Oecologia (1992) 91:481–492

Oecologia

Discrimination between ^{12}C and ^{13}C by marine plants

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Received February 10, 1992 / Accepted in revised form June 4, 1992

Summary. The natural abundance $^{13}C/^{12}C$ ratios (as $\delta^{13}C$) of organic matter of marine macroalgae from Fife and Angus (East Scotland) were measured for comparison with the species' ability to use CO_2 and HCO_3^- for photosynthesis, as deduced from previously published pH-drift measurements. There was a clear difference in $\delta^{13}C$ values for species able or unable to use HCO_3^-. Six species of Chlorophyta, 12 species of Phaeophyta and 8 species of Rhodophyta that the pH-drift data suggested could use HCO_3^- had $\delta^{13}C$ values in the range −8.81‰ to −22.55‰. A further 6 species of Rhodophyta which the pH-drift data suggested could only use CO_2 had $\delta^{13}C$ values in the range −29.90‰ to −34.51‰. One of these six species *(Lomentaria articulata)* is intertidal; the other five are subtidal and so have no access to atmospheric CO_2 to complicate the analysis. For these species, calculations based on the measured $\delta^{13}C$ of the algae, the $\delta^{13}C$ of CO_2 in seawater, and the known $^{13}C/^{12}C$ discrimination of CO_2 diffusion and RUBISCO carboxylation suggest that only 15–21% of the limitation to photosynthesis *in situ* results from CO_2 diffusion from the bulk medium to the plastids; the remaining 79–85% is associated with carboxylation reactions (and, via feedback effects, down-stream processes). This analysis has been extended for one of these five species, *Delesseria sanguinea,* by incorporating data on *in situ* specific growth rates, respiratory rates measured in the laboratory, and applying Fick's law of diffusion to calculate a boundary layer thickness of 17–24 µm. This value is reasonable for a *Delesseria sanguinea* frond *in situ.* For HCO_3^--using marine macroalgae the range of $\delta^{13}C$ values measured can be accommodated by a CO_2 efflux from algal cells which range from 0.306 of the gross HCO_3^- influx for *Enteromorpha intestinalis* ($\delta^{13}C = -8.81‰$) in a rockpool to 0.787 for *Chondrus crispus* ($\delta^{13}C = -22.55‰$). The relatively high computed CO_2 efflux for those HCO_3^--users with the more negative $\delta^{13}C$ values implies a relatively high photon cost of C assimilation; the observed photon costs can be accommodated by assuming coupled, energy-independent inorganic carbon influx and efflux. The observed $\delta^{13}C$ values are also interpreted in terms of water movement regimes and obtaining CO_2 from the atmosphere. Published $\delta^{13}C$ values for freshwater macrophytes were compared with the ability of the species to use CO_2 and HCO_3^- and again there was an apparent separation in $\delta^{13}C$ values for these two groups. $\delta^{13}C$ values obtained for marine macroalgae for which no pH-drift data are available permit predictions, as yet untested, as to whether they use predominantly CO_2 or HCO_3^-.

Key words: Carbon isotope discrimination – Chlorophyta – Inorganic carbon source – Phaeophyta – Rhodophyta

On Earth carbon has two stable isotopes, of which ^{12}C constitutes about 99% and ^{13}C about 1%. The natural abundance of these isotopes in the organic carbon of plants differs from that in the source because a number of kinetic and equilibrium processes discriminate differentially against the heavier isotope (Farquhar et al. 1989). The major potential discriminant in carbon fixation is the carboxylation reaction carried out by ribulose-bisphosphate carboxylase-oxygenase (RUBISCO). Other important steps where discrimination may take place include net diffusion of CO_2 in the gas phase and equilibrium between dissolved CO_2 and HCO_3^-.

In terrestrial C_4 plants, the temporary fixation of inorganic carbon into C_4 product by phosphoenolpyruvate carboxylase (PEPCase), which has a low discrimination against ^{13}C, and the subsequent decarboxylation of the C_4 product, produces high concentrations of CO_2 at the site of carboxylation. This, with relatively limited CO_2 leakage from this site, limits expression of the discriminating effect of RUBISCO. As a consequence, terrestrial C_4 plants discriminate less than

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terrestrial C_3 plants, with $\delta^{13}C$ values in the range -10 to $-14‰$ compared to -21 to $-35‰$ for C_3 plants, where:

$$\delta^{13}C = (R_{\text{sample}} - R_{\text{standard}}/R_{\text{standard}}) \times 1000 \qquad (1)$$

R_{sample} is the ^{13}C:^{12}C ratio of the sample and R_{standard} is the ^{13}C:^{12}C ratio of the standard, calcium carbonate in a fossil belemnite from the Cretaceous Pee Dee formation with a ^{13}C:^{12}C ratio of 0.011237. Because the $\delta^{13}C$ of the source carbon may be variable it is more useful to calculate the overall discrimination (Δ) shown by carbon uptake:

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p) \qquad (2)$$

Where δ_a and δ_p are the values for air (the source) and plant (product) respectively. For terrestrial plants the current atmospheric CO_2 source has a $\delta^{13}C$ of about $-7.8‰$, and C_3 and C_4 plants have Δ values of about 0.013–0.022 and 0.004–0.008 respectively (Ehleringer 1991). This relatively clear-cut distinction between C_3 and C_4 plants has proved to be a useful tool to screen plants for their biochemical pathway (Ehleringer 1991). Plants with obligate Crassulacean acid metabolism (CAM) have Δ values similar to those of C_4 plants but facultative CAM plants show Δ values ranging from those characteristic of C_4 to those of C_3 plants.

The use of $\delta^{13}C$ abundance in aquatic plants is more complicated than for terrestrial plants. First of all, spatial and temporal changes in the $\delta^{13}C$ of the source in the atmosphere are usually fairly small apart from, for example, sites close to power-stations burning fossil fuels, where the release of relatively light CO_2 derived from discrimination by photosynthesis past causes the source CO_2 to have a relatively low $\delta^{13}C$. This is analogous to the problems in many aquatic systems, especially freshwaters, where input of CO_2 from decomposition of autochthonous and allochthonous organic material (e.g. Rebsdorf et al. 1991) produces sources which are relatively depleted in ^{13}C as well as having CO_2 concentrations which are higher than those in equilibrium with the atmosphere. Conversely, where extreme carbon depletion takes place because of rapid photosynthesis, the source carbon may become relatively enriched in ^{13}C as a result of preferential uptake of the lighter isotope (Takahashi et al. 1990). A second source of complexity arises from the availability of free CO_2, HCO_3^- and CO_3^{2-} as potential sources of inorganic carbon, each with a different temperature-dependent $\delta^{13}C$ value relative to the total inorganic carbon. Therefore, in an extreme case, the source $\delta^{13}C$ of a plant restricted to CO_2 will be more negative than that of a plant restricted to HCO_3^- by about 10‰ even though they were growing in the same body of water.

Despite these problems, progress has been made in using $\delta^{13}C$ values of aquatic plants to calculate, for instance, the thickness of the boundary-layer (Raven et al. 1982; Raven et al. 1987; Macfarlane and Raven 1990), the degree of leakiness of the CO_2-concentrating pump in microalgae (Sharkey and Berry 1985) or in marine macroalgae (Surif and Raven 1990). Marine macroalgae show a wide range in δ^{13} values from about $-2.5‰$ to $-35‰$ for different species (Fry and Sherr 1984; Kerby and Raven 1985), despite growing in an environment with a relatively constant $\delta^{13}C$ for inorganic carbon. The reliance on CO_2 or HCO_3^- as a carbon source for aquatic plants is often cited as a factor which could influence the $\delta^{13}C$ value of aquatic plants (e.g. Osmond et al. 1980; Fry and Sherr 1984; Kerby and Raven 1985), but critical data on this point is lacking. In this paper we examine the relationship between the natural abundance of ^{13}C in marine macroalgae and their ability to use CO_2 and HCO_3^-, using the data of Maberly (1990) based on pH-drift measurements.

