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Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses

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Abstract. The literature, and previously unpublished data from the authors' laboratories, shows that the $\delta^{13}\text{C}$ of organic matter in marine macroalgae and seagrasses collected from the natural environment ranges from -3 to -35‰ . While some marine macroalgae have $\delta^{13}\text{C}$ values ranging over more than 10‰ within the thallus of an individual (some brown macroalgae), in other cases the range within a species collected over a very wide geographical range is only 5‰ (e.g. the red alga *Plocamium cartilagineum* which has values between -30 and -35‰). The organisms with very negative $\delta^{13}\text{C}$ (lower than -30‰) are mainly subtidal red algae, with some intertidal red algae and a few green algae; those with very positive $\delta^{13}\text{C}$ values (higher than -10‰) are mainly green macroalgae and seagrasses, with some red and brown macroalgae. The $\delta^{13}\text{C}$ value correlates primarily with taxonomy and secondarily with ecology. None of the organisms with $\delta^{13}\text{C}$ values lower than -30‰ have pyrenoids. Previous work showed a good correlation between $\delta^{13}\text{C}$ values lower than -30‰ and the lack of CO_2 concentrating mechanisms for several species of marine red algae. The extent to which the low $\delta^{13}\text{C}$ values are confined to organisms with diffusive CO_2 entry is discussed. Diffusive CO_2 entry could also occur in organisms with higher $\delta^{13}\text{C}$ values if diffusive conductance was relatively low. The photosynthesis of organisms with $\delta^{13}\text{C}$ values more positive than -10‰ (i.e. more positive than the $\delta^{13}\text{C}$ of CO_2 in seawater) must involve HCO_3^- use.

Keywords: carbon isotope discrimination, Phaeophyceae, Rhodophyta, seagrasses, Ulvophyceae.

Abbreviations used: CA, carbonic anhydrase; CAM, crassulacean acid metabolism; CCM, CO_2 concentrating mechanism; C_i , intercellular gas space CO_2 concentration; C_o , atmospheric CO_2 concentration; D , diffusion coefficient for CO_2 ; J , mean influx rate of CO_2 in the light phase during growth; $K_{0.5}$, substrate concentration at which enzyme achieves half of its substrate saturated rate of catalysis; l , diffusion pathlength for CO_2 /diffusion boundary layer, and within algae; P_{CO_2} , permeability coefficient of a membrane for CO_2 ; PEPC, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; pK_a , negative of the logarithm to the base of the dissociation constant of an acid; S_{rel} , selectivity factor of Rubisco for CO_2 relative to O_2 ; α , ratio of the rate of a process using a molecule containing a light isotope of an element to the rate of that process using a molecule containing a heavy isotope of the element; α_c , weighted mean average for the parallel operation of Rubisco and PEPC, expressed in terms of CO_2 as the substrate and with ^{12}C and ^{13}C as the isotopes; α_{ph} , value of α for diffusion of CO_2 in the aqueous phase with ^{12}C and ^{13}C as the isotopes.

Introduction

Studies of the natural abundance of carbon isotopes (^{13}C and ^{12}C) in terrestrial vascular plants have proved to be of great value in biochemical, physiological and ecological studies (Farquhar *et al.* 1989). The C_3 vascular land plants, relying on diffusion of CO_2 from the bulk atmosphere through the diffusion boundary layer, stomata, intercellular gas spaces, cell walls, and cytoplasm up to Rubisco, have organic matter that is significantly depleted in ^{13}C relative to the atmospheric CO_2 . One reason for this is the more rapid carboxylase activity of Rubisco with $^{12}\text{CO}_2$ than with $^{13}\text{CO}_2$ — α , the ratio of reaction rate with $^{12}\text{CO}_2$ to that with $^{13}\text{CO}_2$, is 1.029 for gaseous CO_2 and 1.030 for dissolved CO_2 (Farquhar *et al.* 1989; Raven and Farquhar 1990). However, there is a small (~ 5%) contribution of phosphoenolpyruvate carboxylase (PEPC) to the carbon in C_3 land plants (Farquhar *et al.* 1989; Raven and Farquhar 1990). Furthermore, the supply of CO_2 to Rubisco is restricted by the boundary layer, stomata, and intercellular gas spaces ($\alpha = 1.0044$ for CO_2 diffusion in the gas phase), and through the aqueous phase ($\alpha = 1.0007$ for CO_2 diffusion in the aqueous phase) (Farquhar *et al.* 1989; Raven and Farquhar 1990). These two effects (i.e. PEPC activity in parallel with Rubisco, and a diffusive conductance to CO_2 in C_3 plants which is about twice that of the carboxylase and subsequent CO_2 assimilation reactions) mean that the $^{13}\text{C}/^{12}\text{C}$ of C_3 plant organic matter is only ^{13}C -depleted by about $2/3$ – $3/4$ of the extent expected for activity of Rubisco (unconstrained by CO_2 diffusion) as the sole carboxylase (Farquhar *et al.* 1989, Raven and Farquhar 1990).

To relate these generalizations to the values for $^{13}\text{C}/^{12}\text{C}$ ratios that are commonly used in studies of the natural abundance of carbon isotopes, we use the $\delta^{13}\text{C}$ terminology:

$$\delta^{13}\text{C} = \left[\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{PDB}}} \right], \quad (1)$$

where ‘sample’ and ‘PDB’ refer to the unknown which is being analysed and the PDB standard (carbonate from the Cretaceous Pee-Dee formation), respectively. The $\delta^{13}\text{C}$ terminology is used because it is much easier to obtain accurate and precise measurements of the $^{13}\text{C}/^{12}\text{C}$ ratio relative to some standard than it is to determine absolute $^{13}\text{C}/^{12}\text{C}$ ratio. The $\delta^{13}\text{C}$ values can be referred to the source (atmospheric CO_2) $\delta^{13}\text{C}$ value by:

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

where ‘source’ and ‘plant’ refer to atmospheric CO_2 and bulk organic C in the plant, respectively. Δ values have the useful property of being related to α by:

$$\alpha = 1 + \Delta, \quad (3)$$

where α is the ‘effective’ discrimination factor operating

over the time period which yields the measured Δ value. The ‘effective’ discrimination is a weighted mean of the *in vivo* carboxylation discrimination (compounded of a predominant Rubisco and a minor PEPC component) and, in series, the discrimination due to CO_2 diffusion. The weighting of the α term for carboxylation, between the high α value for Rubisco and the lower value for PEPC, has been described by Farquhar *et al.* (1989), and Raven and Farquhar (1990). The weighting for the diffusion terms is based on analyses of gas exchange that yield values for the intercellular gas space CO_2 concentration (C_i), during steady-state photosynthesis in the normal atmospheric CO_2 concentration (C_o). If α_c is the weighted mean average α value for the two carboxylases, Rubisco and PEPC (≈ 1.027), and α_d relates to diffusion of CO_2 in the gas phase (≈ 1.0044), then the observed Δ is predicted from:

$$\Delta + 1 = \left[\alpha_d \times \frac{C_o - C_i}{C_o} \right] + \left[\alpha_c \times \frac{C_i}{C_o} \right]. \quad (4)$$

Measurements of C_o and C_i give good estimates of Δ , unless there is a large aqueous phase contribution to the diffusive limitation of photosynthesis. Alternatively, Δ can be used (with α_d , α_c and C_o) to estimate C_i (again with provisos about aqueous phase diffusion).

The measurements of Δ in C_3 vascular plants give important information on the plant’s photosynthetic behaviour over the period in which the organic C was acquired. The analysis can also be applied to astomatal C_3 land plants such as lichens, and moss, liverwort, hornwort (except those with CCMs), and pteridophyte gametophytes, although here the analysis in terms of diffusive and biochemical CO_2 conductances can be complicated by varying thicknesses of external water during a wetting-drying cycle for those (ectohydric) species which do not have a hydrophobic surface.

Terrestrial C_4 and (obligate) crassulacean acid metabolism (CAM) plants have much smaller Δ values than do terrestrial C_3 plants (Farquhar *et al.* 1989). The interpretation of the Δ values of terrestrial vascular C_4 and CAM plants is based on an initial carboxylation reaction catalysed by PEPC with a low α value, followed by decarboxylation of a derivative of the oxaloacetate produced by PEPC-activity releasing CO_2 into the Rubisco-containing compartment. A small leakage of CO_2 back to the bulk medium relative to the rate of pumping means a large fraction of the CO_2 entering the Rubisco-containing compartment is fixed by Rubisco and less opportunity for Rubisco to discriminate between $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ (i.e. a smaller value of Δ), while a more leaky system leads to higher values of Δ (Farquhar *et al.* 1989). Δ values for terrestrial C_4 plants have been used to estimate the extent of leakage of CO_2 from the bundle sheath cells (where Rubisco occurs), to relate this to the leaf anatomy of different C_4 subtypes and, with the estimated

ATP and NADPH costs of the C_3 - C_4 cycle in the C_4 subtype involved, to predict the photon cost of net CO_2 fixation (Farquhar *et al.* 1989).

Analogous uses of Δ apply to obligate CAM plants. For facultative CAM plants, the same analyses can be made provided that the plant is known to be performing CAM with a negligible C_3 component. When a facultative CAM plant is grown under conditions in which it is able to perform either CAM or C_3 photosynthesis, then the use of Δ is mainly to give an indication of the fraction of the sampled bulk organic C which has been assimilated by CAM, and the remainder which has been assimilated by C_3 photosynthesis (Farquhar *et al.* 1989).

This brief overview of the uses of the natural abundance of C isotopes in understanding C acquisition in land plants shows that much qualitative and quantitative information can be obtained from measurements of Δ values. Even more information can be obtained if the $^{13}C/^{12}C$ of individual molecules is examined, or if 'real-time' $^{13}C/^{12}C$ fractionation in photosynthesis is measured (via sampling of the gas phase in a gas exchange study), permitting estimation of (*inter alia*) the contribution of liquid-phase CO_2 conductance in determining the rate of photosynthesis under a given set of conditions. This very significant progress in the use of Δ values in higher plant photosynthesis studies has a number of causes. One is the excellent biochemical and physiological models of photosynthesis in higher land plants into which C stable isotope measurements can be integrated. Another is the constancy, world wide and over the lifetime of most plants, of the $^{13}C/^{12}C$ of the atmosphere, although CO_2 drawdown in canopies during the day and involvement of soil CO_2 in photosynthesis by plants of low stature can partially undermine the universality of the assumption of constant source $^{13}C/^{12}C$.

Application of $^{13}C/^{12}C$ natural abundance techniques to photosynthetic organisms from aquatic habitats is more complicated for a number of reasons (Raven and Farquhar 1990; Raven 1997). Fresh (more generally, inland) water habitats and their biota are especially difficult, with a very wide range of photosynthetic C acquisition mechanisms and of source $^{13}C/^{12}C$ ratios and concentrations (Osmond *et al.* 1981; Raven *et al.* 1982, 1994, 2000b; Keeley 1999). Inland water habitats have C_3 plants (*sensu stricto*, relying on CO_2 diffusion as the C supply to Rubisco), as well as C_4 and CAM vascular plants, while certain freshwater aquatic higher plants obtain most or all of their CO_2 from the root medium (Raven 1984). Many photolithotrophs of inland waters have CCMs based on the external conversion of HCO_3^- (but not CO_3^{2-} ; Maberly 1992) to CO_2 [catalysed by carbonic anhydrase (CA), or by acidification of parts of the surface of the organisms] with subsequent active or passive CO_2 uptake, as well as direct active uptake of HCO_3^- . The properties of water relative to air mean that diffusion boundary layers can be more important determinants of the

rate of photosynthesis in water than on land. Furthermore, there are large variations in the $^{13}C/^{12}C$ of inorganic C species in inland waters as a result of the variable inputs of ^{13}C -depleted C from the catchment as inorganic C from soil respiration and mineral weathering, and as organic C which can be oxidized to inorganic C in the water body (Raven *et al.* 1994, 2000b; Salata *et al.* 2000). These variations, and variability in the chemistry of inorganic C among inland water bodies and during the day or growth season in some water bodies, as well as the diversity of C acquisition mechanisms, greatly complicate the use of $^{13}C/^{12}C$ natural abundance data in the study of inorganic C acquisition by photolithotrophs in inland water bodies.

Some of the problems outlined for inland waters also apply to the study of photosynthesis in the sea using $^{13}C/^{12}C$ natural abundance measurements. Clearly, the general properties of water (rather than air) as a physical and chemical medium for inorganic C supply, apply to the sea as well as to inland waters. Although the equilibrium constants for the CO_2 - H_2CO_3 - HCO_3^- - CO_3^{2-} system are altered by the salinity of the medium, the values for seawater are within the range of inland waters, which covers a range of ion contents from freshwater up to salt contents an order of magnitude higher than that of seawater. Diel variations in inorganic C concentrations and in $^{13}C/^{12}C$ ratios of inorganic species are less marked in the sea (or large inland water bodies) than they are in small inland water bodies (or high intertidal marine rockpools at neap tides). There is also less diversity of inorganic C acquisition mechanisms in marine than in inland water photolithotrophs (Raven 1984, 1997).

As against these apparently simplifying properties of the marine habitat, it must be borne in mind that many marine macrophytes (macroalgae and seagrasses) are intertidal, and so are exposed to the air for some fraction of each day. There is no inland water analogue of this daily (usually twice) emersion. High intertidal organisms can obtain a significant fraction of their carbon from photosynthesis using atmospheric CO_2 while they are emersed, but still retain sufficient water for metabolism (Maberly and Madsen 1990; Madsen and Maberly 1990; Surif and Raven 1990; Mercado and Niell 1999, 2000; Peña *et al.* 1999; Raven 1999). Benthic marine macrophytes are also subject to varying water movement regimes as a function of the stage in the tidal cycle, with effects on supply of inorganic carbon (and other nutrients) via the thickness of the diffusion boundary layer (Smith and Walker 1980; Raven 1984; Wheeler 1988). A number of benthic marine macrophytes are rhizophytes, i.e. have attachment and absorptive organs in fine-grained substrates, rather than haptophytes that are attached to rocks or large stones (Raven 1981, 1984). Rhizophytes include almost all seagrasses and a number of warmer-water green macroalgae (e.g. many *Caulerpa* and *Halimeda* spp.). They obtain N and P, but probably not inorganic C, from the sediment (Raven 1981, 1984, 1998; Chisholm *et al.* 1996;

Chisholm and Jaubert 1997; Terrados and Williams 1997; Lee and Dunton 1999). Nutrient uptake by rhizoids (algae) or roots (seagrasses) in sediment has implications for selective pressures on the surface area per unit volume of the parts of the organism exposed to the water phase, and thus for inorganic C acquisition by the shoots (Raven 1981, 1984).

