New Phytol. (1988), **108**, 125–148

Tansley Review No. 13
The role of CO_2 uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L.

BY JOHN A. RAVEN[1,3], LINDA L. HANDLEY[1,2,3], JEFFREY J. MACFARLANE[3,4], SHONA McINROY[3], LEWIS McKENZIE[3], JENNIFER H. RICHARDS[1] AND GORAN SAMUELSSON[3,5]

[1] *Department of Biological Sciences, Florida International University, Tamiami Campus, Miami, Florida 33176, U.S.A.*
[2] *Drinking Water Research Center, Florida International University, Tamiami Campus, Miami, Florida 33176, U.S.A.*
[3] *Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, U.K.**
[4] *Roseworthy Agricultural College, Roseworthy, S.A. 5371, Australia†*
[5] *Department of Plant Physiology, University of Umea, S-91087 Umea, Sweden‡*

(Received 26 May 1987; accepted 8 October 1987)

CONTENTS

Summary 125
I. Introduction 126
II. Material and Methods 127
III. Results and Discussion 129
1. Weights, areas and chemical composition 129
2. The role of roots in acquisition of inorganic C 131
3. The form in which inorganic C enters the plant 134
4. Measurements of titratable acidity and malate content at dawn and dusk 136
5. Significance of universal occurrence of substantial acquisition of CO_2 by roots in astomatous isoetids 139
6. Evolution of the isoetid life-form 140
IV. Conclusions 142
Acknowledgements 142
References 143

SUMMARY

The isoetid life-form was originally defined on morphological grounds; subsequent physiological investigations showed that all of the isoetids examined took up a large fraction of the inorganic C fixed in their leaves from the root medium under natural conditions, and that some of them carried out much of their assimilation of inorganic C via a CAM-like mechanism. Root-dominated uptake of inorganic C appeared to be unique to, and ubiquitous in, the isoetids. However, a large capacity for CAM-like metabolism in submerged vascular plants is not universal in isoetids, nor is it restricted to this life-form, being also found in *Crassula aquatica*.

The work described here shows that submerged specimens of the North American *Eriocaulon decangulare* have a high fraction of their dry weight in the root system, a trait characteristic of isoetids but uncommon in other submerged vascular plants. *E. decangulare* has vesicular–arbuscular mycorrhizas, as do other flowering plant isoetids but not, generally, submerged *Isoetes* spp. Under conditions of natural supply of inorganic C, *E. decangulare*, like other isoetids, takes up most of its inorganic C through its roots. Uptake of inorganic C by both

Present address:
* J. A. Raven and Linda L. Handley.
† J. J. Macfarlane.
‡ G. Samuelson.

roots and shoots involves CO_2 rather than HCO_3^-: photosynthesis at high external pH values does not exceed the rate of uncatalysed HCO_3^- to CO_2 conversion in the medium and there is no detectable extracellular carbonic anhydrase activity.

Measurements of titratable acidity and of malate content of leaves sampled at dawn and at dusk showed that *E. decangulare*, growing and tested under either emersed or submersed conditions, did not exhibit CAM-like behaviour. CAM was also absent from three non-isoetid aquatic macrophytes (*Amphibolis antarctica*, *Ecklonia radiata* and *Vallisneria spiralis*) which were examined. *E. decangulare* thus resembles all other isoetids tested in acquiring much of its inorganic C via the root system. *E. decangulare* also resembles most of the isoetids which are not members of the Isoetaceae (e.g.) *E. septangulare*, *Lobelia dortmanna* and *Subularia aquatica*) but differs from submerged Isoetaceae and *Littorella uniflora* in lacking CAM.

The ecological significance of uptake of CO_2 via the roots and, where it occurs, of CAM in isoetids may be related to either inorganic C or, via improved N use efficiency, inorganic C as a limiting resource.

The isoetid life-forms has evolved independently in at last five different families of vascular plants; it probably derived fairly immediately from terrestrial or amphibious ancestors with a similar rosette form. Emergent Isoetaceae with acquisition of CO_2 via roots and CAM probably evolved from submerged isoetids.

Key words: Crassulacean Acid Metabolism; carbonic anhydrase; malic acid; permeant C species; titratable acidity.

1. INTRODUCTION

The isoetids (Den Hartog & Segal, 1964; Hageweg & Brenkert, 1969; Hutchinson, 1975; Richardson, *et al.*, 1984; Farmer & Spence, 1985, 1986: Boston, 1986; Boston & Adams, 1987) are a life-form of usually submerged aquatic rhizophytes (Luther, 1949). This life-form was named after the genus *Isoetes* but in this paper, the term is used to mean a plant of the isoetid life-form, regardless of whether or not it is a member of the genus *Isoetes*. Isoetids are characterized by relatively small size, with roots comprising a large fraction of the fresh weight, dry weight, volume and surface area of the plant, and with leaves arranged in a rosette. A large fraction of the volume of the plant is gas space. The plastids in the leaves are mainly in cells surrounding gas spaces, even in submerged specimens. This contrasts with most aquatic macrophytes where plastids are typically aggregated mainly in the epidermis. These morphological and anatomical characteristics can be related fairly readily to a large, and often predominant, supply of CO_2 to the plastids from the sediment *via* the roots and the intercellular gas spaces (see Raven, Osborne & Johnston, 1985). Less readily discerned from their anatomy is the CAM-like behaviour of some submerged, and emergent but astomatous, isoetids (see Raven *et al.*, 1985).

The term 'isoetid' will be used in a limited sense in this paper following the usage of Richardson *et al.* (1984) and Farmer & Spence (1985). The organism considered to be isoetids and which have been used in ecophysiological studies are listed in Table 1. *Juncus bulbosus* L., *Eleocharis acicularis* (L). Roem and Schult and *Ranunculus reptans* L. are not included; this contrasts with the usage of Hutchinson (1975, p. 130) and of Kansanen & Niemi (1974). The status of the Centrolepidaceae and Hydatellaceae (see table 7 of Hutchinson, 1975; Morley & Toelken, 1983; Dahlgren, Clifford & Yeo, 1985) as isoetids is not clear. From the ecophysiological point of view, it is important to remember that isoetids are typically inhabitants of oligotrophic lakes and are evergreen perennials. However, some isoetid inhabitants of oligotrophic lakes are annuals (e.g. *Subularia aquatica*). Furthermore, many members of the genus *Isoetes* which have the isoetid life form are inhabitants of seasonal pools and do not share all of the characteristics discussed in this paper for the inhabitants of oligotrophic lakes (Keeley, 1987 and personal communication).

Relatively little work has been carried out on the submerged isoetid examples of the genus *Eriocaulon* with respect to acquisition of CO_2 by roots and CAM-like metabolism. *Eriocaulon* is a predominantly tropical

Table 1. *Isoetids which have been used in ecophysiological investigations on acquisition of carbon*

Class of Tracheophyta	Family	Organism	Distribution
Lycopsida	Isoetaceae	*Isoetes echinospora* Durieu	Europe, N. America
		Isoetes lacustris L.	Europe
		Isoetes macrospora Durieu	N. America
		Isoetes howellii Englemann	N. America
	Isoetaceae	*Stylites* (= *Isoetes*) *andicola* Amstutz	Peru
Magnoliopsida	Brassicaceae	*Subularia aquatica* L.	Europe
	Plantaginaceae	*Littorella uniflora* (L.) Aschers	Europe, N. America
	Lobeliaceae	*Lobelia dortmanna* L.	Europe, N. America
	Eriocaulaceae	*Eriocaulon septangulare* With.	Europe, N. America
		Eriocaulon decangulare L.	N. America

genus centred in the new world; several species frequently grow submerged and have the isoetid life-form (Tomlinson, 1969; Hutchinson, 1975; Godfrey & Wooton, 1979; Dahlgren & Clifford, 1982; Dahlgren *et al.* 1985). Farmer & Spence (1985) reported the absence of CAM-like behaviour in Scottish populations of *E. septangulare*, while Raven *et al.* (1985) indicated the absence of CAM, and the predominant acquisition of CO_2 through the roots under 'natural' conditions, for Florida populations of *E. decangulare*. This paper gives more details of the two sets of observations on *E. decangulare*, and further data on quantitative anatomy and morphology, C and N contents, occurrence of mycorrhizas, carbonic anhydrase activity and the form of inorganic C that is taken up, which are relevant to the acquisition and handling of inorganic C by the plant.

II. MATERIALS AND METHODS

Submersed and emersed specimens of *E. decangulare* were collected from pine sloughs in Palm Beach County, Florida and stored in natural substratum and overlying water at 22 °C and 200 μmol m^{-2} s^{-1} (400–700 nm) photon flux density in a 12 h light–12 h dark regime. The pH of water above the sediment in which submersed plants were growing and of the water in the sediment or soil in which submersed and emersed plants were growing, was measured using a glass combination electrode; inorganic C was determined by Gran titration (Mackereth, Heron & Talling, 1978).

Plants were sampled for fresh weight of leaves, stems and roots. Subsamples were dried to constant weight at 80 °C; some of the dried samples were analysed for C, H and N in a Carlo-Erba C, H, N analyser. Fresh subsamples were used to find surface area and volume per unit fresh weight, or to measure chlorophyll *a* and *b* by extraction in 90% acetone, using the equations of Jeffrey & Humphrey (1975), or for anatomical investigations.

The contributions of inorganic C from the root medium and the shoot medium under natural conditions were determined by measuring O_2 exchange in the presence of 'natural' and 'unnatural' concentrations of inorganic C around the root and the shoot. Plants of *E. decangulare* which had grown submersed were incubated in filtered water from the plant's natural habitat with the concentration of inorganic C adjusted to the required level; roots and shoots were incubated in separate compartments. The concentration of inorganic C was adjusted by acidification to pH 4·5 with HCl followed by sparging for two hours with CO_2-free air. The pH was then readjusted to 6·0 with 10 m^{-3} mol MES (*N*-morpholino-enthanesulphonate) and inorganic C-free NaOH solution (500 mol m^{-3}). The required volume of 100 mol $NaHCO_3$ m^{-3} was added and the pH readjusted to 6·0 with HCl. The incubations were carried out for two to five h in a glass apparatus which separated the medium around leaves and roots. The 'shoot' and 'root' compartments each had a volume of $15 \cdot 10^{-6}$ m^3 (15 cm^3) and could be manipulated separately *via* sampling ports sealed with 'Suba Seals' (Fig. 1); the space between the stem and the apparatus was sealed with (non-toxic) Blu-Tac, preventing external communication between the two compartments. Incubation was at 20–25 °C in the dark

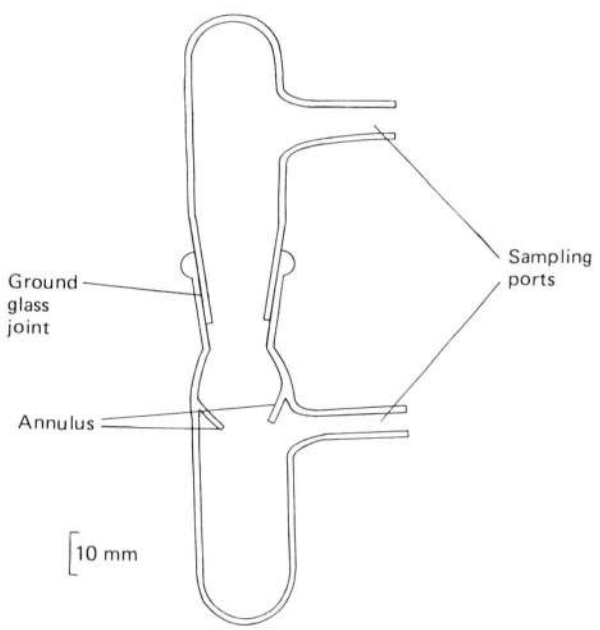


Figure 1. The apparatus used to incubate roots and shoots of *Eriocaulon decangulare* in different solutions.

(aluminium foil around root and shoot compartments) or in the light [250 μmol photon m^{-2} s^{-1} (400–700 nm)] from fluorescent tubes, with no Al foil round the shoot compartment. O_2 concentration in samples of the solution at the start of the incubation, and of the solutions from the two compartments at the end of the incubation, were measured by the Winkler method. This method involves consumption of O_2 from a solution in oxidizing Mn^{II} to Mn^{III} in alkaline conditions. Acidification in the presence of I^- allows the Mn^{III} to oxidize I^- to I_2, which is determined by titration with standard $Na_2S_2O_3$ (see Grasshoff, 1983, for a detailed discussion). No attempt was made to correct the O_2 changes in the bathing media for the accumulation (in the light) or depletion (in the dark) of gaseous O_2 in the intercellular gas spaces so that the rates of photosynthesis and respiration are under-estimates. The presence of these lacunae influence estimates of photosynthesis and respiration in intact submersed vascular plants by net O_2 (Sorrell & Dromgoole, 1986), net inorganic C (Sondergaard, 1979) and ^{14}C (Sondergaard & Sand-Jensen, 1979*b*) methods. The incubation solutions were not stirred.

Computation of the (uncatalysed) rate at which a solution of inorganic C at high pH ($< pK_a$ of the CO_2/HCO_3^- system) can supply CO_2 followed Miller & Colman (1980), Cook, Lanaras & Colman (1986) and Johnston & Raven (1986*a*). Equation (11) of Miller & Colman (1980), with the same numerical values for the first and second dissociation constants for the inorganic carbon system as used by these workers (zero ionic strength, 25 °C), is given here as equation (1)

$$\frac{d[CO_2]}{dt} = \frac{[DIC](1 + 5{\cdot}25 \times 10^8[H^+])}{5000 \times \left(\frac{1 + [H^+] + 4{\cdot}69 \times 10^{-11}}{4{\cdot}45 \times 10^{-7}[H^+]}\right)}, \quad (1)$$

where concentrations and equilibrium constants are expressed in mol dm^{-3} ($\equiv$ kmol m^{-3}), [DIC] is the total inorganic C connection, and $d[CO_2]/dt$ is the maximum rate at which HCO_3^- can be converted to CO_2 in units of kmol CO_2 m^{-3} s^{-1}. Correction of the 'infinite dilution' input rate constants and equilibrium constants for the small ionic strength of the medium was not made (see Johnson, 1982); such corrections are important for seawater and even for carboxylase assay media, as well as for carboxylases *in vivo* (McNeil *et al.*, 1981; Besford, 1984; Yokota & Kitaoka, 1985).

An attempt to define the *upper* limit on the unstirred layer (diffusion boundary layer) thickness for CO_2 uptake by *E. decangulare* roots was made using equation (2) of Raven, Beardall & Griffiths (1982), a relationship obtained from Crank (1956). This equation is given here as equation (2):

$$\ln\left(\frac{r+x}{r}\right) = \frac{2ld(C_b - C_s)}{F}, \quad (2)$$

where r = radius of a cylindrical organ (m), x = thickness of boundary layer round cylindrical organ (m), l = length of organ (m plant^{-1}), D = diffusion coefficient of CO_2/m^2 s^{-1}, C_b = CO_2 concentration in the bulk phase/mol m^{-3}, C_s = CO_2 concentration at surface of organ/mol m^{-3}, F = Rate of net CO_2 uptake the organ/mol CO_2 plant^{-1} s^{-1}.

