  from bulk phase inorganic C, or to changes in dissolved inorganic C and pH, as happened for the inorganic C source for seagrass growth in a lagoon with large inputs of mangrove litter and little exchange of water with the open ocean (Lin et al. 1991). We also note that the range of  $\delta^{13}\text{C}$  values for seagrasses in Table 4 and in Maberly et al. (1992) are at the high end of the 'global' range for seagrasses, all of which seem to be able to use  $\text{HCO}_3^-$  (Abel & Drew 1989, Cooper 1989, Cooper & De Niro 1989, Dauby 1989, Durako & Hall 1992, Durako 1993). Where comparative data for the same species are available in Table 4 and in the references just cited, the agreement is reasonable. The high values of  $\delta^{13}\text{C}$  in seagrasses has been related to  $\text{HCO}_3^-$  use and the effect of diffusion boundary layers, and also to recycling of effluxed inorganic C in the boundary layer and of  $\text{CO}_2$  in intercellular gas spaces (Abel & Drew 1989). The first 3 of these suggestions are the most plausible.

For the Chlorophyta (Table 1) the most negative values are for species of *Caulerpa*, a genus for which Ye et al. (1991) had already reported a  $\delta^{13}\text{C}$  of  $-32.26 \pm 3.80$  (SD)‰ for the Australasian *C. trifaria*. The only other marine chlorophyte with a  $\delta^{13}\text{C}$  more negative than -30‰ in the organic C is *Udotea petiolata* from the Gulf of Calvi in Corsica (Dauby 1989). Another species of *Udotea*, the Caribbean *U. flabellum*, is thought to have a  $\text{C}_4$ -like biochemistry based on initial carboxylation by PEPCK (Reiskind & Bowes 1991). In work with the purified enzyme the  $\alpha_{\text{carb}}$  value (Eq. 1) for PEPCK

from the  $\text{C}_4$  grass *Chloris guyana* (where it functions as a decarboxylase) varies (unlike that of RUBISCO) with the order in which substrates are supplied, but is similar to that of RUBISCO (Arnelle & O'Leary 1992). This means that, despite  $\text{C}_4$ -like biochemistry, *Udotea* could have a negative  $\delta^{13}\text{C}$  with diffusive  $\text{CO}_2$  supply and minimal diffusive limitation on photosynthesis. However, the  $\delta^{13}\text{C}$  of organic material of the (calcified) *Udotea flabellum* is only  $-12.5\text{‰}$  (A. M. Johnston unpubl., using material kindly furnished by Prof. G. Bowes).

For the Phaeophyta (Tables 2 & 5) the most negative  $\delta^{13}\text{C}$  values are slightly lower than the lowest values in the literature for brown marine algae collected from the wild (Maberly et al. 1992, and references therein and in Kerby & Raven 1985). However, these values are much less negative than those for Antarctic macroalgae which were maintained in culture at low temperatures and low photon flux densities, factors known to favour a high  $\Delta$  value, but for which the  $\delta^{13}\text{C}$  of the source inorganic C was uncharacterised (Wiencke & Fischer 1990). More data on wild-collected brown macroalgae and on cultures with defined  $\delta^{13}\text{C}$  of the source inorganic C would be helpful. Values in Table 2 agree with data on the same organism published earlier for *Ecklonia radiata* (Fenton & Ritz 1988, 1989).

The final major taxon is the Rhodophyta (Tables 3, 5 & 6), where the 5 species in Table 3 with  $\delta^{13}\text{C}$  more negative than  $-30\text{‰}$  and  $\Delta$  more positive than  $20\text{‰}$  (one of which, *Plocamium augustum*, had already been shown to have a  $\delta^{13}\text{C}$  more negative than  $-30\text{‰}$ ; Fenton & Ritz 1988) can be added to the 8 species in Table 3 of Maberly et al. (1992). Other reports of  $\delta^{13}\text{C}$  values more negative than  $-30\text{‰}$  for wild-collected red marine macroalgae are those of Black & Bender (1976) for *Halymenia durvillaei*, Fry et al. (1982) for an unidentified organism, Dauby (1989) for *Ceramium rubrum* and Ye et al. (1991) for *Erythroclonium* sp. In addition, there are the 5 species in Table 6, making a total of 22. This cannot be regarded as an unbiased sample of the red macroalgae since 3 of the species in Table 2 were tested because of the relationship to *Bostrychia scorpioides*, already known to have a very negative  $\delta^{13}\text{C}$ . Comparison with other species of genera with low  $\delta^{13}\text{C}$  values (Table 3; Table 3 of Maberly et al. 1992) shows that other species do not always have very negative values (*Plocamium* sp.: Black & Bender 1976; *Jeanerettia* sp.: Fenton & Ritz 1989, although here the difference in  $\delta^{13}\text{C}$  from that of *Jeanerettia pedicillata* is only  $3\text{‰}$ ).