The range of $\delta^{13}\text{C}$ values in marine macrophytes

The values of $\delta^{13}\text{C}$ in seagrasses have been reviewed more frequently and more extensively (McMillan *et al.* 1980; Hemminga and Mateo 1996; Cambridge and Lambers 1998; Hemminga and Duarte 2000) than has been the case for seaweeds. This overview of the range of ^{13}C values emphasizes work on marine macroalgae, using data in the literature and previously unpublished data, including the data referred to as 'unpublished' in Tables 12 and 13 of Raven (1997). The sources of data used here are Black and Bender (1976), Fry *et al.* (1982), Fry (1984), Stephenson *et al.* (1984, 1986), Colman and Cook (1985), Kerby and Raven (1985), Wefer and Killingley (1986), Cook and Colman (1987), Raven *et al.* (1987, 1989, 1990a, b, 1995a, b, 1996, 2001a, b), Cooper and McRoy (1988), Fenton and Ritz (1988, 1989), Cooper (1989), Cooper and De Niro (1989), Raven (1990, 1997), Surif and Raven (1990), Wiencke and Fischer (1990), Lin *et al.* (1991), Raven and Johnston (1991), Ye *et al.* (1991), Durako and Hall (1992), Fischer and Wiencke (1992), Maberly *et al.* (1992), Raven and Osmond (1992), Simenstadt *et al.* (1993), Kübler and Raven (1994, 1995, 1996a, b), Grice *et al.* (1996), Hemminga and Mateo (1996), Brenchley *et al.* (1997, 1998), Cambridge and Lambers (1998), Hammer *et al.* (1998), Hemminga and Duarte (2000), Dunton (2001), Lee and Carpenter (2001), and Moncreiff and Sullivan (2001).

The values of $\delta^{13}\text{C}$ of organic matters of marine macrophytes vary from -2.7‰ for *Codium pomoides* (Chlorophyta: Ulvophyceae) from Sorrento, Vic, Australia (Raven 1997; Raven and Beardall 2002) to -35.3‰ for *Georgiella confluens* (Rhodophyta: Floridiophyceae) from Anvers Island off the Antarctic Peninsula (Dunton 2001).

Marine macrophytes with $\delta^{13}\text{C}$ less than -30‰

Taking $\delta^{13}\text{C}$ values lower than -30‰ as a convenient cutoff for excluding HCO_3^- use (Maberly *et al.* 1992), the data on seaweeds collected from natural habitats show that most of the marine macroalgae (47 species) with such low $\delta^{13}\text{C}$ values are in the class Floridiophyceae of the division Rhodophyta. The class Ulvophyceae of the division Chlorophyta has five known species with a $\delta^{13}\text{C}$ more negative than -30‰ , while in the class Phaeophyceae of the Heterokontophyta only two species are known to sometimes have $\delta^{13}\text{C}$ more negative than -30‰ .

The arguments for regarding marine algae with a $\delta^{13}\text{C}$ more negative than -30‰ as relying on diffusive CO_2

supply to Rubisco are given by Maberly *et al.* (1992). One argument is that, of the algae tested, only those with $\delta^{13}\text{C}$ values more negative than -30‰ (Maberly *et al.* 1992) were unable to increase the pH of seawater to 9.0 or greater in the experiments of Maberly (1990). This was shown for six of the algae tested; two algae with a $\delta^{13}\text{C}$ value -30‰ or less (*Odonthalia dentata* and *Plumaria elegans*) have not been examined in a pH drift experiment. All of the 26 species for which Maberly (1990) had shown final pH values in excess of 9.0, and which Maberly *et al.* (1992) examined, had $\delta^{13}\text{C}$ values much higher than -30‰ , i.e. ranging from -11.03 to -21.40‰ . The final pH argument of Maberly (1990) depends on the CO_2 compensation concentration for photosynthesis based on the S_{rel} (CO_2/O_2 selectivity factor) of Rubisco, and the ratio of CO_2 production in glycolate metabolism to O_2 uptake in phosphoglycolate synthesis. Raven *et al.* (2000a; see Badger *et al.* 1998, 2000) show that the properties of glycolate synthesis and metabolism in red algae might yield a slightly higher final pH value (and hence slightly lower CO_2 compensation concentration) than the C_3 higher plants on which Maberly (1990) and Johnston *et al.* (1992) based their interpretations. Accordingly, the pH drift interpretations of Maberly (1990) and Maberly *et al.* (1992) are sound, with respect to both the question of the inorganic C source and the mode of entry of the inorganic C.

The other argument used by Maberly *et al.* (1992) concerns the $\delta^{13}\text{C}$ value of source inorganic C (Mook *et al.* 1974; Zhang *et al.* 1995) relative to the algal organic C ^{13}C , the Δ value. Eqn (4) shows that, even with the C_i essentially equal to C_o and no account taken of ($\text{C}_3 + \text{C}_1$) anaplerotic carboxylases in parallel with Rubisco, and α_c value of 1.029, Δ relative to the inorganic C source cannot exceed 30‰. With HCO_3^- in seawater at a $\delta^{13}\text{C}$ value of $\approx 0\text{‰}$, the algal organic C cannot be more negative than -30‰ if HCO_3^- is the C source, regardless of the presence or absence of a CCM.

This analysis is subject to a number of conditions. Some of the confounding factors could only increase the $\delta^{13}\text{C}$ of the algal organic C, and so strengthen the case for sole CO_2 use. One such factor is the increased $\delta^{13}\text{C}$ of inorganic C in habitats where the rate of inorganic C uptake exceeds the rate of inorganic resupply. An example is a high intertidal rockpool during neap tides, where flushing with fresh seawater may not occur for several days on end, and CO_2 resupply from the atmosphere to CO_2 -depleted rockpool water is generally not adequate to maintain seawater levels of inorganic C (and of $\delta^{13}\text{C}$; Maberly *et al.* 1992). The red algae with low $\delta^{13}\text{C}$ values do not inhabit rockpools; they are subtidal shade-dwellers in the intertidal, or live in the high intertidal (Maberly *et al.* 1992; Raven *et al.* 1995b). However, subtidal red algae do occur in kelp forests (Lüning 1990) in which photosynthetic inorganic C use, in excess of inorganic C resupply from the atmosphere or by water flow through the forest, can decrease the free CO_2 to less than

10% of the air-equilibrium value, with a much smaller decrease in HCO_3^- concentration (Delille *et al.* 2000). Presumably, the $\delta^{13}\text{C}$ of inorganic C increases as the total inorganic C decreases as a result of photosynthetic uptake by *Macrocystis* (see Appendix), and the diffusion boundary layer thickness may increase as the dense kelp forest decreases water movement in the middle of the forest (Hurd 2000; Stevens *et al.* 2001). Another factor that could increase algal $\delta^{13}\text{C}$ is anaplerotic ($\text{C}_3 + \text{C}_1$) carboxylation via PEPC. The main $\text{C}_3 + \text{C}_1$ carboxylase in red algae is likely to be PEPC. It is less likely to be phosphoenolpyruvate carboxykinase (PEPCK; Raven *et al.* 1990), which would not significantly increase (and could slightly decrease) algal $\delta^{13}\text{C}$, so that ($\text{C}_3 + \text{C}_1$) carboxylase considerations are likely to increase algal $\delta^{13}\text{C}$.

Factors that may decrease $\delta^{13}\text{C}$ of algae include the conversion of photosynthate into lipids, with a low $\delta^{13}\text{C}$ and isotopic mass balance maintained by loss of CO_2 of higher $\delta^{13}\text{C}$. This possibility has apparently not been tested for marine red seaweeds. Another possible cause of decreased $\delta^{13}\text{C}$ is refixation of respired or photorespired CO_2 at the same $\delta^{13}\text{C}$ as bulk organic C (*cf.* the argument about lipids). Then refixation could decrease bulk organic C $\delta^{13}\text{C}$, with efflux of $\delta^{13}\text{C}$ -enriched CO_2 , since the high C_i/C_o allows much of the intrinsic discrimination of Rubisco to be expressed. As in land plants, it is not easy to quantify this effect. However, as far as photorespiration is concerned, the high S_{rel} of red algal Rubisco, combined with the higher plant-like photorespiratory carbon oxidation cycle (Raven *et al.* 2000a), means relatively less substrate for this refixation, and the high C_i/C_o value [eqn (4)] could favour efflux rather than refixation of CO_2 .

A further possible cause of more negative $\delta^{13}\text{C}$ values is a more negative $\delta^{13}\text{C}$ of external inorganic C. Inputs from estuaries have an inorganic C $\delta^{13}\text{C}$ more negative than seawater, as does the CO_2 produced during decomposition of mangrove leaves in coastal waters having limited exchange with the bulk seawater (Lin *et al.* 1991). The $\delta^{13}\text{C}$ of red algae (*Bostrychia*, *Caloglossa*, *Catanelia*) growing on mangrove stems and pneumatophores could be reduced by the $\delta^{13}\text{C}$ -depleted respired CO_2 of the mangroves (Raven *et al.* 1995b). However, the $\delta^{13}\text{C}$ values of *Bostrychia* spp. growing on mangrove pneumatophores are not significantly more negative than those growing on inorganic substrates, and the very closely related *Stictosiphonia* spp. from high intertidal rocks in New Zealand have similar $\delta^{13}\text{C}$ values to those of *Bostrychia* spp. (Raven *et al.* 1995b; Appendix).

Another factor is the fixation of atmospheric CO_2 . This applies only to *Lomentaria articulata* among the algae considered by Maberly *et al.* (1992), but is also important for *Bostrychia*, *Caloglossa*, *Catanelia* and *Stictosiphonia* (Raven *et al.* 1995b; Mercado and Niell 1999, 2000; Peña *et al.* 1999; Raven 1999). Maberly *et al.* (1992) point out that atmospheric CO_2 has a higher (by $\sim 1\%$, depending on

temperature) $\delta^{13}\text{C}$ than CO_2 in seawater, so that the use of atmospheric CO_2 would give a more positive $\delta^{13}\text{C}$ value. However, this effect might be outweighed by the higher diffusive conductance of the atmosphere relative to the aquatic boundary layer (Raven 1984). This point will be revisited when considering the quantitation of boundary layer thicknesses.

A further possibility for decreasing the $\delta^{13}\text{C}$ of the organism relative to source CO_2 is that of HCO_3^- entry, with intracellular conversion of HCO_3^- to CO_2 by CA. Here the $\delta^{13}\text{C}$ of the CO_2 produced from HCO_3^- could be 10.72‰ more negative than that of the HCO_3^- , regardless of whether the equilibration is complete or is kinetically limited, provided that there is essentially complete loss from the cells of the $\delta^{13}\text{C}$ -enriched residual HCO_3^- (Surif and Raven 1990; Maberly *et al.* 1992). Since the transplasmalemma potential difference in most marine algae is ~ 60 mV, inside negative (Raven 1984), in the absence of direct energization of HCO_3^- entry the internal HCO_3^- concentration in the steady state is only 1/10 that of the external concentration, and the equilibrium CO_2 concentration is 1/10 that resulting from only CO_2 fluxes at the plasmalemma. Energized HCO_3^- fluxes could, of course, increase the internal HCO_3^- (and hence CO_2) concentration, but the need for efflux of $\delta^{13}\text{C}$ -enriched HCO_3^- could have a very large energy cost unless much of the efflux is coupled to influx (via the HCO_3^- active transport mechanism) at no energy cost (Raven 1990; Maberly *et al.* 1992). Since the capacity to use HCO_3^- in red algae with very negative $\delta^{13}\text{C}$ values is apparently very limited (Maberly 1990; Johnston *et al.* 1992; Maberly *et al.* 1992; Mercado and Niell 1999, 2000), this mechanism is probably not relevant to red algae with very low $\delta^{13}\text{C}$ values.

A final possible factor contributing to a higher Δ (lower $\delta^{13}\text{C}$) of marine red algae is a larger α_c of Rubisco. While there are no *in vitro* α_c values for red algal Rubisco, *in vivo* estimates on freshwater red algae suggest an α_c value very similar to that of the higher plant value of 1.030 for dissolved CO_2 (Raven *et al.* 1994).

These attempts at quantitation of the various processes, other than those indicated in eqn (4), which could decrease the $\delta^{13}\text{C}$ (and increase Δ) of algae, show that eqn (4) still provides an acceptable quantitative description of Δ .

The notion that the marine red algae with very negative $\delta^{13}\text{C}$ values have C_3 physiology (Maberly *et al.* 1992) is based on pH drift data (Maberly 1990; Sherlock and Raven 2001), direct estimates of the CO_2 compensation concentration (Johnston *et al.* 1992; Mercado and Niell 1999, 2000; Sherlock and Raven 2001), and studies of O_2/CO_2 interactions in photosynthesis at different inorganic C concentrations (Mercado and Niell 1999, 2000; Sherlock and Raven 2001). The gas exchange measurements used *Bostrychia scorpioides* (Mercado and Niell 1999, 2000), *Delesseria sanguinea* and *L. articulata* (Johnston *et al.* 1992), and *Phycodrys rubens* and *P. cartilagineum*

(Sherlock and Raven 2001). While all of these data are consistent with C₃ physiology in these algae, Kübler *et al.* (1999) found no significant CO₂/O₂ interactions in a multifactorial (CO₂ and O₂ levels) growth experiment with *L. articulata*. While growth is less close to photosynthetic reactions than are short-term measurements of gas exchange, it would be expected that the CO₂/O₂ interactions would have some influence on growth (Kübler *et al.* 1999). Perhaps the variability in the growth experiments masked the expected O₂/CO₂ interactions that are small, as a result of the high S_{rel} values of red algal Rubisco, exacerbated by growth at low temperature (Sherlock and Raven 2001).