This equation is used to put an *upper* limit on x by using a value of F which corresponds to a limiting' bulk CO_2 concentration C_b, and setting $C_s = 0$. These assumptions imply that boundary layer diffusion is the *only* process limiting the rate of CO_2 entry to the plant organ. Total carbonic anhydrase activity was measured on homogenates, using the methods of Weaver & Wetzel (1980). Extracellular carbonic anhydrase was determined using the same electrometric assay as for the total enzyme, but on intact plant parts.

The titratable acidity of leaf, stem and root extracts of *E. decangulare* were determined on 1 g samples taken from plants held under natural (outdoor) conditions for four day periods in November 1984 in Miami, i.e. with a photoperiod of $\sim$ 12 h. Samples of the relevant tissue of 1 g were taken at dawn and dusk, cut finely with scissors and ground in distilled water in a pestle and mortar. The homogenate was made up to 5×10^{-5} m^3 with distilled water, its pH was measured, and titration with 10 mol NaOH m^{-3} was conducted to pH 6·0 and pH 7·0. Some measurements were made on material held in the 12 h light:12 h dark regime used for storage. Titratable acidity measurements were also made on the seagrass, *Amphibolis antarctica*, and the phaeophyte macroalga, *Ecklonia radiata* (Laminariales), collected from Victor Harbor and Port Elliot, South Australia, and stored out of doors in seawater at Aldgate, South Australia. Samples of leaves of *A. antarctica* and of the mature, apical, parts of the blade of *E. radiata*, were assayed for titratable acidity as described above for *E. decangulare*. *Vallisneria spiralis* was grown in a heated glasshouse in Dundee; during the experimental period in which titratable acidity was measured it was given 12 h light:12 h dark regime, with day-length extension and supplementary light supplied by sodium lamps giving $\sim$ 100 μmol photon m^{-2} s^{-1} at the water surface during the daylength extension. Malate content of plant extracts was determined by parallel with the titratable acidity, using the spectrophotometric method of Mollering (1974), used also by Johnson & Raven (1986*b*).

Carbon isotope analysis followed the methods used by Richardson *et al.* (1984). Results are expressed as $\delta^{13}C_{PDB}$ (‰), defined in equation (3):

$$\delta^{13}C_{PDB} = \left[\frac{^{13}C/^{12}C_{sample} - 1}{^{13}C/^{12}C_{PDB}}\right] \times 1000, \quad (3)$$

where subscripts 'samples' and PDB' refer to the CO_2 derived from the plant or from the inorganic C in its environment, and the standard carbonate of the Pee-Dee Belemnite respectively.

III. RESULTS AND DISCUSSION

1. *Weights, areas and chemical composition*

Table 2 shows some weight and area values for *E. decangulare*. Comparison with data for other isoetids suggests that *E. decangulare* is fairly typical of this group.

The plants used here are similar in total and organ dry and fresh weight, and number per plant and length per plant of leaves and roots to plants of *Isoetes lacustris* and *Lobelia dortmanna* used by Richardson *et al.* (1984). Too much importance must not be placed on these values which pertain to the larger and older plants in the populations, as is shown by the demographic studies of Moeller (1978) on North American populations of *L. dortmanna*, but they do indicate that the plants approach similar sizes. The emergent, astomatous *Stylites* (= *Isoetes*?) *andicola* investigated by Keeley, Osmond & Raven (1984) is larger than *E. decangulare*.

The larger fraction of the dry and fresh weight of *E. decangulare* which is present in the roots is characteristic of the isoetids (Table 3). Isoetids usually have at least 0·30 of their dry weight in roots, although one report on *Isoetes lacustris* shows that roots *plus stems* may only comprise 0·20 of the total dry weight, while non-isoetids have less than 0·30 of their dry weight in roots or rhizoids. There is thus little overlap between the non-isoetids with the highest root fraction and the isoetids with the lowest root fraction. This point is taken up later in relation to evolution of isoetids (Section III 6).

The data on leaf area per unit dry or fresh weight, and on chlorophyll per unit area or per unit dry or fresh weight in *E. decangulare* (Table 2) may be compared with those for other isoetids (Sand-Jensen, 1978; Keeley & Busch, 1984) and for non-isoetid aquatic plants (Spence & Chrystal 1970). As a non-isoetid baseline, we use the data of Spence & Chrystal (1970) on two species of *Potamogeton* grown under sun and shade conditions; the range of chlorophyll contents is 2·6–12·6 μmol chlorophyll *a* per g d. wt. The data of Keeley & Busch (1984) in *I. howellii* leaves suggest 12·1 μmol chlorophyll *a*+*b* per g d. wt, and 0·66 μmol

Table 2. *Number per plant, freshwater, dry weight, carbon, nitrogen, ash, external surface area, and chlorophyll* a *and* b *content, of leaves, stems and roots of submerged specimens of* Eriocaulon decangulare *L.*

	Leaf	Organ Stem	Root	Total
Number per plant of leaves and roots > 10 mm long	11·25 ± 2·47	1	52·75 ± 11·9814	n.a.
Fresh weight per plant/mg	561 ± 119	379 ± 151	904 ± 195	1844 ± 389
Dry weight per plant/mg	37·0 ± 7·5	41·2 ± 15·7	67·0 ± 13·9	145·2 ± 30·1
Leaf and root length per plant/mm	785 ± 174	n.a.	1797 ± 183	2528
Leaf and root area per plant/mm² (leaf area = both sides)	4121 ± 414	n.a.	7023 ± 703	11 144
Carbon/mmol (g dry wt)$^{-1}$	26·0, 25·5	31·4, 29·9	29·1, 30·3	(28·9)
Nitrogen/mmol (g dry wt)$^{-1}$	1·17, 1·18	1·24, 1·27	1·27, 1·33	(1·26)
Chlorophyll *a*/μmol (g dry wt)$^{-1}$	7·4 ± 0·8	0	0	1·89 ± 0·21
Chlorophyll *b*/μmol (g dry wt)$^{-1}$	2·7 ± 0·3	0	0	0·68 ± 0·07
Chlorophyll *a*+*b*/ μmol (g dry wt)$^{-1}$	0·666 ± 0·073	0	0	0·202 ± 0·022
Chlorophyll *a*+*b* μmol (m² both side leaf)$^{-1}$	91	n.a.	n.a.	n.a.
Leaf area (m² (g f. wt)$^{-1}$ (both sides of leaf)	0·0073	n.a.	n.a.	n.a.
Leaf area/m² (g d. wt)$^{-1}$ (both sides of leaf)	0·111 (per g d. wt of leaf; specific leaf area)	n.a.	n.a.	0·028 (per g d. wt of leaf area ratio)
Leaf + root area/m² (g d. wt)$^{-1}$ (leaf area = both sides)	n.a.	n.a.	n.a.	0·078 (leaf + root area ratio)

n.a., not applicable.

Table 3. *Fraction of total dry weight of plant parts in sediment*, and of roots and rhizoids, in various submersed aquatic rhizophytes (and the partially emersed, astomatous* Stylites andicola)

Plant species†	Fraction of dry weight in plant parts in sediment	Fraction of dry weight in roots or rhizoids	Reference
Clara hispida L.	0·022	0·022	Andrews (1982)
Elodea canadensis Rich in Michx.	?	0·026	Westlake (1965)
Eriocaulon decangulare L.‡	0·74	0·46	Table 1
Heteranthera dubia (Jacq) MacM.	?	0·055	Laczy, *et al.* (1983)
Isoetes lacustris L.‡	0·20–0·63	0·36	Kansanen & Niemi (1974); Sand-Jensen *et al.* (1982); Richardson *et al.* (1984)
Lobelia dortmanna L.‡	0·42–0·73	0·30–0·52	Kansanen & Niemi (1974); Richardson *et al.* (1984)
Myriophyllum spicatum L.	?	0·10–0·13	Waisel *et al.* (1982); Laczy *et al.* (1983)
Najas marina L.	?	0·30	Waisel *et al.* (1982)
Potamogeton lucens L.	?	0·10	Waisel *et al.* (1982)
Potamogeton perfoliatus L.	?	0·10	Waisel *et al.* (1982)
Ruppia occidentalis S. Wats.	< 0·43	< 0·18	Husband & Hickman (1985)
Stylites andicola Amstutz‡	0·75	0·31	Keeley *et al.* (1984)
Syringodium filiforme Kutz.	0·52	0·062	Raven & Handley, unpublished; cf. Raven *et al.* (1985)
Vallisneria americana Michx.	?	0·18	Laczy *et al.* (1983)
Zostera marina L.	0·48–0·56	0·12–0·26	Pregnall *et al.* (1987)

* Plant parts in sediment = roots or rhizoids, with, as appropriate, some or all of the stem of vascular plants.
† All plants except the alga *Chara hispida* L. (Chlorophyta: Charophyceae) are Tracheophytes.
‡ Denotes isoetid life-form.

chlorophyll $a+b$ per g f. wt; Sand-Jensen (1978) found about 0·7 and 1·0 μmol chlorophyll $a+b$ per g leaf f. wt for leaves of *Littorella uniflora* and *I. lacustris*, respectively. These values are similar to the values for *Eriocaulon* (10·1 μmol g^{-1} d. wt and 0·67 μmol g^{-1} f. wt).

The quoted values for leaf total surface area per g dry and fresh weight for *I. howellii* (Keeley & Busch, 1984) are 0·082 m^2 g^{-1} d. wt and 0·0044 m^2 g f. wt, respectively (corrected for a typographical error in the paper: Keeley, personal communication). The data in figure 2 of Sand-Jensen (1978) may be used to calculate surface area g^{-1} leaf d. wt of 0·052 m^2 for *I. lacustris* and 0·049 m^2 for *Littorella uniflora* (note that the specific leaf area quoted, i.e. 0·020 and 0·024 for *Isoetes* and *Littorella* respectively, presumably refer to projected leaf area, not *total* surface area). The differences in m^2 g^{-1} d. wt between the terete-leaved *Isoetes* and *Littorella* and the flat-leaved *Eriocaulon* agree with their anatomical differences. The leaf area (both sides) per unit fresh weight from *E. decangulare* (0·0073 m^2 g f. wt) suggests an average depth of 137 μm of hydrated plant material (density 10^3 kg m^{-3}) under the plant surface, in good agreement with the anatomy of the plant (see below).

The root surface area per plant exceeds the leaf surface area (both sides) in *E. decangulare*, with a root:leaf area ratio of 1·7. Values for *Lobelia dortmanna* range from 1·6 (Sand-Jensen & Prahl, 1982) to 3·6 (Wium-Andersen, 1971). For the non-isoetid alga, *Chara hispida*, the rhizoid area:green shoot area is not greater than 0·3 (from data in Andrews, 1982).

Anatomical investigations (cf. Tomlinson, 1969) showed that stomata were confined to the abaxial leaf surface. The abaxial surface of the leaves of emergent plants had $31{\cdot}3 \pm 0{\cdot}89$ ($n = 10$) stomata mm^{-2}; the submerged plants had only $3{\cdot}89 \pm 1{\cdot}36$ ($n = 10$) stomata mm^{-2}. The stomata of the submerged leaves seemed to be permanently closed; opening could not be induced by light, low external CO_2, or fusicoccin. The leaves of the submerged plants had longitudinal and transverse septa (together with large, colourless, epidermal cells and small, chlorophyllous hypodermal cells) delimiting the large gas spaces. The hypodermal plus epidermal layer is up to 100 μm thick. The longitudinal septa are up to 530 ± 10 ($n = 7$) μm apart and consist of one vascular bundle set in two vertical files of large, colourless cells that lack gas-filled intercellular spaces.

The traverse septa lack vascular bundles, are uniseriate and chlorophyllous, and are perforated by gas spaces that are 'shared' by adjacent cells having adjacent gas-filled invaginations of their longitudinal walls. The transverse septa are 939 ± 30 ($n = 10$) μm apart toward the base of the leaf and 624 ± 13 ($n = 7$) μm apart near the middle. The perforations are ~ 10 μm long (at right angles to the longitudinal cell walls) and 1–2 μm wide in the plane of the cell wall. The leaves are less than 1 mm thick.

Roots of submerged *E. decangulare* are, except at the apex, cylindrical with a diameter of 979 ± 12 ($n = 8$) μm. The root was alternating radial annuli of long longitudinally elongated, and short radially expanded, cortical cells; the former contribute most of the cortical volume and have the large air spaces where the cells are radially and tangentially separated. The short cells form transverse septa similar to those of the leaves, but lacking chlorophyll, with a similar size of pore. The transverse septa are, in 'mature' parts of the root, 403 ± 10 ($n = 16$) μm apart; within 3 mm of the tip they are less than 100 μm apart. The emergent plants have similar, but larger roots (diameter of mature regions 1693 ± 59 ($n = 10$) μm; transverse septa 456 ± 15 ($n = 10$) μm apart).

A large fraction of gas space in the plant body is a common feature of submerged aquatic vascular plants, and especially, of isoetids (Hutchinson, 1975; Sculthorpe, 1967). The occurrence of septate diaphragms across the longitudinal gas lacunae in both leaves and roots of *E. decangulare* may be compared with their presence in leaves (but not roots) of *Isoetes* spp., and in neither organ of *Lobelia dortmanna*. The role(s) of the lacunae, and of the perforate diaphragms, is discussed further in Section III.2 below. An excellent illustrated account of the anatomy of the genus *Eriocaulon*, including *E. decangulare*, may be found in Tomlinson (1969).

When roots of emersed plants of *E. decangulare* were stained (see Brundett, Piché & Peterson, 1984), the presence of vesicular–arbuscular mycorrhizas (VAM) was demonstrated. Farmer (1985) found VAM in *E. septangulare* and in two other British isoetids (*Littorella uniflora* and *Lobelia dortmanna*), but not in *Subularia aquatica* or *I. lacustris*. *S. aquatica* lacked root hairs, but these were present on *I. lacustris*. The observations on *I. lacustris*, *Littorella uniflora* and *Lobelia dortmanna* confirm those on Danish material, (Sondergaard & Laegaard, 1977). The occurrence of a low level of infection with VAM fungi in submerged *Isoetes* has, however, been reported for New Zealand material of *I. kirkii* A. Braun (Clayton & Bagyaraj, 1984). It should be noted that submerged aquatic plants of a variety of life forms are more generally mycorrhizal than was thought a decade ago (Harley & Smith, 1983; Clayton & Bagyaraj, 1984; Harley & Harley, 1987).