Materials and methods

Seaweeds were collected on the coast around St. Andrews between Tentsmuir to the north (2° 48′ W, 56° 25′ N) and Fife Ness (2° 35′ W, 56° 17′ N) to the south. They were identified and sorted prior to drying on aluminium foil overnight at 80° C. The calcareous species *Corallina officinalis* was treated with acid (100 mol m^{-3} HCl at 22° C for 1 h) to remove carbonates. Mass spectrometric measurements were made with 1 mg of dry material using a VG SIRA II Isotope Ratio Mass Spectrometer with a Carlo Erba CHN analyser as a combustion unit to generate CO_2 for the mass spectrometer inlet. Results are presented as the mean of at least duplicate measurements, with an average standard deviation of 1.7% of the mean for replicate samples.

Results

There is a large range in $\delta^{13}C$ values for the marine macroalgae studied here and published elsewhere. The values obtained in this study, and previously for seaweeds in Angus (Broughty Ferry 2° 53′ W, 56° 28′ N; Arbroath 2° 35′ W, 56° 33′ N) range from -8.8 to $-21.2‰$ for the Chlorophyta (Table 1) with a mean of $-15.74‰$, from -10.5 to $-20.8‰$ for the Phacophyta (Table 2) with a mean of $-17.09‰$ and -11.0 to $-34.7‰$ for the Rhodophyta (Table 3) with a mean of $-22.94‰$. Where more than one sample is available per species, there is sometimes little variation, as for *Delesseria sanguinea* or *Phycodrys rubens,* which grow in a relatively stable environment, while in other species, such as *Enteromorpha sp.* there is a very large range. In the case of *Enteromorpha,* although it is possible that taxonomic uncertainty is a contributing factor, the variation may largely reflect differences in carbon availability brought about by the temporally and spatially variable habitat in which these species live. Factors such as the relative availability of CO_2 and HCO_3^-, the degree of water movement, carbon depletion, and photosynthesis in air may all influence the $\delta^{13}C$ value of a plant, and the effect of these will be addressed in the discussion.

Discussion

Relationship between $\delta^{13}C$ and use of CO_2 and HCO_3^-

If the average $\delta^{13}C$ value for species where pH-drift data are available (Maberly 1990) is plotted against the mean

Table 1. $\delta^{13}C$ values and final pH in drift experiments (Maberly 1990), for Chlorophyta collected from the east coast of Scotland, at or around St. Andrews unless otherwise stated

Species	Date or reference	Site details	$\delta^{13}C$ (‰)	$\bar{x}\delta^{13}C$ (‰)	Final pH
Bryopsis plumosa[a]	2/10/91	Tentsmuir drift on *C. rubrum*	−19.35	−19.35	–
Cladophora sericea[b]	3/4/91	Mid-shore	−16.66	−16.66	10.43
Cladophora rupestris[c]	3/7/91	Fringing sheltered rockpool	−13.33	−14.17	10.49
Cladophora rupestris[c]	3/4/91	Rockpool	−15.01		
Codium fragile[d]	3/4/91	Rockpool	−14.08	−12.59	9.67
Codium fragile[d]	3/7/91	Sheltered rockpool	−10.23		
Codium fragile[d]	3/7/91	Sheltered rockpool	−10.66		
Codium fragile[d]	29/11/90	Fife Ness	−15.38		
Enteromorpha[e] sp.	3/7/91	Sheltered rockpool	− 9.36	−13.92	10.71
Enteromorpha[e] sp.	3/7/91	Sheltered rockpool	−13.33		
Enteromorpha intestinalis[f]	3/4/91	Rockpool	− 8.81		
Enteromorpha intestinalis[f]	1	Broughty Ferry, intertidal	−20.3		
Enteromorpha linza[g]	3/7/91	Rockpool, not sheltered	−17.80		
Prasiola stipitata[h]	3/4/91	Upper-shore	−18.70	−19.94	9.95
Prasiola stipitata[h]	2	Upper shore	−21.18		
Ulva lactuca[i]	3/7/91	Sheltered rockpool	−13.88	−13.51	10.61
Ulva lactuca[i]	3/7/91	Drift	−17.80		
Ulva lactuca[i]	3/4/91	Rockpool	− 8.86		

[1] Raven et al. (1987)
[2] Raven and Johnston (1991a)
Authorities:
[a] (Huds.) C. Ag.
[b] (Huds.) Kütz.
[c] (L.) Kütz.
[d] (Sur.) Hariot subsp. *atlanticum* (Cotton) Silva
[e] Link in Ness
[f] (L.) Link
[g] (L.) J. Ag.
[h] Suhr. in Jessen
[i] L.

Table 2. $\delta^{13}C$ values and final pH in drift experiments (Maberly 1990), for Phaeophyta collected from the east coast of Scotland, at or around St. Andrews unless otherwise stated

Species	Date or reference	Site details	$\delta^{13}C$ (‰)	$\bar{x}\delta^{13}C$ (‰)	Final pH
Alaria esculenta[a]	3/7/91	Low-shore, 40 cm long	−17.80	−16.70	9.61
Alaria esculenta[a]	1	Arbroath, lower shore	−15.6		
Ascophyllum nodosum[b]	3/4/91	Mid-shore	−18.93	−18.31	10.23
Ascophyllum nodosum[b]	1	Arbroath, upper shore	−17.6		
Ascophyllum nodosum[b]	3	Mid-shore	−18.4		
Asperococcus fistulosus[c]	3/7/91	Sheltered rockpool	−11.87	−11.87	–
Chorda filum[d]	3/7/91	Drift	−19.89	−19.89	–
Fucus serratus[e]	1	Arbroath, lower shore	−17.6	−17.60	10.31
Fucus spiralis[e]	1	Arbroath, upper shore	−18.4	−18.40	10.05
Fucus vesiculosus[e]	1	Arbroath, upper-mid shore	−19.5	−19.50	10.26
Halidrys siliquosa[f]	1	Arbroath, rockpool	−17.1	−17.10	9.97
Himanthalia elongata[g]	29/11/90	'Button', mid-shore	−15.39	−14.97	9.88
Himanthalia elongata[g]	2	'Button', mid-shore	−14.7		
Himanthalia elongata[g]	5/11/91	'Button', mid-shore	−14.81		
Himanthalia elongata[g]	29/11/91	'Thong', mid-shore	−16.37	−13.62	–
Himanthalia elongata[g]	2	'Thong', mid-shore	−10.5		
Himanthalia elongata[g]	5/11/91	'Thong', mid-shore	−13.99		
Laminaria digitata[h]	3/7/91	Mid-shore rockpool	−19.64	−18.65	9.67
Laminaria digitata[h]	1	Arbroath, rockpool	−18.5		
Laminaria digitata[h]	3	Broughty Ferry, subtidal	−17.8		
Laminaria hyperborea[i]	1	Arbroath, rockpool	−16.0	−17.00	9.20
Laminaria hyperborea[i]	3	Broughty Ferry, subtidal	−18.0		
Laminaria saccharina[j]	3/7/91	Mid-shore rockpool	−20.02	−20.02	9.76
Leathesia difformis[k]	3/7/91	Mid-shore on *L. pinnatifida*	−14.16	−14.16	10.11
Pelvetia canaliculata[l]	3/4/91	Upper-shore	−19.44	−20.12	9.78
Pelvetia canaliculata[l]	1	Arbroath, upper shore	−20.8		
Pilayella[m] sp.	3/7/91	On *Fucus vesiculosus*	−15.57	−15.57	–