Before considering the quantitative implications for CO₂ diffusion of the δ¹³C values of the red algae with C₃ physiology, it is necessary to point out that a species of *Palmaria*, *P. decipiens*, has a variable, but sometimes low, δ¹³C value (Fischer and Wiencke 1992; Dunton 2001; Kübler and Hurd, data in Appendix), yet is able to use HCO₃⁻ and has a CCM (Beardall and Roberts 1999). The HCO₃⁻-use experiments were performed on Antarctic specimens of the alga, for which the higher δ¹³C values were obtained. The capacity to use HCO₃⁻ and exhibit a CCM had previously been shown for *P. palmata* (as *Rhodymenia palmata*) by Colman and Cook (1985) and Cook and Colman (1987), and confirmed by Maberly (1990), Maberly *et al.* (1992), and Kübler and Raven (1994, 1996a, b). Beardall and Roberts (1999) point out that the high δ¹³C value found for Antarctic *P. decipiens* by Fischer and Wiencke (1992) is consistent with HCO₃⁻ use. The much lower δ¹³C (-31.39‰) found by Kübler and Hurd (Appendix) from a drift specimen (from a subantarctic island?) in New Zealand, is only with difficulty accommodated by HCO₃⁻ use. It is of interest that the higher values for δ¹³C of *P. decipiens* (-9.7‰, Fischer and Wiencke 1992; -19.9‰, Dunton 2001) come from Antarctic specimens, as do the gas exchange data of Beardall and Roberts (1999).

Most of the data considered so far are consistent with the view that the low δ¹³C of certain subtidal, shaded intertidal, and high-intertidal red algae, is associated with diffusive CO₂ entry to Rubisco. It is possible to see if the measured growth rates (and hence C acquisition rates) *in situ* are consistent with diffusive CO₂ entry, using eqn (4) to compute the C_i/C_o weighted to the periods of major C gain. Maberly *et al.* (1992) used the δ¹³C of algal organic C in *D. sanguinea* (see also the slightly wider range of Δ values in Table 1), and the known value of C_o, to compute C_i from eqn (4). These values are then used in a version of Fick's equation, with the diffusion coefficient for CO₂ and an estimate of the rate of C gain during the growth period, to compute a diffusion pathlength through diffusion boundary layers and within the alga, i.e.

$$l = D(C_o - C_i)/J \quad (5)$$

where *D* is the diffusion coefficient for CO₂ (1.3 × 10⁻⁹ m² s⁻¹), *J* is the mean influx rate of CO₂ in the light phase during growth (mol m⁻² s⁻¹), and *l* is the diffusion pathlength for CO₂/diffusion boundary layer and within the algae (m).

For *J* the specific growth rate data of Kain (1984) were used, with conversion from dry matter to carbon influx on an area basis and correction for respiration at night using data of Johnston *et al.* (1992). Any losses of dissolved organic C were not accounted for. The data suggested [eqn (5)] an *l* value of 20 μm, including the diffusion boundary layer as well as the intracellular pathway. Raven and Beardall (2002) point out that the analysis by Maberly *et al.* (1992) does not take into account the low conductance of the plasmalemma indicated by the best currently available estimate, that for *Chlamydomonas reinhardtii* plasmalemma (Sültemeyer and Rinast 1996). Assuming the P_{CO2} of 1.5 × 10⁻⁵ m s⁻¹ from Sültemeyer and Rinast (1996) for both the plasmalemma and the inner plastid envelope membrane (the outer membrane has a higher P_{CO2} as a result of porins), the aqueous diffusion pathlength can only be 2.4 μm. This is unacceptably small as

Table 1. Specific growth rates, free CO₂ concentrations, and Δ values for freshwater and marine benthic red macroalgae

Organism	Habitat	Specific growth rate (d ⁻¹)	[CO ₂] (mmol m ⁻³)	Δ value (‰)	Reference
<i>Delesseria sanguinea</i>	Marine subtidal (often under kelp)	0.043	16	20.52–23.90	Kain (1984) Maberly <i>et al.</i> (1992) Appendix
<i>Odonthalia dentata</i>	Marine subtidal (often under kelp)	0.049	16	(6.37-) 12.59–21.60	Kain (1984) Maberly <i>et al.</i> (1992) Appendix
<i>Plocamium cartilagineum</i>	Marine subtidal (often under kelp)	0.026 – 0.039	16	19.06–24.71	Kain (1984) Maberly <i>et al.</i> (1992) Appendix
<i>Lemanea mamillosa</i>	Near surface in fast-flowing fresh water	≤ 0.51 (Dighty Burn)	66 (Dighty Burn) 49 (Ardeonaig Burn)	22.8–32.1 (Dighty Burn) 11.3–18.4 (Ardeonaig Burn)	MacFarlane and Raven (1989, 1990) Raven and Beardall (1981) Raven <i>et al.</i> (1982, 1987, 1994, 2000b)

an extracellular (cell wall plus diffusion boundary layer) diffusion pathlength. The P_{CO_2} of the plasmalemma and inner chloroplast membrane could be increased by the presence of those variants of aquaporins that can transport CO_2 (Nakhoul *et al.* 1998; Prasad *et al.* 1998). Such an increase in P_{CO_2} , together with intracellular CA facilitating inorganic C fluxes within the cytosol and/or stroma by permitting HCO_3^- fluxes as well as those of CO_2 , could increase CO_2 conductance within the cells (Raven and Glidewell 1981; Raven 1991; von Caemmerer and Evans 1991; Price *et al.* 1994; Evans and von Caemmerer 1996; Raven 1997) and leave 10–15 μm of diffusion boundary layer within the overall conductance to CO_2 . As Maberly *et al.* (1992) point out, a 10–15 μm diffusion boundary layer would be appropriate for the habitat of *D. sanguinea* in the subtidal.

Similar calculations may be made for *Plocamium cartilagineum*, for which Kain (1987) provides *in situ* specific growth rate data. Sherlock and Raven (2001) give respiratory rate and other relevant data to permit calculation of area-based values of J [eqn (5)]. Using C_o and C_i computed from Δ (Table 1) using eqn (4), very similar l values can be computed to those for *D. sanguinea* (Maberly *et al.* 1992). Kain (1984) also gives *in situ* specific growth rates for *O. dentata*, although no respiratory rate, surface area per unit fresh or dry matter measurements, are available. While similar values of l are likely using the lowest of the range of $\delta^{13}\text{C}$ values for *Odonthalia* (Table 1), the higher of the measured values for $\delta^{13}\text{C}$ (i.e. a lower Δ) yields a larger $(C_o - C_i)$ [eqn (4)], so that eqn (5) would yield a larger value of l (Table 1).

It is clear that the rate of light-saturated photosynthesis (rather than the photosynthetic rate during light-limited growth *in situ*) in seawater of red algae with low final pH values and $\delta^{13}\text{C}$ values yields, via eqns (4) and (5), unacceptably low values for l even if very high intracellular conductances to CO_2 are assumed. Light-saturated photosynthetic rates and final pH value, as well as *in situ* $\delta^{13}\text{C}$ values, are known for *D. sanguinea* and *L. articulata* (Maberly *et al.* 1992), and for *P. rubens* and *P. cartilagineum* (Sherlock and Raven 2001). Eqns (4) and (5) yield l values of only 3–6 μm : these are rather small values for diffusion boundary layers, and would be further lowered if a lower intracellular CO_2 conductance is assumed. Similar conclusions can be drawn for *Griffithsia ovalis* with a $\delta^{13}\text{C}$ lower than -30‰ , assuming that the photosynthetic rates at light saturation in seawater are similar to those for *G. flabelliformis* and *G. monile* (Lilley and Hope 1971). Finally, the data of Mercado and Niell (1999, 2000) for *B. scorpioides* also indicate rather low l values, based on light-saturated photosynthetic rates in seawater (or air). Of course, the ^{13}C values used here are from *in situ* rather than laboratory estimates. The freshwater red macroalga *Lemanea mamilliosa* has a higher specific growth rate *in situ*

than do the three marine red algae considered, at higher photon flux density and CO_2 concentration, and with an overlapping range of Δ values (Table 1).

For Antarctic algae, photosynthetic rates have been measured at 1°C , where the diffusion coefficient for CO_2 is lower but the CO_2 concentration is greater, as computed from the inorganic C system (higher pK_a values) and from equilibration with air (greater solubility; Beardall and Roberts 1999; J. Raven, A. Johnston, J. Kübler, R. Korb, S. McInroy, L. Handley, C. Scrimgeour, D. Walker, J. Beardall, M. Clayton, J. Chudek, M. Vanderklift, S. Fredricksen, K. Dunton, unpublished data). The only overlap of gas exchange measurements and $\delta^{13}\text{C}$ measurements are for *Phycodrys antarctica* (Drew 1977; Dunton 2001, noting that Drew was uncertain about the identity of the species which he referred to as *Pseudophycodrys* sp. but which could have been *P. antarctica*). Here again the $\delta^{13}\text{C}$ values indicate a rather small l value.

These data suggest that the light-saturated rate of photosynthesis and $(C_o - C_i)$ computed from *in situ* $\delta^{13}\text{C}$ values by red algae which rely on diffusive CO_2 entry, yield implausibly low l values. It is likely that the $(C_o - C_i)$ values are larger during light-saturated photosynthesis than those predicted from the Δ value for algae *in situ* during light-limited growth. This could be checked by measuring real-time $^{13}\text{C}/^{12}\text{C}$ discrimination during photosynthesis as a function of incident photon flux density. Data on marine macrophytes in nature (Wefer and Killingley 1986; Cooper and De Niro 1989; Durako and Hall 1992; Grice *et al.* 1996) and in the laboratory (Kübler and Raven 1994, 1995, 1996a, b) show that low photon flux densities correlate with lower $\delta^{13}\text{C}$ values. However, almost all of these data relate to organisms using CCMs (see Beardall 1991): the exception is *L. articulata* (Kübler and Raven 1994).

Another correlate of low $\delta^{13}\text{C}$ values in marine photolithotrophs is temperature, as seen with lower $\delta^{13}\text{C}$ values at higher latitudes in marine phytoplankton (Kopenskaya *et al.* 1995; Johnston and Kennedy 1998). This has been attributed to higher CO_2 concentrations in the colder, higher latitude waters, using a model relating Δ to CO_2 concentration and growth rate. However, this simple model fails to take into account the general occurrence of CCMs in microalgae. It is not clear if the $\delta^{13}\text{C}$ of the red algal species with $\delta^{13}\text{C}$ values less than -30‰ are even lower for populations growing at low temperatures. The red macroalgal species for which most data are available for $\delta^{13}\text{C}$ over a latitudinal range is *P. cartilagineum*, with data from 62°N to 33°N and from 37°S and 63°S (Appendix): there is no significant latitudinal trend. However, the sampling at the highest latitudes was relatively restricted, and there are significant non-latitudinal variations in seawater temperatures as a result of, for example, the North Atlantic Conveyer and the California Current.

Another aspect of the biogeography of red algae with diffusive CO₂ entry concerns their abundance in different environments. Again, the $\delta^{13}\text{C}$ data sets are not as comprehensive as would be wished, especially for low latitudes. The expectation that the diffusive CO₂ entry mechanism would be favoured at low temperatures comes from the increased air-equilibrium, and increased inorganic C system-equilibrium, CO₂ concentrations in cooler waters (J. Raven, A. Johnston, J. Kübler, R. Korb, S. McInroy, L. Handley, C. Scrimgeour, D. Walker, J. Beardall, M. Clayton, J. Chudek, M. Vanderklift, S. Fredricksen, K. Dunton, unpublished data). CO₂ diffusive entry would also be favoured by the increased S_{rel} of Rubisco at low temperature (at least for terrestrial higher plant Rubisco; Sherlock and Raven 2001), and by the decreased $K_{0.5}$ for the carboxylation function of Rubisco at low temperatures in some algae (Beardall and Roberts 1999). These three interacting factors, that make CO₂ diffusive entry at low temperatures more effective than at high temperatures, more than outweigh the effect of low temperature in decreasing the diffusion coefficient for CO₂ and the diffusion boundary layer thickness in water (Raven 1984; Beardall and Roberts 1999; J. Raven, A. Johnston, J. Kübler, R. Korb, S. McInroy, L. Handley, C. Scrimgeour, D. Walker, J. Beardall, M. Clayton, J. Chudek, M. Vanderklift, S. Fredricksen, K. Dunton, unpublished data). In this context, it is of interest that the data set of Dunton (Appendix) from the warmer-water habitat at 20–40 m depth in the Gulf of Mexico has two of the six species of red algae examined with a $\delta^{13}\text{C}$ at or equal to -30‰ (*Wrangelia* sp. and *Wrightiella blodgettii*), and that the deep-growing *Bostrychia* has a relatively high $\delta^{13}\text{C}$ value.

In terms of the number of species in a regional flora that have low $\delta^{13}\text{C}$ values (putative diffusive entry of CO₂), there are no data sets which have even half of the total number of red algal species. The most intensively examined flora is that of the North-East Atlantic, especially Scotland, England and Norway (Table 2). While we would not claim that our sampling (one-fifth of the total) was necessarily representa-

tive of the regional flora, it would appear that more than a third of the red algal species have $\delta^{13}\text{C}$ values more negative than -30‰ , and hence presumably diffusive CO₂ entry. However, in terms of biomass and productivity, the red algae with diffusive CO₂ entry probably contribute less to the total biomass and productivity of red algae than their species representation suggests. This conclusion is based, *inter alia*, on the preferential occurrence of low $\delta^{13}\text{C}$ species in low-light environments, which outweighs the effect of emersion in the light on intertidal, non-rockpool species. It is also clear that brown and green algae contribute less to the total number of species in the flora (about 79 and 76 species, respectively; Parke and Dixon 1976), but together dominate productivity and biomass. No brown or green algae examined from this area have $\delta^{13}\text{C}$ values less than -30‰ (Table 2).