2. *The role of roots in acquisition of inorganic C*

Table 4 shows O_2 exchange by roots and shoots, in darkness and in saturating light, as a function of inorganic C concentration around roots and shoots. Net photosynthesis is less rapid than the dark respiration rate when both roots and shoots are supplied with 0·1 mol inorganic C m^{-3}, i.e. the concentration appropriate to the shoot medium in nature. The inorganic carbon-saturated rate of net photosynthesis is not quite achieved when 2 mol inorganic C m^{-3} i.e. the concentration appropriate to the root medium in nature, is supplied to both roots and shoots, with almost ten times the rate of O_2 evolution than when 0·1 mol inorganic C m^{-3} is supplied to both roots and shoots. Five mol inorganic C m^{-3} round both root and shoot saturates net photosynthesis at $24{\cdot}2 \pm 2{\cdot}5$ ($n = 4$) nmol g^{-1} d. wt s^{-1}. When the 'natural' inorganic C levels are supplied (0·1 mol m^{-3}) around the shoots, 2 mol m^{-3} around the roots) almost as high a net photosynthetic rate is achieved as with 2 mol m^{-3} around both roots and shoots. Comparison of the whole-plant rate with that seen with only 0·1 mol m^{-3} inorganic C around both roots and shoots shows that, under 'natural' conditions, the plant obtains most of its inorganic C (more than 85 %, assuming that the photosynthetic quotient i.e. mol O_2 evolved mol CO_2 fixed of O_2 evolved in net photosynthesis is independent of the supplied inorganic C concentration) via the roots. It is, however, clear that substantial inorganic C uptake and assimilation can occur from the medium round the shoot, since the whole-plant rate of photosynthesis with 2·0 mol m^{-3} inorganic C round the shoots and 0·1 mol m^{-3} round the roots is little lower than when the concentrations round root and shoot are reversed. However, the high CO_2 concentration round the shoot but low concentration round the root is not likely to occur in nature!

Neither root or shoot medium was stirred (cf. fig. 4 of Sondergaard & Sand-Jensen, 1979). While a stagnant medium around the roots is an acceptable approximation for the roots in their natural habitat, there is likely to be more movement of the medium around the shoots under natural conditions. However, the sloughs from which submersed *E. decangulare* was collected flowed but slowly ($\leqslant 10$ mm s^{-1}); this would reduce the diffusive restriction on uptake of inorganic C from the limiting concentrations of inorganic C found in nature by lowering unstirred layer thicknesses, although the reduction would not be saturated at the *in situ* flow rate (see Madsen & Sondergaard, 1983). Thus the conclusion as to the fraction of inorganic C acquired, under natural conditions, by the roots of *Eriocaulon* may be slight over-estimates.

The fraction of inorganic C acquired by *E. decangulare* which enters through the roots under the near-natural conditions (~ 85 %) is compared in Table 5 with values for other astomatous submerged or amphibious isoetids, and with values for submerged aquatic plants of other life forms. The values were obtained by measuring inorganic C-dependent O_2 evolution or, more usually, ^{14}C-labelled inorganic C fixation. It will be seen that the isoetids acquire the majority of their inorganic C from the sediment; the exception was *I. lacustris*, where flooding of the (damaged) roots probably restricted access of root-medium

Table 4. *Rates of O_2 exchange in light and darkness by roots and shoots of* Eriocaulon decangulare *supplied with various ($CO_2 + HCO_3^-$) concentrations in the medium surrounding roots and shoots*

	O_2 exchange [nmol (g d. wt of whole plant)$^{-1}$ s^{-1}] by roots, shoots and whole plants when roots (R) and shoots (S) are supplied with ($CO_2 + HCO_3^-$) at 0·1 or 2·0 mol m^{-3} at pH 6·0			
	R 0·1 S 0·1	R 0·1 S 2·0	R 2·0 S 0·1	R 2·0 S 2·0
Net photosynthetic O_2 evolution by				
Shoot	0·8 ± 0·4	4·8 ± 0·6	7·0 ± 0·8	8·6 ± 1·1
Root	1·4 ± 0·4	6·2 ± 0·7	8·8 ± 1·0	10·0 ± 1·3
Shoot + Root	2·2 ± 0·6	11·0 ± 0·9	15·8 ± 1·3	18·6 ± 1·7
Dark respiratory O_2 uptake by				
Shoot	1·2 ± 0·2	1·4 ± 0·3	1·7 ± 0·3	1·3 ± 0·2
Root	1·8 ± 0·3	1·5 ± 0·3	2·0 ± 0·2	1·9 ± 0·3
Shoot + Root	3·0 ± 0·4	2·9 ± 0·4	3·7 ± 0·4	3·2 ± 0·4
Gross photosynthetic O_2 evolution by				
Shoot	2·0 ± 0·6	6·2 ± 0·8	8·7 ± 1·0	9·9 ± 1·2
Root	3·2 ± 0·7	7·7 ± 0·9	10·8 ± 1·1	11·9 ± 1·4
Shoot + Root	5·2 ± 0·9	13·9 ± 1·2	19·5 ± 1·5	21·8 ± 1·9

Experimental conditions: Filtered slough water acidified to pH 4·5 with HCl, sparged for 2 h with CO_2-free air, readjusted to pH 6·0 with 10 mol m^{-3} MES + NaOH, and the appropriate volume of 100 mol $NaHCO_3$ m^{-3} added. Light 250 μmol photon m^{-2} s^{-1} (400 – 700 nm) from fluorescent tubes. 4 plants per treatment.

inorganic C to the shoots. The non-isoetids, by contrast, obtain most of their inorganic C from the shoot medium (contrast the early work of Brown, 1913). The highest root contribution ($\leqslant 35\%$) is for *Juncus bulbosus* which Wetzel *et al.* (1985) term a cyperid but which is regarded by some others as an isoetid (Hutchinson, 1975; cf. Farmer & Spence, 1985). It is not certain what fraction of the biomass of *J. bulbosus* is present in roots; published data appears to include some stem material in the 'roots' and the rest of the stem material in the 'leaves' (Roelefs *et al.*, 1984). The 'root':'leaf' ratio (dry weight) is 1·3–7·5 in the isoetid *Littorella uniflora* while, by similar criteria, it is only 0·15–0·76 in *J. bulbosa* (Roelefs *et al.*, 1984). For those parts which are definitely in the non-isoetid group listed in Table 4, the fraction of inorganic C entering via the roots is less than 5%.

Turning to the absolute rate of photosynthesis, light and CO_2-saturated photosynthesis at 22 °C is 27·6 ± 2·9 ($n = 4$) mol O_2 g^{-1} d. wt s^{-1} in *E. decangulare*. This compares with rates of 27 nmol O_2 g^{-1} d. wt s^{-1} for *Lobelia dortmanna*, and 15·5 nmol O_2 g^{-1} d. wt s^{-1} for *I. lacustria*, at 15 °C (Sand-Jensen *et al.* 1982); rather lower C fixation rates were recorded for these two species (probably not light-saturated) at 7·5–8 °C (Richardson *et al.*, 1984). On a chlorophyll basis, the rate of light- and CO_2-saturated photosynthesis at 22 °C is 2·73 mmol O_2 (mol chlorophyll)$^{-1}$ s^{-1} in *E. decangulare*. For comparison, at 10 °C, *Littorella uniflora* and *Isoetes lacustris* achieve 8·6 mmol O_2 (mol chlorophyll)$^{-1}$ s^{-1} and 3·44 mmol O_2 (mol chlorophyll)$^{-1}$ s^{-1} respectively (Sand-Jensen, 1978), while *I. howellii* reaches 20·8 mmol CO_2 (mol chlorophyll)$^{-1}$ s^{-1} at 25 °C (Keeley & Bowes, 1982). With the exception of the high *I. howellii* values, the *E. decangulare* data agree with the other isoetid data. On a total plant area (leaves + roots) basis, the maximum rate of gross photosynthesis in 5 mol CO_2 m^{-3} is 354 nmol O_2 m^{-2} s^{-1}; this is much less than the values for submerged aquatic macrophytes of other life forms (see table 5·7 of Raven, 1984*a*) when the appropriate plant surface area values are used.

Thus the rate of photosynthesis by *E. decangulare* resembles that of many other isoetids, but is lower (on various bases) than that of many other submerged aquatic macrophytes of other life-forms.

A further aspect of the O_2 exchange data relates to the fraction of total O_2 evolved in the light which emanates from the roots. Tables 5 and 6 show that this varies from 0·54–0·64 of net photosynthesis for *E. decangulare* when air-equilibrium levels of O_2 were initially present around both root and shoot. Table 6 shows data from the literature on the fraction of total O_2 production which is lost via the roots in a number of isoetid and non-isoetid submerged rhizophytes. External O_2 concentrations used in the experiments ranged from 0 to 625 mmol m^{-3}. Where asymmetric O_2 concentrations were used, i.e. low around roots, high around shoots, O_2 release by roots would be favoured relative to symmetrical O_2 treatments since the concentration difference between plant and medium which drives diffusive O_2 efflux is increased for roots relative to shoots. Furthermore, the data quoted in Table 6 are not corrected for root respiration in the light; such corrections increase the low values more than the high ones (Sand-Jensen *et al.*, 1982) but still leave the large gap between the low, non-isoetid and high, isoetid values seen in Table 6. Thus the plants classified

Table 5. *Fraction of inorganic C assimilated by aquatic plants which enter via the roots or rhizoids when near-natural concentrations of inorganic C are supplied to roots or rhizoids and to shoots. Life-forms listed are IR (rhizophytes of the isoetid life-form), NR (rhizophytes not of the isoetid life-form) and AP (acropleustophytes, i.e. plants free floating at the water surface)*

Organism (Life-form)	Fraction of inorganic C entering via roots or rhizoids	Reference
Isoetes lacustris L. (IR)	> 0·39	Richardson *et al.* (1984)
Stylites andicola Amstutz (IR)	> 0·95	Keeley *et al.* (1984); Sternberg *et al.* (1985)
Eriocaulon decangulare L. (IR)	> 0·85	Table 3 of this paper.
Littorella uniflora (L.) Aschers (IR)	> 0·95	Sand-Jensen & Sondergaard (1978); Sondergaard & Sand-Jensen (1979*a*); Roelefs *et al.* (1984); Madsen (1987*a*)
Lobelia dortmanna L. (IR)	> 0·99	Wium-Anderson (1971); Richardson *et al.* (1971)
Chara hispida L. (NR)*	< 0·001	Andrews *et al.* (1984)
Heteranthera dubia (Jacq.) MacM. (NR)	0·003	Laczy *et al.* (1983)
Juncus bulbosus, L. (NR)†	< 0·15	Roelefs *et al.* (1984)
	< 0·35	Wetzel *et al.* (1985)
Myriophyllum spicatum L. (NR)	0·002–0·011	Laczy *et al.* (1985)
Penicillus capitatus Lamarc. (NR)*	< 0·05	Raven and Handley, quoted by Raven *et al.* (1985)
Syringodium filiforme Kutz. (NR)	< 0·05	Raven and Handley, quoted by Raven (1986)
Thalassia testudinum Koenig (NR)	0·017	Wetzel & Penhale (1979); Penhale & Thayer (1980)
Vallisneria americana Michz. (NR)	0·013	Laczy *et al.* (1983)
Zostera marina L. (NR)	0·01–0·03	Penhale & Thayer (1980); Wetzel & Penhale (1979)
Eichhornia crassipes (Mart.) Solms. (AP)‡	0·08–0·11	Ultsch & Anthony (1973)
Spirodela polyrrhiza (L.) Schleid (AP)§	< 0·05	Eshel & Beer (1986)

* Apart from the two algae (*Chara hispida* L.: Chlorophyta: Charophyceae and *Penicillus capitatus* Lamarc: Chlorophyta: Ulvophyceae), all of the organisms are Tracheophytes.

† This organism is classified by some authorities as an isoetid (see Text, Section 2 of Results and Discussion).

‡ Data for adult, free-floating plant, obtaining the bulk of its C from the atmosphere. Classified as a pleusto-helophyte by Den Hartog & Segal (1964).

§ Eshel & Beer (1986) discuss other data on the Lemnaceae; most of this favours as low a contribution of uptake of inorganic C by roots as is quoted for *Spirodela polyrrhiza*.

Table 6. *Fraction of total O_2 produced by submerged rhizophytes in the light which is lost from the plant via the roots*

Plant	Initial O_2 concentration (mmol m^{-3}) around		Fraction lost via roots	Reference
	Roots	Shoots		
Eriocaulon decangulare L. (I)*	~ 250	~ 250	0·54–0·64	Table 3
Isoetes lacustris L. (I)	~ 0	~ 250	0·39	Sand-Jensen *et al* (1982)
Littorella uniflora (L.) Aschers (I)	~ 0	~ 250	0·28	Sand-Jensen *et al.* (1982)
	~ 30	~ 30	0·23–0·39	Roelefs *et al.* (1984)
Lobelia dortmanna L. (I)	~ 0	~ 0	1·0	Sand-Jensen *et al.* (1982)
	90–625	90–625	0·90	Sand-Jensen & Prahl (1982)
Juncus bulbosus L. (I?)	~ 30	~ 30	0·025–0·03	Roelefs *et al.* (1984)
Potamogeton L. spp. (4 species, 5 experiments)	~ 0	~ 250	0·02–0·04	Sand-Jensen *et al.*
Sparganium simplex Huds.	~ 0	~ 250	0·03	Sand-Jensen *et al.* (1982)
Zostera marina L.	~ 0	~ 250	0·01	Sand-Jensen *et al.* (1982)

* (I) denotes isoetid.

here as isoetids evolve 0·23–1·0 of their total photosynthetic O_2 produced via their roots, while the non-isoetids only evolve 0.04 of their O_2 via their roots.

It is clear, from a comparison of Table 5 and 6, that the high fraction of CO_2 uptake via the roots in isoetids broadly parallels the high fraction of O_2 evolution via the roots. *Juncus bulbosus* is a difficult case. It is classified by Hutchinson (1975) as an isoetid, yet the data of Roelefs *et al.* (1984) show relatively low O_2 efflux, and CO_2 influx via the roots during photosynthesis. However, the data of Wetzel *et al.* (1985) show a much greater role for roots in CO_2 uptake. None of the clearly non-isoetid plants have a large CO_2 influx or O_2 efflux via their roots during photosynthesis (Tables 5 and 6).

While a broad correlation between fractional CO_2 influx and O_2 efflux via roots in illuminated plants is to be expected, a perfect correlation under near-natural conditions is not. If all of the gas exchange is by diffusion, and HCO_3^- is not involved in CO_2 transport (see Section III. 3) we can apply Fick's law to transport between the root and shoot media which serve as CO_2 sources and O_2 sinks and the leaf plastids which are CO_2 sinks and O_2 sources. With an assumed O_2/CO_2 quotient of 1·0 for net photosynthesis, and an assumed D_{CO_2}/D_{O_2} of 0·85, for both the gas and aqueous phase parts of the pathways, the very large difference between the solubility of O_2 and CO_2 means that solution-phase concentration differences which are in the same ratio for roots

$$\frac{[CO_2]\text{ root medium} - [CO_2]\text{ plastid})}{[O_2]\text{ plastid} - [O_2]\text{ root medium}},$$

and leaves

$$\frac{[CO_2]\text{ shoot medium} - [O_2]\text{ plastid}}{[O_2]\text{ plastid} - [O_2]\text{ root medium}},$$

would not lead to an equal fraction of total plant exchange via roots for O_2 as for CO_2. Since the gas-phase is likely to be a larger fraction of the total pathway for root than for shoot exchange, the case outlined above would give roots a greater fractional share in whole-plant O_2 efflux than in whole-plant CO_2 influx (see Samish, 1975; Raven, 1977*a*; Sand-Jensen & Prahl, 1982). The 'natural' CO_2 and O_2 concentrations in water around roots and shoots are, moreover, unlikely to yield the symmetrical situation indicated above (Wium-Anderson, 1971; Wium-Anderson & Andersen, 1972*a*, *b* Roelefs *et al.*, 1984; Christensen & Sørenson, 1986).