[1] Surif and Raven (1990)
[2] Raven (1991)
[3] Raven et al. (1987)

Authorities:
[a] (L.) Grev.
[b] (L.) Le Jol.
[c] (Huds.) Hook.
[d] (L.) Stackh.
[e] L.
[f] (L.) Lyngb.
[g] (L.) S. F. Gray
[h] (Huds.) Lamour.
[i] (Gunn.) Fosl.
[j] (L.) Lamour.
[k] (L.) Aresch.
[l] (L.) Dcne et Thur.
[m] Bory

Table 3. δ^{13}C values and final pH in drift experiments (Maberly 1990) for Rhodophyta collected from the east coast of Scotland at or around St. Andrews unless otherwise stated

Species	Date or reference	Site details	δ^{13}C(‰)	$\bar{x}$ δ^{13}C(‰)	Final pH
Ceramium rubrum[a]	3/4/91	Rockpool	−13.09	−16.33	9.77
Ceramium rubrum[a]	2/10/91	Tentsmuir, drift	−19.56		
Chondrus crispus[b]	3/4/91	Lower shore	−22.55	−18.25	9.54
Chondrus crispus[b]	1	Winter	−14.2		
Chondrus crispus[b]	1	Summer	−18.0		
Corallina officinalis (organic C)[c]	3/4/91	Rockpool	−15.40	−15.40	10.07
Corallina officinalis (whole)[c]	3/4/91	Rockpool	−9.61	−9.61	
Delesseria sanguinea[d]	3/4/91	Drift	−34.29	−33.37	8.86
Delesseria sanguinea[d]	3/7/91	Drift	−32.91		
Delesseria sanguinea[d]	3/7/91	Drift	−32.91		
Dilsea carnosa[e]	3/4/91	Lower-shore	−18.81	−18.81	–
Dumontia contorta[f]	3/4/91	Rockpool	−11.03	−11.03	9.97
Laurencia pinnatifida[d]	3/4/91	Mid-shore	−19.81	−17.35	9.73
Laurencia pinnatifida[d]	3/4/91	Mid-shore	−18.15		
Laurencia pinnatifida[d]	15/10/91	Mid-shore	−14.09		
Lomentaria articulata[g]	3/4/91	Mid-shore	−29.90	−30.04	8.97
Lomentaria articulata[g]	15/10/91	Mid-shore	−30.17		
Mastocarpus stellatus[h]	3/7/91	Mid-shore	−17.50	−17.64	9.75
Mastocarpus stellatus[h]	3/4/91	Mid-shore	−17.78		
Membranoptera alata[i]	3/4/91	Drift	−32.77	−32.91	8.95
Membranoptera alata[i]	29/11/90	Drift	−33.04		
Odanthalia dentata[j]	3/4/91	Drift	−30.57	−30.57	–
Palmaria palmata[k]	3/4/91	Lower-shore	−19.2	−16.48	10.50
Palmaria palmata[k]	29/11/90	Lower-shore	−17.74		
Palmaria palmata[k]	15/10/91	Drift on *F. vesiculosus*	−14.56		
Palmaria palmata[k]	15/10/91	Drift on *L. hyperborea*	−14.41		
Phycodrys rubens[l]	3/4/91	Drift	−34.74	−33.23	8.98
Phycodrys rubens[l]	3/7/91	Drift	−32.49		
Phycodrys rubens[l]	29/11/90	Drift	−32.47		
Plocamium cartilagineum[m]	3/4/91	Drift	−34.51	−34.51	8.97
Plumaria elegans[n]	3/4/91	Drift	−31.98	−31.98	–
Polyides rotundus[o]	3/4/91	Rockpool	−15.01	−15.01	–
Polysiphonia lanosa[p]	3/4/91	On *A. nodosum*	−21.40	−21.40	9.79
Polysiphonia[q] sp.	3/4/91	Rockpool	−16.11	−16.11	–
Porphyra purpurea[r]	3/4/91	Mid-shore	−20.27	−20.27	–
Ptilota plumosa[s]	3/4/91	Drift	−33.66	−33.66	8.94
Rhodochorton floridulum[t]	15/11/91	Mid-shore	−16.39	−16.39	–
Rhodomela confervoides[u]	3/4/91	Rockpool	−23.85	−23.85	–

[1] Raven (1991)

Authorities:
[a] (Huds.) C. Ag.
[b] Stackh.
[c] L.
[d] (Huds.) Lamour.
[e] (Schmidel) O. Kuntz.
[f] (S.G. Gmel.) Rupr.
[g] (Huds.) Lyngb.
[h] (Stackh. in Withering) Guiry.
[i] (Huds.) Stackh.
[j] (L.) Lyngb.
[k] (L.) O. Kuntze
[l] (L.) Batt.
[m] (L.) Dixon
[n] (Bonnem.) Schmitz
[o] (Huds.) Grev.
[p] (L.) Tandy
[q] Greville
[r] (Roth.) C. Ag.
[s] (Huds.) C. Ag.
[t] (Dillw.) Nag.
[u] (Huds.) Silva

final pH achieved, (Fig. 1) there is a striking separation into two groups. One comprises six rhodophytes which have very negative δ^{13}C values (−30.0 to −34.5‰) and which fail to raise the pH above about pH 9.0. Since the calculated final concentration of free CO_2 is more than 1 mmol m^{-3} and one of these species, *Lomentaria articulata,* failed to photosynthesise faster than the maximal rate of supply of CO_2 from the dehydration of HCO_3^-, Maberly (1990) suggested that these species are restricted to free CO_2. More recent work (Johnston et al. 1992) found similar characteristics for another of these species *Delesseria sanguinea.* Although low rates of HCO_3^- uptake cannot be ruled out, these species largely rely on CO_2 as a carbon source for photosynthesis. The second group, comprising 26 species, display a range of abilities to raise pH and are all believed to be able to use HCO_3^- (Maberly 1990). Species in this group have average δ^{13}C values between −11.03 and −21.40‰ (Fig. 1). There is no apparent relationship between ability to raise pH, and hence deplete inorganic carbon, and the δ^{13}C of the plant within this group (linear regression of δ^{13}C against final pH was not significant).