The red marine macroalgae seem to have a larger fraction of species with very

low ( $<-30\text{‰}$ )  $\delta^{13}\text{C}$  values than do the green marine macroalgae, with no very negative  $\delta^{13}\text{C}$  representatives in seagrasses or brown marine macroalgae. However, it must be emphasised that coverage of local algal and seagrass floras for  $\delta^{13}\text{C}$  analysis is incomplete (Table 8), and many more measurements are needed. Despite this, even with the present data, the 21 identified species of red marine algae with  $\delta^{13}\text{C}$  values more negative than  $-30\text{‰}$  ( $\Delta$  in excess of  $20\text{‰}$ ) are from 18 genera in 4 orders (Table 9) of the 10 orders of red marine macroalgae given in by Table 18.1 of Garbary & Gabrielson (1990).  $\Delta$  values in excess of  $20\text{‰}$  are also common in the freshwater Batrachospermales, albeit growing in  $\text{CO}_2$  levels well above air-equilibrium (MacFarlane & Raven 1990). It appears that the absence of  $\text{HCO}_3^-$  use under natural conditions is taxonomically widespread in red marine algae.

Turning to the ecophysiological correlates of low  $\delta^{13}\text{C}$  values in marine macroalgae, we can divide the habitats of these algae into sublittoral, littoral and supralittoral. Of the rhodophyte genera in Table 9, *Bostrychia* and *Stictosiphonia* are from the supralittoral fringe, *Asparagopsis* and *Lomentaria* are littoral and the remainder are sublittoral, as are the chlorophyte marine macroalgae with very low  $\delta^{13}\text{C}$  values (*Caulerpa trifaria*, *C. longifolia* f. *crispata* and *Udotea petiolata*).

The sublittoral group are perhaps the most readily interpreted in ecophysiological terms (Maberly et al. 1992). The relatively low photon flux density to which these organisms are naturally exposed imposes a restriction on the rate of photosynthesis on a thallus area basis which can be predicted from thallus absorbance and the photon yield of photosynthesis (Lüning 1990, MacFarlane & Raven 1990, Frost-Christensen & Sand-Jensen 1992, Markager & Sand-Jensen 1992). Thus, a photon flux density of  $42 \mu\text{mol photon m}^{-2} \text{s}^{-1}$

Table 8. Extent of sampling of the local marine macroalgal and seagrass floras for northeast Fife and southern Tayside (Maberly et al. 1992) and for Penguin Island (Tables 1 to 4)

Location	Taxon	Species in local flora	Species examined	Species with $\delta^{13}\text{C} \leq -30\text{‰}$
Fife and Tayside	Chlorophyta	54 <sup>a</sup>	7	0
	Phaeophyta	71 <sup>a</sup>	7	0
	Rhodophyta	222 <sup>a</sup>	15	8
	Seagrasses	3 <sup>b</sup>	1	0
Penguin Island	Chlorophyta	55 <sup>c</sup>	9	1
	Phaeophyta	86 <sup>c</sup>	15	0
	Rhodophyta	115 <sup>c</sup>	22	5
	Seagrasses	9 <sup>d</sup>	5	0

<sup>a</sup> Laverack & Blackler (1974); <sup>b</sup> Perring & Walters (1976);

<sup>c</sup> Huisman & Walker (1990); <sup>d</sup> Walker (1991)

Table 9. Ordinal and familial assignments of genera of red marine algae with  $\delta^{13}\text{C}$  values more negative than  $-30\text{‰}$  (data from Black & Bender 1976; Maberly et al. 1992, Tables 3 & 5; Tables 3 & 5 of this paper)

Order	Family	Genus with $\delta^{13}\text{C} \leq -30\text{‰}$
Bonnemaisoniales	Bonnemaisoniaceae	<i>Asparagopsis</i>
Cerariales	Ceramiaceae	<i>Ceramium</i> , <i>Plumaria</i> , <i>Ptilota</i> , <i>Wrangelia</i>
	Delesseriaceae	<i>Delesseria</i> , <i>Membranoptera</i> , <i>Phycodrys</i>
	Rhodomelaceae	<i>Jeanerettia</i> , <i>Odonthalia</i>
	Rhodomelaceae: Bostrychioideae	<i>Bostrychia</i> , <i>Stictosiphonia</i>
Gigartinales	Grateloupiaceae	<i>Halymenia</i>
	Kalymeniaceae	<i>Kallymenia</i>
	Plocamiaceae	<i>Plocamium</i>
	Rhabdoniaceae	<i>Erythroclonium</i>
	Solieraceae	<i>Callophycus</i>
Rhodymeniales	Champiaceae	<i>Lomentaria</i>