The anatomy of the gas spaces in *E. decangulare* is not as obviously capable of supporting a low-resistance gas-phase root-to-leaf CO_2 flux and leaf-to-root O_2 flux as is the case of *Lobelia dortmanna*, which lacks septa or diaphragms in the lacunae, or even *Isoetes* spp. which have diaphragms in their leaves, but not their roots. The presence of diaphragms with pores of the order of 1 μm diameter may represent a compromise between a low resistance to gas flow and damage limitations. Flooding of one compartment at the maximum depths at which isoetids occur (< 10 m) would not allow liquid water to pass through the pores to the adjacent compartments in view of the < 100 kPa (above atmospheric) pressure of water, the pore diameter, and the surface properties of the walls (see Williams & Barber, 1961; Milburn, 1979). The situation in *Eriocaulon* relative to that in *Lobelia* with respect to putative 'efficiency' and 'safety' of gas transport tempts comparison with liquid water transport in, respectively, xylem consisting entirely of tracheids, and xylem with long and wide vessels (Zimmermann, 1983).

Direct evidence for *gas-phase* movement of CO_2 from roots to leaves, or of O_2 in the opposite direction, in the lacunae of illuminated isoetids is, apparently, lacking, although movement in solution in xylem or phloem seems very unlikely (chapter 9 of Raven, 1984*a*; cf. Kemp & Murray, 1986). While Smith, Dennison & Alberte (1984; cf. Pregnall, Smith & Alberte, 1987) find that O_2 movement from leaves to roots at the onset of illumination of the non-isoetid *Zostera marina* is, apparently, too fast to be accounted for by gaseous diffusion alone, the thermo-osmotic mechanism of mass flow of gases would not seem to be feasible for an astomatous submerged aquatic, even though the presence of diaphragms and pores of an appropriate size would otherwise favour it (Schroder, Grosse & Woerman, 1986). Furthermore, mass flow, unless it was bidirectional in a single plant, could not accommodate the net movement of CO_2 and O_2 in opposite directions which occurs in isoetids. Further investigation of CO_2 and O_2 transport within isoetids is clearly required to determine if diffusion in the gas phase is adequate to account for the observed fluxes.

3. *The form in which inorganic C enters the plant*

The question asked here is 'does inorganic C enter *E. decangulare* during photosynthesis as CO_2, as HCO_3^-, or as both'. In view of the pH values around roots and leaves of *E. decangulare in situ* (i.e. less than the pK_a value of CO_2/HCO_3^-) which makes CO_2 the dominant external inorganic C species in the medium,

Table 7. *Net rates of photosynthesis by submersed* Eriocaulon decangulare *with limiting inorganic C concentrations supplied to roots and leaves at pH 6·0 and at pH 8·0, and the rate at which* HCO_3^- *can be converted to* CO_2 *in the bathing medium at the higher pH value*

Inorganic C supply*		Net O_2 produced by the whole plant†	Rate of CO_2 production from HCO_3^-‡
S 1·0 mol m^{-3}	pH 6	1·08 ± 0·12	—
R 0·05 mol m^{-3},	pH 6		
S 0·05 mol m^{-3},	pH 6	1·67 ± 0·19	—
R 1·0 mol m^{-3},	pH 6		
S 5 mol m^{-3},	pH 8	0·25 ± 0·03	91
R 0·1 mol m^{-3},	pH 8		
S 0·1 mol m^{-3},	pH 8	0·42 ± 0·05	91
R 5 mol m^{-3},	pH 8		

* (Total inorganic C concentration/mol m^{-3}) at the specified pH value, to shoot (S) or root (R) compartment.

† Mean dry weight of plants = 169·7 ± 12·6 (n = 16), nmol O_2 $plant^{-1}$ s^{-1}

‡ Assuming all CO_2 produced is immediately assimilated, in media where $[HCO_3^-]$ exceeds $[CO_2]$, in the compartment with the higher inorganic C concentration/nmol $compartment^{-1}$ s^{-1}.

and the high CO_2 permeability of many biological membranes (chapters 5 of Raven, 1984*a*), it might be expected that CO_2 is the C species entering the plant.

The data in Table 7 do not require HCO_3^- to enter the plant in order to account for the observed O_2 evolution which is dependent on the provision of exogenous inorganic C (Table 4). The inorganic C assimilation rate, assumed to be equal to the rate of O_2 efflux, is lower at high (pH 8·0) than at low (pH 6·0) external pH values. This conclusion is independent of whether inorganic C is supplied via the roots or the shoots. While the *total* inorganic C concentration is higher in the pH 8 treatment, the CO_2 concentration, and the net rate of photosynthesis, is lower. However, per unit total CO_2 concentration, the net photosynthetic rate is rather higher (about 1·5 times the low pH value) at the higher pH value, although inorganic C supply restricts the rate of photosynthesis at both pH values (cf. Table 4). Such data are frequently found for plants unable to use HCO_3^-, i.e. in which inorganic C enters the cells as CO_2, and no catalysis of the HCO_3^- to CO_2 conversion occurs outside the cell. Plants which use HCO_3^- have increases of about 10 fold in the photosynthesis per unit (limiting) CO_2 concentration increase at high pH 8 relative to that at pH 6.

The third column in Table 7, when compared to the entries in the same row in the Table, shows that there should be no shortage of CO_2 production in the medium: in each compartment the rate of HCO_3^- conversion to CO_2 is $\sim$ 100 times greater than the rate of C assimilation at pH 8·0. The CO_2 produced in the whole of the compartment may not be available to the root or leaf; what might occur in the *minimal* thickness of unstirred layer around the organ (see Johnston & Raven, 1986*b*). However, attempts to estimate the unstirred layer thickness by the method described in Materials and Methods, assuming that *all* of the limitation on CO_2 fixation from inorganic C supplied to the roots resides in the root unstirred layer (C_s in equation (2) = 0) yielded values which, when converted to volumes of solution by multiplying by total root area, very substantially exceeded the volume of the root compartment when the data in row (2) of Table 7 was employed. This at least confirms that, under the experimental conditions, most of the limitation of photosynthesis occurs within the plant when inorganic C is supplied to the roots (Section (2) above).

Thus the rate of photosynthesis at high external pH values does not require the use of HCO_3^-, either by HCO_3^- influx *or* by external, catalysed, conversion of HCO_3^- to CO_2. Intact leaves and roots of *E. decangulare* do not exhibit carbonic anhydrase activity (cf. Price *et al.*, 1985). The activity of carbonic anhydrase in *extracts* of the *E. decangulare* leaves (Table 8), corresponding to intracellular activity (*plus* any extracellular activity), are much lower than those of many terrestrial C_3 plants, exemplified by *Spinacia oleracea*. Such differences are commonly found when submerged aquatic macrophytes are compared with terrestrial C_3 plants (Weaver & Wetzel, 1980; Graham, 1982; Salvucci & Bowes, 1983). The mixing experiment, with a mixture of equal fresh weight of leaves of *Eriocaulon* and *Spinacia*, suggests that the low activity in the extracts of aquatic leaves are not a result of the presence of endogenous inhibitors (see table II of Weaver & Wetzel, 1980). The very low carbonic anhydrase activity of *Eriocaulon* roots (Table 8) is typical for the roots of terrestrial plants (p. 226 of Graham, 1982); other published data on carbonic anhydrase activity of the roots

Table 8. *Activity of carbonic anhydrase of whole organs, and of homogenates of the organs, of submerged plants of* Eriocaulon decangulare *and, for comparison, of homogenates of leaves of* Spinacia oleracea

Organism	Organ and treatment	Carbonic anhydrase	
		10^6 E.U.* (mol chl)$^{-1}$	E.U. (g f. wt)$^{-1}$
Eriocaulon decangulare L.	Intact leaf	3·0±7·5	2±5
Eriocaulon decangulare L.	Leaf homogenate	132±18	88±12
Eriocaulon decangulare L.	Intact root	– –	3±6
Eriocaulon decangulare L.	Root homogenate	– –	13±6
Spinacia oleracea L.	Leaf homogenate	3650±440	4880±590
Spinacia oleracea L. plus *Eriocaulon decangulare* L. (equal fresh weight of each)	Leaf homogenate	2440±340	2240±310

* The enzyme unit (E.U.) quoted above is derived from the equation E.U. $= 10(t_b/t_e - 1)$, where t_b is the time taken for the solution to change from pH 8·0 to pH 7·0 using a boiled extract (or a boiled plant part), and t_e is the time taken for the pH change using a non-inactivated extract (or plant part). The terminology of enzyme for carbonic anhydrase is discussed by Yang, Tsuzuki & Miyachi (1985).

of aquatic plants are scarce. The low activity in the roots of *Eriocaulon* may be involved in inorganic C ($CO_2 + HCO_3^-$) transfer across tissues in the root which separate the CO_2 source in the medium and the gas phase in the cortex (sections III.1 and 4; and Raven & Glidewell, 1981). In seeking an explanation for a lower permeability to CO_2 than to O_2 in tissues separating gas lacunae and medium in *Littorella uniflora* and *Lobelia dortmanna*, Madsen (1987*a*, *b*) suggests that interconversion of CO_2 and HCO_3^- *reduces* the CO_2 permeability coefficient, an unlikely occurrence even in non-steady-state conditions (cf. Raven & Glidewell, 1981; Cowan, 1986).

The lack of 'HCO_3^- use' in *E. decangulare* accord with the data on other isoetids. i.e. *Lobelia dortmanna* (Wium-Anderson, 1971), *Littorella uniflora* (Sondergaard & Sand-Jensen, 1979*a*; Maberly & Spence, 1983; Boston & Adams, 1986; Madsen, 1987*a*). *I. howellii* (Keeley, 1981*a*, 1983) and *I. macrospora* (Boston & Adams, 1986). Of these reports, Sondergaard & Sand-Jensen (1979*a*), Boston & Adams (1986) and Madsen (1987*a*) provide no details of their experimental techniques. Wium-Andersen (1971) used split leaves of *Lobelia dortmanna* and, as he points out, only showed that the leaf cells were apparently unable to use HCO_3^- supplied *directly* to those cells in the intercellular spaces. These data do not bear directly on the use of HCO_3^- supplied to the outer surface of the plant. The other data generally use the criterion of more rapid photosynthesis, at a given, limiting, total inorganic C concentration at low relative to high pH, or relatively small effects of pH on the rate of photosynthesis at a given, limiting, free CO_2 concentration. The strictest criteria previously applied were those used by Maberly & Spence (1983) in their work on *Littorella uniflora*. The data on *E. decangulare* (Tables 7 and 8) provide evidence based on the strictest of the criteria of HCO_3^- use (see Walker, Smith & Cathers, 1980); no HCO_3^- use was discernible.

For comparison with root uptake of inorganic C by isoetids, little evidence is available on the form in which inorganic C enters other non-green plant cells; work on *Avena* coleoptiles, using an isotope disequilibrium technique, suggests that inorganic C enters as CO_2 (Johnson & Rayle, 1976).

A footnote to this work on the form in which C enters the plant relates to the $\delta^{13}C$ values for the *E. decangulare* plants and for their source of inorganic C. The leaves of submerged *E. decangulare* have $\delta^{13}C = -30{\cdot}0 \pm 0{\cdot}65‰$ ($n = 5$), while roots + stems have $\delta^{13}C = -28{\cdot}8 \pm 0{\cdot}12‰$ ($n = 3$). These values are not unexpected for isoetids (e.g. Richardson *et al.*, 1984; Keeley, *et al.*, 1984; Sternberg *et al.*, 1985; Keeley, de Niro & Sternberg, 1986; Raven, MacFarlane & Griffiths, 1987). [Problems with sampling (by J.A.R.) of the source inorganic C means that no reliable $\delta^{13}C$ values for the source CO_2 are available, so little can be made of the plant $\delta^{13}C$ values as an indication of limiting processes in inorganic carbon acquisition (cf. Raven, MacFarlane & Griffiths, 1987)].

4. *Measurements of titratable acidity and malate content at dawn and dusk*

This section presents results on the lack of Crassulacean acid metabolism in *E. decangulare* and discusses the occurrence of CAM in other isoetids and in non-isoetid aquatics.

A significantly higher titratable acidity and malate content at dawn than at disk is a *sine qua non* of classic Crassulacean acid metabolism (Cockburn, 1985; Winter, 1985). Table 9 records that neither submersed nor emersed specimens of *E. decangulare* show diel variations in either titratable acidity or malate content of their leaves. *E. decangulare* thus resembles *E. septangulare* (Farmer & Spence, 1985), *Lobelia dortmanna* (Boston

Table 9. *Measurements at dawn and dusk of pH, titratable acidity, and malate content, of homogenates of leaves of* Eriocaulon decangulare *L.*

Material conditions	Initial pH of extract	Titratable acidity (to pH 7·0) [μmol H^+ (g f. wt)$^{-1}$]	Malate content [μmol (g f.wt)$^{-1}$]
Submerged plants held outdoors; measurements at dawn, 4 and 5 November 1984	5·30 ± 0·06 (8)*	13·8 ± 0·5 (8)	11·1 ± 0·8 (8)
Submersed plants held outdoors; measurements at dusk, 4 and 5 November 1984	5·33 ± 0·04 (8)	13·0 ± 0·5 (8)	12·2 ± 0·9 (8)
Emersed plants held outdoors; measurements at dawn, 4 and 5 November 1984	5·39 ± 0·05 (8)	13·5 ± 0·8 (8)	10·5 ± 0·9 (8)
Emersed plants held outdoors; measurements at dusk, 4 and 5 November 1984	5·28 ± 0·05 (8)	14·6 ± 0·9 (8)	9·8 ± 0·9 (8)

* Figures within parentheses are number of replicates.

& Adams, 1983; Richardson *et al*, 1984; Farmer & Spence, 1985) and *Subularia aquatica* (Farmer & Spence, 1985) in being an isoetid which, when growing submerged (as well, wherever tested, as when growing emerged) do not exhibit CAM. Submerged specimens of *Isoetes* spp. and *Littorella uniflora*, in contrast, show substantial CAM-like diel variations in titratable acidity and malate content of their leaves (Keeley, 1981*a*, *b*, 1982, 1983, 1987, Keeley *et al.*, 1981; Keeley & Morton, 1982; Boston & Adams, 1983; Aulio, 1985, 1986; Farmer & Spence, 1985; Madsen, 1985, 1987*a*; Boston & Adams, 1985; Smith, Boston & Adams, 1985). It is clear that CAM-like metabolism can make a substantial contribution to net C acquisition under natural conditions in *Isoetes* spp. and *Littorella uniflora* (Madsen, 1985; Boston & Adams, 1986). When growing in air unsaturated with water vapour, most genotypes of *Isoetes* spp. and *Littorella uniflora* do not exhibit CAM-like behaviour (Keeley & Busch, 1984; Aulio, 1985, 1986; Farmer & Spence, 1985). This is not the case for *Stylites* (= *Isoetes*?) *andicola* and various *Isoetes* species which resemble *Stylites* in habitat and morphology; these organisms have CAM, but never produce stomata and always obtain most of their CO_2 via the roots (Keeley *et al.*, 1984; Keeley, 1987).

