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Inorganic C-Sources for *Lemanea, Cladophora* and *Ranunculus* in a Fast-Flowing Stream: Measurements of Gas Exchange and of Carbon Isotope Ratio and their Ecological Implications

John Raven, John Beardall and Howard Griffiths
Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland

Summary. CO_2- and O_2-exchange characteristics and $\delta^{13}C$ values have been measured in a rhodophycean haptophyte (*Lemanea mamillosa*), a chlorophycean haptophyte (*Cladophora glomerata*) and a magnoliophyte rhizophyte (*Ranunculus* sp.) from a 5 m stretch of the Dichty Burn near Dundee. Light- and CO_2-saturated rates of photosynthesis are greatest on a dry weight basis for *Cladophora* and lowest for *Lemanea*; the order is reversed on a surface area basis. The CO_2 concentration at pH 6.5 at which photosynthesis is half-saturated is 25–40 μM, with *Lemanea* rather lower than *Cladophora* or *Ranunculus*; these half-saturation values are similar to the free CO_2 concentration in the Burn water. *Lemanea* cannot use HCO_3^- in photosynthesis, while *Cladophora* and *Ranunculus* can. Despite being within a factor or two of saturation with free CO_2 in terms of the bulk water concentration, the growth habit of *Cladophora* and, particularly, *Ranunculus* means that the high water velocity in the Burn does not necessarily prevent C depletion effects around the plants, thus providing a possible role for HCO_3^- use by these plants. *Lemanea* lives in the fastest-growing parts of the Burn, and its growth habit insures that it is exposed to this high water velocity, thus minimising CO_2 depletion during photosynthesis despite the low surface/volume ratio for this plant. $\delta^{13}C$ measurements on the inorganic C in the Burn water are consistent with at least part of its excess (above air-equilibrium) inorganic C levels coming from heterotrophic activity. *Lemanea* has the most negative $\delta^{13}C$ value of the three plants, consistent with CO_2 use and small diffusion resistances. *Ranunculus* has the least negative $\delta^{13}C$ value, consistent with some CO_2 depletion and/or HCO_3^- use in situ related to a high diffusion resistance in a rhizophyte which does not have to obtain all of its N and P from the bulk water but can obtain some from the sediments. *Cladophora* is intermediate, suggesting some CO_2 depletion and/or HCO_3^- use in this densely growing haptophyte.

Introduction

The supply of inorganic carbon to submerged benthic macrophytes is conditioned by the concentration of the various inorganic carbon species in the water and by its flow rate, and by the characteristics of the macrophytes, such as their morphology and their net photosynthesis – free CO_2 concentration and net photosynthesis – HCO_3^- concentration relationships (e.g. Gessner 1959; Steeman-Nielsen 1960; Sculthorpe 1967; Raven 1970; Westlake 1975a; Smith and Walker 1980; Raven 1981). In this paper we describe an investigation of three macrophytes of contrasting taxonomy from a stream (the Dichty Burn) near Dundee which attempts to take all of these factors into account in analysing how the plants obtain their inorganic carbon under natural conditions.

The three plants we have used are found within a few meters of each other at the site we investigated; two are algal haptophytes (Luther 1949; cf. Raven 1981), namely the chlorophyte *Cladophora glomerata* Kützing and the rhodophyte *Lemanea mamillosa* Kützing, and the third is the flowering plant rhizophyte (Luther 1949; cf. Raven 1981) *Ranunculus* sp. (probably *R. penicillatus* (Dumort.) Bab var. *calcareus* (R.W. Butcher) Cook). Previous work on the distribution of these plants shows them to favour relatively fast-flowing waters of above-neutral pH and quite high alkalinity, with *Lemanea* in the fastest-flowing waters, *Ranunculus* in slower flow regimes, and *Cladophora* in intermediate flow rates (Whitton 1970, 1975; Westlake 1975a; Haslam 1978; Raven and Beardall 1981a).

The biochemistry of inorganic C assimilation in photosynthesis by these three plants is probably of the 'C_3' type, i.e. involves RuBPc-o as the primary carboxylase. The evidence that this is the case for *Lemanea* is discussed by Raven and Beardall (1981a). For *Cladophora* species there does not seem to have been any analysis of sufficiently short-term $^{14}CO_2$ fixation in vivo to unequivocally identify the primary carboxylase: Kremer (1980a) reports 3 *minute* fixation products of the marine *Cladophora rupestris*. Kremer and Kuppers (1977) showed that *Cladophora rupestris* had a very high ratio of in vitro RuBPc activity to PEPc and PEPck activity, and that similar activities were found in *Ulva lactuca* which short-term labelling studies (2–5 s exposure to $^{14}CO_2$) showed to be a 'C_3' plant. The situation is even worse for *Ranunculus*, where arguments as to its C_3 nature have to be based on experiments on other submersed freshwater vascular plants which generally seem to have C_3-type fixation in the light (see Raven and Glidewell 1978; Benedict 1978; Hough and Wetzel 1977; Holaday and Bowes 1980; Browse et al. 1979a, 1980). In terms of the kinetics of RuBPc-o which determine the CO_2 compensation concentration in the absence of any 'CO_2 concentrating mechanism', the scant data available suggests that the submerged vascular plants are like the C_3 land plants, while chlorophyte algae would have a higher CO_2 compensation concentration (Laing et al. 1974; Jordan and

Offprint requests to: J.A. Raven

Abbreviations. RuBPc-o = Ribulose bisphosphate carboxylase-oxygenase (E.C. 4.1.1.39); PEPc = Phosphenolpyruvate carboxylase (E.C. 4.1.1.31); PEPck = Phosphoenolpyruvate carboxykinase (ATP) (E.C. 4.1.1.48)

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Ogren 1981; Raven and Beardall 1981b; Salvucci and Bowes 1981; Yeoh et al. 1981); no data seem to be available for the rhodophyte enzyme.

Previous physiological work on these plants suggests that *Lemanea* cannot 'use' HCO_3^- in photosynthesis (Raven and Beardall 1981a). There are strong indications that *Cladophora glomerata*, like most other fresh water and marine species of *Cladophora*, can use HCO_3^- in photosynthesis (Gessner 1959; Dahm 1926; Steeman-Nielsen 1947, 1949; Lindahl 1963; cf. Blinks 1963). Similarly, the majority of submerged *Ranunculus* species also appear to be able to use HCO_3^- (Ruttner 1947; Gessner 1959; Hutchinson 1975; cf. Bristow 1969). The work presented here provides further evidence as to the ability of *Cladophora* and *Ranunculus* to use HCO_3^- in photosynthesis, and also presents evidence as to the relationship between photosynthetic rate and CO_2 concentration; measurements of the CO_2 compensation concentration in *Cladophora glomerata* suggests that a 'CO_2 concentrating mechanism' is operative in this alga (Birmingham and Colman 1979; cf. Raven 1980; Beardall and Raven 1981), while fragmentary data (Turner et al. 1956; Westlake 1967; Kremer 1980b), indicates a relatively small O_2 inhibition of net photosynthesis in *Cladophora glomerata* and in *Ranunculus pseudofluitans*, at least at relatively high CO_2 concentrations.