In Tables 1, 2 and 3 δ^{13}C values are presented for 12 species for which there is no corresponding pH-drift data. Of these 9 *(Asperoccus fistulosus, Bryopsis plumosa, Chorda filum, Dilsea carnosa, Pilayella sp. Polyides rotundus, Polysiphonia sp. Porphyra purpurea, Rhodochorton floridulum)* have δ^{13}C values between −11.90 and

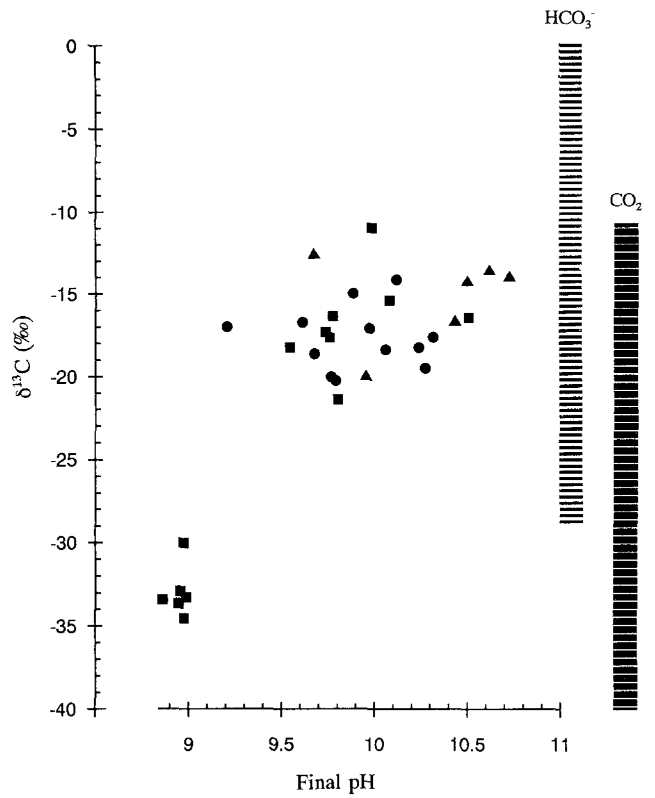

Fig. 1. Relationship between average $\delta^{13}C$ value (‰) for a species and final pH in drift experiments (Maberly 1990), for Chlorophyta (▲), Phaeophyta (●) and Rhodophyta (■). The *bars* to the right delimit the range of possible values for species restricted wholly to CO_2 or HCO_3^- (see text)

−20.27‰ and so fall within the range of species able to use HCO_3^-. *Rhodomela confervoides* is slightly more negative than this at −23.85‰, but still close to the range for HCO_3^-use. Two species, *Odanthalia dentata*, and *Plumaria elegans* with $\delta^{13}C$ values of −30.57‰ and −31.98‰ respectively fall within the range for species restricted to CO_2. These species are ecologically similar to the other species restricted to CO_2, except for *L. articulata*, as they are small, subtidal rhodophytes growing in low light. On the basis of their $\delta^{13}C$ values, we predict that these species are likely to be restricted to CO_2 as a source of inorganic carbon for photosynthesis.

Athough marine macroalgae using CO_2 and HCO_3^- appear to have different $\delta^{13}C$ values, there is a large degree of overlap in the values theoretically possible based on these two carbon sources. Thus the values for a species restricted to HCO_3^- with a source $\delta^{13}C$ value of 0.08‰ (see Appendix) could vary from 0.08‰ if all the HCO_3^- entering the cell without discrimination between ^{13}C and ^{12}C was fixed and there was no leakage, to −29‰ if the degree of leakage of pumped HCO_3^- was high (Sharkey and Berry 1985; Surif and Raven 1990) (Fig. 1). The possibility that HCO_3^--entry could yield even lower (more negative) $\delta^{13}C$ values, as a result of the combination of large fractional leakage of inorganic carbon and refractionation of the pumped inorganic carbon to yield internal CO_2 with a very low $\delta^{13}C$, is discussed further under "How are estimated variations in leakage reconciled with the measured photon cost?" below.

For a species restricted to CO_2 with a $\delta^{13}C$ of −10.64‰ (see Appendix) complete limitation of photosynthetic rates by RUBISCO carboxylation would produce carbon with a $\delta^{13}C$ of −39.6‰ (−10.6+ −29‰) while complete diffusion limitation would produce carbon with a $\delta^{13}C$ of −11.3‰ (−10.6+ −0.7‰) (Fig. 1). Based on this analysis, the $\delta^{13}C$ values for the species previously identified as being restricted to CO_2 are too negative to have been produced with a substantial amount of HCO_3^- as the carbon-source. Conversely, seven species have individual values where the $\delta^{13}C$ is more positive than the upper limit of −11.3‰ possible if CO_2 was the only source of inorganic carbon (Tables 1, 2 and 3), although the average value for a species is more positive for only one species, *Dumontia contorta* (Fig. 1). Three individuals of the seagrass *Zostera* cf. *angustifolia* (Hornem.) Rchb. from littoral mud at Tentsmuir had average $\delta^{13}C$ values of −9.96‰ (SD = 0.32‰) which also imply the ability to use HCO_3^-.

The difference between the mean value for putative CO_2-users of −32.95‰ (SD = 1.39‰) and −16.82‰ (SD = 2.48‰) for putative HCO_3^--users is 16.13‰. This is greater than the difference between the sources which is 10.72‰ at 10° C (Mook et al. 1974) which suggests that use of HCO_3^- reduces the discriminating effect of RUBISCO.

Limitation of photosynthesis by diffusion or carboxylation

The discrimination (Δ) can be calculated for plants restricted to CO_2 using Eq. 2 adapted so that the $\delta^{13}C$ value of the source is that for CO_2 calculated to be −10.64‰ at 10° C for seawater with a salinity of 35 kg m^{-3}, a carbonate alkalinity of 2.3 equiv m^{-3}, and in equilibrium with 350 μmol CO_2 mol^{-1} air at standard pressure (see Appendix). The value for the discrimination can be used to partition limitation of photosynthetic rate between diffusion and carboxylation using the equation in Raven and Farquhar (1990):

$$\Delta = 1 - [\alpha_{\mathrm{diffusion}}(C_o - C_i/C_o) + \alpha_{\mathrm{carboxylation}}(C_i/C_o)] \quad (3)$$

where $\alpha_{\mathrm{diffusion}}$ = ratio of diffusion coefficient of $^{12}CO_2$ in solution to that for $^{13}CO_2$ which has a value of 1.0007 (O'Leary 1984) and $\alpha_{\mathrm{carboxylation}}$ is the ratio of rate constants for $^{12}CO_2$ fixation to that for $^{13}CO_2$ fixation by RUBISCO in terms of dissolved CO_2 which has a value of 1.029 (Farquhar et al. 1989); C_o is the concentration of CO_2 in the external bulk solution; C_i is the concentration of CO_2 at the site of RUBISCO activity during steady-state photosynthesis. For the five species which appear to be restricted to CO_2 and excluding *L. articulata* with access to atmospheric CO_2, C_o equals 15.7 mmol m^{-3} (Appendix) and with Δ ranging from 0.0230 to 0.0247, C_i can be calculated to be between 0.789 and 0.849 times C_o and hence to be 12.36–13.29 mmol m^{-3}. Consequently, between 79 and 85% of the limitation of rate of photosynthesis can be attributed to carboxylation rather than diffusion. This may be the result of growth at low light, since Wiencke and Fischer (1990) have shown that this makes $\delta^{13}C$ values become more negative, possibly as a result of a decreased boundary-layer resistance, and hence increased expression of RUBISCO discrimination. For species which habitually grow in low light, this may reflect genotypic adaptation on the part of the alga to ensure that capacity to fix inorganic carbon per unit area is commensurate with the low rates of light absorption. These points are discussed in more detail below.