We thus see that more genera of isoetids (defined morphologically, and in terms of substantial root uptake of CO_2) lack CAM-like behaviour than have it, even in *Stylites* is recognized as distinct from *Isoetes*. (cf. Fuchs-Eckert, 1982). Outside the isoetids, CAM-like behaviour involving diel changes in titratable acidity in excess of 30 μmol H^+ g^{-1} f. wt is apparently limited, in submerged aquatic plants, to *Crassula aquatica* (Keeley & Morton, 1982; Raven *et al.*, 1985; Table 10 of this paper). In many cases, reports of the absence of CAM are based entirely on measurements of titratable acidity while in some (notably Keeley & Morton, 1982; Boston & Adams, 1983, 1986) malate content was also measured. The absence of diel changes of malate as well as of titratable acidity rules out the possibility that dark CO_2 fixation into malate, followed by CO_2 release from malate in the light with subsequent refixation via RUBISCO can occur *without* changes in titratable acidity via a mechanism resembling that in Figure 2(B). In this (hypothetical) variant on CAM the 2H* generated, along with malate^{2-}, when dark CO_2 fixation occurs, is exported to the medium in exchange for $2K^+$; malate^{2-} is then stored in the vacuole along with $2K^+$ rather than with the $2H^+$ in 'normal' CAM. In light deacidification, the scheme in Figure 2(B) involves $2K^+$ plus malate^{2-} efflux from vacuole to cytoplasm, with $2K^+$ efflux at the plasmalemma in exchange for $2H^+$ permitting (malate^{2-} + $2H^+$) metabolism as in conventional CAM (Fig. 2(A)). The absence of diel malate^{2-} changes when titratable acidity does not change suggests that the mechanism of CAM indicated in Figure 2(B) does not occur in the plants investigated by Keeley & Morton (1982) and in the species listed in Tables 9 and 10. We note that the suggestion of CAM-like phenomena in *Vallisneria spiralis*, based on ^{14}C-labelled inorganic C assimilation patterns (Helder & Harmelan, 1982) and stable isotope studies (Sternberg, De Niro & Keeley, 1984) is not supported by the data presented here. CAM-like behaviour was also absent from the brown macroalga *Ecklonia radiata* (Laminariales) (Table 10). This finding accords with data for British brown macro-algae (Laminariales, and the fucoid *Halidrys siliquosa*) which do not normally suffer air exposure, and contrasts with data for British and Australian fucoids which are usually air-exposed during the tidal cycle and which exhibit low-amplitude CAM-like behaviour (Johnston & Raven, 1986*b*; Raven *et al.* 1985).

The possibility that CAM-like C fixation can occur without changes in the titratable acidity of aquatic, but not of terrestrial, plant extracts, relates to the presence of a large extracellular (extraplant) aqueous sink

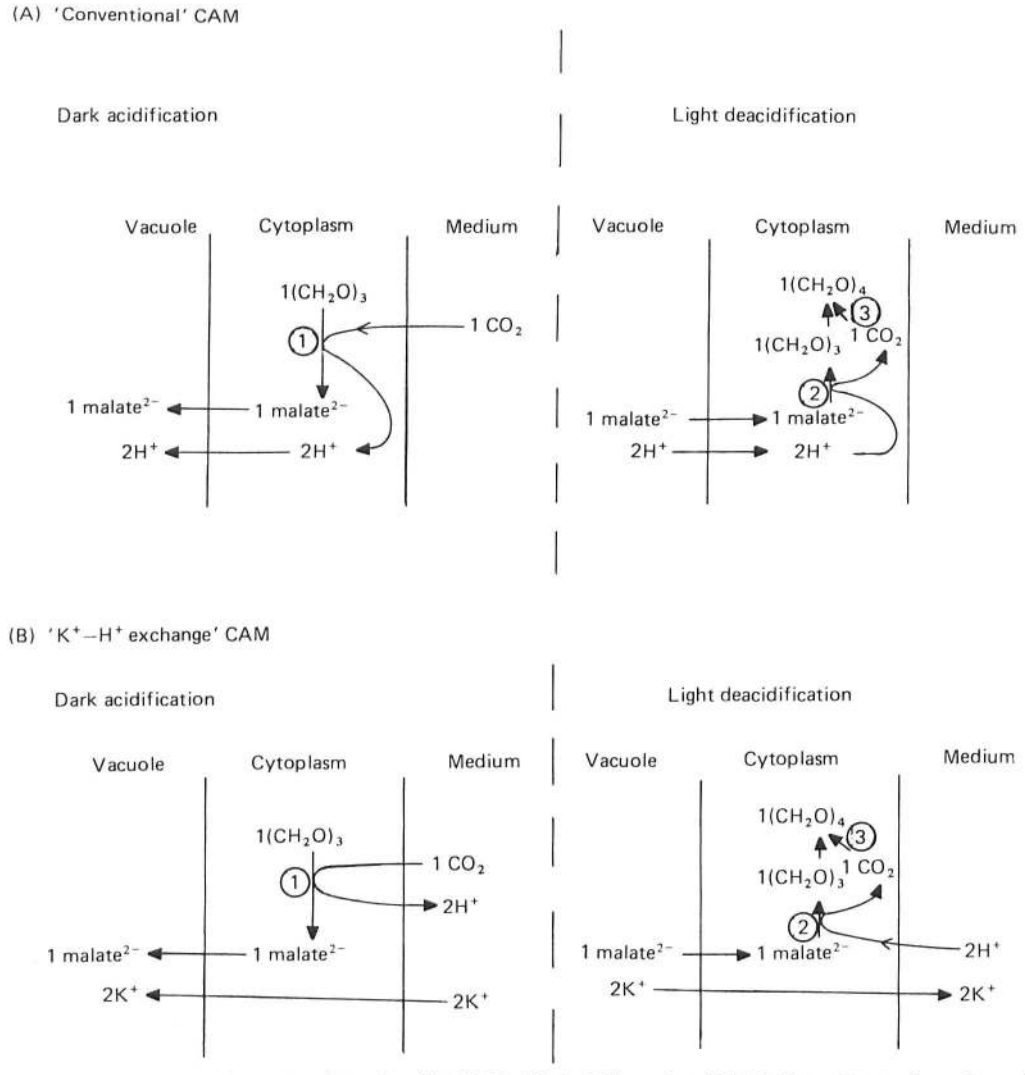


Figure 2. Representations of a 'conventional' CAM (Fig. 2A) and a CAM-like process based on H^+–K^+ exchange with the bathing medium (Fig. 2B).

around the green tissue with which H^+ exchange could take place. Raven (1977*b*, 1986) discusses the lack of biochemical or phloem transport mechanisms for the disposal of H^+ produced at rates appropriate to primary ammonium assimilation in the shoots; these arguments apply *a fortiori* to H^+ production in terrestrial CAM-plants. We note that suggestions of the occurrence of a 'within-leaf' variant on the scheme of Figure 2(B) (Bloom, 1979) has not been upheld by subsequent work (Winter, Edwards & Holtum, 1981). In aquatic plants there *is* the possibility of K^+/H^+ exchange between leaves and their aqueous medium, although, in at least *some* isoetids, the diffusion barrier at the leaf surface which restricts, O_2 and CO_2 exchange might well also act as a barrier to ion transport and exchange (Sand-Jensen & Prahl, 1982; Keeley *et al.*, 1984; Schuurkes, Kok & Den Hartog, 1986; cf. Madsen, 1987*b*).

Measurements were also made of the titratable acidity and malate content of the stems and roots of *E. decangulare* (data not shown). Diel changes were not detected for either organ, thus eliminating the possibility of a spatial separation of the acidification/deacidification cycle from photosynthetic refixation of the type recently found to account for 30% or more of CAM acid fluctuation in three terrestrial plants (Earnshaw, Carver & Charlton, 1987; Nishio *et al.*, 1987).

On a historical note, Allsop (1951), investigating the sugars and non-volatile organic acids of Archegoniates, noted that *I. echinospora* Durieu had malate levels similar to those found in the terrestrial CAM plant *Bryophyllum calycinum* Salisb. However, he did not investigate diel changes and it was left to Keeley (1981*a*, *b*; 1982, 1987; Keeley *et al.*, 1981) to discover the CAM-like behaviour of submerged species of *Isoetes*. Allsop (1951) also noted that *Platycerium alicorne* (= *P. bifuratum* (Cav) C. Chr.) had high concentrations of malate; however, *Platycerium* spp. lack CAM-like characteristics (Winter *et al.*, 1983; Winter, 1985).

Table 10. *Measurements at dawn and dusk of pH, titratable acidity, and malate content, of homogenates of leaves or blades of* Amphibolis antarctica, Ecklonia radiata *and* Vallisneria spiralis *(see also table 2 of Raven* et al., *1985; Farmer & Spence, 1985; Boston & Adams, 1983; Keeley, 1982; Keeley & Morton, 1982; Aulio, 1986)*

Materials and conditions	Initial pH of extract	Titratable acidity (to pH 7·0) [μmol H^+ (g f. wt)$^{-1}$]	Malate content [μmol (g f. wt)$^{-1}$]
Amphibolis antarctica (Labill) Sond. & Aschers (Tracheophyta, submerged in seawater) Measurements at dawn, 11, 12, September, 1986	6·11 ± 0·03 (6)*	7·14 ± 0·42 (6)	5·3 ± 0·5 (6)
Amphibolis antarctica (Labill) Sond. & Aschers Measurements at dusk, 10, 11 September, 1986	6·16 ± 0·03 (6)	6·80 ± 0·34 (6)	4·5 ± 0·4 (6)
Ecklonia radiata (Turn.) J. Agardh (Phaeophyta, submerged in seawater) Measurements at dawn, 11, 12 September, 1986	6·36 ± 0·07 (6)	5·90 ± 0·22 (6)	6·9 ± 0·8 (6)
Ecklonia radiata (Turn.) J. Agardh Measurements at dusk, 10, 11 September, 1986	6·44 ± 0·02 (6)	5·40 ± 0·25 (6)	5·2 ± 0·8 (6)
Vallisneria spiralis L. (Tracheophyta, submerged in freshwater) Measurements at dawn, 17, 18 December, 1986	6·48 ± 0·05 (6)	9·22 ± 0·55 (6)	3·2 ± 0·3 (6)
Vallisneria spiralis L. Measurements at dusk, 17, 18 December, 1986	6·05 ± 0·05 (6)	8·21 ± 0·55 (6)	2·8 ± 0·3 (6)

* Figures within parenthesis are number of replicates.

5. *Significance of universal occurrence of substantial acquisition of CO_2 by roots in astomatous isoetids*

The CO_2 concentration in the root medium of isoetids is higher than that in the shoot medium; this, however, is also true of essentially all rhizophytes under natural growth conditions. Gravity leads to the accumulation of particulate organic mater in the sediment in which the roots (or rhizoids) occur; microbial activity produces CO_2 which, due to restricted mass flow of fluid (water or, for terrestrial soiled, water + air) also accumulates in the sediment. This relative lack of mass flow in the sediment (or soil) decreases the availability of CO_2 there by comparison with the bulk fluid phase round the shoot. The relative lack of bulk phase fluid movement in the environments of astomatous isoetids (stagnant or slowly flowing water or, for emergent *Stylites* which has leaves almost flush with the soil surface, the atmospheric boundary layer) imposes a constraint on CO_2 supply from the leaf medium qualitatively similar to that seen for the root medium. This constraint on the bulk phase CO_2 supply is greater than that found (per unit plant biomass) for many non-isoetid rhizophytes, especially in a non-stagnant environment; a further increment in inorganic C supply could arise from use of HCO_3^- in alkaline media. Isoetid environments are generally, but not necessarily, biased toward a high CO_2 availability in the sediment relative to that in the bulk water when compared with habitats *not* dominated by isoetids (cf. Brown, 1911; Farmer & Spence, 1985; Boston, 1986). The greater availability (potential flux *sensu* Raven, 1984*a*, chapter 1) of CO_2 to roots relative to leaves on a total plant biomass basis is, then, to a substantial degree a product of the larger root area per unit plant biomass than is the case for other life-forms of rhizophytes in a similar environment.

It is worth asking if the large root surface area per unit biomass in the isoetids, with the concomitant increase in potential flux of CO_2 to the root surface per unit biomass, might have some other, selective, rationale. Two obvious possibilities relate to acquisition of other resources (N, P, K, Fe: Christiansen, Fries & Sondergaard, 1985) or to oxygenation of the rhizosphere; (Sand-Jensen *et al.*, 1982; Carpenter & Lodge, 1986; Christensen & Sørensen, 1986; Kemp & Murray, 1986). Clearly these two possibilities are not mutually exclusive: mineralization of organic matter might occur faster in the presence of higher O_2 concentrations, although P may thereby be immobilised (see Richardson *et al.*, 1984). At all events, the presence of mycorrhizas, a condition which favours acquisition of P, but is probably not effective in inorganic C acquisition (Harley & Smith, 1983; Raven 1984*a*; Tanner & Clayton, 1985), does *not* seem to be notably lower in isoetids than in other submerged aquatics (Sondergaard & Laegaard, 1977; Clayton & Bagyaraj, 1984; Farmer, 1985).

A third possible function of the large root surface area per unit biomass of isoetids is in anchoring the plant in the sediment (Williams & Barber, 1961; Westlake, 1965). The large fraction of the plant occupied by air spaces gives the whole plant a density well below 10^6 g m^{-3}, and the root surface provides frictional interaction preventing the rhizophyte from becoming a pleustophyte (Williams & Barber, 1965). Any water movement relative to the substratum would also tend to uproot the plants (cf. the occurrence of *Isoetes* spp.

in tidal creeks: Keeley, 1987). However, non-isoetid rhizophytes in similar habitats clearly survive uprooting despite the larger buoyancy or water movement forces per unit root surface area; attachment is therefore likely to be a general role of this increased root surface area per unit biomass in isoetids relative to non-isoetids.

The absence of CAM from many genera of isoetids requires consideration. The presence of CAM in submersed *Littorella uniflora* and *Isoetes* spp., and in terrestrial evergreen *Isoetes* spp. (Keeley & Morton, 1982; Keeley, 1987) represents a temporal extension to net C gain, as well as a potential means of increasing the N use efficiency of C acquisition (see above) and is additional to the spatial extension of net C gain and, perhaps, of increasing N use efficiency, suggested for the root acquisition of CO_2 in almost all isoetids. Additionally, CAM could help the C economy by recycling respiratory CO_2 as well as by fixing exogenous CO_2 in the dark. What resource costs, or other constraints, could be invoked to explain the lack of CAM in many isoetids? Are there selectively unacceptable costs of possessing, transcribing, translating and regulating extra genes (e.g. for the catalyst of malate transport at the tonoplast, or for the enzyme phosphate, pyruvate dikinase) which are invoked in CAM? Has the previous evolutionary history of the non-CAM isoetids resulted in some genetic change inimical to the evolution of CAM? We do not know the answer to these questions.

We suggest that the high root area per unit biomass in isoetids is probably related, selectively, to increasing the inorganic C supply to photosynthetic cells by increasing the input from sediments. A similar argument could be made for the selective advantage of CAM-like behaviour in those isoetids which possess this trait. This suggestion is supported by Madsen (1987*c*) who showed that the capacity for CAM in *Littorella uniflora* was reduced by 'high CO_2' treatment; the added CO_2 also reduced the *in vivo* affinity of the plants for exogenous CO_2.