The study of stable carbon isotope discrimination (commonly expressed as '$\delta^{13}C$'[1]) has given valuable information on the mechanism of inorganic carbon transport and fixation in terrestrial plants (see O'Leary 1981, for a recent authoritative review). The application of this technique to submerged freshwater plants can also give useful insights into the mechanism of inorganic carbon assimilation in these organisms, and can also indicate the sources of the inorganic carbon in their environment, provided that the $\delta^{13}C$ value of the source inorganic C is also measured (see Benedict 1978; Bhaskaran and Benedict 1980; Bhaskaran et al. 1981; Osmond et al. 1981; Park and Epstein 1960; Rau 1978; Raven 1970, 1972, 1980; Smith and Walker 1980; Wong et al. 1979). A number of measurements of the $\delta^{13}C$ values of freshwater plants are rendered very difficult to interpret by the absence of data on source $\delta^{13}C$ (e.g. Craig 1954; Rundel et al. 1979; Smith and Epstein 1971; Wickman 1952; Ziegler 1979). We present data on the $\delta^{13}C$ values of inorganic C in the Burn water and in the three plants and discuss the significance of these measurements in relation to the uptake of inorganic C by the plants in situ.

Materials and Methods

The source of the plants was the same as that for the investigation of Raven and Beardall (1981a), i.e. the Dichty Burn near Dundee at O.S. Grid Reference NO 473 327. *Cladophora* was collected from a concrete surface laid about six years ago at the southern end of the eastern span of the road bridge; at the time of collection in April 1981 the water depth was about 100 mm, and the linear flow rate (measured by timing the passage of an orange over a measured distance) was about 0.8 m s^{-1}; this area was also used to calibrate a home-made flow meter (the angle of displacement from the vertical of a flat surface at the end of a pivoted rod, tensioned by a rubber band). The *Lemanea* occurred at the end of this concrete platform where water velocity was higher (1 m s^{-1}) and the depth was lower (about 50 mm) before the water spilled over the edge of the platform onto a stream bed consisting of rocks and stones grading into a gravel bottom where *Ranunculus* was rooted. The water depth at the *Ranunculus* site about 2 m down stream from the end of the platform was some 300 mm, and the velocity some 0.65 m s^{-1}. *Ranunculus* was collected as non-rooted 'free' shoot apices 0.3–0.5 m long. pH was determined on water samples from the Burn in the field; water samples for alkalinity determination were brought back to the laboratory. Precipitation of inorganic carbon in Burn water as $BaCO_3$ for subsequent analysis of the $\delta^{13}C$ was carried out in the field as described by Osmond et al. (1981); after filtration through a Whatman GF/C filter (using a hand operated pump) an aliquot (0.5–1.0 cm^3) was injected into a bottle, sealed with a suba seal, containing 10 cm^3 of filtered saturated $Ba(OH)_2$ solution. Plants collected from the Burn were stored in aerated Burn water under a 14 h light, 10 h dark illumination cycle in a growth cabinet at 11° C for up to two days before use in metabolic experiments, or for determination of wet weight, surface/volume ratio, and chlorophyll content; samples for dry weight and for $\delta^{13}C$ determinations were dried in an oven at 70° C immediately upon return to the laboratory.

Wet and dry weight determinations were as described by Raven and Beardall (1981a). Chlorophylls *a* and *b* were determined in 90% acetone extracts using the equations given by Strickland and Parsons (1972). Surface/volume ratios of *Cladophora* filaments were determined by measuring microscopically the diameter of filaments, assuming that filaments were cylinders of diameter equal to the mean measured diameter; for *Ranunculus*, the diameters of different orders of branches of the leaves were weighted according to the total length of the various branches. Relating this to wet weight (and hence to dry weight) by assuming a density of 1 g cm^{-3} introduces an error due to surface water films in wet weight measurements, i.e. surface area/dry weight is over-estimated; this error is presumably greater for *Cladophora* than for *Ranunculus* due to the larger S/V for *Cladophora*. The presence of intercellular air spaces in *Ranunculus* (but not in *Cladophora* or *Lamanea*) reduces the density of *Ranunculus* leaves; however, the fraction of the leaf tissue occupied by air spaces is small (Fig. 8.14 of Sculthorpe 1967; unpublished anatomical investigations, cf. Cook, 1969), so this does not introduce a large error into the values for cm^2 surface area/g dry weight.

Infra-red gas analysis measurements of dark respiration rates, of light-saturated net photosynthesis at various CO_2 concentrations, and of the CO_2 compensation concentration at light saturation, were performed as described by Raven and Beardall (1981a). Water from the Burn was adjusted to pH 6.5 with 10 mM MES-NaOH, or to pH 8.0 with 10 mM HEPES-NaOH. Typically 0.5 g *Cladophora*, 1 g *Lemanea* or 1 g *Ranunculus* were incubated in 30 ml of the buffered natural water. The irradiance was 50 W m^{-2} (400–700 nm); experiments were carried out at 11° C (approximately the temperature of the Burn).

'pH-drift' measurements of the CO_2 compensation concentration were made as described by Raven and Beardall (1981a) at 11° C and with an irradiance of 50 W m^{-2} (400–700 nm).

Measurements of O_2 exchange in light and dark were carried out as described by Raven and Beardall (1981a) using the Winkler technique, and Burn water adjusted to pH 6.5 with 10 mM MES-NaOH after supplementation with 2 mM $NaHCO_3$, except that the same light source was employed as was used for the IRGA and pH drift experiments, and the experimental period was only 1 h. Typically 0.1 g of *Cladophora* or 0.3 g of *Ranunculus* (both fresh weights) were incubated in 36 ml bottles. The O_2 exchange measurements were conducted in late

[1] $\delta^{13}C\ (‰) = \left[\frac{^{13}C/^{12}C\ \text{sample}}{^{13}C/^{12}C_{PDB}} - 1\right] \times 1{,}000$,

where PDB refers to the belemnite from the Pee Dee Formation in South Carolina with a $^{13}C/^{12}C$ of 0.01124 (O'Leary 1981)

June at a temperature of 12° C (the temperature of the Burn at that time); the other measurements were made in late April.

$\delta^{13}C$ analyses were performed with a VG MM 601 monopole mass spectrometer with 6 cm radius and magnetic deflection (VG Gas Analysis, Winsford, Cheshire, UK). Samples were generated directly into the inlet system with the same pressure used for each analysis, so that pressure variations were prevented. The critical masses 44 and 45 were scanned manually six times during the linear phase of sample decay, and a reading of the m/e 46 peak was taken at the mid-point of the measurement. Thus all peaks could be read directly from the same sample, careful timing being the key to accurate analysis of 3 peaks with a single collector. Peak overlap between the 44 and 45 m/e peaks was 0.04% and each sample was corrected against the background of the whole inlet system prior to sample generation, and against reagent blanks. The machine was calibrated with N.B.S. oxalic acid (kindly supplied by Dr. D. Harkness, Scottish Universities Research and Reactor Centre, East Kilbride) at $-19^0/_{00}$ relative to the PDB standard.