The Δ value of HCO_3-users: the role of "leakiness"

Our starting point here is the model of C_4 photosynthesis in relation to $^{13}C/^{12}C$ discrimination derived independently by Peisker (1982) and Farquhar (1983) and its modification by Sharkey and Berry (1985) for HCO_3^--using microalgae. Sharkey and Berry (1985) wrote (with some symbols modified to accord with our usage):

$$\Delta = d + (1 - \alpha_{\mathrm{carboxylation}} F_3/F_1) \qquad (4)$$

where Δ is defined in Eq. 2

d is the equilibrium discrimination associated with hydration of CO_2 (−10.72‰ at 10° C see Appendix)

$\alpha_{\mathrm{carboxylation}}$ is as defined in Eq. 3

F_3 is the unidirectional efflux of CO_2 out of the cell and

F_1 is the unidirectional influx of HCO_3^- into the cells.

F_2 $(=F_1 - F_3)$ is the rate of net CO_2 fixation by the illuminated cells; assuming that CO_2 release in respiratory and photorespiratory processes has the same $\delta^{13}C$ value as the organic substrate, F_2 can be effectively taken as the rate of fixation of CO_2 by RUBISCO as far as our analysis is concerned.

For the limiting case of $F_3 = 0$ (no "leakiness") $\Delta = d$, i.e. −10.72‰. With the value of $\delta^{13}C$ for dissolved CO_2 at 10° C of −10.64‰ (Appendix), the $\delta^{13}C$ is 0.08‰ (i.e. equivalent to the $\delta^{13}C$ of HCO_3^-). None of the $\delta^{13}C$ values which we measured were as positive as this; *Ulva lactuca* had a minimum value of −8.86‰, and *Enteromorpha intestinalis* −8.81‰, when growing in sheltered rockpools (Table 1). Application of Eq. 4 using $\alpha_{\mathrm{carboxylation}}$ of 1.029, yields a value for F_3/F_1 of 0.306 for *E. intestinalis* $(\Delta = -0.00183)$ and 0.308 for *U. lactuca* $(\Delta = -0.00178)$. At the other extreme, the most negative $\delta^{13}C$ value for HCO_3^-users which we measured was −22.55‰ for *Chondrus crispus* (Table 3). This yields a Δ value in terms of dissolved CO_2 of 0.01218 and an F_3/F_1 of 0.787. Recent work by Henderson et al. (1992) on 11 species of terrestrial C_4 plants has shown that discrimination by dry matter was greater than discrimination shown during on-line measurement of photosynthesis by, on average, 1.7‰. They suggest that this may result from non-photosynthetic processes, possibly discrimination during respiration. If this effect occurs in marine macroalgae, it will reduce our estimates of "leakiness".

This analysis assumes that HCO_3^--uptake completely saturates the carboxylation reactions of RUBISCO i.e. that F_1 is greater than F_2 so that there is an efflux rather than a diffusive influx of CO_2. The range of final pH values and $K_{1/2}$ for HCO_3^- exhibited by a range of marine macroalgae (Maberly 1990) suggests that there is room for variation in the efficiency of HCO_3^- uptake. Even for a species such as *Ulva fasciata* Delile, which appears to be an extremely effective user of HCO_3^-, Beer and Eshel (1983) estimate that up to 27% of carbon uptake could result from CO_2 rather than HCO_3^- uptake at air-equilibrium. The variation in $\delta^{13}C$ exhibited by species identified as being able to use HCO_3^- could result, in part, from a variation in the contribution of CO_2 and HCO_3^- to carbon-uptake under natural conditions. This view is strengthened by the relatively negative ^{13}C value of *Chondrus crispus* (Table 3), a low ability to raise pH in drift experiments and the inability of Smith and Bidwell (1989a, b) to demonstrate internal accumulation of inorganic carbon in fragments or protoplasts of this species using the silicone-oil technique. Furthermore, a linear regression of $\delta^{13}C$ against $K_{1/2}HCO_3^-$ using data from Maberly (1990) for 26 species able to use HCO_3^- revealed more negative $\delta^{13}C$ values for species with higher $K_{1/2}HCO_3$-, significant at the 5% level [$\delta^{13}C = a + b \times K_{1/2}HCO_3^-$; where $a = -11.62$ (SEM 2.04) and $b = -5.61$ (SEM 2.15)], suggesting a reduced reliance on HCO_3^- for species with negative $\delta^{13}C$ values. Further analysis of this possibility, and its implications for the analysis of the photon cost of "leakiness" (see below), is not possible with the data available.

How are estimated variations in leakage reconciled with the measured photon costs of photosynthesis?

Leakiness has implications for the photon cost of photosynthesis (Raven and Lucas 1985). Assuming that the HCO_3^--transport system yields an intracellular CO_2 concentration which completely suppresses the oxygenase activity of RUBISCO, then the cofactor requirements for the biochemistry of CO_2 fixation by the photosynthetic carbon reduction cycle are 3ATP and 2NADPH per CO_2 fixed. With an H^+/e^- of non-cyclic electron transport of 3, and an H^+/ATP of the ATP synthetase of 3, CO_2 fixation could proceed with 8 absorbed photons per mol CO_2 fixed since the 8 mol photons could yield 2 mol NADPH and 4 mol ATP. To this must be added the costs of HCO_3^- pumping. A minimum thermodynamic ATP cost of HCO_3^- influx is 0.5 mol ATP per mol C; if extra ATP is supplied by cyclic photophosphorylation with H^+/e^- and H^+/ATP of 3, each mol ATP produced needs 1.5 mol photons. *Enteromorpha* with F_3/F_1 equal to 0.306 has an ATP cost of HCO_3^- pumping of $(F_1/F_2) \times 0.5$, i.e. $[1/(1-0.306)] \times 0.5$ or 0.720 mol per net mol CO_2 fixed. Since the stoichiometry quoted for non-cyclic photophosphorylation has 1 mol extra ATP per mol CO_2 fixed, the ATP required for HCO_3^- influx could be accommodated within the 8 mol photons per mol CO_2 fixed. With the F_3/F_1 of 0.787 for *Chondrus*, the ATP requirement for HCO_3^- influx is $[1/(1-0.787)] \times 0.5$ or 2.35 mol ATP per mol (net) of CO_2 fixed. 1 mol of ATP could be supplied by

Table 4. Comparison of $\delta^{13}C$ values (‰) for marine macroalgae collected from rockpools or the sea. Values from Tables 1, 2 and 3

Species	Rockpool	Sea or intertidal	Average Rockpool-open sea
Ceramium rubrum	−13.09	−19.56	6.47
Codium fragile	−14.08, −10.22, −10.66	−15.38	3.73
Enteromorpha sp.	−9.36, −13.33, −8.81, −17.80	−20.30	7.97
Laminaria digitata	−19.64, −18.5	−17.8	−1.27
Laminaria hyperborea	−18.0	−16.0	−2.09
Ulva lactuca	−13.88, −8.86	−17.80	6.43

non-cyclic photophosphorylation within the 8 mol photon per mol CO_2 fixed mentioned above, leaving 1.35 mol ATP to be produced at a cost of a further 2.03 mol photon if cyclic photophosphorylation is employed. This gives a total cost of 10.03 mol photon per mol CO_2 fixed. Using the more mechanistically reasonable value of 1 mol ATP consumed per mol HCO_3^- the cost of HCO_3^- influx for *Enteromorpha* would be 1.44 mol ATP per mol net CO_2 fixed, with a total photon cost of $[8+(0.44\times1.55)]$ or 8.66 mol photons per mol CO_2 fixed, while for *Chondrus* the 4.7 mol ATP per mol net CO_2 fixed implies a total photon cost of $[8+(3.7\times1.5)]$ or 13.55 photons per mol CO_2 fixed.