A further potential indicator of the ecological role of the macroalgae with $\delta^{13}\text{C}$ values more negative than -30‰ , comes from $\delta^{13}\text{C}$ measurements on consumer organisms. The $\delta^{13}\text{C}$ of herbivores or detritivores is 1‰ or less different from the weighted mean $\delta^{13}\text{C}$ of the live or dead algae, and dead algae in various stages of decomposition have $\delta^{13}\text{C}$ values which are within 1‰ of that of the living algae (Fenton and Ritz 1989; J. Raven, unpublished results). This means that herbivores and detritivores that specialize (stenophagy) in the red algal species with very low $\delta^{13}\text{C}$ values, should themselves have very low $\delta^{13}\text{C}$ values. The same applies to specialist (stenophagous) carnivores that consume these grazers and decomposers. A potential complication is the presence of other food sources with $\delta^{13}\text{C}$ values of -30‰ or less. This is especially a problem at very high latitudes where phytoplankton $\delta^{13}\text{C}$ values can reach -30‰ (Rau *et al.* 1982; Johnston and Kennedy 1998; Dunton 2001), and algae relying on CCMs can also have $\delta^{13}\text{C}$ values as negative as -25 to -27‰ (Fischer and Wiencke 1992; Dunton 2001). At least the Antarctic does not suffer from problems of input of large quantities of terrestrial C₃ plant material with a $\delta^{13}\text{C}$ of -25 to -30‰ , as do many coastal regions at lower latitudes. Even with red macroalgae as the

Table 2. Occurrence of organisms with $\delta^{13}\text{C}$ values equal to or more negative than -30‰ among red, green and brown macroalgae in the North-East Atlantic (England, Scotland and Norway) (Maberly *et al.* 1992; Raven *et al.* 1994, 1995b; Raven 1997; Appendix)

Data on the number of species in the flora are from Parke and Dixon (1976) assuming that there are no species in Norway that do not also occur in Britain

Division/class	Total species in flora	Total species examined	Species with $\delta^{13}\text{C} \leq -30\text{‰}$	% Flora examined	% Species examined with $\delta^{13}\text{C} \leq -30\text{‰}$
Rhodophyta/ Bangiophyceae plus Florideophyceae	290	59	21	20.3	35.6
Heterokontophyta/ Phaeophyceae	199	28	0	14.1	0
Chlorophyta/ Ulvothamniophyceae	76	13	0	17.1	0

sole input of organic material with a $\delta^{13}\text{C}$ of less than -30% in the Antarctic, Fischer and Wiencke (1992) and Dunton (2001) did not find consumers with a $\delta^{13}\text{C}$ more negative than -30% , and the more negative $\delta^{13}\text{C}$ values for a consumer group were found for organisms from outside the benthic macroalgal beds, i.e. those dependent on phytoplankton rather than macroalgae. Clearly the impact of the productivity of red algae with low $\delta^{13}\text{C}$ values on consumer $\delta^{13}\text{C}$ is very small: any consumers specializing in these low $\delta^{13}\text{C}$ red algae were not sampled.

Grazers that are specific for one or a few species of red algae are sacoglossans (*Hermea* spp.) living on *Griffithsia* spp. The *Griffithsia* sp. examined has a $\delta^{13}\text{C}$ lower than -30% . *Hermea* is kleptoplastidic and fixes CO_2 photosynthetically using the *Griffithsia* plastids (Williams and Walker 1999). While *Griffithsia* organic matter that contributes to *Hermea* biomass reflects the $\delta^{13}\text{C}$ of *Griffithsia*, photosynthesis by plastids in the animals may discriminate less between ^{13}C and ^{12}C than those in the alga (see Raven *et al.* 2001a).

The cell structural correlates of low ^{13}C values in red algae are few. The only obvious one is that red algae with low $\delta^{13}\text{C}$ values never have pyrenoids (Raven 1997; Badger *et al.* 1998). However, while all algae with pyrenoids seem to be capable of expressing CCMs, some algae without pyrenoids can also express CCMs (Raven 1997; Badger *et al.* 1998; Morita *et al.* 1998, 2000).

The second, smaller category of marine macroalgae with very negative $\delta^{13}\text{C}$ values, are certain species of coenocytic green algae from tropical to temperate waters. Of the *Caulerpa* species examined, four species from Australia have $\delta^{13}\text{C}$ values below -30% , while three species from Australia and one from the USA have higher $\delta^{13}\text{C}$ values (Raven *et al.* 2001a; Appendix). Two species of *Udotea* have been examined; a Mediterranean species has a $\delta^{13}\text{C}$ less than -30% , while a species from Florida has a high $\delta^{13}\text{C}$ (Raven 1997a). There seem to be no gas exchange data (such as that obtained by Garcia *et al.* 1996) for the *Caulerpa* spp. with the most negative $\delta^{13}\text{C}$ values. Accordingly, we cannot say that the four *Caulerpa* spp. with low $\delta^{13}\text{C}$ values have different C acquisition mechanisms from the four species with higher $\delta^{13}\text{C}$ values. It would appear that the *Caulerpa* spp. with very low $\delta^{13}\text{C}$ values lack pyrenoids, while some of the others have pyrenoids (see Vroom *et al.* 1998). There are no measurements of the S_{rel} or $K_{0.5}\text{CO}_2$ of Rubisco in *Caulerpa* spp., but in other Ulvophyceae, the S_{rel} is lower than in higher land plants and the $K_{0.5}\text{CO}_2$ is higher (Raven 1997). *Caulerpa* spp. with low $\delta^{13}\text{C}$ values grow in low intertidal or subtidal locations (as do those with higher $\delta^{13}\text{C}$ values), i.e. some of them grow in rather high light levels. Furthermore, they grow in warm temperate waters, while the subtidal red algae with very low $\delta^{13}\text{C}$ values range from warm temperate to polar waters. Clearly, much more work is

needed to understand the high discrimination between ^{13}C and ^{12}C found in these four *Caulerpa* spp.

Caulerpa spp. with high and low $\delta^{13}\text{C}$ are grazed on by sacoglossan gastropods. Some of the plastids obtained by ingestion of the food alga are retained by the animal and continue to photosynthesise. Most evidence suggests that sacoglossans in nature are stenophagous, i.e. feed mainly on a single algal species (Williams and Walker 1999; Cavagnin *et al.* 2000; Raven *et al.* 2001a; cf. Trowbridge and Todd 2001). The $^{13}\text{C}/^{12}\text{C}$ ratio of the sacoglossan is the weighted mean of the $^{13}\text{C}/^{12}\text{C}$ ratio of the organic C derived directly from the food alga and the $^{13}\text{C}/^{12}\text{C}$ ratio of the organic C derived from photosynthesis by the kleptoplastids. If the kleptoplastids were fixing CO_2 with the same $^{13}\text{C}/^{12}\text{C}$ discrimination as the plastids *in situ*, then the sacoglossans would have the same $\delta^{13}\text{C}$ value as the food alga. However, this is never the case (Raven *et al.* 2001a), although the sacoglossans are clearly in the same light, exogenous inorganic C, and hydrodynamic environments as the food algae. Perhaps it is not surprising that the plastids behave differently in the sacoglossan relative to the alga, and much more work is needed to determine the causes of the differences.

In the case of *Udotea*, the Mediterranean *U. petiolatum* has a $\delta^{13}\text{C}$ of less than -30% , while the Caribbean *U. flabellum* has a much higher $\delta^{13}\text{C}$ (Raven 1997). Interpretation here is complicated by the occurrence of C_4 -like photosynthesis involving PEPCK as the initial carboxylase in the cytosol, in the only *Udotea* species, *U. flabellum*, which has been examined (see Raven 1997). This C_4 -like metabolism could explain the low $\delta^{13}\text{C}$ of *U. petiolata*, with diffusive CO_2 entry through a high conductance. However, the pathway of CO_2 fixation in *U. petiolata* has not been investigated, and *U. petiolata* has a high $\delta^{13}\text{C}$ value (Raven 1997). Further work is needed on this genus.

Marine macrophytes with $\delta^{13}\text{C}$ greater than -10%

At the other extreme are the marine macrophytes with $\delta^{13}\text{C}$ values more positive than -10% , i.e. with a higher $\delta^{13}\text{C}$ than can be accommodated by CO_2 use without discrimination in favour of $\delta^{13}\text{C}$. As well as some specimens of most of the seagrasses species tested, there are organisms with high $\delta^{13}\text{C}$ values in the brown, red, and green marine macroalgae (Appendix). Certainly the algae with high ^{13}C values that have been tested have the capacity to use HCO_3^- (Raven *et al.* 1995b; Raven 1997). These very positive $\delta^{13}\text{C}$ values can be considered in the context of many of the factors considered to influence very negative ^{13}C values.

It does not seem possible that diffusive CO_2 entry (Fig. 1 of Raven *et al.* 1995) could, even with inorganic C drawdown with increasing $^{13}\text{C}/^{12}\text{C}$ of inorganic C (see below), account for organic $\delta^{13}\text{C}$ in excess of -10% . It is just possible that a CCM based on active CO_2 uptake with minimal leakage of inorganic C could give an organic $\delta^{13}\text{C}$

value very close to that of the external CO_2 (see Sharkey and Berry 1986). With such small discrimination during CO_2 uptake, the extent of ^{13}C -enrichment of the residual external inorganic C would be small, so that imposing the same (negligible relative to CO_2) discrimination during uptake on the higher $^{13}\text{C}/^{12}\text{C}$ ratio in inorganic C would only give a small increase in organic $\delta^{13}\text{C}$ relative to the $\delta^{13}\text{C}$ of the original source CO_2 . All in all, the use of HCO_3^- , other than by catalysed external conversion to CO_2 , seems the most likely explanation of these very positive $\delta^{13}\text{C}$ values. Another possible cause of a high $\delta^{13}\text{C}$ value is ($\text{C}_3 + \text{C}_1$) carboxylation via PEPc. However, the extent of such fixation could not cause a $\delta^{13}\text{C}$ significantly lower than -10% , unless it was part of a C_4 -like photosynthetic mechanism, rather than simply being involved in anaplerotic inorganic C fixation (Raven 1997). No evidence of C_4 -like photosynthesis is known for any of the organisms with very high $\delta^{13}\text{C}$ values (Kerby and Raven 1985; Raven 1997).

The factors that could decrease $\delta^{13}\text{C}$ are those discussed for organisms with very low $\delta^{13}\text{C}$ values. Clearly any of these effects would mean that the underlying cause of the high organic C $\delta^{13}\text{C}$ value would have to be even more significant in increasing the $^{13}\text{C}/^{12}\text{C}$ value. The most likely explanation of very high $\delta^{13}\text{C}$ values is HCO_3^- uptake as part of a CCM, with very little inorganic C efflux relative to inorganic C influx or to the rate of carboxylation (Sharkey and Berry 1986; Keller and Morel 1999; Tortell *et al.* 2000).

These considerations apply to marine macrophytes growing in an essentially unrestricted supply of inorganic C. However, some marine macrophytes spend at least part of their time in a small volume of seawater relative to photosynthetic demand for inorganic C, and with very limited resupply of inorganic C. A habitat of this kind is in upper intertidal rockpools: at neap tides such rockpools are not flushed with fresh seawater for several days on end, and the rate of photosynthesis can very substantially exceed the rate of CO_2 invasion from the atmosphere. Under these conditions, the total inorganic C can be drawn down to less than half of the seawater concentration, with a corresponding increase in pH and a shift in speciation of inorganic C away from CO_2 and toward CO_3^{2-} . Under such conditions, the $^{13}\text{C}/^{12}\text{C}$ ratio of the inorganic C system increases, as photosynthesis removes inorganic C with a $^{13}\text{C}/^{12}\text{C}$ ratio less than that of the source inorganic C. This means that the $^{13}\text{C}/^{12}\text{C}$ of the macrophytes using HCO_3^- will be higher than that when growing in continuously replaced seawater, since the macrophytes will take up inorganic C with a $^{13}\text{C}/^{12}\text{C}$ ratio lower than that of the ^{13}C -enriched poolwater, but higher than what would be assimilated from bulk seawater. This is readily seen when HCO_3^- is transported into cells. The effect is qualitatively the same if CO_2 is used. It is likely that diffusive CO_2 entry in C_3 -physiology plants could not remove sufficient inorganic C from a rockpool to lower the inorganic C level and increase the inorganic C $^{13}\text{C}/^{12}\text{C}$ ratio

sufficiently to give a total inorganic C $\delta^{13}\text{C}$ in excess of -10% . Furthermore, the rate of photosynthesis by macrophytes relying on CO_2 diffusion is very low if diffusive restriction is high enough to give very low $^{13}\text{C}/^{12}\text{C}$ discrimination. It is more likely that $\delta^{13}\text{C}$ of macrophytes could exceed -10% if CO_2 use involved active CO_2 transport. For this to occur, the organism would have to remove CO_2 from seawater with negligible discrimination between $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$. Re-equilibration of HCO_3^- and CO_2 would produce CO_2 with rather more ^{13}C than the original CO_2 . The macrophyte $^{13}\text{C}/^{12}\text{C}$ would then gradually decrease in parallel with that of the pool water. Active CO_2 transport would allow more inorganic C removal than would diffusive CO_2 entry, and a greater potential for production of biomass with a $\delta^{13}\text{C}$ higher than -10% . The greater potential for inorganic C drawdown, pH increase, and decreased CO_2 concentration with HCO_3^- use than with CO_2 use, and the lower $^{13}\text{C}/^{12}\text{C}$ of CO_2 than of HCO_3^- , means that the potential for producing biomass with a $^{13}\text{C}/^{12}\text{C}$ more than -10% is not as great for organisms with active CO_2 transport as in those with active HCO_3^- transport. The order of likelihood of producing organic $^{13}\text{C}/^{12}\text{C}$ higher than -10% in a rockpool is CO_2 diffusion $<$ active CO_2 transport $<$ active HCO_3^- influx.