(400 to 700 nm) incident normal to one side of the thallus would permit a photosynthetic rate (relative to projected area of thallus) of  $1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  assuming an absorptance of 0.8 and a photon yield of  $0.06 \text{ mol CO}_2 \text{ fixed mol}^{-1} \text{ photon absorbed}$  (Raven 1984, Frost-Christensen & Sand-Jensen 1992, Markager & Sand-Jensen 1992). The photosynthetic rate on the basis of both sides of the thallus can also be predicted from  $\Delta$  and  $l$  values using Eqs. (4) & (5) for diffusive  $\text{CO}_2$  entry. Fig. 1 includes such estimates of photosynthetic rate as a function of  $\Delta$ , based on the following assumptions. In Eq. (4),  $\alpha_{\text{diff}}$  is taken as 1.0004 (O'Leary 1984), while  $\alpha_{\text{carb}}$  is taken as 1.028. The rationale for  $\alpha_{\text{carb}} = 1.028$  is that  $\alpha_{\text{RUBISCO}}$  for red and green algae is, on the basis of dissolved  $\text{CO}_2$ , 1.029 (Raven et al. 1994). These values are not known to be influenced by pH, salinity or temperature (O'Leary 1984, Faraquhar et al. 1989, Raven et al. 1994). Correction for the contribution of PEPC and CPS to  $\alpha_{\text{carb}}$  is based on their  $\alpha$  values, and the assumption that, in algae, essentially all of the CPS and PEPC activity relates to the synthesis of C skeletons for N assimilation for a (molar) C:N ratio in the algae of 10 (see Raven & Farquhar 1990). Eq. (4) then allows  $C_c/C_b$  to be computed from  $\alpha_{\text{diff}} = 1.0004$ ,  $\alpha_{\text{carb}} = 1.028$ , and the value of  $\Delta$  calculated from the  $\delta^{13}\text{C}$  values for plant organic C and the estimated seawater  $\delta^{13}\text{C}$  of inorganic C. For Eq. (5),  $C_b$  is  $11.36 \text{ mmol m}^{-3}$  as computed for air-equilibrated seawater at  $20^\circ\text{C}$  in 'Materials and methods' and  $C_c$  is computed from  $C_b$  and  $C_c/C_b$  derived from Eq. (4).  $D_{\text{CO}_2}$  is taken as  $1.7 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$  (Table 5.1 of Raven 1984). The values of  $J$  (left-hand axis) in Fig. 1 as a function of  $(C_b - C_c)/C_b$  are shown for various values of  $l$ , the diffusion boundary layer thickness plus length of the internal diffusion pathway.

Since the total diffusion path-length,  $l$ , for  $\text{CO}_2$  in a macrophyte is unlikely to be less than  $10^{-5} \text{ m}$ , the upper line in Fig. 1 represents an upper limit on the photosynthetic rate for the organisms with  $\Delta$  greater than  $20\text{‰}$ , i.e. relying on  $\text{CO}_2$  diffusion (MacFarlane & Raven 1990, Maberly et al. 1992). This means a maximum photosynthetic rate on a projected area basis of  $1.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , twice the rate on a total thallus area (one side of thallus) basis, requiring some  $25 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$  incident on the thallus. The area-based primary productivity of such a population could, of course, be increased by a thallus area index in excess of 1 (see Raven 1984). The area-based

rates predicted in Fig. 1 for  $l = 10^{-5} \text{ m}$  slightly exceed measured rates (Johnston et al. 1992, Maberly et al. 1992; also Drew 1977 for *Plocamium* and *Pseudophycodrys*, with corrections for temperature effects on solubility of  $\text{CO}_2$  and on diffusion coefficients). In terms of relative growth rates [ $\text{mol C increase (mol thallus C)}^{-1} \text{ s}^{-1}$ ] the relatively low area-based rate of photosynthesis is partially offset by the high thallus area per mol thallus C (Maberly et al. 1992, Markager & Sand-Jensen 1992). This large area per unit thallus C is important both in light interception and in inorganic C acquisition by  $\text{CO}_2$  diffusion (Raven 1984, Maberly et al. 1992, Markager & Sand-Jensen 1992).