Oxalate (used as standard) was decarboxylated by adapting the method of Mapstone (1971), using excess $KMnO_4$, H_2SO_4, and catalyst $FeCl_3$ to generate CO_2. Using the technique of Preston (1981), the reagents were frozen with liquid nitrogen on to the side of the sample tube above the sample. The tube was then transferred to the mass spectrometer for evacuation and sample generation. CO_2 was thus generated in vacuo by gently warming the sample tube, and the tube was re-frozen with dry-ice/ethanol to trap water. Similarly, the method of Osmond et al. (1981) was used to generate CO_2 from $BaCO_3$ with concentrated phosphoric acid. CO_2 generation was complete after 5 min incubation. Reagent blanks were less than 0.5% of the sample for both methods.

The dried plant samples were combusted in the combustion train of Preston (1981) modified to include an oxygen supply from which any CO_2 impurities were removed with a column of indicating soda asbestos ("Carbasorb", BDH Chemicals, UK). Quartz-glass combustion tubes with 3 mm by 20 mm inner quartz tubes were precombusted to reduce carbon blanks and a 1 mg sample of the ground, dried plant material was sandwiched between two portions of precombusted tungstic oxide catalyst. Reagent blanks were less than 0.2% of the sample. The sample was combusted for 2.5 min at 900° C, those gases evolved being drawn over an oxidation furnace of CuO (wire) and MnO_2 (10–20 mesh) by an in-line, in vacuo liquid nitrogen trap. Non-condensing gases were pumped away and the trap was then replaced with a dry-ice/ethanol bath after freeing CO_2. For comparison, oxalate standards were also run using the combustion system. All reagents were purchased from BDH Chemicals, Poole, UK, and were micro-analytical grade where available. Results were calculated using the formulae of Craig (1957) to correct for the ^{17}O contribution to the m/e 45 peak, and are presented as $^0/_{00}$ relative to the PDB standard.

Results

The data shown in Table 1 permit the rates of metabolism measured on a fresh weight basis to be expressed on other bases for comparison with other aquatic phototrophs (see Raven et al. 1979; Raven 1981; Raven and Beardall 1981a).

The data for *Lemanea mamillosa* are in part taken from Raven and Beardall (1981a); the independent measurements reported in Table 1 are in reasonable agreement with those in Raven and Beardall (1981a). For fresh weight/dry weight ratio the data are in good agreement with Müller (1978) who examined

Table 1. Relationship between wet weight, dry weight, chlorophylls *a* and *b* and total chlorophyll, and thallus surface area for *Cladophora*, *Lemanea* and *Ranunculus*

Ratio	*Cladophora*	*Lemanea*	*Ranunculus*
$\frac{\text{g wet weight}}{\text{g dry weight}}$	6.36 ±0.22	8.88 ±1.48	10.36 ±0.05
$\frac{\text{mg chlorophyll } a}{\text{g dry weight}}$	3.28 ±0.67	2.28 ±0.38	10.28 ±1.47
$\frac{\text{mg chlorophyll } b}{\text{g dry weight}}$	1.35 ±0.30	0	2.98 ±0.52
$\frac{\text{mg chlorophyll } (a+b)}{\text{g dry weight}}$	4.63 ±0.97	2.28 ±0.38[a]	13.26 ±1.99
$\frac{\text{chlorophyll } a}{\text{chlorophyll } b}$	2.45 ±0.06	∞[a]	3.46 ±0.11
$\frac{\text{cm}^2 \text{ surface area}^c}{\text{g dry weight}}$	5,552 ±192	394 ±17[b]	1,207 ±96
$\frac{\mu\text{g chlorophyll } (a+b)}{\text{cm}^2 \text{ surface area}}$	0.833 ±0.175	3.83 ±0.64[a]	11.0 ±1.65

[a] Chlorophyll *a* only
[b] From Raven and Beardall 1981a
[c] From S/V ratio and wet weight/dry weight, assuming density of 1 g cm^{-3}

L. fluviatilis, although Müller's (1978) chlorophyll a levels are much lower than our values for *L. mamillosa* (Table 1; Raven and Beardall 1981a).

The data for *Cladophora glomerata* are also in reasonable agreement with other data on this and other *Cladophora* species, both freshwater and marine (Craigie et al. 1966; Müller 1978; Odum et al. 1958; Seybold and Egle 1938). The chlorophyll *a*/dry weight is higher than that found by Müller (1978) for *C. glomerata*, while total chlorophyll/dry weight is lower than in some reports for marine *Cladophora* spp. (Seybold and Egle 1938). The chlorophyll *a*/*b* ratio is at the high end of the range for marine *Cladophora* spp. (Seybold and Egle 1938; Yokohama 1973; Yokohama et al. 1977; Nakamura et al. 1976); this may be related to the high-irradiance habitat in the shallow, unshaded water of the Dichty Burn, and to the tendency for freshwater chlorophytes to have a higher chlorophyll *a*/*b* ratio than their marine relatives (see Hager and Stransky 1970; Yokohama 1973; Nakamura et al. 1976). The total chlorophyll/surface area of *C. glomerata* is lower than for most chlorophyte and charophyte algae for which data are available (see Drew 1977; Ramus 1978; Raven et al. 1979; Seybold and Egle 1938), although the algae mentioned in these references either have a more complex thallus structure with less membrane area exposed to the macroscopic cell surface, or have larger cells than *C. glomerata*. Raven (1980, Table 2B) suggests that *Chlorella* spp. may have some 1 μg chlorophyll cm^{-2} cell surface.

Many fewer data seem to be available in the literature for submersed *Ranunculus* spp. with which the data in Table 1 can be compared. The chlorophyll $(a+b)$ content on an area or dry weight basis, and the chlorophyll *a*/*b* ratio, are at the high end of the range of values reported in the literature (Boyd 1970; Brown et al. 1980; Egle 1937; Ikusima 1966; Marcus 1980; Nakamura et al. 1976; Seybold and Egle 1937; Spence and Chrystal 1970; Van et al. 1977). The high wet weight/dry weight ratio (Table 1) is common in submerged freshwater vascular

Table 2. Rates of CO_2 uptake by *Cladophora, Lemanea* and *Ranunculus* at pH 6.5 and pH 8.0 at 300 ppm in the gas phase at light saturation

Parameter	*Cladophora*	*Lemanea*	*Ranunculus*
CO_2 uptake at pH 6.5, μmol (g.d.wt)$^{-1}$ h^{-1}	146.2 ± 2.9 (5)	97.2±3.9 (3)	53.9 ± 3.5 (5)
CO_2 uptake at pH 8.0, μmol (g.d.wt)$^{-1}$ h^{-1}	162.8 ±11.1 (9)	91.9±8.8 (4)	89.4 ±13.9 (6)
CO_2 uptake at pH 6.5, μmol (g.f.wt)$^{-1}$ h^{-1}	23.0 ± 0.5 (5)	10.9±0.5 (3)	5.20± 0.34 (5)
CO_2 uptake at pH 8.0, μmol (g.f.wt)$^{-1}$ h^{-1}	25.6 ± 1.8 (9)	10.4±1.0 (4)	8.63± 1.34 (6)
CO_2 uptake at pH 6.5, μmol (mg chl $a+b$)$^{-1}$ h^{-1}	31.6 ± 0.6 (5)	42.6±1.7 (3)	4.06± 0.26 (5)
CO_2 uptake at pH 8.0, μmol (mg chl $a+b$)$^{-1}$ h^{-1}	35.2 ± 2.4 (9)	40.3±3.9 (4)	6.74± 1.04 (6)
CO_2 uptake at pH 6.5, pmol cm^{-2} s^{-1}	7.31± 0.15 (5)	68.5±2.8 (3)	12.4 ± 0.8 (5)
CO_2 uptake at pH 8.0, pmol cm^{-2} s^{-1}	8.14± 0.56 (9)	64.8±7.0 (4)	20.6 + 3.2 (6)