Similar arguments apply to non-rockpool intertidal algae with some seawater with its inorganic C left in the cell walls at low tide (e.g. some specimens of *Codium spongiosum*), although here the likelihood of use of atmospheric CO_2 with a constant $\delta^{13}\text{C}$ is greater than for rockpools. In these two cases in which photosynthesis is at the expense of a restricted volume of seawater, some of the photosynthesis takes place in bulk seawater, thereby reducing the potential for CO_2 use to give a macrophyte $^{13}\text{C}/^{12}\text{C}$ above -10% .

Marine macrophytes with $\delta^{13}\text{C}$ between -30 and -10%

The final, and largest, category of marine macrophytes are those with $\delta^{13}\text{C}$ between -30 and -10% , i.e. within the range in which both CO_2 and HCO_3^- use could occur without any special arguments being made (Maberly *et al.* 1992; Raven *et al.* 1995b; Raven 1997). These ^{13}C measurements cannot be used to distinguish HCO_3^- transport from CO_2 transport, or from a mixture of HCO_3^- and CO_2 use (Raven 1997). However, with other physiological information, the ^{13}C could be used to give insights into the extent of use of atmospheric CO_2 by intertidal organisms, involvement of diffusion restrictions in the photosynthesis of organisms which rely on CO_2 diffusion (rather few in this -30 to -10% category), and the extent of inorganic C efflux from organisms with CCMs based on active influx of CO_2 or on HCO_3^- .

Values for cyanobacterial symbioses with sponges (*Chondrilla*; *Cymbastella*: Cheshire *et al.* 1995) have $\delta^{13}\text{C}$ values in the -30 to -10% range. Inadequate knowledge of the value of α_c for cyanobacterial Rubisco, and the physiol-

ogy of symbiotic sponges (see Raven *et al.* 1995b; Raven 1997), hinders interpretation of these $\delta^{13}\text{C}$ values. However, Raven (2000) suggested that the water flow through the sponge caused by the choanocytes is involved in inorganic C supply to the cyanobionts, thereby possibly decreasing the $\delta^{13}\text{C}$ relative to what would occur without the water movement, as well as increasing the photosynthetic rate. The cyanobacterial intertidal lichen *Lichina*, with no flagella or enhanced water movement within the thallus, has a more positive $\delta^{13}\text{C}$. Raven *et al.* (1990a) showed that *Lichina* could use HCO_3^- in photosynthesis, and point out that the supply of HCO_3^- to the cyanobiont *Calothrix* through the mycobiont may limit photosynthesis in this lichen.

Comparison with freshwater macrophytes

Freshwater habitats have much greater variations in inorganic C $\delta^{13}\text{C}$, total inorganic C concentration, and pH, and hence inorganic C speciation, than do marine habitats, so that the interpretation of $\delta^{13}\text{C}$ values is even more complicated for freshwater macrophytes than for marine macrophytes (Raven *et al.* 1994, 2000b). Using Δ values relative to CO_2 as source [eqn (2)], some freshwater red macroalgae and bryophytes have Δ values in excess of 20‰ correlated with diffusive CO_2 influx, while the green algae and aquatic angiosperms with CCMs have lower Δ values (Osmond *et al.* 1981; Raven *et al.* 1982, 1994, 2000b; Keeley 1999). There are no examples of negative Δ values (equivalent to marine $\delta^{13}\text{C}$ values more positive than -10‰) in freshwaters, and no Δ values for aquatic angiosperms (e.g. many *Callitriche* species) that rely on diffusive CO_2 entry (Madsen and Maberly 1991).

Conclusions

The marine red macroalgae with $\delta^{13}\text{C}$ values lower than -30‰ are subtidal, shaded intertidal, or in the uppermost littoral, and those that have been tested in the laboratory rely on diffusive CO_2 entry. The growth rate *in situ* of the low $\delta^{13}\text{C}$ red algae which have been tested are consistent with diffusive entry of CO_2 with plausible diffusion pathlengths computed from the $\delta^{13}\text{C}$ measurements. Of the 20.3% of the North-East Atlantic red algal flora which have been examined, 21 species had very negative $\delta^{13}\text{C}$ values, while 38 had $\delta^{13}\text{C}$ values > -30‰. Most of the red algae in the North-East Atlantic with very low $\delta^{13}\text{C}$ values live at low photon flux densities at relatively low temperatures. These two environmental factors, combined with the kinetic characteristics of Rubisco of red algae, favour diffusive CO_2 entry. However, some red algae with diffusive CO_2 entry and very low $\delta^{13}\text{C}$ values live in warmer, high intertidal habitats, with significant CO_2 gain from the atmosphere.

Marine green macroalgae with $\delta^{13}\text{C}$ values lower than -30‰ are certain species of *Caulerpa* and *Udotea*. There is no evidence as to whether the *Caulerpa* spp. with very low $\delta^{13}\text{C}$ values have diffusive CO_2 entry, and the interpretation

of the data on *Udotea* spp. is complicated by the occurrence of C_4 -like metabolism.

Marine benthic organisms with $\delta^{13}\text{C}$ values more positive than -10‰ include at least some specimens of one or more species of seagrass, red algae, green algae, and brown algae, and the cyanobacterial lichen, *Lichina*. All of these organisms are able to use HCO_3^- , and the specimens with these very positive $\delta^{13}\text{C}$ values must be using this ability.

The great majority of species of marine benthic photolithotrophs have $\delta^{13}\text{C}$ values in the range -10 to -30‰. Here, the $\delta^{13}\text{C}$ value cannot distinguish between HCO_3^- and CO_2 use, or the presence or absence of a CCM, although other lines of evidence suggest that most of these organisms can use HCO_3^- and have CCMs. Furthermore, the $\delta^{13}\text{C}$ values can give additional information to that found by gas exchange and biochemical measurements.

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Appendix. Natural abundance $^{13}\text{C}/^{12}\text{C}$ ratios of marine macrophytes

Specimens which were calcified were treated with HCl prior to preparation for analyses. Methodology follows Maberly *et al.* (1992), Raven *et al.* (1994, 1995a, b, 1996, 2001a, b) and Dunton (2001). Asterisks indicate specimens for which the $\delta^{13}\text{C}$ is based on a single mass spectrometric estimate rather than measurements on duplicate samples

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
Green				
<i>Caulerpa cactoides</i>		Coobowie Bay, SA, Australia	1 July 1997	-21.35
<i>Caulerpa flexilis</i>		Coobowie Bay, SA, Australia	1 July 1997	-32.98
<i>Caulerpa microphysa</i>	Winter	West Flower Garden, Gulf of Mexico		-22.79
<i>Caulerpa microphysa</i>	Spring	West Flower Garden, Gulf of Mexico		-20.3
<i>Caulerpa microphysa</i>	Summer	Sonnier, Gulf of Mexico		-19.6
<i>Caulerpa microphysa</i>	Autumn	Stetson, Gulf of Mexico		-19.94
<i>Caulerpa microphysa</i>	Winter	Stetson, Gulf of Mexico		-20.97
<i>Caulerpa microphysa</i>	Winter	East Flower Garden, Gulf of Mexico		-19.99
<i>Caulerpa obscura</i>		Stragglers, WA, Australia	7 July 1999	-30.33 \pm 0.9 ($n = 3$)
<i>Caulerpa obscura</i>		Carnac Island, WA, Australia	14 January 2000	-30.18; -29.49
<i>Caulerpa obscura</i>		Hamelin Bay, WA, Australia	1 December 1999	-31.33 \pm 0.27 ($n = 4$)
<i>Caulerpa obscura</i>		Mewstone, WA, Australia	20 January 2000	-28.99; -28.59
<i>Caulerpa obscura</i>		Stragglers, WA, Australia	27 January 2000	-30.12; -28.39
<i>Caulerpa obscura</i>		The Lumps, WA, Australia	1 January 2000	-25.92; -31.38
<i>Chaetomorpha coliformis</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-14.6
<i>Chaetomorpha linum</i> *	From mussel beds	East Haven, Scotland, UK	13 May 1994	-14.73
<i>Chaetomorpha linum</i>	Drift	Eden Estuary, Scotland, UK	8 January 1995	-15.75; -15.52
<i>Chlorodesmis</i> sp.	Epiphyte on <i>Gracilaria</i> sp.	Singapore	1 April 1996	-16.88; -17.39
<i>Cladophora albida</i> *		Filey, England, UK	22-24 July 1994	-10.83
<i>Cladophora hutchinsonia</i> *		Filey, England, UK	22-24 July 1994	-12.15
<i>Cladophora rupestris</i>	Intertidal/attached	FifeNess, Scotland, UK	2 March 1991	-14.02
<i>Cladophora rupestris</i> *		Filey, England, UK	22-24 July 1994	-18.09
<i>Cladophora rupestris</i>	Emersed	East Sands, St Andrews, Scotland, UK	8 January 1995	-15.00 \pm 0.62 ($n = 3$)
<i>Cladophora rupestris</i>	Submersed	East Sands, St Andrews, Scotland, UK	8 January 1995	-15.37 \pm 1.11 ($n = 3$)
<i>Cladophora rupestris</i> *		Helmsdale, Scotland, UK	26 July 1998	-14.33
<i>Cladophora</i> sp.		Gran Canaria	1 February 1991	-16.12
<i>Codium convolutum</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-14.54
<i>Codium fragile</i>		CA, USA	1 August 1995	-11.70; -11.15
<i>Codium fragile</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-12.04
<i>Codium fragile</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-15.46
<i>Codium fragile</i> *		Helmsdale, Scotland, UK	26 July 1998	-12.87
<i>Codium fragile</i> *		St Andrews, Scotland, UK	31 May 1999	-13.26
<i>Codium hubsii</i> *		Catalina Island, CA, USA	1 February 2000	-8.17
<i>Codium pomoides</i>	HCl-treated	Sorrento, Vic, Australia	4 November 1995	-2.47 \pm 0.12 ($n = 4$)
<i>Codium</i> sp.	Sheltered rockpool	FifeNess, Scotland, UK	2 March 1991	-10.86
<i>Codium</i> sp.	Adherent	Gran Canaria	1 February 1991	-9.86
<i>Codium</i> sp.	Erect	Gran Canaria	1 February 1991	-14.47
<i>Codium</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-15.76
<i>Dictyosphaeria sericea</i>	HCl-treated	Nancy Cove, Rottneest Island, WA, Australia	10 April 1998	-6.33
<i>Enteromorpha intestinalis</i>		Eden Estuary, Scotland, UK	8 January 1995	-14.49; -13.70
<i>Enteromorpha linza</i>		Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-14.54
<i>Enteromorpha linza</i> *		Helmsdale, Scotland, UK	26 July 1998	-12.47
<i>Enteromorpha prolifera</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-17.3
<i>Enteromorpha</i> sp. 1		Gran Canaria	1 February 1991	-12.53
<i>Enteromorpha</i> sp. 2		Gran Canaria	1 February 1991	-15.41
<i>Enteromorpha</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-17.57 \pm 0.68 ($n = 3$)

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Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Enteromorpha</i> sp.	Emersed	East Sands, St Andrews, Scotland, UK	8 January 1995	-14.47 ± 0.13 (<i>n</i> = 3)
<i>Enteromorpha</i> sp.	Submersed	East Sands, St Andrews, Scotland, UK	8 January 1995	-12.33 ± 0.21 (<i>n</i> = 3)
<i>Enteromorpha</i> sp.		South Coast of Devon, UK		-10.98
<i>Enteromorpha</i> sp.		Kristineberg/Fiskebacksil, Sweden		-9.69
<i>Enteromorpha</i> sp.	HCl-treated	Singapore	1 April 1996	-19.78 ± 0.72 (<i>n</i> = 3)
<i>Enteromorpha</i> sp.*		Helmsdale, Scotland, UK	26 July 1998	-18.21
<i>Enteromorpha</i> sp. (<i>intestinalis</i>)*		Helmsdale, Scotland, UK	26 July 1998	-15.69
<i>Halimeda</i> sp.	HCl-treated	Gran Canaria	1 February 1991	-11.33
<i>Halimeda</i> sp.	HCl-treated	Singapore	1 April 1996	-6.83 ± 0.71 (<i>n</i> = 4)
<i>Monostroma</i> sp.		FifeNess, Scotland, UK		-13.39
<i>Prasiola stipitata</i>		St Andrews, Scotland, UK	5 October 1993	-18.71
<i>Rhipiliopsis peltata</i>		Coobowie Bay, SA, Australia	1 July 1997	-14.01
<i>Spongomorpha aeruginosa</i> *		Filey, England, UK	22–24 July 1994	-16.98
<i>Ulva lactuca</i> (<i>rigida</i>)		Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-15.93
<i>Ulva lactuca</i>	Drift	Eden estuary, Scotland, UK	8 January 1995	-11.05; -11.35
<i>Ulva lactuca</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-15.5
<i>Ulva lactuca</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-17.65
<i>Ulva</i> sp.		Gran Canaria	1 February 1991	-15.64
<i>Ulva</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-16.79
<i>Ulva</i> sp.		Singapore	1 April 1996	-17.72
<i>Ulva</i> sp.*		Helmsdale, Scotland, UK	26 July 1998	-15.91
<i>Valonia clavata</i>		Gran Canaria	1 February 1991	-14.83
Brown				
<i>Adenocystis utricularis</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-5.35
<i>Alaria esculenta</i> *		Helmsdale, Scotland, UK	26 July 1998	-16.44
<i>Alaria marginata</i>		Point Lobos and Big Sur, California, USA	28 June 1992	-13.32
<i>Ascophyllum nodosum</i>		Cobblers Brook, Newfoundland, Canada	17 July 1992	-13.37
<i>Ascophyllum nodosum</i>		FifeNess, Scotland, UK		-17.69
<i>Ascophyllum nodosum</i>	Vegetative tissue, not bearing	St Andrews, Scotland, UK	5 October 1993	-17.84
<i>Ascophyllum nodosum</i>	<i>Polysiphonia lanosa</i> Receptacle, not bearing	St Andrews, Scotland, UK	5 October 1993	-18.77
<i>Ascophyllum nodosum</i>	<i>Polysiphonia lanosa</i> Vegetative tissue, bearing	St Andrews, Scotland, UK	5 October 1993	-18.28
<i>Ascophyllum nodosum</i>	<i>Polysiphonia lanosa</i> Receptacles, one bearing	St Andrews, Scotland, UK	5 October 1993	-19.17
<i>Ascophyllum nodosum</i>		Arnolds Cove, Newfoundland, Canada	17 July 1992	-16.26 ± 1.11 (<i>n</i> = 3)
<i>Ascophyllum nodosum</i> *		Helmsdale, Scotland, UK	26 July 1998	-16.23
<i>Chondria dasyphylla</i>		South Coast of Devon, UK		-16.11
<i>Chorda filum</i>	Drift	Broughty Ferry, Scotland, UK	1 June 1992	-15.50 ± 0.46 (<i>n</i> = 4)
<i>Chorda filum</i>	Drift	St Andrews, Scotland, UK	19 July 1995	-14.54; -15.31
<i>Chorda filum</i>		East Haven, Scotland, UK	13 July 1995	-13.88
<i>Chorda filum</i> *		Helmsdale, Scotland, UK	26 July 1998	-18.18
<i>Cladostephus (verticillatus)</i> <i>spongiosus</i>		South Coast of Devon, UK		-21.44
<i>Cladostephus spongiosus</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-17.91
<i>Cladostephus spongiosus</i> *		Flamborough, England, UK	22–24 July 1994	-16.96
<i>Colpomenia peregrina</i>	HCl-treated	WA, Australia	1 April 1995	-6.14; -6.18
<i>Colpomenia perigrina</i> *		Filey, England, UK	22–24 July 1994	-11.51

Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Colpomenia</i> sp.		Gran Canaria	1 February 1991	-10.91
<i>Colpomenia</i> sp.	HCl-treated	Singapore	1 April 1996	-11.21; -11.68
<i>Cystophora scalaris</i>	Attached	Brighton/Papatowai Beach, New Zealand	1 February 1996	-13.4
<i>Cystophora torulosa</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-15.79
<i>Cystoseira nodicaulis</i>		South Coast of Devon, UK		-19.88
<i>Cystoseira osmundacea</i>		Point Lobos and Big Sur, CA, USA	28 June 1992	-16.76
<i>Cystoseira</i> sp.		Gran Canaria	1 February 1991	-18.77
<i>Cystoseira tamariscifolia</i>		South Coast of Devon, UK		-16.18
<i>Desmarestia aculeata</i>	Drift	Monifieth, Scotland, UK	12 September 1993	-23.87; -23.29
<i>Desmarestia aculeata*</i>	Drift	East Haven, Scotland, UK	13 May 1994	-21.65
<i>Desmarestia aculeata*</i>	W/hairs	Filey, England, UK	22-24 July 1994	-26.93
<i>Desmarestia aculeata*</i>	Smooth	Filey, England, UK	22-24 July 1994	-19.34
<i>Desmarestia aculeata</i>	Drift	St Andrews, Scotland, UK	19 July 1995	-19.64 \pm 0.58 ($n = 8$)
<i>Desmarestia aculeata</i>	Drift	East Haven, Scotland, UK	13 July 1995	-17.94 \pm 0.31 ($n = 28$)
<i>Desmarestia aculeata</i>		Broughty Ferry, Scotland, UK	20 November 1995	-25.34 \pm 0.76 ($n = 4$)
<i>Dictyomenia sonderi</i>	HCl-treated	WA, Australia	1 April 1995	-17.07; -17.44
<i>Dictyosiphon hippuroides*</i>		Flamborough, England, UK	22-24 July 1994	-13.24
<i>Dictyota cervicornis</i>	Spring	Stetson, Gulf of Mexico		-15.06
<i>Dictyota dichotoma</i>	Drift	North of Oban, Argyll, Scotland, UK	17 September 1994	-18.43; -19.68
<i>Dictyota dichotoma*</i>		Filey, England, UK	22-24 July 1994	-17.81
<i>Dictyota dichotoma</i>		Finnoy, Norway		-17.49
<i>Dictyota menstrualis</i>	Winter	West Flower Garden, Gulf of Mexico		-19.05
<i>Dictyota menstrualis</i>	Spring	Stetson, Gulf of Mexico		-17.26
<i>Dictyota menstrualis</i>	Autumn	Stetson, Gulf of Mexico		-19.45
<i>Dictyota menstrualis</i>	Winter	Stetson, Gulf of Mexico		-17
<i>Dictyota pfaffi</i>	Winter	Stetson, Gulf of Mexico		-19.6
<i>Dictyota pfaffi</i>	Winter	East Flower Garden, Gulf of Mexico		-15.38
<i>Dictyota pulchella</i>	Spring	Stetson, Gulf of Mexico		-17.72
<i>Dictyota menstrualis</i>	Winter	East Flower Garden, Gulf of Mexico		-19.25
<i>Dilophus</i> sp.	Spring	Stetson, Gulf of Mexico		-16.75
<i>Durvillaea antarctica</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-14.09
<i>Durvillea antarctica</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-12.32
<i>Durvillaea willana</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-15.61
<i>Durvillea willana</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-12.59
<i>Ectocarpus siliquosa</i>		Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-15.26
<i>Ecklonia radiata</i>		Carnac Island, WA, Australia	14 January 2000	-19.26 \pm 0.72 ($n = 6$)
<i>Ecklonia radiata</i>		Hamelin Bay, WA, Australia	1 December 1999	-18.10 \pm 0.28 ($n = 8$)
<i>Egregia menziesii</i> 1	Stipe, HCl-treated	CA, USA	1 August 1995	-17.38
<i>Egregia menziesii</i> 1	Bladder, HCl-treated	CA, USA	1 August 1995	-16.61
<i>Egregia menziesii</i> 2	Stipe, HCl-treated	CA, USA	1 August 1995	-14.27
<i>Egregia menziesii</i> 2	Whiskers	CA, USA	1 August 1995	-19.35
<i>Egregia menziesii</i> 1	Blade, HCl-treated	CA, USA	1 August 1995	-17.55
<i>Elachista fucicola*</i>		Filey, England, UK	22-24 July 1994	-15.38
<i>Fucus serratus</i>		FifeNess, Scotland, UK		-18.54
<i>Fucus serratus*</i>	Vegetative	Flamborough, England, UK	22-24 July 1994	-16.23
<i>Fucus serratus*</i>	Reproductive	Flamborough, England, UK	22-24 July 1994	-14.37
<i>Fucus serratus*</i>		Helmsdale, Scotland, UK	26 July 1998	-16.69
<i>Fucus serratus*</i>	Vegetative	Filey, England, UK	22-24 July 1994	-15.34
<i>Fucus serratus*</i>	Reproductive	Filey, England, UK	22-24 July 1994	-15.91
<i>Fucus spiralis</i>		FifeNess, Scotland, UK		-17.72

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Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Fucus spiralis</i>		FifeNess, Scotland, UK	26 July 2000	-17.91
<i>Fucus spiralis</i>	Fertile	Cobblers Brook, Newfoundland, Canada	17 July 1992	-13.7
<i>Fucus spiralis</i>	Sterile	Cobblers Brook, Newfoundland, Canada	17 July 1992	-10.67
<i>Fucus spiralis</i> *		Helmsdale, Scotland, UK	26 July 1998	-16.88
<i>Fucus vesiculosus</i>		FifeNess, Scotland, UK		-17.58
<i>Fucus vesiculosus</i>	Bearing <i>Polysiphonia lanosa</i>	St Andrews, Scotland, UK	5 October 1993	-18.32
<i>Fucus vesiculosus</i>		Arnolds Cove, Newfoundland, Canada	17 July 1992	-15.37
<i>Fucus vesiculosus</i>	On rock, mainly receptacles	Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-16.32
<i>Fucus vesiculosus</i>		Hampton Bay, Long Island, NY, USA	16 November 1994	-15.00; -16.17
<i>Fucus vesiculosus</i> *		Helmsdale, Scotland, UK	26 July 1998	-15.12
<i>Giffordia granulosa</i>		Flamborough, England, UK	22-24 July 1994	-16.81
<i>Halidrys dioica</i> 'Black'	HCl-treated	CA, USA	1 August 1995	-12.18
<i>Halidrys dioica</i> 'Red'	HCl-treated	CA, USA	1 August 1995	-13.91
<i>Hesperophycus harveyanus</i>		Point Lobos and Big Sur, CA, USA	28 June 1992	-12.17
<i>Hesperophycus harveyanus</i> *		Catalina Island, CA, USA	1 February 2000	-15.22
<i>Himantalia elongata</i>	Vegetative	Flamborough, England, UK	22-24/7/94	-16.22
<i>Himantalia elongata</i>	Reproductive	Filey, England, UK	22-24/7/94	-13.5
<i>Himantalia elongata</i>	Reproductive	Flamborough, England, UK	22-24/7/94	-10.44
<i>Himantalia elongata</i> *		Helmsdale, Scotland, UK	26 July 1998	-15.61
<i>Himantalia elongata</i> *		FifeNess, Scotland, UK	23 May 1998	-17.76
<i>Hormosira banksii</i>	Subtidal, attached	Coobowie Bay, SA, Australia	1 July 1997	-10.88
<i>Hormosira banksii</i>	Intertidal, attached, HCl-treated	Coobowie Bay, SA, Australia	1 July 1997	-10.19
<i>Hormosira banksii</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-11.09
<i>Laminaria digitata</i> *		Helmsdale, Scotland, UK	26 July 1998	-15.49
<i>Laminaria hyperborea</i> blade*		Kingsbarns, Scotland, UK	31 May 1999	-15.91; -14.06
<i>Laminaria hyperborea</i> *	Juvenile on holdfast of <i>Laminaria hyperborea</i>	Kingsbarns, Scotland, UK	31 May 1999	-20.35
<i>Laminaria hyperborea</i> *		Helmsdale, Scotland, UK	26 July 1998	-18.28
<i>Laminaria saccharina</i> *		Helmsdale, Scotland, UK	26 July 1998	-17.12
<i>Leathesia intermedia</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-11.4
<i>Lobophora variegata</i>	Summer	West Flower Garden, Gulf of Mexico		-16.71
<i>Lobophora variegata</i>	Spring	West Flower Garden, Gulf of Mexico		-15.87
<i>Lobophora variegata</i>	Autumn	West Flower Garden, Gulf of Mexico		-15.74
<i>Lobophora variegata</i>	Winter	West Flower Garden, Gulf of Mexico		-16.09
<i>Lobophora variegata</i>	Summer	Sonnier, Gulf of Mexico		-16.28
<i>Lobophora variegata</i>	Spring	Sonnier, Gulf of Mexico		-14.78
<i>Lobophora variegata</i>	Autumn	Sonnier, Gulf of Mexico		-16.68
<i>Lobophora variegata</i>	Autumn	Stetson, Gulf of Mexico		-17.55
<i>Lobophora variegata</i>	Summer	East Flower Garden, Gulf of Mexico		-15.12
<i>Lobophora variegata</i>	Autumn	East Flower Garden, Gulf of Mexico		-16.27
<i>Lobophora variegata</i>	Winter	East Flower Garden, Gulf of Mexico		-16.92
<i>Macrocystis pyrifera</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-16.61
<i>Macrocystis pyrifera</i>	Blade	Point Lobos and Big Sur, CA, USA	28 June 1992	-15.78
<i>Macrocystis pyrifera</i> 1	Blade, HCl-treated	CA, USA	1 August 1995	-15.98
<i>Macrocystis pyrifera</i> 1	Bladder, HCl-treated	CA, USA	1 August 1995	-14.37
<i>Macrocystis pyrifera</i> 2	Young stipe	CA, USA	1 August 1995	-12.21
<i>Macrocystis pyrifera</i> 2	Young blade	CA, USA	1 August 1995	-11.12
<i>Macrocystis pyrifera</i> 2	Young stipe	CA, USA	1 August 1995	-20.73
<i>Macrocystis pyrifera</i> 2	Young blade	CA, USA	1 August 1995	-19.25
<i>Macrocystis pyrifera</i> 2	Young holdfast	CA, USA	1 August 1995	-20.58

Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Macrocystis pyrifera</i> 2	Young bladder	CA, USA	1 August 1995	-11.61
<i>Macrocystis pyrifera</i> 2	Young bladder	CA, USA	1 August 1995	-19.58
<i>Macrocystis pyrifera</i> *		Catalina Island, CA, USA	1 February 2000	-18.4
<i>Nereocystis luetkeana</i>	Blade	Point Lobas and Big Sur, CA, USA	28 June 1992	-15.19
<i>Padina sanctae-crucis</i>	Spring	Stetson, Gulf of Mexico		-13.21
<i>Padina</i> sp.	Subtidal, HCl-treated	Gran Canaria	1 February 1991	-10.61
<i>Padina</i> sp.	intertidal, HCl-treated	Gran Canaria	1 February 1991	-9.77
<i>Pelagophycus porra</i> 1	Young stipe	CA, USA	1 August 1995	-23.67
<i>Pelagophycus porra</i> 1	Young blade	CA, USA	1 August 1995	-28.91
<i>Pelagophycus porra</i> 1	Young holdfast	CA, USA	1 August 1995	-26.06
<i>Pelagophycus porra</i> 1	Young bladder	CA, USA	1 August 1995	-24.8
<i>Pelvetia canaliculata</i>		FifeNess, Scotland, UK	26 July 2000	-18.66
<i>Pelvetia canaliculata</i> *		Helmsdale, Scotland, UK	26 July 1998	-18.39
<i>Pelvetia fastigiata</i>	Now <i>Silvetia fastigiata</i>	Point Lobas and Big Sur, CA, USA	28 June 1992	-11.82
<i>Petalonia</i> sp.		Gran Canaria	1 February 1991	-14.05
<i>Pilayella</i> sp.	On <i>Ascophyllum nodosum</i>	Arnolds Cove, Newfoundland, Canada	17 July 1992	-16.18
<i>Rosenvignea intricata</i>	Spring	Stetson, Gulf of Mexico		-15.92
<i>Saccorhiza polyschides</i> 1	Blade	Isle of Man, UK	18 June 1996	-15.15
<i>Saccorhiza polyschides</i> 1	Stipe	Isle of Man, UK	18 June 1996	-18.56
<i>Saccorhiza polyschides</i> 1	Holdfast	Isle of Man, UK	18 June 1996	-14.72
<i>Saccorhiza polyschides</i> 2	Blade	Isle of Man, UK	18 June 1996	-19
<i>Saccorhiza polyschides</i> 2	Stipe	Isle of Man, UK	18 June 1996	-19.67
<i>Saccorhiza polyschides</i> 2	Holdfast	Isle of Man, UK	18 June 1996	-17.76
<i>Sargassum aghardhianum</i> *		Catalina Island, CA, USA	1 February 2000	-19.97
<i>Sargassum muticum</i>		South Coast of Devon, UK		-19.67
<i>Sargassum</i> sp.		Port Aransas, TX, USA	29 April 1997	-17.36 \pm 0.33 ($n = 3$)
<i>Sargassum</i> sp.		Singapore	1 April 1996	-16.93; -17.28
<i>Sargassum vulgare</i>		Gran Canaria	1 February 1991	-15.82
<i>Scytosiphon</i> sp.		Gran Canaria	1 February 1991	-25.62
<i>Scytosiphon</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-14.07
<i>Scytothamnus australis</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-18.36
<i>Silvetia fastigiata f. gracilis</i> *		Catalina Island, CA, USA	1 February 2000	-23.33
<i>Sphacelaria</i> sp.*		Helmsdale, Scotland, UK	26 July 1998	-17.85
<i>Splachnidium rugosum</i>		Sorrento, Vic, Australia	4 November 1995	-10.69 \pm 0.42 ($n = 4$)
<i>Splachnidium rugosum</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-13.28
<i>Splachnidium rugosum</i>	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-11.27
<i>Xiphophora chondrophylla</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-15.05
Red				
<i>Aglaothamnion sepositum</i>		Finnoy, Norway		-28.96
<i>Ahnfeltia plicata</i>		Finnoy, Norway		-20.82
<i>Ahnfeltia plicata</i> *		Flamborough, England, UK	22-24 July 1994	-17.59
<i>Apophlaea lyallii</i> *	Attached ?	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-11.32
<i>Asparagopsis armata</i>	Rhizoid, HCl-treated	Strickland Bay, Rottneest Island, WA, Australia	10 April 1998	-29.71
<i>Asparagopsis armata</i>	Stipe, HCl-treated	Strickland Bay, Rottneest Island, WA, Australia	10 April 1998	-29.55
<i>Asparagopsis taxiformis</i> *		Catalina Island, CA, USA	1 February 2000	-28
<i>Audouinella floridula</i> *		Flamborough, England, UK	22-24 July 1994	-17.13
<i>Bonnemaisonia hamifera</i>		South Coast of Devon, UK		-28.98
<i>Bostrychia</i> sp.	Winter	East Flower Garden, Gulf of Mexico		-21.03
<i>Bostrychia</i> sp.	Plus epiphytes, HCl-treated	Singapore	1 April 1996	-28.61

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Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Brongniartella byssoides</i> *		Filey, England, UK	22–24 July 1994	–26.48
<i>Callithamnion corymbosum</i>		Bergen, Norway		–30.42
<i>Callithamnion tetragonum</i> *		Filey, England, UK	22–24 July 1994	–24.82
<i>Callithamnion tetragonum</i>		Finnoy, Norway		–30.47
<i>Callophyllis cristata</i>		Bergen, Norway		–33.61
<i>Callophyllis laciniata</i>		Finnoy, Norway		–34.12
<i>Callophyllis laciniata</i>		Bergen, Norway		–34.29
<i>Callophyllis calliblephroides</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–16.71
<i>Callophrysus hombroneana</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–31.86
<i>Catenella caespitosa</i>		Bergen Espeland, Norway		–29.51
<i>Ceramium rubrum</i>		Bergen, Norway		–18.29; –18.29
<i>Ceramium shuttleworthianum</i>		Bergen Store Kalsoy, Norway		–19.26
<i>Ceramium shuttleworthianum</i> *		Flamborough, England, UK	22–24 July 1994	–19.13
<i>Chondrus crispus</i>		Finnoy, Norway		–22.66
<i>Chondrus crispus</i> *		Flamborough, England, UK	22–24 July 1994	–18.09
<i>Chondrus</i> sp.		Arnolds Cove, Newfoundland, Canada	17 July 1992	–19.95
<i>Chylocladia verticillata</i>		Bergen Store Kalsoy, Norway		–10.22
<i>Corallina officinalis</i>		Finnoy, Norway		–11.67
<i>Corallina pinatifolia</i> *	HCl-treated	Catalina Island, CA, USA	1 February 2000	–7.45
<i>Corallina vancouverensis</i> *	HCl-treated	Catalina Island, CA, USA	1 February 2000	–5.69
<i>Cryptopleura ramosa</i> *		Flamborough, England, UK	22–24 July 1994	–31.64
<i>Cystoclonium purpureum</i> *		Flamborough, England, UK	22–24 July 1994	–13.88
<i>Dasya</i> sp.		Nancy Cove, Rottnest Island, WA, Australia	10 April 1998	–24.55
<i>Delesseria sanguinea</i>		East Haven, Scotland, UK	14 July 1995	–32.59 ± 0.26 (n = 4)
<i>Delesseria sanguinea</i>	Midribs, HCl-treated	Llandudno, Wales, UK	1 October 1996	–31.51 ± 0.50 (n = 3)
<i>Delesseria sanguinea</i>		Finnoy, Norway		–32.37
<i>Delesseria sanguinea</i>		Bergen, Norway		–33.74
<i>Delesseria sanguinea</i> *		Flamborough, England, UK	22–24 July 1994	–32.09
<i>Dilsea carnosa</i>		Finnoy, Norway		–22.75
<i>Dilsea carnosa</i> *		Filey, England, UK	22–24 July 1994	–14.33
<i>Dumontia contorta</i> *		East Haven, Scotland, UK	13 May 1994	–13.16
<i>Dumontia contorta</i>		South Coast of Devon, UK		–19.1
<i>Dumontia contorta</i> *		Flamborough, England, UK	22–24 July 1994	–14.98
<i>Dumontia contorta</i>		Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	–13.74
<i>Echinothamnion lyallii</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–22.32
<i>Endarachne bighameiae</i> *		Catalina Island, CA, USA	1 February 2000	–15.95
<i>Euptilota formosissima</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–33.12
<i>Furcellaria lumbricalis</i>		Finnoy, Norway		–24.87
<i>Furcellaria lumbricalis</i> *		Flamborough, England, UK	22–24 July 1994	–17.1
<i>Gelidiopsis</i> sp.	Autumn	Stetson, Gulf of Mexico		–19.68
<i>Gelidium latifolium</i>		Bergen Store Kalsoy, Norway		–15.86
<i>Gigartina acicularis</i>		South Coast of Devon, UK		–14.51
<i>Gigartina circumcincta</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–21.68
<i>Gigartina clavifera</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	–12.56
<i>Gigartina clavifera</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–14.51
<i>Gigartina dilitata</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	–19.1

Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Gigartina livida</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-15.73
<i>Gigartina livida</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-16.18
<i>Gigartina marginifera</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-16.15
<i>Gracilaria chilensis</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-17
<i>Gracilaria gracilis</i>		Bergen Kviturvikspollen, Norway		-17.87
<i>Gracilaria secundata</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-14.69
<i>Gracilaria</i> sp.	HCl-treated	Singapore	1 April 1996	-21.84 ± 0.23 (n = 8)
<i>Gracilaria</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-17.22
<i>Grateloupia</i> sp.		Gran Canaria	1 February 1991	-12.54
<i>Halilptilon cuvieri</i>	As <i>H. roseum</i> , alias <i>Jania</i> <i>cuvieri</i>	Nancy Cove, Rottneest Island, WA, Australia	10 April 1998	-11.96
<i>Halopitys uncinatus</i>		South Coast of Devon, UK		-19.41
<i>Halurus equisetifolius</i>		South Coast of Devon, UK		-28.81
<i>Heterosiphonia concinna</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-28.98
<i>Heterosiphonia</i> sp.	Sand contaminated	Nancy Cove, Rottneest Island, WA, Australia	10 April 1998	-14.99
<i>Hildenbrandia</i> sp.	From rockpool	Broughty Ferry, Scotland, UK	2 March 1991	-18.04
<i>Hymenena palmata</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-29.79
<i>Hymenena durvilleai</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-28.8
<i>Hypnea volubilis</i>	Spring	Stetson, Gulf of Mexico		-19.42
<i>Hypnea volubilis</i>	Winter	Stetson, Gulf of Mexico		-21.09
<i>Hypnea volubilis</i>	Winter	East Flower Garden, Gulf of Mexico		-18.56
<i>Hypnea</i> sp.		Gran Canaria	1 February 1991	-21.75
<i>Iridaea flaccida</i>		Point Lobos and Big Sur, CA, USA	28 June 1992	-19.01
<i>Jania micrarthandia</i>		Nancy Cove, Rottneest Island, WA, Australia	10 April 1998	-22.55
<i>Jania rubens</i>		South Coast of Devon, UK		-12.57
<i>Laurencia pinnatifida</i>		South Coast of Devon, UK		-16.39
<i>Laurencia pinnatifida</i>		Bergen Store Kalsoy, Norway		-9.72
<i>Laurencia hybrida</i>		South Coast of Devon, UK		-13.55
<i>Laurencia hybrida</i> *		Filey, England, UK	22-24 July 1994	-14.57
<i>Laurencia pinnatifida</i>	Intertidal/attached	Broughty Ferry, Scotland, UK	2 March 1991	-15.98
<i>Laurencia pinnatifida</i>		Finnoy, Norway		-9.92
<i>Laurencia pinnatifida</i> *		Flamborough, England, UK	22-24 July 1994	-11.73
<i>Lomentaria articulata</i>		Finnoy, Norway		-31.87
<i>Lomentaria articulata</i> *		Flamborough, England, UK	22-24 July 1994	-28.23
<i>Lomentaria articulata</i> *		Filey, England, UK	22-24 July 1994	-29.41
<i>Lomentaria articulata</i> *		Helmsdale, Scotland, UK	26 July 1998	-24.25
<i>Lomentaria articulata</i> *		St Andrews, Scotland, UK	31 May 1999	-29.22
<i>Lomentaria</i> sp.	Intertidal/attached	Broughty Ferry, Scotland, UK	2 March 1991	-29.04
<i>Mastocarpus argardhii</i> (?)		Point Lobos and Big Sur, CA, USA	28 June 1992	-13.04
<i>Mastocarpus</i> sp.		Arnolds Cove, Newfoundland, Canada	17 July 1992	-20.89
<i>Mastocarpus stellata</i> *		Fife Ness, Scotland, UK	23 May 1998	-19.76
<i>Mastocarpus stellatus</i>		Finnoy, Norway		-19.14
<i>Melanosiphon</i> sp.		Arnolds Cove, Newfoundland, Canada	17 July 1992	-17.55
<i>Membranoptera alata</i>		East Haven, Scotland, UK	14 July 1995	-32.53; -30.77
<i>Membranoptera alata</i>	Drift	Broughty Ferry, Scotland, UK	20 November 1995	-31.8