It is also possible to argue (cf. Richardson *et al.*, 1984) that the increased CO_2 supply to the photosynthetic apparatus is related to nitrogen use efficiency (mol C fixed (mol N in plant)$^{-1}$ s^{-1}; see Agren, 1985). The rationale here is that RUBISCO is one of the largest individual N users among the plant catalysts, so that increasing the $[CO_2]$, and $[CO_2]/[O_2]$, at the site of RUBISCO activity would, by increasing the net CO_2 fixation rate per unit N in RUBISCO, permit a given rate of CO_2 fixation per unit biomass at a lower [N] per unit biomass. This suggestion has been made for terrestrial C_4 and CAM plants (Osmond, Winter & Ziegler, 1982; Pearcy & Ehleringer, 1984; Edwards, Ku & Monson, 1985; Winter, 1985). For aquatic CAM plants, Madsen (1987*c*) showed that sub-optimal nutrient (including N) supply to *Littorella uniflora* slightly *decreased* the capacity for CAM; this could be construed as contradicting the above suggestion.

It is not possible to critically evaluate the 'N suggestion' on the basis of present data. However, we note that the N use efficiency of photosynthesis (adjusted to 20 °C assuming a Q_{10} of 2) with 'natural' CO_2 supply at light saturation is lower for *E. decangulare* (C_3) (15 μmol C (mol N)$^{-1}$ s^{-1}), and for (perhaps not light-saturated) data of Richardson *et al.* 1984, on *I. lacustris* (CAM) ($\sim$ 12 μmol C (mol N)$^{-1}$ s^{-1}) and *Lobelia dortmanna* (C_3) ($\sim$ 20 μmol C (mol N)$^{-1}$ s^{-1}) than for the haptophytic freshwater red alga *Lamanea mamillosa* Kutz. (C_3) from a fast-flowing stream (24–36 μmol C (mol N)$^{-1}$ s^{-1}) (Raven *et al.*, 1987).

While root CO_2 uptake (with or without CAM) may be important in allowing isoetids to exploit their habitat, these C-acquisition mechanisms do not result in high productivities, or high relative growth rates *in situ* (Boston & Adams, 1987; Farmer & Spence, 1987).

6. *Evolution of the isoetid life-form*

Conventional wisdom on the evolution of submerged aquatic vascular plants holds that they were derived from terrestrial vascular plants via emergent wetland plants (e.g. Raven, 1984*a*). This return to the aquatic environment occupied by their algal ancestors (cf. Stebbins & Hill, 1980) meant that their stomata, cuticle and mechanical (compression-resistant) tissue were not very selectively useful, so were either lost or (in the case of stomata of some aquatic vascular plants) became non-functional. Gas spaces, initially used in the intraplant gas transport of fully terrestrial plants, became more important as the root medium become more anoxic, so increasing the demand for O_2 supply to the roots. We can thus envisage an 'isoetid precursor' with roots in sediment and shoots with non-functional (or no) stomata in the bulk water phase, and a large fraction of its volume occupied by gas spaces. As with all submerged vascular rhizophytes, these plants would have had not only phloem but also xylem tissue. Phloem would have been necessary to supply photosynthate from leaves to root. Xylem would have been necessary for the root to shoot transfer, using a root pressure mechanism, of sediment-derived N, P, K, Fe etc. (see chapter 9 of Raven, 1984*a*). The xylem's original major role in transfer of water from roots to shoots, with much of it lost in transpiration, would no longer be required; nor would its later role in mechanical support as a compression-resistant element of plants with their shoots growing up into the air (see Raven, 1977*c*, 1984*b*, 1985). It is not easy to see how the xylem, with its wall containing lignin which, in emergent plants, resists collapse of the xylem elements when the

solution in the lumen is under tension due to transpiration, could have evolved except in a transpiring, emergent plant. A similar case can be made for the cuticle and, especially, the stomata. Even for astomatous isoetids (e.g. the often emergent *Stylites*) the occurrence of cuticle and xylem is more consistent with an ancestry embracing a previous terrestrial existence as a homoiohydric vascular plant (Raven, 1977*c*, 1984*b*, 1985). The astomatous isoetids are thus considered to be secondarily aquatic or, in the case of *Stylites*, *secondarily terrestrial* following a second aquatic interlude (cf. Keeley *et al.*, 1984; see Keeley, Osmond & Raven, 1985).

Knowledge of extant and fossil relatives of the present-day isoetids shows that at last some have close relatives which are (or were) emergent plants of wetlands. This is certainly true of *E. septangulare* and *E. decangulare* (see references in Introduction). *Lobelia dortmanna* also has relatives which grow in wetlands (e.g. the arborescent Lobeliaceae of Hawaii). *Littorella uniflora* has *Plantago maritima* L. as a coastal wetland (as well as an upland) member of the same family (Godwin, 1975), while the Andean *Plantago rigida* H.B.H. (classified as a cushion plant by Hedberg & Hedberg, 1979) can be found in close association with the emergent astomatous isoetid *Stylites* (*Isoetes*) *andicola* (Sternberg *et al.*, 1985). Finally, *Subularia aquatica* is related to a number of Brassicacious emergent hydrophytes, as well as to the almost conspecific Afro-alpine *S. monticola* which grows in East Africa with a number of large *Lobelia* spp. (Hellberg, 1957, 1969; Hiltner, 1986); cf. the circumboreal isoetids *S. aquatica* and *Lobelia dortmanna*. However, it is also clear that most tracheophyte families with many wetland or submerged aquatic members have *not* produced representatives with the isoetid life-form (see Hutchinson, 1975). Indeed, it is the exception rather than the rule for a genus containing one or more isoetid species to have also a non-isoetid submerged aquatic species in it; such an exception is *Eriocaulon* with the Australian *E. setacea* (Aston, 1973). It is, however, also clear that many aquatic plants with non-isoetid adults have isoetid-like seedlings (see Sculthorpe, 1967; Hutchinson, 1975; Godfrey & Wooton, 1979, 1981). An example is *Eichhornia crassipes*, in which the seeds germinate at the margin of bodies of freshwater (either submersed or emersed) to produce an attached rosette-form seedling. This seedling produces 4–8 ligulate leaves in 30–40 days, followed (30–40 days from germination) by 'float' leaves which, apparently, ultimately make the plant so buoyant that, if submerged, *either* the roots can no longer attach the plant to the sediment, or root abscission is enhanced, thus producing the free-floating interfacial pleustophytic adult (Gopal & Sharma, 1981; Richards, 1983). Figure 3 shows (for emersed seedlings) that the roots of *Eichhornia crassipes* occupied over 30% of the dry weight of the plant at the youngest developmental stage investigated, and that this fraction fell as the seedling continued its rhizophytic (as opposed to its adult, pleustophytic) stage (see Fig. 3). There appear to be no data on the role of roots in C acquisition by young rhizophytic submersed *Eichhornia crassipes*; the adult pleustophyte may be able to fix as much C via the root medium as it produced in root respiration (Table 5). It is possible that the isoetid life-form in adult plants can, in some cases, represent the variant of paedomorphosis known as progenesis (Guerrant, 1982). It is not clear if the habitat of isoetid-like seedling of aquatic plants which do not have this growth form as adults is, in general, more like the oligotrophic habitat usually occupied by isoetids than are the habitats of the adult plant. The possible selective advantage of isoetid-like seedlings has not been explored.

This speculation as to the origin of isoetids is not, as far as the flowering plants go, constrained by the

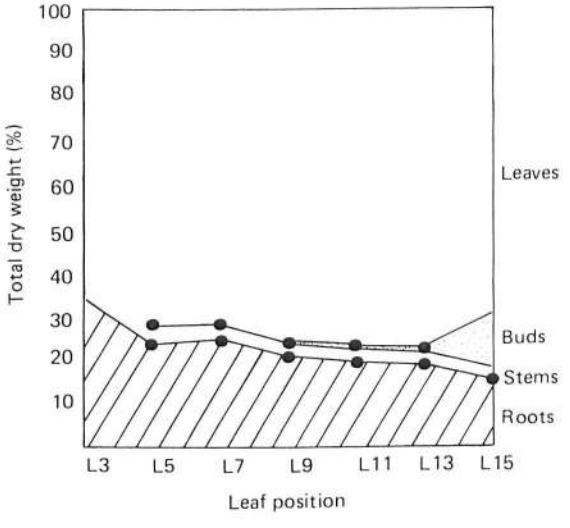


Figure 3. Changes in the percentage of total dry weight in different organs during the growth of the rhizophytic seedling stage of *Eichhornia crassipes* (from Richards, 1980).

occurrence of a sufficiently lengthy and detailed fossil history. This is not to denigrate the utility for other purposes of the existing fossil record, e.g. the quarternary remains of *Littorella uniflora*, *E. septangulare* and *S. aquatica* in the British Isles (Godwin, 1975).

The most detailed fossil record of an isoetid genus is that of *Isoetes* (including *Stylites*: Fuchs-Eckert, 1982). It has long been recognised that there are important anatomical similarities between *Isoetes* and many Carboniferous, arborescent Lycopsids, especially with respect to their roots which may be more nearly homologous with leaves than with roots of other Lycopsids (Karrfalt, 1980; Rothwell & Erwin, 1985). However, it is likely that the closest fossil relatives of the extant *Isoetes* are not the trees with *Stigmaria* Brongniart, 1822, underground organs, but such organisms as *Protostigmaria* Jennings, 1975 (Lower Carboniferous), *Chaloneria* Pigg and Rothwell, 1983 (upper Carboniferous), *Pleuromeia* Conda 1852, (Triassic), *Isoetites* Munster 1842 (upper Triassic onwards) and *Nathorstiana* Richter 1909 (Cretaceous) (see Ash, 1980; Karrfalt, 1980, 1984*a*, *b*; Paolillo, 1982; Jennings *et al.*, 1983; Pigg & Rothwell, 1983*a*, *b*; Rothwell, 1984; Thomas & Brack-Hanes, 1984; Thomas & Spicer, 1987). *Protostigmaria*, *Chaloneria* and *Pleuromeia* were small arborescent plants; at least *Protostigmaria* and *Chaloneria* grew in rather wet habitats. *Chaloneria* had well-characterized stomata; the preservation of *Protostigmaria* does not, alas, permit the presence of stomata to be assessed, and the same is true for *Isoetites*. On the basis of fossil evidence, then, arborescent relatives of *Isoetes* with secondary thickening, cuticle and stomata *preceded* the more *Isoetes* – like *Isoetites*; this evidence favours the secondarily aquatic nature of the predominant, submersed of *Isoetes* and the secondarily terrestrial nature of such members of the Isoetaceae as *Isoetes* (= *Stylites*) *andicola* (evergreen, astomatous) and *Isoetes durieu* (summer deciduous, with stomata which are presumably functional: see Keeley (1987) for details of the habit and habitat of members of the Isoetaceae). Dr J. E. Keeley (personal communication) points out that the ubiquitous occurrence of four lacunae in the leaves of all *Isoetes* species (including all of the terrestrial species) further suggests an aquatic origin for the terrestrial species. It is tempting to relate the four leaves lacunae of the Isoetaceae to the (two) parichnos strands (aerenchymatous) entering the leaves of Lepidodendrids (see references in Thomas & Brack-Hanes, 1984; Thomas & Spicer, 1987).

IV. CONCLUSIONS

E. decangulare is, when growing submerged, morphologically a typical isoetid. It also shares with all the isoetids which have been investigated a very substantial contribution of the roots to net inorganic C acquisition under natural C supply conditions. CO_2 rather than HCO_3^- is the form in which inorganic C enters both roots and shoots. *E. decangulare* lacks external carbonic anhydrase activity and does not exhibit CAM.

It seems most likely that the selective significance of root C acquisition relates to a limited availability of C in the bulk water phase relative to the availability of other resources. An additional factor could be an increased N use efficiency consequent on the improved C supply to the significant N-sink, RUBISCO.

Isoetids may have arisen by progenesis as derivatives of wetland vascular plants of larger stature, a view for which fossil evidence is only available for the Isoetaceae. The astomatous isoetid *Stylites* (= *Isoetes*) *andicola* is almost certainly derived from submerged ancestors which had, in turn, originated from conventional terrestrial vascular plants with functional stomata.

ACKNOWLEDGEMENTS

J. A. Raven is grateful for the excellent hospitality of Dr M. Tracey and his colleagues at the Florida International University, Department of Biological Sciences during the sabbatical term generously granted by the University of Dundee; he is particularly grateful for the supply of fusicoccin. J. A. Raven and L. L. Handley also wish to acknowledge the splendid hospitality extended by Dr F. A. Smith (Department of Botany at the University of Adelaide). L. L. Handley acknowledges receipt of research support from N.S.F. (U.S.A.) under Grant numbers INT-8501918 and CEE-83-15819. J. J. McFarlane was supported by Grant GR3/5037 from N.E.R.C. (U.K.) to J. A. Raven. G. Samuelsson thanks the British Council for assistance with funding his visit to Dundee. We thank Dr H. Griffiths, University of Newcastle upon Tyne, for the indefinite loan of the chambers used to distinguish acquisition of inorganic C by roots from that by shoots of *E. decangulare* and Dr J. E. Keeley, Occidental College, Los Angeles, for provision of unpublished information.

This paper is based on a Tansley Fund Lecture given by J. A. Raven in the Department of Botany, University of Sheffield, on 28 May 1985; the hospitality of Professor D. H. Lewis, and the generosity of the *New Phytologist Trust*, are gratefully acknowledged.

NOTE ADDED IN PROOF

Since this paper was accepted for publication, two very significant papers concerning CAM and root CO_2 uptake in aquatic vascular plants have appeared. These are:

BOSTON, H. L., ADAMS, M. S. & PIENKOWSKI, T. P. (1987*a*). Utilization of sediment CO_2 by selected North American isoetids. *Annals of Botany* **60**, 485–494.

BOSTON, H. L., ADAMS, M. S. & PIENKOWSKI, T. P. (1987*b*). Model of the use of root-zone CO_2 by selected North American isoetids. *Annals of Botany* **60**, 495–503.

REFERENCES

AGREN, G. I. (1985). Theory of the growth of plants derived from the nitrogen productivity concept. *Physiologia Plantarum* **64**, 17–28.

ALLSOP, A. (1951). The sugars and non-volatile organic acids of some archegoniates: a survey using paper chromatography. *Journal of Experimental Botany* **2**, 121–124.

ANDREWS, M. (1982). *Ecological and Physiological Studies on* Chara hispida *L.* Ph.D. Thesis, University of Dundee.

ANDREWS, M., BOX, R., FYSON, A. & RAVEN, J. A. (1984). Source-sink characteristics of carbon transport in *Chara hispida*. *Plant, Cell and Environment* **7**, 683–687.

ASH, S. R. (1980). Upper Triassic Floral Zones of North America. *Biostratigraphy of Fossil Plants: Successional and Palaeoecological Analyses*. (Ed. by T. N. Dilcher and T. N. Thomas), pp. 153–170. Dowden, Hutchinson and Ross, Stroudsberg, Pa.

ASTON, H. I. (1973). *Aquatic Plants of Australia*. University Press, Melbourne.

AULIO, K. (1985). Differential expression of diel acid metabolism of two life forms of *Littorella uniflora* (L) Aschers. *New Phytologist* **100**, 533–536.

AULIO, K. (1986). CAM-like photosynthesis in *Littorella uniflora* (L) Aschers: the role of humidity. *Annals of Botany* **58**, 273–275.