Table 3. Rates of O_2 evolution (net) at light and CO_2 saturation at pH 6.5, and the ratio of P_{net} at light and CO_2 saturation to P_{net} at light saturation and 300 ppm CO_2 in the gas phase at pH 6.5 for *Cladophora, Lemanea* and *Ranunculus*. (Data for *Lemanea* from Raven and Beardall, 1981a)

Parameter	*Cladophora*	*Lemanea*	*Ranunculus*
O_2 evolution, μmol (g.d.wt)$^{-1}$ h^{-1}	681 ±88 (3)	122 ±14 (4)	243 ±24 (3)
O_2 evolution, μmol (g.f.wt)$^{-1}$ h^{-1}	107 ±14 (3)	20.7± 2.4 (4)	23.5± 2.3 (3)
O_2 evolution, μmol (mg chl $a+b$)$^{-1}$ h^{-1}	147 ±19 (3)	65.9± 7.7 (4)	18.3± 1.8 (3)
O_2 evolution, pmol cm^{-2} s^{-1}	34.1± 4.4 (3)	117 ±13 (4)	55.9± 5.5 (3)
P_{net} (CO_2 saturation)/ P_{net} (300 ppm CO_2)	4.66	3.34	4.51

plants, including species of *Ranunculus* (Westlake 1965; Sculthorpe 1967). Uspenskij (1913; cf. Arber 1920) comments on the surface-volume relations of the submerged cylindrical leaves of *Ranunculus trichophyllus*.

Table 2 shows the rate of CO_2 fixation at light saturation in air (300 ppm CO_2-equilibrated) solution on a number of bases (cf. Table 1). The value for *Lemanea* are rather higher than those found by Raven and Beardall (1981a; their Table I and Fig. 1). The rates on a dry weight basis are considerably higher than those found by Müller (1978) at (probably) a higher CO_2 concentration for *Lemanea fluviatilis* but are much lower on a chlorophyll basis (cf. the discussion of Müller's (1978) chlorophyll levels above).

The values (Table 2) for the photosynthetic rates of *Cladophora* and *Ranunculus* at 300 ppm CO_2 do not appear to have counterparts in the literature: Steeman-Nielsen's (1947, 1949) work on *Cladophora 'insignis'* presents photosynthesis *versus* inorganic C concentration at pH 5.6 with photosynthesis as 'percent optimum', while Westlake (1967) used water with more than air-equilibrium levels of CO_2. Comparisons of the rates in Table 2 between plants, pH values and bases for expression shows that *Cladophora* and *Lemanea* have similar rates at pH 6.5 and pH 8.0, while *Ranunculus* photosynthesises markedly faster at pH 8.0 than at pH 6.5. Our method of measurement is likely to under-estimate any contribution of HCO_3^- uptake to photosynthesis. *Cladophora* has the highest rate of photosynthesis on a dry weight basis and *Ranunculus* the lowest; the low chlorophyll/dry weight in *Lemanea* gives it the highest chlorophyll-based rate, while the chlorophyll-rich *Ranunculus* has a much lower rate. Even on a chlorophyll *a* basis (to take into account the 'replacement' of chlorophyll *b* by phycobilins in *Lemanea*) *Ranunculus* fares little better, although *Cladophora* then has a slightly higher rate than *Lemanea*. Perhaps most significant from the viewpoint of inorganic C supply in the Dichty Burn is the rate of photosynthesis on an *area* basis. The very low surface area/ dry weight ratio for *Lemanea* (Table 1) means that this alga has a much higher area-based rate of photosynthesis at air levels of CO_2 than does *Ranunculus* or *Cladophora*; the very *high* surface area/dry weight ratio for *Cladophora* means that it has the *lowest* area-based rate of photosynthesis despite having the *highest* rate based on dry weight.

Table 3 shows rates of net O_2 evolution at pH 6.5 at inorganic carbon and light saturation. Other data on light-saturated rates of photosynthesis with greater-than-air-equilibrium CO_2 levels in *Cladophora glomerata* and *Ranunculus pseudofluitans* were generally obtained at higher temperatures which may explain why the rates obtained were somewhat higher: up to 2,500 μmol O_2 (g.d.wt)$^{-1}$ h^{-1} for *Cladophora glomerata* (Turner et al. 1956; Wallentinus 1976; Wood 1968) and 500 μmol (g.d.wt)$^{-1}$ h^{-1} for *Ranunculus* (Westlake 1967, 1975a, b).

Table 3 also shows the ratio of the net O_2 evolution rate at light and inorganic C saturation to the net CO_2 uptake at light saturation and 300 ppm CO_2; if the photosynthetic quotient is assumed to be 1.0, then these values can be used to estimate the CO_2 concentration at which net photosynthesis achieves half its CO_2-saturated rate (assuming linearity of the rate/CO_2 relationship between the CO_2 compensation concentration and the air-equilibrium CO_2 concentration). Using the CO_2 compensation concentration from Tables 5–7, these half-saturation values are 39 μM for *Cladophora*, 26 μM for *Lemanea* and 37 μM for *Ranunculus*. The *Cladophora* value is rather higher than the 30 μM obtained for *Cladophora 'insignis'* at pH 5.6 by Steeman-Nielsen (1947, 1949), while the *Ranunculus* value is rather lower than most values for submerged vascular plants obtained at pH values around 6.5 and at air-equilibrium O_2 concentrations under a variety of conditions of stirring (Allen and Spence,

Table 4. Rate of respiratory O_2 uptake in the dark at pH 6.5, and of respiratory CO_2 evolution in the dark at pH 8.0, in *Cladophora* and *Ranunculus*

Parameter	*Cladophora*	*Ranunculus*
O_2 uptake, pH 6.5, μmol (g.d.wt)$^{-1}$ h^{-1}	188 ±14 (3)	132 ±14 (3)
CO_2 evolution, pH 8, μmol (g.d.wt)$^{-1}$ h^{-1}	29.6± 2.2 (3)	14.8± 1.6 (2)

Table 5. Measurements of the CO_2 compensation concentration by the IRGA technique at two fixed external pH values, and by the 'pH-drift' technique, for *Lemanea*