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Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Membranoptera alata</i>	Drift	Barnhill, Scotland, UK	25–26 January 1996	-32.95 ± 0.37 ($n = 3$)
<i>Membranoptera alata</i>		South Coast of Devon, UK		-19.07
<i>Membranoptera alata</i>		Finnoy, Norway		-33.156; -32.09
<i>Membranoptera alata</i> *		Helmsdale, Scotland, UK	26 July 1998	-31.25
<i>Membranoptera alata</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-34.43; -33.51
<i>Membranoptera alata</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-32.69; -33.99
<i>Metamastophora flabellata</i>	Could be mixed species	Nancy Cove, Rottneest Island, WA, Australia	10 April 1998	-15.04
<i>Microzonia velutina</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-18.92
<i>Myriogrammae crispata</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-31.06
<i>Nitophyllum punctatum</i>		Finnoy, Norway		-30.55
<i>Nitophyllum punctatum</i>	Carposporic	South Coast of Devon, UK		-32
<i>Nitophyllum punctatum</i>	Tetrasporangic	South Coast of Devon, UK		-32.16
<i>Odonthalia dentata</i>	Drift	Isle of Man, UK	18 June 1996	-25.85; -23.21
<i>Odonthalia dentata</i>		Finnoy, Norway		-28.8
<i>Odonthalia dentata</i>		East Haven, Scotland, UK	14 July 1995	-24.83
<i>Odonthalia dentata</i> *		Flamborough, England, UK	22–24 July 1994	-22.94
<i>Odonthalia dentata</i> *		Fife Ness, Scotland, UK	23 May 1998	-16.90
<i>Odonthalia dentata</i> *		East Coast, Scotland, UK	3 January 1999	-26.10
<i>Odonthalia dentata</i> *		Kingsbarns, Scotland, UK	31 May 1999	-31.03
<i>Osmundaria prolifera</i>		Coobowie Bay, SA, Australia	1 July 1997	-25.98
<i>Pachymenia lusoria</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-14.08
<i>Pachymenia lusoria</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-13.64
<i>Palmaria decipiens</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-31.39
<i>Palmaria palmata</i>	Intertidal/attached	Broughty Ferry, Scotland, UK	2 March 1991	-17.82
<i>Palmaria palmata</i>	Drift	Barnhill, Scotland, UK	25–26 January 1996	-17.65
<i>Palmaria palmata</i>		Finnoy, Norway		-19.82
<i>Palmaria palmata</i>		Bergen, Norway		-20.9
<i>Palmaria palmata</i>	On <i>Laminaria hyperborea</i> , subtidal/drift	Broughty Ferry, Scotland, UK	2 March 1991	-16.88
<i>Palmaria palmata</i>	On <i>Fucus serratus</i>	Broughty Ferry, Scotland, UK	2 March 1991	-16.51
<i>Palmaria palmata</i> *	Ruffled morphology	Filey, England, UK	22–24 July 1994	-16.59
<i>Palmaria palmata</i> *	Flat morphology	Filey, England, UK	22–24 July 1994	-18.79
<i>Palmaria palmata</i> *		Helmsdale, Scotland, UK	26 July 1998	-17.80
<i>Palmaria palmata</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-19.66; -20.00
<i>Palmaria palmata</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-21.16
<i>Peyssonnelia</i> sp.	Autumn	Stetson, Gulf of Mexico		-15.84
<i>Peyssonnelia</i> sp.	Winter	Stetson, Gulf of Mexico		-19.02
<i>Peyssonnelia</i> sp.		Coobowie Bay, SA, Australia	1 July 1997	-12.7
<i>Phacelocarpus</i> sp.		WA, Australia	1 March 1995	-34.16
<i>Phycodryes rubens</i>		Broughty Ferry, Scotland, UK	2 March 1991	-31.14
<i>Phycodryes rubens</i>	No bryozoans, drift	Barnhill, Scotland, UK	25–26 January 1996	-34.06; -34.14
<i>Phycodryes rubens</i>	Plus bryozoans, drift	Barnhill, Scotland, UK	25–26 January 1996	-30.89; -31.60
<i>Phycodryes rubens</i>		Finnoy, Norway		-33.6
<i>Phycodryes rubens</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-33.05
<i>Phycodryes rubens</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-34.81; -33.75
<i>Phyllophora crispa</i>		Finnoy, Norway		-34.76
<i>Plocamium cartilagineum</i>		East Haven, Scotland, UK	14 July 1995	-32.00 ± 0.37 ($n = 3$)
<i>Plocamium cartilagineum</i>		Barnhill, Scotland, UK	25–26 January 1996	-29.14; -31.48
<i>Plocamium cartilagineum</i>		Finnoy, Norway		-32.23
<i>Plocamium cartilagineum</i>		Mission Bay, CA, USA	1 August 1995	-31.04 ± 0.22 ($n = 8$)
<i>Plocamium cartilagineum</i>		Sorrento, Vic, Australia	1 March 1995	-31.93
<i>Plocamium cartilagineum</i> *	Drift	East Haven, Scotland, UK	13 May 1994	-32.57

Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Plocamium cartilagineum</i> *		FifeNess, Scotland, UK	23 May 1998	-32.72
<i>Plocamium cartilagineum</i> *		Helmsdale, Scotland, UK	26 July 1998	-32.86
<i>Plocamium cartilagineum</i> *		Kingsbarns, Scotland, UK	31 May 1999	-34.27; -31.99
<i>Plocamium costata</i>		Coobowie Bay, SA, Australia	1 July 1997	-33.39
<i>Plocamium mertensii</i>		Coobowie Bay, SA, Australia	1 July 1997	-34.72
<i>Plocamium microcladioides</i> *	Drift	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-30.95
<i>Plocamium preissianum</i>		Coobowie Bay, SA, Australia	1 July 1997	-33.74
<i>Plocamium preissianum</i>	Tetrasporophyte	Coobowie Bay, SA, Australia	1 July 1997	-34.55
<i>Plocamium</i> sp.		Broughty Ferry, Scotland, UK	2 March 1991	-33.34
<i>Plocamium</i> sp.		Mission Bay, CA, USA	1 August 1995	-31.14 \pm 0.36 ($n = 5$)
<i>Plocamium violacum</i> *		Catalina Island, CA, USA	1 February 2000	-30.96
<i>Plumaria plumosa</i> *		Filey, England, UK	22-24 July 1994	-31.1
<i>Plumaria plumosa</i> *		Helmsdale, Scotland, UK	26 July 1998	-30.53
<i>Polyides rotundus</i> *		Filey, England, UK	22-24 July 1994	-15.71
<i>Polyides rotundus</i> *		Helmsdale, Scotland, UK	26 July 1998	-15.69
<i>Polyides rotundus</i> ,		Bergen Store Kalsoy (5M), Norway		-17.62
<i>Polysiphonia brodiaei</i>		Bergen Store Kalsoy, Norway		-17.77
<i>Polysiphonia elongata</i>		Finnoy, Norway		-18.4
<i>Polysiphonia</i> from <i>Fucus vesiculosus</i>	HCl-treated	St Andrews, Scotland, UK	5 October 1993	-19.89
<i>Polysiphonia fucoides</i> *		Filey, England, UK	22-24 July 1994	-14.47
<i>Polysiphonia lanosa</i>		Finnoy, Norway		-19.59
<i>Polysiphonia lanosa</i>	On <i>Ascophyllum nodosum</i>	Arnolds Cove, Newfoundland, Canada	17 July 1992	-21.76
<i>Polysiphonia nigra</i> *		Flamborough, England, UK	22-24 July 1994	-17.49
<i>Polysiphonia</i> on <i>Fucus vesiculosus</i>	HCl-treated	St Andrews, Scotland, UK	5 October 1993	-20.41
<i>Polysiphonia</i> sp.	On <i>Codium</i> sp.	Hampton Bay, Long Island, NY, USA	16 November 1994	-20
<i>Polysiphonia stricta</i>		Finnoy, Norway		-29.32
<i>Polysiphonia stricta</i>		Bergen, Norway		-26.85
<i>Polysiphonia stricta</i>		Finnoy, Norway		-29.39
<i>Porphyra umbilicalis</i> *		Flamborough, England, UK	22-24 July 1994	-18.54
<i>Porphyra leucosticta</i> *		Flamborough, England, UK	22-24 July 1994	-18.75
<i>Porphyra perforata</i>		Barnhill, Scotland, UK	25-26 January 1996	-18.02
<i>Porphyra perforata</i>		Point Lobos and Big Sur, CA, USA	28 June 1992	-13.89
<i>Porphyra</i> sp.		Hampton Bay, Long Island, NY, USA	16 November 1994	-20.74
<i>Porphyra</i> sp.		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-19.98
<i>Porphyra</i> sp.		Barnhill, Scotland, UK	25-26 January 1996	-16.41
<i>Porphyra umbilicalis</i>		Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-21.69
<i>Porphyra umbilicalis</i>		Barnhill, Scotland, UK	25-26 January 1996	-19.5
<i>Porphyra umbilicalis</i>		Finnoy, Norway		-20.41
<i>Porphyra umbilicalis</i>		Bergen Store Kalsoy, Norway		-20.88
<i>Pterocladia capillacea</i> *		Catalina Island, CA, USA	1 February 2000	-15.36
<i>Pterocladia</i> sp.		Gran Canaria	1 February 1991	-18.86
<i>Ptilota gunneri</i>		Finnoy, Norway		-31.88
<i>Ptilota gunneri</i>		East Haven, Scotland, UK	14 July 1995	-32.07
<i>Ptilota gunneri</i>	Drift	Barnhill, Scotland, UK	25-26 January 1996	-32.54; -33.57
<i>Ptilota gunneri</i> *		Flamborough, England, UK	22-24 July 1994	-27.02
<i>Ptilota gunneri</i> *		Helmsdale, Scotland, UK	26 July 1998	-33.83
<i>Ptilota gunneri</i> *	On <i>Laminaria hyperborea</i> stipe	Kingsbarns, Scotland, UK	31 May 1999	-33.46; -34.63
<i>Rhodomela lycopodoides</i>		Finnoy, Norway		-23.15
<i>Rhodomenia australis</i>		WA, Australia	1999-2000	-31.72; -32.68
<i>Schizymenia pacifica</i> *		Catalina Island, CA, USA	1 February 2000	-18.46
<i>Sphaerococcus coronopifolius</i>	Possibly new name?	South Coast of Devon, UK		-32.87

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Appendix. Continued

Species	Details	Origin	Date collected	$\delta^{13}\text{C}$
<i>Stictosiphonia arbuscula</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-30.65; -30.05
<i>Stictosiphonia arbuscula</i> *	Attached	Brighton Beach/Papatowai Beach, New Zealand	1 February 1996	-29.58
<i>Trailliella intricata</i>	Tetrasporophytic stage of	Finnoy, Norway		-30.15
<i>Trailliella intricata</i>	<i>Bonnemaisonia hamifera</i>	Bergen Store Kalsoy, Norway		-27.43
<i>Wrangelia</i> sp.	Winter	East Flower Garden, Gulf of Mexico		-30
<i>Wrightiella blodgettii</i>	Spring	Stetson, Gulf of Mexico		-32.24
Lichen				
<i>Lichina pygmaea</i>	HCl-treated	FifeNess, Scotland, UK		-15.47
<i>Lichina pygmaea</i> *	Lichen with cyanobacterium (<i>Calothrix</i>)	Fife Ness, Scotland, UK	1 February 2000	-9.72 \pm 0.02 (<i>n</i> = 3)
<i>Lichina pygmaea</i>	Lichen with cyanobacterium (<i>Calothrix</i>), HCl-treated	FifeNess, Scotland, UK	26 July 2000	-9.81
<i>Cyanobacterium</i> <i>Oscillatoria-like</i>	Cyanobacterium, HCl-treated	Singapore	1 April 1996	-17.34
Sponge				
<i>Chondrilla</i> sp.		Carnac Island, WA, Australia	14 January 2000	-17.66 \pm 0.48 (<i>n</i> = 3)
<i>Chondrilla</i> sp.		Hamelin Bay, WA, Australia	1 December 1999	-20.20*
<i>Chondrilla</i> sp.		Mewstone, WA, Australia	23 July 1999	-19.22 \pm 0.13 (<i>n</i> = 4)
<i>Chondrilla</i> sp.		Mewstone, WA, Australia	20 January 2000	-18.90 \pm 0.19 (<i>n</i> = 3)
<i>Chondrilla</i> sp.		Stragglers, WA, Australia	7 July 1999	-19.40*
<i>Chondrilla</i> sp.		Stragglers, WA, Australia	27 January 2000	-18.84 \pm 0.31 (<i>n</i> = 3)
<i>Chondrilla</i> sp.		The Lumps, WA, Australia	1 January 2000	-18.28 \pm 0.11 (<i>n</i> = 3)
<i>Cymbastela notiana</i>	Sponge with cyanobacteria	Coobowie Bay, SA, Australia	1 July 1997	-21.4
Seagrass				
<i>Amphibolis</i> sp.	Leaves	Mewstone, WA, Australia	23 July 1999	-14.46 \pm 0.98 (<i>n</i> = 4)
<i>Amphibolis</i> sp.	Stems	Mewstone, WA, Australia	23 July 1999	-12.94 \pm 0.96 (<i>n</i> = 4)
<i>Phyllospadix scouleri</i>	Leaves, bases, stem + roots	Point Lobas and Big Sur, CA, USA	28 June 1992	-15.37
<i>Phyllospadix scouleri</i>	Leaves	Point Lobas and Big Sur, CA, USA	28 June 1992	-16.15
<i>Phyllospadix scouleri</i>	Leaves	CA, USA	1 August 1995	-13.2
<i>Thalassia testudinum</i>		Port Aransas, TX, USA	29 April 1997	-9.06
<i>Zostera marina</i>	Fruits	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-10.66 \pm 0.63 (<i>n</i> = 3)
<i>Zostera marina</i>	Bract (= fertile leaf)	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-11.57 \pm 0.50 (<i>n</i> = 4)
<i>Zostera marina</i>	Leaf (sterile)	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-12.07 \pm 0.37 (<i>n</i> = 4)
<i>Zostera marina</i>	Roots	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-12.04; -13.63
<i>Zostera marina</i>	Rhizome	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-10.93 \pm 0.26 (<i>n</i> = 4)
<i>Zostera marina</i>	Stem	Tayport, Tentsmuir, Scotland, UK	15 October 1991	-11.61 \pm 0.23 (<i>n</i> = 4)
<i>Zostera marina</i>	Leaves, from intertidal	Monifieth, Scotland, UK	12 September 1993	-11.94
<i>Zostera marina</i>	Rhizome, from intertidal	Monifieth, Scotland, UK	12 September 1993	-11.83
<i>Zostera marina</i>	Rhizome	Monifieth, Scotland, UK	24 May 1993	-10.92 \pm 0.20 (<i>n</i> = 10)
<i>Zostera marina</i>	Leaves	Monifieth, Scotland, UK	24 May 1993	-11.46 \pm 0.08 (<i>n</i> = 10)
<i>Zostera marina</i>	Leaves	Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-9.83
<i>Zostera marina</i>	Stems/rhizomes	Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-9.05
<i>Zostera marina</i>	Roots	Invergowrie Bay/Monifieth/Barnhill, Scotland, UK	29 May 1995	-9.75
<i>Zostera nova - zelandiae</i>		Brighton Beach, South of Dunedin, New Zealand	1 July 1998	-14.11
<i>Zostera</i> sp.	Root	Cobblers Brook, Newfoundland, Canada	17 July 1992	-8.26
<i>Zostera</i> sp.	Leaf	Cobblers Brook, Newfoundland, Canada	17 July 1992	-11.87
<i>Zostera</i> sp.	Stem (rhizome)	Cobblers Brook, Newfoundland, Canada	17 July 1992	-8.31
<i>Zostera</i> sp.	Leaf	Hampton Bay, Long Island, NY, USA	16 November 1994	-7.95