BESFORD, R. T. (1984). Some properties of ribulose bisphosphate carboxylase extracted from tomato leaves. *Journal of Experimental Botany*, **35**, 495–504.

BLOOM, A. J. (1979). Diurnal ion fluctuations in the mesophyll cells of the Crassulacean Acid Metabolism plant *Mesembryanthemum crystallinum*. *Plant Physiology* **64**, 919–923.

BOSTON, H. L. (1986). A discussion of the adaptations for carbon acquisition in relation to the growth strategy of aquatic isoetids. *Aquatic Botany* **26**, 258–270.

BOSTON, H. L. & ADAMS, M. S. (1983). Evidence of Crassulacean Acid Metabolism in two North American Isoetids. *Aquatic Botany* **15**, 381–386.

BOSTON, H. L. & ADAMS, M. S. (1985). Seasonal diurnal acid rhythms in two aquatic CAM plants. *Oecologia* **65**, 573–579.

BOSTON, H. L. & ADAMS, M. S. (1986). The contribution of Crassulacean Acid Metabolism to the annual productivity of two aquatic vascular plants, *Oecologia* **68**, 615–622.

BOSTON, H. L. & ADAMS, M. S. (1987). Productivity, growth and photosynthesis of two small 'isoetid' plants, *Littorella uniflora* and *Isoetes macrospora*. *Journal of Ecology* **75**, 333–350.

BROWN, W. H. (1911). The plant life of Ellis, Great, Little and Long Lakes in North Carolina. *Contributions of the U.S. National Herbarium* **13**, 323–341.

BROWN, W. H. (1913). The relations of the substratum to the growth of *Elodea*. *Philippine Journal of Science. C. Botany* **8**, 1–20.

BRUNDETT, M. C., PICHÉ, Y. & PETERSON, R. L. (1984). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Canadian Journal of Botany* **62**, 2128–2134.

CARPENTER, S. R., & LODGE, D. M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany* **26**, 341–370.

CHRISTIANSEN, R., FRIES, N. J. S. & SONDERGAARD, M. (1985). Leaf production and nitrogen and phosphorus content of *Littorella uniflora* (L.) Aschers in relation to N and P enrichment of the sediment in oligotrophic Lake Hampden, Denmark. *Aquatic Botany* **23**, 1–11.

CHRISTENSEN, P. B. & SØRENSEN, J. (1986). Temporal variation of denitrification activity in plant-covered, littoral sediment from Lake Hampden, Denmark. *Applied and Experimental Microbiology* **51**, 1174–1179.

CLAYTON, J. S. & BAGYARAJ, D. J. (1984). Vesicular–arbuscular mycorrhizas in submerged aquatic plants of New Zealand. *Aquatic Botany* **19**, 251–262.

COCKBURN, W. (1985). Variations in photosynthetic acid metabolism in vascular plants: crassulacean acid metabolism and related phenomena. *New Phytologist* **101**, 3–24.

COOK, C. M., LANARAS, T. & COLMAN, B. (1986). Evidence for bicarbonate transport in species of red and brown macrophytic marine algae. *Journal of Experimental Botany* **37**, 977–984.

COWAN, I. R. (1986). Economics of carbon fixation in higher plants. In *On the Economy of Plant Form and Function* (Ed. by T. J. Givnish), pp. 133–170. Cambridge University Press, Cambridge.

CRANK, J. (1956). *The Mathematics of Diffusion*. Oxford University Press, Oxford.

DAHLGREN, R. M. T. & CLIFFORD, H. T. (1982). *The Monocotyledons: A Comparative Study*. Academy Press, London.

DAHLGREN, R. M. T., CLIFFORD, H. T. & YEO, P. F. (1985). *The Families of the Monocotyledons. Structure, Evolution and Taxonomy*. Springer, Berlin.

DEN HARTOG, C. & SEGAL, C. (1964). A new classification of the water-plant communities. *Acta Botanica Neerlandica* **13**, 367–393.

EARNSHAW, M. J., CARVER, K. A. & CHARLTON, W. A. (1987). Leaf anatomy, water relations and crassulacean acid metabolism in the chlorenchyma and colourless internal water-storage tissue of *Carpobrotus edulis* and *Senecio mandraliscae*. *Planta* **170**, 421–432.

EDWARDS, G. E., KU, M. S. B. & MONSON, R. K. (1985). C_4 Photosynthesis and its regulation. In: *Photosynthetic Mechanisms and the Environment* (Ed. by J. Barber and N. R. Baker), pp. 287–327. Elsevier, Amsterdam.

ESHEL, A. & BEER, S. (1986). Inorganic Carbon Assimilation by *Spirodela polyrrhiza*. *Hydrobiologia* **132**, 149–153.

FARMER, A. M. (1985). The occurrence of vesicular–arbuscular mycorrhiza in Isoetid-type submersed aquatic macrophytes under naturally varying conditions. *Aquatic Botany* **21**, 245–249.

FARMER, A. M. & SPENCE, D. H. N. (1985). Studies on diurnal acid fluctuations in British Isoetid-type submerged aquatic rhizophytes. *Annals of Botany* **56**, 347–350.

FARMER, A. M. & SPENCE, D. H. N. (1986). The growth strategies and distribution of isoetids in Scottish Freshwater Lochs. *Aquatic Botany* **26**, 246–258.

FARMER, A. M. & SPENCE, D. H. N. (1987). Environmental control of the seasonal growth of the submersed aquatic macrophyte *Lobelia dortmanna* L. *New Phytologist* **106**, 289–299.

FUCHS-ECKERT, H. P. (1982). Zur heutigen Kenntnis von Vorkammen und Verbmeitung der Sudamerikanischen *Isoetes*-arten. *Proceedings Koniklijke nederlandse akadiemie von Wetenschappen* C **85**, 205–260.

GODFREY, R. K. & WOOTON, J. W. (1979). *Aquatic and Wetland Plants of the Southeastern United States. Monocotyledons*. University of Georgia Press, Athens.

GODFREY, R. K. & WOOTON, J. W. (1981). *Aquatic and Wetland Plants of Southeastern United States. Dicotyledons*. University of Georgia Press, Athens.

GODWIN, H. (1975). *History of the British Flora. A factual basis for phytogeography. Second Edition*. Cambridge University Press, Cambridge.

GOPAL, B. & SHARMA, K. P. (1981). *Water-hyacinth (Eichhornia crassipes), the Most Troublesome Weed in the World*. Hindsia, Delhi.

GRAHAM, D. (1982). Carbonic anhydrase (carbonate dehydratase) from plants. In: *C.R.C. Handbook of Biosolar Resources* (Ed. by A. Mitsui and C. C. Black), vol. 1, part 1, pp. 215–229. C.R.C. Press, Boca Raton.

GRASSHOFF, K. (1983). Determination of oxygen. In *Methods of Seawater analysis* (Ed. by K. Grasshoff, M. Ehrhardt and K. Kremling), pp. 61–72. Verlag Chemie, Weinheim.

GUERRANT, E. O., JR. (1982). Neotenic evolution of *Delphinium hudicaule* (Ranunculaceae): a humming bird-pollinated larkspur. *Evolution* **36**, 699–712.

HAGEWEG, P. & BRENKERT, A. L. (1969). Structure of aquatic vegetation: a comparison of aquatic vegetation in India, the Netherlands and Czechoslovakia. *Tropical Ecology* **10**, 139–162.

HARLEY, J. L. & HARLEY, E. L. (1987). A check-list of mycorrhiza in the British Flora. *New Phytologist* **105** (supplement), 1–102.

HARLEY, J. L. & SMITH, S. E. (1983). *Mycorrhizal Symbiosis*. Academic Press, London.

HEDBERG, O. (1957). Afroalpine vascular plants. A taxonomic revision. *Symbolae Botanicea Upsallienses* **15**(1), 1–411.

HEDBERG, O. (1969). Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society* **1**, 135–148.

HEDBERG, I. & HEDBERG, O. (1979). Tropical alpine life-forms of vascular plants. *Oikos* **33**, 297–307.

HELDER, R. J. & HARMELAN, M. V. (1982). Carbon assimilation patterns in the submerged leaves of the aquatic angiosperm: *Vallisneria spiralis* L. *Acta Botanica Neerlandica* **31**, 281–295.

HILTNER, L. (1886). Untersuchungen uber die Gattung *Subularia*. *Botanische Jahrbuch* **7**, 264–272.

HUSBAND, B. C. & HICKMAN, M. (1985). Growth and biomass allocation of *Ruppia occidentalis* in three lakes, differing in salinity. *Canadian Journal of Botany* **63**, 2004–2014.

HUTCHINSON, G. E. (1975). *A Treatise on Limnology, Volume 3, Limnological Botany*. John Wiley, New York.

JEFFREY, S. W. & HUMPHREY, G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, **167**, 191–194.

JENNINGS, J. R., KARRFALT, E. E. & ROTHWELL, G. W. (1983). Structure and affinities of *Protostigmaria eggertiana*. *American Journal of Botany* **70**, 963–974.

JOHNSON, K. D. & RAYLE, D. L. (1976). Enhancement of CO_2 uptake in *Avena* coleoptiles by fusicoccin. *Plant Physiology* **57**, 806–811.

JOHNSON, K. S. (1982). Carbon dioxide hydration and dehydration kinetics in seawater. *Limnology and Oeanography* **27**, 849–855.

JOHNSTON, A. M. & RAVEN, J. A. (1986*a*). The utilization of bicarbonate ions by the macroalga *Ascophyllum nodosum* (L.) Le Jolis. *Plant, Cell and Environment* **9**, 175–184.

JOHNSTON, A. M. & RAVEN, J. A. (1986*b*). Dark fixation studies on the intertidal macroalga *Ascophyllum nodosum* (Phaeophyta). *Journal of Phycology* **22**, 78–83.

KANSANEN, A. & NIEMI, R. (1974). On the production ecology of isoetids, especially *Isoetes lacustris* and *Lobelia dortmanna* in Lake Paajarvi, Southern Finland. *Annales Botanici Fennici* **11**, 178–187.

KARRFALT, E. E. (1980). A further comparison of *Isoetes* roots and stigmarian appendages. *Canadian Journal of Botany* **58**, 2318–2322.

KARRFALT, E. (1984*a*). The origin and early development of the root-forming meristem of *Isoetes andicola* L. D. Gomex. *Botanical Gazette* **145**, 372–377.

KARRFALT, E. (1984*b*). Further observations on *Nathorstiana* (Isoetaceae). *American Journal of Botany* **71**, 1023–1030.

KEELEY, J. E. (1981*a*). Diurnal acidity fluctuations in vernal pool *Isoetes* (Isoetaceae). *Madrona* **28**, 167–171.

KEELEY, J. E. (1981*b*). *Isoetes howellii*: a submerged aquatic CAM plant? *American Journal of Botany* **68**, 420–424.

KEELEY, J. E. (1982). Distribution of diurnal acid metabolism in the genus *Isoetes*. *American Journal of Botany* **69**, 254–257.

KEELEY, J. E. (1983). Crassulacean acid metabolism in the seasonally submerged aquatic *Isoetes howellii*. *Oecologia* **58**, 57–62.

KEELEY, J. E. (1987). The adaptive radiation of photosynthetic modes in the genus *Isoetes*. In: *Plant Life in Aquatic and Amphibious Habitats*. British Ecological Society Special publication (Ed. by R. M. M. Crawford), pp. 113–128. Blackwell Scientific Publications, Oxford.

KEELEY, J. E. & BOWES, G. (1982). Gas exchange characteristics of the submerged aquatic crassulacean acid metabolism plant, *Isoetes howellii*. *Plant Physiology* **70**, 1455–1459.

KEELEY, J. A. & BUSCH, G. (1984). Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. *Plant Physiology* **76**, 525–530.

KEELEY, J. E. & MORTON, B. A. (1982). Distribution of diurnal acid metabolism in submerged aquatic plants outside the genus *Isoetes*. *Photosynthetica* **16**, 546–553.

KEELEY, J. E., DE NIRO, M. J. & STERNBERG, L. O. (1986). The use of stable isotopes in the study of photosynthesis in freshwater plants, *Aquatic Botany* **26**, 213–223.

KEELEY, J. E., OSMOND, C. B. & RAVEN, J. A. (1984). *Stylites*, a vascular land plant without stomata absorbs CO_2 via its roots. *Nature* **310**, 694–695.

KEELEY, J. E., OSMOND, C. B. & RAVEN, J. A. (1985). Stomatal mechanisms as the basis of evolution of crassulacean acid metabolism. *Nature* **314**, 200.

KEELEY, J., MORTON, B., BABCOOK, B., CASTILLO, P., FISH, B., JERAULD, E., JOHNSON, B., LANDRE, L., LUM, H., MILLER, C., PARKER, A. & VAN STEENWYK, G. (1981). Dark CO_2 fixation and diurnal malic acid fluctuations in the submerged aquatic *Isoetes storkii*. *Oecologia* **48** 332–333.

KEMP, W. M. & MURRAY, L. (1986). Oxygen release from roots of the submersed macrophyte *Potamogeton perfoliatus* L: regulating factors and ecological implications. *Aquatic Botany* **26**, 271–283.

LACZY, S., CARIGNAN, R. & PENHALE, D. (1983). The role of roots in carbon uptake by the submersed macrophytes *Myriophyllum spicatum*, *Vallisneria americana* and *Heteranthera dubia*. *Hydrobiologia* **98**, 3–7.

LUTHER, H. (1949). Vorschlag zu einer okologischen Grundeinteilung der Hydrophyten. *Acta Botanica Fennica* **44**, 1–15.

MCNEIL, P. H., FOYER, C. H., WALKER, D. A., BIRD, I. F., CORNELIUS, M. J. & KEYS, A. J. (1981). Similarity of RuBPCs of isogenic diploid and tetraploid ryegrass (*Lolium perenne* L.) cultivars. *Plant Physiology* **67**, 530–534.

MABERLY, S. C. & SPENCE, D. H. N. (1983). Photosynthetic inorganic carbon use by freshwater plants. *Journal of Ecology* **71**, 705–724.

MACKERETH, F. J. H., HERON, J. & TALLING, J. F. (1987). *Water Analysis: Some Revised Methods for Limnologists*. Scientific publications of the Freshwater Biological Association No. 36, pp. 120.

MADSEN, T. V. (1985). A community of submerged aquatic CAM plants in Lake Kalgaard, Denmark. *Aquatic Botany* **23**, 97–108.

MADSEN, T. V. (1987*a*). Sources of inorganic carbon acquired through CAM in *Littorella uniflora* (L.) Aschers. *Journal of Experimental Botany* **38**, 367–377.

MADSEN, T. V. (1987*b*). Interactions between internal and external CO_2 pools in the photosynthesis of the aquatic CAM plants *Littorella uniflora* (L.) Aschers and *Isoetes lacustris* L. *New Phytologist* **106**, 35–50.

MADSEN, T. V. (1987*c*). The effect of different growth conditions on dark and light carbon assimilation in *Littorella uniflora*. *Physiologia Plantarum* **70**, 183–188.

MADSEN, T. V. & SUNDERGAARD, M. (1983). The effects of current velocity on the photosynthesis of *Callitriche stagnalis* Scopt. *Aquatic Botany* **15**, 187–193.

MILBURN, J. A. (1979). *Water Flow in Plants*, Longman, London.