Method	Initial pH	Final pH	Final CO_2 concentration	
			μM in solution	ppm in gas phase
IRGA	6.5	6.5	1.92±0.03	35.8±0.6
IRGA	8.0	8.0	1.99±0.02	37.1±0.4
pH drift	8.87±0.03	8.83±0.02	1.87±0.13	34.9±2.4

Table 6. Measurements of the CO_2 compensation concentration by the IRGA technique at two fixed external pH values, and by the 'pH-drift' technique, for *Cladophora*

Method	Initial pH	Final pH	Final CO_2 concentration	
			μM in solution	ppm in gas phase
IRGA	6.5	6.5	0.88 ±0.05	16.4 ±0.9
IRGA	8.0	8.0	0.46 ±0.05	8.6 ±0.9
pH drift	7.79±0.05	10.31±0.08	0.011±0.001	0.21±0.02
pH drift	7.98±0.02	10.42±0.95	0.013±0.003	0.24±0.05
pH drift	8.05±0.02	10.55±0.03	0.008±0.001	0.15±0.01
pH drift	7.97±0.03	10.49±0.03	0.008±0.001	0.14±0.01

Table 7. Measurements of the CO_2 compensation concentration by the IRGA technique at two fixed external pH values, and by the 'pH-drift' technique, for *Ranunculus*

Method	Initial pH	Final pH	Final CO_2 concentration	
			μM in solution	ppm in gas phase
IRGA	6.5	6.5	1.37 ±0.06	25.5 ±1.1
IRGA	8.0	8.0	0.67 ±0.08	12.5 ±1.5
pH drift	7.54±0.05	9.27±0.08	0.27 ±0.03	5.0 ±0.5
pH drift	8.00±0.01	9.89±0.11	0.132±0.010	2.5 ±0.3
pH drift	7.90±0.01	10.34±0.04	0.014±0.001	0.26±0.02

1981; Betts, 1979; Browse et al. 1979a, b; Lloyd et al. 1977; Lucas et al. 1978; Prins 1974; Smith and Walker 1980; Steeman-Nielsen 1947). The great majority of these values relate to plants associated with slower water flow regimes than *Ranunculus* (Haslam 1978; cf. Ledger 1981).

Table 4 shows the rates of respiration of *Cladophora* and *Ranunculus* obtained by the CO_2 method (pH 8.0) and by the O_2 method (pH 6.5). The rates obtained by the two methods show much less good agreement than was the case for *Lemanea* and *Batrachospermum* (Raven and Beardall 1981a); the measurements on these rhodophytes were made at the same pH and at the same time of year which was not the case for the work on *Cladophora* and *Ranunculus* presented here. The much higher inorganic C levels present in the O_2 measurements may be a significant factor in view of the results of Wood (1968) on *Cladophora glomerata* in which the rates of dark O_2 uptake were increased 2.5 fold by an (unspecified) increase in the inorganic C concentration. Further investigation of the respiratory quotient in these plants is called for, since a genuinely high ratio of dark O_2 uptake to dark CO_2 evolution would be consistent with the occurrence of 'dark acidification-light deacidification' in these plants (cf. Holaday and Bowes 1980; Beer and Wetzell 1981; Keeley 1981; Keeley et al. 1981). Previous measurements of the dark respiration rate of *Cladophora glomerata* (e.g. Turner et al. 1956; Müller 1978; Wood 1968) and *Ranunculus pseudofluitans* (Westlake 1967; cf. Sculthorpe 1967) are within the range of values in Table 4.

Tables 5, 6 and 7 show the CO_2 compensation concentrations for the three plants at various external pH values. The results for *Lemanea* (Table 5) confirm and extend (by means of an IRGA estimation of the CO_2 compensation concentration at pH 8.0) those reported by Raven and Beardall (1981a), and are consistent with the conclusion that *Lemanea* is a plant with C_3 biochemistry which accounts for the observed CO_2 compensation concentration at air-equilibrium concentrations of O_2, and that CO_2 diffusion is the mechanism of inorganic C exchange between the site of RuBPc-o activity and the environment at all pH values (6.5–8.9) tested (Raven and Beardall 1981a). *Lemanea* is unable to 'use' directly the most abundant inorganic C source, i.e HCO_3^-, in its environment in the Dichty Burn.

For the other two plants (Table 6 and 7) the situation is different; the CO_2 compensation concentration is slightly (*Ranunculus*) or considerably (*Cladophora*) lower than the value found for *Lemanea* at the lowest pH (6.5) which was tested, and decreases with increasing pH. The CO_2 compensation concentration found at the highest pH tested is less than 1/10 (*Ranunculus*) and about 1/100 (*Cladophora*) respectively of the value found at the lowest pH tested. In view of the kinetic properties of the RuBPc-o from submerged freshwater vascular plants (Salvucci and Bowes 1981) and for chlorophyte freshwater algae (Jordan and Ogren 1981; Raven and Beardall 1981b) it is likely that the CO_2 compensation concentration observed at the lowest pH tested might (*Ranunculus*) and almost certainly (*Cladophora*) require the operation of a 'CO_2 concentrating mechanism'; both plants clearly need such a mechanism at the highest pH values (above 9) which were tested. The findings for *Cladophora glomerata* confirm and extend earlier studies on the CO_2 compensation concentration in this plant at pH values below 8.0 (Birmingham and Colman 1979) and above 8.0 (Gessner 1959), while the *Ranunculus* data agree with most previous measurements of 'pH drifts' in this genus (Ruttner 1947; Gessner 1959; Hutchinson 1965; cf. Bristow 1969).

Evidence that the low CO_2 compensation concentrations achieved in the pH drift experiments with *Cladophora* (Table 6) and *Ranunculus* (Table 7) involve HCO_3^- 'use' (cf. Walker et al. 1980) at the plasmalemma rather than CO_2 entry at the plasmalemma with a 'CO_2 concentrating mechanism' at the chloroplast envelope is provided in Table 8. Here the increment of photosynthesis per unit increase in CO_2 concentration (determined at limiting CO_2 concentrations at pH 6.5) is compared with the photosynthetic C fixation in a pH drift experiment (computed

Table 8. Comparison of the measured initial slope of the photosynthetic rate/CO_2 concentration relationship at pH 6.5 (μmol C fixed (g.d.wt)$^{-1}$ h^{-1} (μM CO_2)$^{-1}$ with the inorganic C uptake in a pH drift experiment (computed from the rate of pH change according to Lindahl, 1963) divided by the mean CO_2 concentration present during the pH increase (μMol C fixed (g.d.wt)$^{-1}$ h^{-1} (μM CO_2)$^{-1}$

	Net inorganic C fixation per unit concentration of CO_2 (μmol C (g.d.wt)$^{-1}$ h^{-1} (μM CO_2)$^{-1}$)		
	Cladophora	*Lemanea*	*Ranunculus*
Calculated from slope of C fixation vs. CO_2 concentration relationship between the CO_2 compensation concentration and air-equilibrium CO_2 concentration at pH 6.5 (from Tables 2, 5, 6 and 7).	8.71 ± 0.19 (5)	6.13 ± 0.27 (3)	3.32 ± 0.21 (5)
Calculated from net C loss from solution corresponding to pH increase specified in brackets, and mean CO_2 concentration present over this pH range (from experiments reported in Table 5, line 3; Table 6, line 5; and Table 7 line 5)	169 ± 57 (pH 10.29–10.46)	0.323 ± 0.097 (6) (pH 8.65–8.78)	42.5 + 10.4 (6) (pH 9.87–10.12)