These computations suggest that the varying degrees of leakiness implied by Eq. 4 for seaweeds with $\delta^{13}C$ values between −8.81‰ and −22.55‰ involve significant effects on the photon cost of net CO_2 fixation. Comparison with observed photon costs of O_2 evolution in marine macroalgae, including *Ulva lactuca* and *Chondrus crispus*, suggest that it is likely that the predicted photon cost of carbon fixation by *Chondrus* may be significantly greter than the photon cost of carbon fixation consistent with the photon cost of O_2 evolution (Lüning and Dring 1985). Until data are available on the photon cost of an alga of known $^{13}C/^{12}C$ discrimination in photosynthesis measured *during* the photon cost measurement by assay of extracellular carbon (see Sharkey and Berry 1985) no firm conclusions can be drawn, but the available data suggest that photon costs may be lower than those predicted from Δ values and the concept of leakiness. This possibility has been addressed by Raven (1990) who suggested that some of the inorganic carbon efflux could be via a catalysed, non-energy-requiring exchange of inorganic carbon, probably using the HCO_3^- influx mechanism. This suggestion is consistent with the large Δ value and the relatively low photon cost of photosynthesis by *Chondrus*, but the hypothesis has not been tested. It is of interest that Lüning and Dring (1985) also measured the photon cost of photosynthesis in *Delesseria sanguinea*, a seaweed which appears to use only CO_2 as its exogenous source of inorganic carbon, and found no significant difference from that of the HCO_3^- using algae. This suggests similar energy costs of HCO_3^- transport and of the photorespiration which occurs in its absence (see Raven 1984).

An implicit assumption of the model of Sharkey and Berry (1985) is that the $\delta^{13}C$ values of intracellular CO_2 and HCO_3^- are essentially equal. This must be effectively the case when $F_3=0$ (no leakiness in Eq. 4), but the condition is relaxed when the system has a finite value of F_3 and especially then the efflux involves HCO_3^- (see above). If the catalysis, by carbonic anhydrase, of HCO_3^- and CO_2 interconversion achieves both chemical *and* isotopic equilibrium the $\delta^{13}C$ of intracellular CO_2 would be 10.72‰ more negative than that of intracellular HCO_3^- (see Appendix). Even if the conversion of HCO_3^- to CO_2 is kinetically constrained the steady-state CO_2 pool would have a $\delta^{13}C$ value also about 10.72‰ more negative than HCO_3^- (O'Leary 1989). Such a circumstance would make the value of Δ larger for a given value of F_3/F_1 in Eq. 3a and would expand the HCO_3^- histogram of Fig. 1 to a lower (more negative) value. This point is taken up again below when considering literature values for marine macroalgal $\delta^{13}C$ values.

The finding, discussed later in relation to rockpools, that a seaweed generally has a less negative $\delta^{13}C$ in a less agitated than in a more agitated environment (Table 4), can be related to Eq. 4 for HCO_3^- using algae; all of the algae listed in Table 4 *can* use HCO_3^-. Raven and Johnston (1991b) suggest that a restricted inorganic carbon supply to the plant surface reduces Δ (as shown in Table 4) by increasing the extent of recycling of ^{13}C-enriched inorganic C effluxed from the alga (flux F_3; Eq. 4) *via* the influx F_1. This effect (Table 4) outweighs any effect of the restricted inorganic carbon supply to the algal surface in reducing F_1 relative to F_3 (Levavasseur et al. 1991).

Boundary-layer thickness

Raven et al. (1982) have demonstrated how Δ values can be used to calculate boundary layer thickness in freshwater macrophytes restricted to CO_2. Similar calculations for marine macroalgae (e.g. Surif and Raven 1990) are complicated by any contribution from HCO_3^- in these species. However, the apparent lack of ability of the rhodophyte *Delesseria sanguinea* to use HCO_3^- coupled with physiological and morphological measurements (Johnston et al. 1992) allows an estimate of boundary layer thickness to be made.

In order to translate Δ of 0.0235 into a boundary-layer thickness, the mean net rate of photosynthetic CO_2 influx over the photoperiod is needed. Kain (1984) quotes a maximum specific growth rate for *D. sanguinea* fronds off the Isle of Man, at the time of year at which we have physiological and morphological measurements, as $0.043\ d^{-1}$. The measurements of $0.386\ g\ C\ g^{-1}$ dry weight and a two-sided area of $0.195\ m^2\ g^{-1}$ dry weight

(Johnston et al. 1992) allow this to be converted to a rate of 1.383 mmol C m^{-2} d^{-1}. Since the respiration rate of the *D. sanguinea* fronds corresponds to 0.30 mmol C m^{-2} day^{-1} in a 12-h photoperiod (Johnston et al. 1992), the net rate of photosynthesis is 0.20 μmol C m^{-2} s^{-1}.

These values can be used to compute the diffusion boundary-layer thickness by applying Fick's equation for planar diffusion:

$$l = D_{CO_2}(C_o - C_i)/J \qquad (5)$$

where l = the diffusion boundary layer thickness/m; D_{CO_2} the diffusion coefficient for $CO_2/m^2 s^{-1}$ and J = net A^{CO_2} fixation rate/mol $m^{-2} s^{-1}$. With $D_{CO_2} = 1.3 \times 10^{-9}$ m^2 s^{-1} (Table 5.1 of Raven 1984), $(C_o - C_i) = 3.04$ mmol m^{-3} and $J = 0.20$ μmol m^{-2} s^{-1}, $l = 20$ μm. If the concentration of inorganic carbon in the bulk medium was half that at air-equilibrium, i.e. 7.8 mmol m^{-3} the boundary-layer estimate is reduced to 10 μm; if the concentration was twice that at air-equilibrium the estimate increases to 40 μm. Changes in growth rate from 0.033 to 0.053 day^{-1} will generate estimates of the boundary-layer thickness between 24 and 17 μm. Furthermore, if reserve organic material surviving overwinter is used in part to support growth, (cf. Lüning and Schmitz 1988), the estimate of thickness will be increased. Nevertheless, these estimates are not unreasonable for a boundary layer for a frond of *Delesseria* in its natural environment (cf. Macfarlane and Raven 1985, 1989, 1990).

Carbon depletion in rockpools

All but one of those samples with relatively positive $\delta^{13}C$ values were collected from rockpools. Many rockpools experience substantial depletion of inorganic carbon (e.g. Truchot and Duhamel-Jouve 1980; Maberly 1992), and if most of the available inorganic carbon is removed, the potential for discrimination will be reduced. This may be balanced to some extent by the resupply of inorganic carbon from CO_2 in the atmosphere which is more negative at −7.8‰ than the inorganic carbon in seawater at 0‰ although there is a further fractionation of about −1.13‰ at 10° C for dissolved versus gaseous CO_2 (Mook et al. 1974) which means that dissolved CO_2 derived from an atmospheric source will have a $\delta^{13}C$ of −8.93‰. The approximate magnitude of this effect can be gauged roughly for a hypothetical rockpool with an area of 1 m^2 and a volume of 0.25 m^3. Assuming a pH of 10.0 generated by carbon-depletion, a stagnant layer thickness of 1 mm and an atmospheric CO_2 concentration of 350 μmol mol^{-1}, then the chemically-enhanced rate of influx of CO_2 from the atmosphere can be estimated from the equations of Smith (1985) to be 2×10^{-5} mol m^{-2} s^{-1}. If pH 10 is maintained for 6 h (e.g. Maberly 1992) the total influx of CO_2 will equal 0.43 mol which compares with an initial carbon concentration of about 0.54 mol at air-equilibrium. The effect of any modification of the $\delta^{13}C$ in rockpools by invasion of $\delta^{13}C$ from the atmosphere on the $\delta^{13}C$ of organic carbon will depend *inter alia* on the extent of photosynthesis under the different conditions and the duration of carbon-depletion. Common denizens of rockpools such as *Ulva lactuca* and species of *Enteromorpha* are able to maintain high rates of photosynthesis even at pH 9.5 (Maberly 1990) and so could fix substantial amounts of carbon derived indirectly from the atmosphere.