It must be emphasised that not all of the macroalgae in the sublittoral have high  $\Delta$  values. Fig. 1 shows that the possible photosynthetic rate for  $\text{CO}_2$  diffusion as the inorganic C acquisition mechanism are, for a given diffusion pathlength  $l$ , much higher at low  $\Delta$  values. However, the very high  $(C_b - C_c)/C_b$  required to account for these low  $\Delta$  values for  $\text{CO}_2$  diffusion, and the very high biochemical capacity for  $\text{CO}_2$  fixation which is also required, with further implications for a high N cost of photosynthesis (i.e. a low rate of C fixation per unit thallus N), make diffusive  $\text{CO}_2$  entry very unlikely in, for example, the seagrasses (Fig. 1).  $\text{HCO}_3^-$  use seems a much more plausible explanation (Maberly et al. 1992). How the energetics of  $\text{CO}_2$  use with diffusive  $\text{CO}_2$  entry (no cost of making and operating inorganic C pumps, but with the energy cost of photorespiratory production and metabolism of phosphoglycolate) compare with those of  $\text{HCO}_3^-$  use via an inorganic C pump (cost of making and operating inorganic C pumps, but little energy cost of photorespiration) is not clear (Maberly et al. 1992). We note that these energy costs are likely to be important in

under-storey or deep-water sublittoral phototrophs living at low photon flux densities, and that the inorganic C pump might be less energetically efficient at low photon flux densities since it is operating against a fixed (light-independent) leak, whereas the photo-respiratory energy cost should be a constant increment at any incident photon flux density.

For the littoral and infra-littoral fringe species with high  $\Delta$  values the interpretation of these values in terms of diffusive  $\text{CO}_2$  entry involves a low achieved photosynthetic rate per unit area of thallus despite the small limitation of photosynthesis by  $\text{CO}_2$  diffusion indicated by the high  $(C_b - C_c)/C_b$  computed from the  $\Delta$  values (Fig. 1). This in turn means a low incident photon flux density requirement for light-saturation of achieved photosynthesis (Fig. 1) in an ostensibly high-light environment, unless implausibly low tissue absorptances and photon yields on an absorbed photon basis are assumed. These findings are consistent with a low area-based capacity for light-saturated photosynthesis in seawater, or a low incident photon flux density, or to both of these factors (i.e. 'shade adaptation'). It appears that some of these algae do indeed occupy low-light micro-environments (e.g. *Lomentaria*, Johnston et al. 1992, Maberly et al. 1992; members of the *Bostrychietum* on mangrove pneumatophores under the mangrove leaf canopy), and that those tested have relatively low maximum (light-saturated) photosynthetic rates and relatively low specific growth rates (*Bostrychia*: Karsten & Kirst 1989; *Caloglossa*: Karsten & West 1993; *Lomentaria*: Johnston et al. 1992), i.e. a combination of the 2 factors mentioned above ('shade adaptation'). Whether these low rates are a result of the inability to use  $\text{HCO}_3^-$ , or whether dependence on  $\text{CO}_2$  diffusion is permitted by a low metabolic rate imposed by some other cause, is not clear. Furthermore, for the infra-littoral fringe *Bostrychia* and *Stictosiphonia*, and the 'damp when the tide is out' *Lomentaria* (Johnston et al. 1992, Maberly et al. 1992), atmospheric  $\text{CO}_2$  may be a significant inorganic C source and it may be relevant that most terrestrial plant species, and most global terrestrial productivity, involve diffusive  $\text{CO}_2$  flux to RUBISCO (Raven et al. 1992a). At least the gas-phase part of the  $\text{CO}_2$  diffusion pathway is through a medium in which the  $\text{CO}_2$  diffusion coefficient is  $10^4$  times that in solution; the thicker diffusion boundary layers around an object in air relative to those around a similar object in water may reduce this superiority of  $\text{CO}_2$  diffusion in air to a factor of  $10^3$  or so (Raven 1984). It is possible to rationalise the dependence of the high- $\Delta$  species of marine macroalgae on  $\text{CO}_2$  diffusion in ecophysiological terms. However, more information is needed on the taxonomic and ecological diversity of these high- $\Delta$  seaweeds, the robustness of the correlation of high

$\Delta$  values with diffusive  $\text{CO}_2$  entry (currently the correlation is 100%, but with a sample size of  $n = 6$ ; Maberly et al. 1992), and the implications of diffusive  $\text{CO}_2$  entry for the photon and nitrogen cost of photosynthesis and growth.

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