Table 9. $\delta^{13}C$ for organic C from *Cladophora, Lemanea* and *Ranunculus* from the Dichty Burn, for total inorganic C from the Dichty Burn (measured), and for HCO_3^- and CO_2 from the Dichty Burn (calculated from the measured $\delta^{13}C$ for total inorganic carbon at pH 8, 11° C, according to Mook et al. 1974)

Source of C	$\delta^{13}C$
Organic C of *Lemanea*	−38.9 ± 0.8
Organic C of *Cladophora*	−30.6 ± 1.6
Organic C of *Ranunculus*	−22.5 ± 0.9
Total inorganic C	− 5.5 ± 0.5
HCO_3^-	− 5.2 ± 0.5
CO_2	−15.9 ± 0.5

from the pH change and the alkalinity) divided by the mean CO_2 concentration present over the pH interval used to calculate the photosynthetic rate.

It will be seen from Table 8 that *Lemanea* has a much lower net photosynthesis per unit of (limiting) CO_2 concentration in the 'pH drift' experiment than in the 'IRGA' experiment. Since the pH in the pH drift experiment was only 8.65–8.78 and the photosynthetic rate of *Lemanea* is the same at pH 8.0 as it is at pH 6.5 (Table 2), it seems more reasonable to attribute this 19-fold difference to the much poorer CO_2 supply in the stagnant 'pH drift' experiment than in the well-stirred medium used in the 'IRGA' experiment. The relatively high rate of photosynthesis of *Lemanea* on an area basis (Tables 2 and 3) would make the absence of stirring a severe constraint on CO_2 supply. At all events the achieved rate of photosynthesis in the 'pH drift' experiment can be readily explained in terms of the characteristics of CO_2 use at pH 6.5; there is no need to invoke HCO_3^- use. By contrast, *Cladophora* and *Ranunculus* show respectively a 19-fold and a 13-fold *greater* rate of photosynthesis per unit of (limiting) CO_2 concentration at high pH in the 'pH drift' experiment than at pH 6.5 in the 'IRGA' experiment. It is unlikely, in view of the kinetics of HCO_3^- to CO_2 conversion at high pH (cf. Lucas 1975; Walker et al. 1980), that the very high rate of photosynthesis per unit free CO_2 at high pH values results from CO_2 supply to the plasmalemma by virtue of HCO_3^- dissociation and diffusion. Accordingly, the 13–19 fold greater rate of carbon assimilation at high pH cannot be explained in terms of CO_2 uptake by the cells, and HCO_3^- use must be occurring – either directly by HCO_3^- active transport, or indirectly (see discussion) by the production of localised acid regions (cf. Walker et al. 1980).

Table 9 shows the $\delta^{13}C$ values for organic C from the three plant species, together with that for total inorganic C in the Dichty Burn, and the calculated values for the HCO_3^- and CO_2 components of the total inorganic C. The inorganic C $\delta^{13}C$ values give clear evidence for an input of inorganic C from heterotrophic metabolism into the Burn water, since the $\delta^{13}C$ value for dissolved free CO_2 is significantly more negative (−15.9‰) than would be expected for the dissolution at 11° C of atmospheric CO_2 with a $\delta^{13}C$ value of −7‰ i.e. −8.1‰ (Mook et al. 1974). Such an input is consistent with the finding (from measurements of alkalinity, pH and total inorganic C in the Burn water) that the free CO_2 concentration in the Burn water is 2–3 times that expected for air-equilibrium at pH 8 (cf. Browse et al. 1977; Rau 1978; Bristow 1969; Skirrow 1975; Golterman 1975). Assuming the major heterotrophic inorganic C input occurs at a $\delta^{13}C$ value of −25‰ (from the dominant phototroph in the Burn, i.e. *Ranunculus*: Table 9; and from C_3 plants growing on its banks) it can be computed that some 0.31 of the inorganic C comes from heterotrophic metabolism of organic C at −25‰, with the remaining 0.69 coming from sedimentary $CaCO_3$ ($\delta^{13}C$ of about 0‰) and from atmospheric CO_2 ($\delta^{13}C = -7$‰). This would give a total inorganic C in the Burn which is rather lower (1.5 times air-equilibrium values of inorganic C at pH 8) than the 2–3 times which is observed. At all events these data lend support to the notion that there is substantial allochthonous organic input and metabolism in most streams (cf. Hynes 1975). We note that, even in rapidly-flowing streams, the equilibration of metabolic gases with the atmosphere is relatively slow (rate constants 0.2–0.6 h^{-1} for CO_2: Schurr and Ruchti 1975, 1977) unless white water is found (cf. Ledger, 1981). For the Dichty with a mean velocity of perhaps 1 km h^{-1} it is clear that the heterotrophically derived 'excess inorganic C' will, on average, be carried past a lot of plants before it is lost to the atmosphere. By the same token, even the *Lemanea* habitat (with rapid water movement and shallow, but not markedly white, water) will not benefit from an input of atmospheric CO_2 (cf. Raven and Beardall 1981a); rather it will tend to be a site of more rapid CO_2 *loss* to the atmosphere.

Free CO_2 is presumably the C source for *Lemanea* (Raven and Beardall 1981a; see above), so the $\Delta\delta^{13}C$ ($\delta^{13}C$ sample *minus* $\delta^{13}C$ source) of −23% (Table 9) is consistent with fixation by RuBPc-o with a relatively low diffusion resistance between source CO_2 and enzyme (O'Leary 1981; Smith and Walker 1980). Unless the discrimination against $\delta^{13}C$ by the carboxylase were abnormally high (i.e. giving products with a very negative $\Delta\delta^{13}C$ value under non-transport-limited conditions) it is unlikely that

a substantial contribution from HCO_3^- to CO_2 conversion in an unrenewed boundary layer, generating CO_2 with a less negative $\delta^{13}C$ value is significant for *Lemanea* (cf. Smith and Walker 1980); this is consistent with the high water flow rates in the *Lemanea* habitat.