A comparison of the six species where samples are available from rockpools and the open sea shows in all but two cases less negative values for samples from rockpools (Table 4). This indicates that combinations of the effect of carbon-depletion on preventing discrimination, increased diffusion limitation in the relatively calm waters of rockpools, and increased reliance on HCO_3^-; is larger than the effect of ^{12}C depletion of the source carbon by invasion of CO_2 from the atmosphere.

Carbon supply during photosynthesis in air

The arguments used above for the influence of the invasion of atmospheric CO_2 are even more relevant for a thallus photosynthesising in air and surrounded by only a thin film of seawater. In this case, almost all the available carbon for photosynthesis when emerged will be derived from atmospheric CO_2. Of the six species restricted to CO_2, 5 are subtidal and one, *L. articulata*, is intertidal. This species species has the least negative $\delta^{13}C$ of the group (Table 3). For a species restricted to CO_2 the source carbon (dissolved CO_2) derived from atmospheric CO_2 is more positive at about −8.93‰ (−7.8 + −1.13‰; Mook et al. 1974) than the source carbon in seawater at about −10.64‰ by about −1.71‰. Applying this difference to the mean $\delta^{13}C$ value for *L. articulata* of −30.04‰ (Table 3) gives it a value of −31.75‰ which is more similar to the mean for the other 5 species of −33.54‰. This analysis implies that photosynthesis in air is a major contributor to the carbon balance for this species; an analysis of the relative contribution of air and water to carbon uptake could only be made, however, if the relative limitation by diffusion and carboxylation is known for both media.

For a species restricted predominantly to HCO_3^-, the carbon for photosynthesis in air is still derived largely from atmospheric CO_2, given the low amount of inorganic carbon in the water-film, and so the source (HCO_3^-) has a $\delta^{13}C$ of +1.80‰ (−7.8 for atmospheric CO_2 +9.6‰, the isotope fractionation between HCO_3^- and atmospheric CO_2 at 10° C; Mook et al. 1974) which is slightly more positive than the source of HCO_3^- at 0.08‰ (assuming complete equilibrium between atmospheric CO_2 and water-film CO_2, and between water-film CO_2 and water-film HCO_3^- using extracellular carbonic anhydrase). Using the classic zonation sequence shown by different species of the Fucales, and excluding values for samples collected from rockpools, there is a trend for less negative $\delta^{13}C$ values with decreasing height on the shore (Table 2). Thus *Pelvetia canaliculata* at the top of the zonation sequence has a $\delta^{13}C$ of −20.12‰, *Fucus spiralis, Ascophyllum nodosum* and *F. vesiculosus* in the

Table 5. Literature values of marine macroalgae with $\delta^{13}C$ values lower than $-30‰$ when grown in seawater in nature or in culture

Alga	Growth in nature or culture	$\delta^{13}C + SD(‰)$	Reference
Chlorophyta: Ulvophyceae			
Caulerpa trifaria	nature	-32.36 ∓ 3.80	Ye et al. (1991)
Udotea petiolata	nature	-30.80	Dauby (1989)
Phaeophyta: Desmarestiales			
Desmarestia anceps	culture	-34.33	Wiencke & Fischer (1990)
Desmarestia antarctica	culture	-34.66	Wiencke & Fischer (1990)
Himantothallus grandifolius	culture	-37.64	Wiencke & Fischer (1990)
Phaeurus antarcticus	culture	-30.66	Wiencke & Fischer (1990)
Phaeophyta: Laminariales			
Laminaria digitata	culture	-37.52	Wiencke & Fischer (1990)
Laminaria solidungula	culture	-38.29	Wiencke & Fischer (1990)
Rhodophyta: Florideophyceae			
Ceramium rubrum	nature	-30.00	Dauby (1989)
Erythroclonium sp.	nature	-30.20 ∓ 0.22	Ye et al. (1991)
Plocamium angustum	nature	-30.90	Fenton & Ritz (1989)
unidentified	nature	-34.70	Fry et al. (1982)

middle of the shore have values of -18.40, -18.31 and $-19.50‰$ respectively, while *F. serratus* at the foot of the intertidal has a $\delta^{13}C$ value of $-17.60‰$. A similar decrease in $\delta^{13}C$ value with height on the shore is seen for *A. nodosum* (Raven et al. 1987). Increased exposure to air is correlated with more negative $\delta^{13}C$ values which implies that any effect of the $\delta^{13}C$ of the carbon source is less than the effect of other processes such as higher boundary-layer conductance, leading to a reduction in diffusion limitation, in air (Raven et al. 1987; Surif and Raven 1990). An alternative explanation is direct uptake of dissolved CO_2 at $-8.93‰$ derived from, air. However, we note that while the external carbonic anhydrase present in upper-shore algae (Giordano and Maberly 1989; Surif and Raven 1989) would enhance CO_2 movement from the gas-water interface to the cell surface by enlisting HCO_3^- as a diffusing inorganic carbon species, it would also (see above) increase the availability of HCO_3^- to transporters in the plasmalemma.

Comparison with data on other marine macroalgae

This brief discussion relates mainly to literature values of $\delta^{13}C$ of marine macroalgae which are less than $-30‰$ because they may indicate restriction to CO_2. Such low values are found in all three of the divisions contributing to the seaweed flora (Table 5). *Plocamium* is the only genus which our works suggests has species which could only use CO_2 under natural conditions; the $\delta^{13}C$ of *Plocamium angustum* is similar to that of *Plocamium cartilagineum* (Table 5). By contrast, the HCO_3^--using *Laminaria digitata* and *Ceramium rubrum* have $\delta^{13}C$ values in Table 5 which are substantially more negative than those reported in Tables 2 and 3. For the other organisms in Table 5 there appears to be no evidence as to their ability to use HCO_3^-. For *Udotea*, the relatively negative value of $\delta^{13}C$ for *Udotea petiolata* (Turra) Boergesen (Table 5) is not readily reconciled with the C_4-like metabolism reported for *Udotea flabellum* (Ellis et Solander) Howe by Reiskind et al. (1988) and Reiskind and Bowes (1991) assuming that the $C_3 + C_1$ carboxylase used in this alga, i.e. phosphoenolpyruvate carboxykinase (Reiskind and Bowes 1991) has little discrimination between ^{13}C and ^{12}C (Descolas-Gros and Fontugne 1990; Farquhar 1983; Peisker 1982).