MILLER, A. G. & COLMAN, B. (1980). Evidence for HCO_3^- transport by the blue-green alga (Cyanobacterium) *Coccochloris peniocystis*. *Plant Physiology* **65**, 397–402.

MOELLER, R. E. (1978). Seasonal changes in biomass, tissue chemistry and net production of the evergeen hydrophyte, *Lobelia dortmanna*. *Canadian Journal of Botany* **56**, 1425–1433.

MOLLERING, H. (1974). Determination with malate dehydrogenase and glutamate-oxaloacetate transaminase. In: *Methods in Enzymic Analysis* (Ed. H. V. Bergmeyer), pp. 1589–1593. Academic Press, New York.

MORLEY, B. D. & TOELKEN, H. R. (eds). (1983). *Flowering Plants in Australia*. Rigby, Adelaide.

NISHIO, J. N., WEBBER, A. N., GURALNIK, L. J., HEATH, R. L. & TING, I. P. (1987). Photosynthetic properties of the three major layers of the CAM plant, *Peperomia camptotricha*. In: *Progress in Photosynthesis Research*, vol. 4: (Ed. by J. Biggins), pp. 523–526. Nijhoff, Dordrecht.

OSMOND, C. B., WINTER, K. & ZIEGLER, H. (1982). Functional significance of different pathways of CO_2 fixation in photosynthesis. In: *Encyclopedia of Plant Physiology, New Series*, vol. 12B (Ed. by O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler), pp. 345–372. Springer, Berlin.

PAOLILLO, D. J., JR (1982). Meristems and evolution: developmental correspondence among the rhizomorphs of the lycopsids. *American Journal of Botany* **69**, 1032–1043.

PEARCY, R. W. & EHLERINGER, J. (1984). Comparative ecophysiology of C_3 and C_4 plants. *Plant, Cell and Environment* **7**, 1–13.

PENHALE, P. A. & THAYER, G. W. (1980). Uptake and transfer of carbon and phosphorus by eelgrass (*Zostera marina* L.) and its epiphytes. *Journal of Experimental Marine Biology and Ecology* **42**, 113–123.

PIGG, K. B. & ROTHWELL, G. W. (1983*a*). *Chaloneria* gen.nov.; heterosporous lycophytes from the Pennsylvanian of North America. *Botanical Gazette* **144**, 132–147.

PIGG, K. B. & ROTHWELL, G. W. (1983*b*). Megagametophyte development in the Chaloneriaceae Fam. Nov., permineralized Palaeozoic Isoetales (Lycopsida). *Botanical Gazette* **144**, 295–302.

PREGNALL, A. M., SMITH, R. D. & ALBERTE, R. S. (1987). Glutamine synthetase activity and free amino acid pools of eelgrass (*Zostera marina* L.) roots. *Journal of Experimental Marine Biology and Ecology* **106**, 208–211.

PRICE, G. D., BADER, M. R., BASSETT, M. E. & WHITECROSS, M. I. (1985). Involvement of plasmalemmasomes and carbonic anhydrase in photosynthetic utilization of bicarbonate in *Chara corallina*. *Australian Journal of Plant Physiology* **12**, 241–256.

RAVEN, J. A. (1977*a*). Ribulose biphosphate carboxylase activity in terrestrial plants: significance of O_2 and CO_2 diffusion. *Commentaries in Plant Science* **9**, 579–580.

RAVEN, J. A. (1977*b*). H^+ and Ca^{2+} in phloem and symplast: relation of relative immobility of the ions to the cytoplasmic nature of the transport paths. *New Phytologist* **79**, 465–480.

RAVEN, J. A. (1977*c*). The evolution of vascular land plants in relations to supracellular transport processes. *Advances in Botanical Research* **5**, 153–219.

RAVEN, J. A. (1984*a*). *Energetics and Transport in Aquatic Plants*. A. R. Liss, New York.

RAVEN, J. A. (1984*b*). Physiological correlates of the morphology of early vascular plants. *Botanical Journal of the Linnean Society* **88**, 105–126.

RAVEN, J. A. (1985). The comparative physiology of the adaptation of plants and arthropods to life on land. *Philosophical Transactions of the Royal Society of London* B**309**, 273–288.

RAVEN, J. A. (1986). Biochemical disposal of H^+ in plants? *New Phytologist* **104**, 175–206.

RAVEN, J. A. & GLIDEWELL, S. M. (1981). Processes limiting photosynthetic conductance. In: *Physiological Processes Limiting Plant Productivity* (Ed. by C. B. Johnson), pp. 109–136. Butterworths, London.

RAVEN, J. A., BEARDALL, J. & GRIFFITHS, H. (1982). Inorganic C-sources of *Lemanea*, *Cladophora* and *Ranunculus* in a fast-flowing stream: measurements of gas exchange and a carbon isotope ratio and their ecological implications. *Oecologia* **53**, 68–78.

RAVEN, J. A., OSBORNE, B. A. & JOHNSTON, A. M. (1985). Uptake of CO_2 by aquatic vegetation. *Plant, Cell and Environment* **8**, 417–425.

RAVEN, J. A., JOHNSTON, M., MACFARLANE, J. J., BIN SURIF, M. & MCINROY, S. (1987). Diffusion and active transport of inorganic cabon species in freshwater and marine macroalgae. In: *Progress in Photosynthesis Research*, vol. 4 (Ed. by J. Biggins), pp. 333–340. Nijhoff, Dordrecht.

RAVEN, J. A., MACFARLANE, J. J. & GRIFFITHS, H. (1987). The application of carbon isotope discrimination techniques. In: *Plant Life in Aquatic and Amphibious Habitats*. British Ecological Society Special Symposium (Ed. by R. M. M. Crawford), pp. 129–149. Blackwell Scientific Publications, Oxford.

RICHARDS, J. H. (1980). *The developmental basis of morphological plasticity in the water hyacinth*, Eichhornia crassipes *Solms*. Ph.D. Dissertation, University of California, Berkeley.

RICHARDS, J. H. (1983). Heteroblastic development in the water hyacinth *Eichhornia crassipes* Solms. *Botanical Gazette* **144**, 247–259.

RICHARDSON, K., GRIFFITHS, H., REED, M. L., RAVEN, J. A. & GRIFFITHS, N. A. (1984). Inorganic C assimilation in the Isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L. *Oecologia* **61**, 115–121.

ROELEFS, J. G. M., SCHUURKES, J. A. A. R. & SMITS, A. J. M. (1984). Impact of acidification and eutrophication on macrophyte communities in soft water. II. Experimental studies. *Aquatic Botany* **18**, 389–411.

ROTHWELL, G. W. (1984). The apex of *Stigmaria* (Lycopsida), rooting and organ of Lepidodendrales. *American Journal of Botany* **71**, 1031–1034.

ROTHWELL, G. W. & ERWIN, D. M. (1985). The rhizomorph apex of *Paurodendron*: implications for homologies among the rooting organs of Lycopsida. *American Journal of Botany* **72**, 86–98.

SALVUCCI, M. E. & BOWES, G. (1983). Ethoxyzolamide repression of the low photorespiratory state in two submersed angiosperms. *Planta* **158**, 27–34.

SAMISH, Y. B. (1975). Oxygen build-up in photosynthesising leaves and canopies in small. *Photosynthetica* **9**, 372–375.

SAND-JENSEN, K. (1978). Metabolic adaptation and vertical zonation of *Littorella uniflora* (L.) Aschers. and *Isoetes lacustris* L. *Aquatic Botany* **4**, 1–10.

SAND-JENSEN, K. & PRAHL, C. (1982). Oxygen exchanges with the lacunae and across leaves and roots of the submerged vascular macrophyte, *Lobelia dortmanna* L. *New Phytologist* **91**, 103–120.

SAND-JENSEN, K., PRAHL, C. & STOKHOLM, H. (1982). Oxygen release from roots of submerged aquatic macrophytes. *Oikos* **38**, 349–354.

SAND-JENSEN, K. & SONDERGAARD, M. (1978). Growth and production of isoetids in oligotrophic Lake Kalgaard, Denmark. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **20**, 659–666.

SCHRODER, P., GROSSE, W. & WOERMAN, D. (1986). Localization of thermosmotically active partitions in young leaves of *Nuphar lutea*. *Journal of Experimental Botany* **37**, 1450–1461.

SCHUURKES, J. A. A. R., KOK, C. J. & DEN HARTOG, C. (1986). Ammonium and nitrate uptake by aquatic plants from poorly buffered and acidified waters. *Aquatic Botany* **24**, 131–146.

SCULTHORPE, C. D. (1967). *The Biology of Aquatic Vascular Plants*. Edward Arnold, London.

SMITH, R. D., DENNISON, V. C. & ALBERTE, R. S. (1984). Role of seagrass photosynthesis in root aerobic processes. *Plant Physiology* **74**, 1055–1058.

SMITH, R. G., BOSTON, H. L. & ADAMS, M. S. (1985). A preliminary study of the source of fate of carbon acquired via CAM in *Littorella uniflora* (var. *Americana* (Fern) Gl. *Journal of Freshwater Ecology* **3**, 203–209.

SONDERGAARD, M. (1979). Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submerged aquatic plants. *Aquatic Botany* **6**, 269–284.

SONDERGAARD, M. & LAEGAARD, S. (1977). Vesicular–arbuscular mycorrhiza in some aquatic vascular plants. *Naure* **268**, 232–233.

SONDERGAARD, M. & SAND-JENSEN, K. (1979*a*). Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Botany* **6**, 1–12.

SONDERGAARD, M. & SAND-JENSEN, K. (1979*b*). The delay in ^{14}C fixation rates by 3 submerged macrophytes. A source of error in the ^{14}C technique. *Aquatic Botany* **6**, 111–119.

SORRELL, B. K. & DROMGOOLE, F. I. (1986). Errors in measurements of aquatic macrophyte gas exchange due to oxygen storage in internal air-spaces. *Aquatic Botany* **24**, 103–114.

SPENCE, D. H. N. & CHRYSTAL, J. (1970). Photosynthesis and zonation of freshwater macrophytes. II. Adaptability of species to deep and shallow water. *New Phytologist* **69**, 217–227.

STEBBINS, G. L. & HILL, G. J. C. (1980). Did multicellular plants invade the land? *American Naturalist* **115**, 342–353.

STERNBERG, L., DE NIRO, M. J. & KEELEY, J. A. (1984). Hydrogen, oxygen and carbon isotope ratios of cellulose from submerged aquatic crassulacean acid metabolism and non-crassulacean acid metabolism plants. *Plant Physiology* **76**, 68–70.

STERNBERG, L. D. A. S. L., DE NIRO, M. J., MCJUNKIN, D., BERGER, R. & KEELEY, J. E. (1985). Carbon, oxygen and hydrogen isotope abundances in *Stylites* reflect its unique physiology. *Oecologia* **67**, 598–600.

TANNER, C. C. & CLAYTON, J. S. (1985). Effects of vesicular-arbuscular mycorrhizas and growth on nutrition of a submerged aquatic plant. *Aquatic Botany* **22**, 377–386.

THOMAS, B. A. & BRACK-HANES, S. E. (1984). A new approach to family groupings in the Lycophytes. *Taxon* **33**, 247–255.

THOMAS, B. A. & SPICER, R. A. (1987). *The Evolution and Palaeobiology of Land Plants*. Croom Helm, London.

TOMLINSON, P. B. (1969). *Anatomy of the Monocotyledons*. vol. III: *Commelinales-Zingiberales*. Clarendon Press, Oxford.

ULTSCH, G. R. & ANTHONY, D. S. (1973). The role of the aquatic exchange of carbon dioxide in the ecology of the water hyacinth. *Florida Scientist* **36**, 16–22.

WAISEL, Y., AGAMI, M. & SHAPIRA, Z. (1982). Uptake and transport of ^{86}Rb, ^{32}P, ^{36}Cl and ^{22}Na by four submerged hydrophytes. *Aquatic Botany* **13**, 179–186.

WALKER, N. A., SMITH, F. A. & CATHERS, I. R. (1980). Bicarbonate assimilation by freshwater charophytes and higher plants. I. Membrane transport of HCO_3^- is not proven. *Journal of Membrane Biology* **57**, 51–58.

WEAVER, C. L. & WETZEL, R. G. (1980). Carbonic anhydrase levels and internal lacunar CO_2 concentrations in aquatic macrophytes. *Aquatic Botany* **8**, 173–186.

WESTLAKE, D. F. (1965). Some basic data for investigations of the productivity of aquatic macrophytes. *Memorie Instituto Italie Idrobiologia* **18** (suppl.) 229–248.

WETZEL, R. G. & PENHALE, P. A. (1979). Transport of carbon and excretion of dissolved organic C by leaves and roots/rhizomes of seagrasses and their epiphytes. *Aquatic Botany* **6**, 149–158.

WETZEL, R. G., BRAMMER, E. S., LINDSTROM, K. & FORSBERG, C. (1985). Photosynthesis of submersed macrophytes in acidified lakes. II. Carbon limitation and utilization of benthic CO_2 sources. *Aquatic Botany* **22**, 107–120.

WILLIAMS, W. T. & BARBER, D. A. (1961). The functional significance of aerenchyma in plants. *Symposium of the Society for Experimental Biology* **15**, 132–144.

WINTER, K. (1985). Crassulacean acid metabolism. In: *Photosynthetic Mechanisms and the Environment* (Ed. by J. Barber and N. R. Baker), pp. 329–387. Elsevier, Amsterdam.

WINTER, K., EDWARDS, G. E. & HOLTUM, J. A. M. (1981). Nocturnal accumulation of malic acid occurs in mesophyll tissue without proton transport to epidermal tissue in the inducible crassulacean acid metabolism plant *Mesembryanthemum crystallinum*. Evidence against a previous hypothesis. *Plant Physiology* **68**, 355–357.

WINTER, K., WALLACE, B. J., STOCKER, G. C. & ROKSANDIC, Z. (1983). Crassulacean acid metabolism in Australian vascular epiphytes and some related species. *Oecologia* **57**, 129–141.

WIUM-ANDERSEN, S. (1971). Photosynthetic uptake of free CO_2 by the roots of *Lobelia dortmanna*. *Physiologia Plantarum* **25**, 245–248.

WIUM-ANDERSEN, S. & ANDERSEN, J. M. (1972*a*). Carbon dioxide content of the interstitial water in the sediment of Grane Langso, a Danish *Lobelia* lake. *Limnology and Oceanography* **17**, 943–947.

WIUM-ANDERSEN, S. & ANDERSEN, J. M. (1972*b*). The influence of vegetation on the redox profile of the sediment of Grane Langso, a Danish *Lobelia* lake. *Limnology and Oceanography* **17**, 948–952.

YANG, S. Y., TSUZUKI, M. & MIYACHI, S. (1985). Carbonic anhydrase of *Chlamydomonas*: Purification and studies on its induction using antiserum against *Chlamydomonas reinhardtii*. *Plant and Cell Physiology* **26**, 25–34.

YOKOTA, A. & KITAOKA, S. (1985). Correct pK values for dissocation constant of carbonic acid lower the reported K_m values of ribulose bisphosphate carboxylase to half. Presentation of a nomogram and an equation for determining the pK values. *Biochemical and Biophysical Research Communications* **131**, 1075–1079.

ZIMMERMANN, M. H. (1983). *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.