At pH 8 in the Burn it is possible that HCO_3^- ($\delta^{13}C$ of −5.2) can contribute inorganic C to photosynthesis by *Cladophora* and *Ranunculus*, thus helping to account for the less negative $\delta^{13}C$ values of organic C in these plants than in *Lemanea* (Table 9; cf. Raven 1970; Smith and Walker 1980; Osmond et al. 1981). The lower water velocities around *Cladophora* and *Ranunculus* may also be involved (see Discussion). It is unlikely that CO_2 from the sediment passing through the plant (in the gas phase) rather than outside the plant is a significant feature of photosynthesis by *Ranunculus*, in contrast to the situation in rosette (and some other) plants in waters containing relatively little CO_2, for the following reasons: (1) the diffusion pathlength in the gas phase is relatively large – the flower-bearing shoots may be almost a metre from the nearest root in sediment from which CO_2 can be obtained; (2) while the stems have large gas canals, these seem to function mainly to bring the flowers up to the water surface (Wilson 1947); there are diaphragms at the nodes which impede transport, and (Cook 1969, notwithstanding) the ultimate ramuli of the leaves are not well provided with intercellular air spaces: our leaves resemble those depicted in Fig. 8.14B of Sculthorpe, 1967; (3) there is no 'special relationship' of chlorophyllous cells with the boundaries of the major intercellular air spaces of stems and petioles (unpublished anatomical investigations). All of these points contrast with the well-established cases of the 'internal' transport of CO_2 from sediments to sites of photosynthesis (Wium-Anderson 1971; Wium-Anderson and Anderson 1972; Sondergaard and Sand-Jensen 1979a, b; cf. Raven 1970), and suggest that this sedimentary CO_2, which probably has an even more negative $\delta^{13}C$ than the bulk Burn-water, is not a significant contributor to net CO_2 assimilation or to whole-plant $\delta^{13}C$ values.

The $\delta^{13}C$ value for our *Ranunculus* (Table 9) is within the (rather wide) range for submerged *Ranunculus* species quoted by Osmond et al. (1981); part of the variation in the $\delta^{13}C$ values obtained by Osmond et al. (1981) can be related to water velocity, with more negative values in faster-flowing streams.

Discussion

The data presented in this paper suggest that there are substantial differences between the three plants studied with respect to their capacity to use HCO_3^- ions in photosynthesis (i.e. to transport HCO_3^- into the cells, or to produce an acidic extracellular compartment which can give superficially similar behaviour: Walker et al. 1980). While *Lemanea* (cf. Raven and Beardall 1981a) cannot use HCO_3^-, it appears that both *Ranunculus* and *Cladophora* have this capacity. Not only is the CO_2 compensation concentration at pH 6.5 lower for these two plants than for *Lemanea*, but the CO_2 compensation concentration decreases with increasing external pH. The low CO_2 compensation concentration at low extracellular pH values (pH 6.5) could be explained in terms of a CO_2 accumulation mechanism based on the chloroplast envelope (cf. Raven and Glidewell 1978; Beardall 1981; Beardall and Raven 1981); the decrease in CO_2 compensation concentration with increasing pH, and the rate at which inorganic C is consumed in these pH drift experiments (i.e. the rate at which the equilibrium pH is reached) seem only to be explicable in terms of a plasmalemma mechanism for HCO_3^- use (active HCO_3^- influx or localised H^+ efflux). In *Cladophora* there does not seem to be morphological scope for the 'localised H^+ efflux' mechanism to work (cf. Raven and Beardall 1981a): in *Ranunculus* the paradermal occurrence of 'transfer cell'-type cell wall and plasmalemma invaginations in the epidermal cells of the leaves (Gunning and Pate 1969; Pate and Gunning 1972) may be involved in the separation of acidic and alkaline extracellular regions. This has been mentioned (in the context of a different mechanism of HCO_3^- use from that proposed by Walker et al. 1980) by Raven and Smith (1980).

It is of interest that these differences in CO_2 compensation concentration at low extracellular pH values (Tables 5–7) are not parallelled by differences in the half-saturation value of CO_2 concentration; it might be anticipated that, if the low CO_2 compensation concentration in *Ranunculus* and, particularly, *Cladophora* represents the activity of a CO_2 concentrating mechanism, then these organisms should show a higher in vivo affinity for CO_2. However, the data available (see discussion of Table 3) suggest that, if anything, the affinity is higher for *Lemanea* than for *Ranunculus* or *Cladophora*. However, it is clear that the resistance to inorganic C diffusion and/or the presence of a CO_2 concentrating mechanism is not the sole determinant of the half-saturation CO_2 concentration in vivo. In an elegant analysis Farquhar and Von Caemmerer (1981) have shown that the imposition of a 'ceiling' on the rate of photosynthesis at CO_2 saturation by the capacity of redox reactions is lower than the 'roof' imposed by the capacity of RuBPc-o and associated reactions. It is of great interest that this transition from a 'carboxylation' limitation to a 'light reaction capacity' limitation as the inorganic C concentration is increased gives a similar 'Blackman-type' relationship to that produced by rate-limitation by CO_2 diffusion at low CO_2 concentrations and by carboxylation (or the capacity of light reactions) at higher CO_2 concentrations. Thus the slightly lower half-saturation CO_2 concentration in *Lemanea* could result from a higher RuPBc-o capacity in this organism which outweighs the effect of any CO_2-concentrating mechanism which may be present in *Cladophora* and *Ranunculus*.

At pH 8 (in the Burn) it is possible that HCO_3^- use can be significant in photosynthesis by *Ranunculus* and *Cladophora*, but not in *Lemanea*; since the CO_2 concentration in the Burn is some 35–50 μM (see discussion of Table 8) none of the organisms will be saturated with CO_2 in vivo unless the stirring in vivo is much better than in in vitro determinations of the photosynthetic rate at 300 ppm CO_2 in the gas phase (Table 3; see below). Thus the capacity for HCO_3^- use may help to offset the higher half-saturation constant for CO_2 in the two HCO_3^--users.

The $\delta^{13}C$ measurements (Table 9) may be used to provide quantitative information about limitations on photosynthetic CO_2 assimilation in situ. For a system in which CO_2 is supplied by diffusion from bulk phase CO_2 to RuBPc-o, the relative limitations to photosynthesis attributable to diffusion of CO_2 and to RuBPc-o activity can be estimated from a relationship based on Eq. (6) of Farquhar (1980) with modifications for use in aquatic environments. The appropriate Eq. (1) is:

$$c_c/c_o = \frac{\delta\,\text{plant} - \delta\,\text{solution} + a}{-(b-a)} \qquad (1)$$

where c_o is the CO_2 concentration (mol cm^{-3}) in the bulk medium;
c_c is the CO_2 concentration at the site of RuBPc-o activity during steady-state photosynthesis (mol cm^{-3});
δ plant is the $\delta^{13}C$ value of the plant material (‰ relative to PDB);

a is the δ value associated with CO_2 diffusion in solution from a source to a sink ($^0/_{00}$ relative to source CO_2);
b is the δ value associated with CO_2 fixation by RuBPc-o ($^0/_{00}$ relative to the CO_2 supplied to the enzyme active centre).

a may be taken as equal to zero (Table 1 of O'Leary 1981) and b equal to 30% (Table 1 of O'Leary 1981; cf. Farquhar 1980). For a c_o value in the Dichty Burn of 30 μM, equation 1 give c_c values of 23 μM (*Lemanea*), 15 μM (*Cladophora*) and 7 μM (*Ranunculus*). This suggests that (granted the assumptions that all CO_2 supply is by diffusion, and b is $30^0/_{00}$, are correct) the major resistance to *Lemanea* photosynthesis is associated with biochemical reactions, that in *Ranunculus* is associated with diffusion, while in *Cladophora* the two reactions are more nearly equal.