The very low values for the Phaeophyta in Table 5 relate to culture at low photon flux densities (1.0–4.1 μmol photon $m^{-2}\,s^{-1}$), with the exception of *Phaeurus antarcticus* Skottberg where 50 μmol photon $m^{-2}\,s^{-1}$ was the only photon flux density tested, in air-equilibrated seawater at 0–10° C (Wiencke and Fischer 1990). Higher $\delta^{13}C$ values are found at higher photon flux densities for growth. Although the absolute $\delta^{13}C$ values are higher, the decreasing values of $\delta^{13}C$ with increasing depth of growth of populations of the seagrass *Posidonia oceanica* (L.) Delile (Cooper and De Niro 1989), the marine ulvophycean *Halimeda incrassata* (Ellis et Solander) Lamx. (Wefer and Killingley 1986) and the freshwater characean *Chara ceratophylla* Wallr. (Ziegler 1979) are also consistent with phenotypic decreases in $\delta^{13}C$ with decreasing photon flux density for growth. For the Phaeophyta with very negative values of $\delta^{13}C$ when grown at low photon flux densities and hence low specific growth rates (Wiencke and Fischer 1990), the arguments used above as to larger Δ values when supply of inorganic carbon to the plant surface is relatively less limiting could explain the reduced Δ values with reduced photon flux density when either CO_2 diffusion or HCO_3^- transport account for inorganic carbon entry. It is, however, of interest that many of the phaeophyte values are more negative than the lowest values for HCO_3^--using organisms shown in the histogram in Fig. 1; these low values include that for *Laminaria digitata*, an alga capable of using HCO_3^- under the conditions employed by Maberly (1990) and Surif and Raven (1989, 1990). Two possible

Table 6. Comparison of discrimination by freshwater macrophytes with known ability to use HCO_3^- or restricted to CO_2 using $\delta^{13}C$ or $\Delta^{13}C$ values (‰) in Osmond et al. (1980). Mean values are corrected for any effects of site after analysis of variance

Comparison	CO_2 $\delta^{13}C$	$\Delta^{13}C$	HCO_3^- $\delta^{13}C$	$\Delta^{13}C$	Difference	P
Comparison for 10 sites with both CO_2- and HCO_3^--users	−30.45 (15)	–	−24.65 (25)	–	5.80	$P < 0.001$
Comparison for 7 sites with $\delta^{13}C$ of source carbon	–	−28.42 (9)	–	−21.06 (17)	7.36	$P < 0.01$

explanations of such negative $\delta^{13}C$ values in a HCO_3^--using alga suggest themselves. One is that, when grown and examined at low photon flux densities, the HCO_3^--transport mechanism is partially repressed (see Beardall 1991) and the value for F_1 in Eq. 4 is relatively low, converting the alga into an organism relying on diffusive entry of CO_2. The second possibility is that, with low values of F_2 (Eq. 4) imposed by light limitation, both catalysed, energy-independent exchange fluxes of inorganic carbon across the plasmalemma (F_1, F_3; Eq. 4) and the carbonic anhydrase-catalysed interconversion of HCO_3^- and CO_2, proceed very rapidly relative to F_2, thus permitting very large Δ values without invoking diffusive entry of CO_2 (see above). In both cases, the fractional limitation of photosynthesis by diffusion of inorganic carbon to the whole plant is minimal.

It is clear, however, that different species of seaweed growing in the same light regime can have very different $\delta^{13}C$ values, implying that other factors such as differential use of CO_2 and HCO_3^- may be involved. One example is *Plocamium angustum* at 5 m depth off Tasmania with a $\delta^{13}C$ of −30.90‰, consistent with restriction to CO_2 as found for *Plocamium cartilagineum,* while co-occurring red, brown and green seaweeds had $\delta^{13}C$ values in the range −14.58‰ to −27.80‰ (Fenton and Ritz 1989) consistent with HCO_3^--use. Similarly, Ye et al. (1991) found that *Caulerpa trifaria* and *Erythroclonium* sp. had $\delta^{13}C$ values lower than −30‰, while the co-occurring *Ulva* sp. had a $\delta^{13}C$ of −19.82‰. For the data presented here, *Laurencia pinnatifida* and *Lomentaria articulata* were collected close together on 3/4/91 yet their $\delta^{13}C$ values differ by more than 10‰ (Table 3). Furthermore, seaweeds which occur both intertidally and subtidally can have similar $\delta^{13}C$ values. An example is *Palmaria palmata* where an intertidal population from St. Andrews had a $\delta^{13}C$ value of $-16.18+0.94$‰ (SEM; $n=4$) while a subtidal population (drift on *Laminaria hyperborea*) had a $\delta^{13}C$ value of $-15.32+0.69$‰ (SEM; $n=4$) (unpublished information).

CO_2 and HCO_3^- use by freshwater macrophytes

Data provided by Osmond et al. (1980) can be re-interpreted in the light of the difference between users of CO_2 and HCO_3^- found here, and the known ability or inability of many of the species studied to use HCO_3^- (e.g. Spence and Maberly 1985). An analysis of variance showed that for sites where both users of CO_2 and users of HCO_3^- are found the $\delta^{13}C$ of species restricted to CO_2 are significantly more negative by, on average, −5.8‰ (Table 6). For sites where the $\delta^{13}C$ of the carbonate was measured a comparison of $\Delta^{13}C$ for all the CO_2-users versus all the HCO_3^--users can be made, and again an analysis of variance showed that CO_2-users are more negative with a mean $\Delta^{13}C$ value of −28.4‰ than HCO_3^--users with a mean $\Delta^{13}C$ value of −21.1‰ by about −7.4‰ (Table 6). The difference between users of CO_2 and HCO_3^- is less than the difference between sources which is probably because of substantial use of CO_2 by freshwater macrophytes even when they have additional access to HCO_3^- (Allen and Spence 1981). A similar trend is apparent in the $\delta^{13}C$ values for three species in the Dichty Burn (Raven et al. 1987). *Lemanea mamillosa* Kützing and *Fontinalis antipyretica* Hedw. are restricted to CO_2 and have $\delta^{13}C$ values of −39.9 and −43.5‰ respectively while *Ranunculus penicillatus* (Dumort) Bab. var. *calcareous* (R.W. Butcher) which also has access to HCO_3^- is less negative at −22.5‰ although more recent work (Raven, unpublished) shows values closer to −30.0‰.

Acknowledgements. Work on inorganic carbon acquisition in marine algae by AMJ and JAR in Dundee is supported by NERC under GR3/7291; NERC also funded the VG SIRA II mass spectrometer under GR3/7967 to JAR and Prof. J.I. Sprent. Dr. C.M. Scrimgeour has given invaluable advice and assistance with mass spectrometric measurements.

Appendix

Calculation of concentration and $\delta^{13}C$ value of CO_2 in seawater

Assuming a $\delta^{13}C$ for seawater of 0‰, a temperature of 10° C and water in equilibrium with an atmosphere of 350 μmol CO_2 mol^{-1} at standard pressure, then the concentration of CO_2 will be 15.66 mmol m^{-3} using a value for the solubility of CO_2 in seawater of 45.2 mol m^{-3} atm^{-1} for a salinity of 35 kg m^{-3} (Skirrow 1975) and a water vapour pressure of 0.012 atm (Benson and Krause 1984). If the carbonate alkalinity of the seawater is 2.3 equiv m^{-3} then the pH of the water can be calculated to be 8.10 using values from the first and second dissociation constants (Goyet and Poisson 1989). At this pH CO_2 comprises 0.73% of the total inorganic carbon. Combining this with the equilibrium isotopic fractionation between dissolved CO_2 and dissolved HCO_3^- of −10.72 at 10° C (Mook et al. 1974), and assuming no fractionation between HCO_3^- and CO_3^{2-}, gives a $\delta^{13}C$ value of −10.64‰ for CO_2 and +0.08‰ for HCO_3^-.

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