These values, together with morphological and photosynthetic data from Table 1 and 2, may be used to compute apparent thicknesses of unstirred layers at the plant surface, using the equation for diffusion through an annular layer surrounding a cylinder (cf. Crank 1967). The appropriate Eq. (2) is:

$$\ln(r_2/r_1)=\frac{2\cdot\pi\cdot l\cdot D_{CO_2}(c_o-c_c)}{F} \quad (2)$$

where r_1 is the radius of the (cylindrical) photosynthetic organ (cm);
r_2 is the radius of the cylindrical organ plus the external unstirred layer (cm)
c_o is the CO_2 concentration in the bulk phase, i.e. at and further from the photosynthetic organ than r_2 (mol cm^{-3})
c_c is the CO_2 concentration at the site of RuBPc-o activity, assumed to be at r_1 (mol cm^{-3})
D_{CO_2} is the diffusion coefficient for CO_2 in water ($cm^2\ s^{-1}$)
F is the total net C fixation by the organ (mol s^{-1})
l is the length of the photosynthetic organ (cm).

F/l is determined from the measured rates of photosynthesis on an area basis (Table 2) and the morphometric data in Table 1, with the rate of CO_2 fixation being adjusted to that which would be found in Dichty Burn water (30 μM CO_2) by assuming a linear relationship between CO_2 fixation rate and CO_2 concentration between 15.7 and 30 μM (cf. discussion above); D_{CO_2} was taken as $1.7\cdot10^{-5}\ cm^2\ s^{-1}$; r_1 was the measured mean radius of the photosynthetic organ. Using these values in Eq. (2) gives the following values for the thickness of the unstirred layer (r_2-r_1): *Lemanea* (r_2-r_1) 11 μm (r_1 is 450 μm); *Ranunculus* (r_2-r_1) 132 μm (r_1 is 178 μm); *Cladophora* (r_2-r_1) $2.8\cdot10^4$ μm (r_1 is 23 μm).

We may note that the very high figure for *Cladophora* probably indicates the inapplicability of Eq. (2) when $r_2 \gg r_1$. An alternative approach is to assume planar rather than radial diffusion through the unstirred layer, an assumption which leads to increasing *over*-estimation of thickness of the unstirred layers as the radius r_1 is decreased. The appropriate equation here is a form of Fick's law [Eq. (3)]:

$$u=\frac{(c_o-c_c)\cdot D_{CO_2}}{P} \quad (3)$$

where u is the unstirred layer thickness (cm);
P is the area-based rate of photosynthesis (mol $cm^{-2}\ s^{-1}$) and other parameters are as defined earlier.

Equation (3) gives values of u [analagous to (r_2–r_1) derived from Eq. (2)] of 11 μm for *Lemanea*, 99 μm for *Ranunculus* and 164 μm for *Cladophora*.

Granted the assumptions made, we can conclude that the unstirred layer thickness is about 11 μm for *Lemanea*, while for *Ranunculus* and *Cladophora* the values are respectively rather below and rather above 100 μm. These estimates of unstirred layer thickness in situ are under-estimates if the achieved photosynthetic rate in situ is lower than that found in the laboratory. This effect is likely to be smallest for *Lemanea* where the rapid water flow rate of 1 m s^{-1} considerably exceeds estimates of 'velocity saturation' for minimising unstirred layers around submerged macrophytes (e.g. Wheeler 1980; Parker 1981), and the sparse plant population at the *Lemanea* site means that all of the *Lemanea* 'bristles' are exposed to the rapidly-flowing water. These factors minimise CO_2 depletion around the plant, and account for the low unstirred layer thickness computed for *Lemanea* despite its having a higher area-based rate of CO_2 fixation than *Ranunculus* and *Cladophora* under either CO_2-limiting or CO_2-saturating.

The other two plants have substantially larger unstirred layer thicknesses computed from Eqs. (2) and (3) than does *Lemanea*. For both *Ranunculus* and *Cladophora* it is likely that unstirred layers of 100 μm or so around the photosynthetic elements would overlap in situ, thus reducing F [Eq. (2)] or P [Eq. (3)] and further increasing the computed value of unstirred layer thickness. A similar effect would result from any mutual shading of the densely bunched photosynthetic elements of *Ranunculus* and *Cladophora*. Acordingly we may relate the large unstirred layer thicknesses in these two plants to the density at which the plants occur in the Burn, with very low water velocities over photosynthetic elements in the centre of the plant despite high velocities over the plant as a whole.

However, two factors suggest that the unstirred layer thicknesses computed from $\delta^{13}C$ values may be over-estimates for *Cladophora* and *Ranunculus*. One factor is the re-equilibration of CO_2 and HCO_3^- following photosynthetic removal of CO_2 from the solution bathing the inner parts of a plant; replacement of CO_2 from HCO_3^- rather than from bulk phase CO_2 in this incompletely mixed system would lead to a more positive value for $\delta^{13}C$ of the CO_2 used by the plants than obtains in the bulk medium. This means that Eq. (1) would over-estimate (c_o-c_c), and hence Eq. (2) and (3) would over-estimate (r_2-r_1) and u respectively. However, such a depletion in the effective CO_2 concentration means that the local c_o (and hence c_c) would be so reduced as to decrease F [Eq. (2)] and P [Eq. (3)], thus countering the above-mentioned decrease in (c_o-c_c); further, the extent of extracellular HCO_3^- to CO_2 conversion relative to photosynthetic CO_2 demand is likely to be small, in view of the limited volume of the unstirred layer and the small rate constant for HCO_3^- to CO_2 conversion in solutions of pH value in excess of 8.0 (cf. Walker et al. 1980). The other factor is the ability of both *Ranunculus* and *Cladophora* to use HCO_3^- in photosynthesis. Isotopic discrimination during HCO_3^- uptake is unknown, but if it is small (cf. Raven 1970; Smith and Walker 1980) and leakage of CO_2 generated within the cell from HCO_3^- is limited, then the $\delta^{13}C$ of plant material produced by the use of external HCO_3^- would be close to that of external HCO_3^-; intermediate values would obtain from the simultaneous use of exogenous CO_2 and HCO_3^-. Substantial HCO_3^- use could mean that the unstirred layer thicknesses were substantially over-estimated for *Cladophora* and *Ranunculus* in our analysis above, with corresponding reductions in the problems which these plants would encounter in acquiring other nutrients (e.g. P, N) from low concentrations in the bulk medium; this would be of particular significance for the haptophyte *Cladophora* which, unlike the rhizophyte *Ranunculus*, has no direct access to P and N from sediments via a root or rhizoid system (cf. Raven 1981).

However, this problem is somewhat mitigated by the low area-based N and P fluxes required to give the observed C:N:P ratio (cf. Table 2).

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Recent work (Beardall, Griffiths and Raven, in preparation) shows that growth of *Chlorella emersonii* under conditions in which the CO_2 accumulating mechanism is operative (i.e. grown under CO_2^- or N-limitation) results in $\Delta\delta^{13}C$ values which are less negative than those of cells in which the CO_2 accumulating mechanism is repressed (grown with high CO_2^+ high N). This data supports our suggestion that the operation of a CO_2 concentration mechanism can lead to a less negative $\delta^{13}C$ value in plant organic matter relative to that of source CO_2.