Physiological and photosynthetic plasticity in the amphibious, freshwater plant, *Littorella uniflora*, during the transition from aquatic to dry terrestrial environments

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ABSTRACT

The physiological and photosynthetic responses of *Littorella uniflora* (L.) Ascherson, an amphibious macrophyte of isoetid life form, to rapid and prolonged emersion onto dry land, was studied at a reservoir. Water relations were little affected in the short term, but declining water potential and turgor pressure indicated water stress after flowering. High leaf lacunal CO_2 concentrations suggested continued CO_2 uptake from sediments. In contrast, a switch from Crassulacean acid metabolism (CAM) to C_3 photosynthesis was indicated by much lower levels of ΔH^+ (down minus dusk titratable acidity) and phosphoenolpyruvate carboxylase (PEPC) activity in new terrestrial leaves, 7–8-fold higher activity of ribulose bisphosphate carboxylase oxygenase (Rubisco), and increased chlorophyll and soluble protein contents. Accumulated nitrate and amino acid pools were depleted, whereas storage of carbohydrates as soluble sugars, fructan and starch increased. Plant carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) declined, perhaps reflecting changes in C fixation processes, N metabolism, and source C and N. In leaves of plants grown half-emersed for an extended period, contrasting activities of PEPC and Rubisco were found in submersed and emersed portions. Overall, *L. uniflora* showed considerable phenotypic plasticity, yet seemed to remain poised for re-submersion; these characteristics could be adaptive in the unpredictable water margin habitat.

Key-words: *Littorella uniflora*; amphibious macrophyte; isoetid; Crassulacean acid metabolism; Rubisco; stable isotopes

INTRODUCTION

Amphibious plants form a significant part of temperate and tropical herbaceous floras (e.g. Sand-Jensen & Frost-Christensen 1999), and are an important component of aquatic and wetland ecosystems (see Sculthorpe 1967;

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Haslam 1978; Polunin & Walters 1985). They show a range of degrees of specialization for aquatic life, which are thought to reflect stages in their evolution from terrestrial and amphibious ancestors (see Arber 1920; Sculthorpe 1967; Raven *et al*. 1988; Keeley 1998a,b). In response to changing water levels, some show extraordinary plasticity in leaf and growth form, developing aquatic or terrestrial leaves depending on whether they are submersed or emersed, and the stimulus seems to be provided by a variety of environmental and internal signals (e.g. changes in photoperiod, light quality, CO_2 and O_2 concentration, water status) (see Arber 1920; Sculthorpe 1967; Anderson 1978; Deschamp & Cook 1983; Goliber & Feldman 1989; Bruni, Young & Dengler 1996). Recently, in a few species, emersion and re-submersion have also been found to induce photosynthetic pathway switches, e.g. from C_3 to C_4 (Ueno 1996), or from C_4 (or CAM) to C_3 as the CO_2-concentrating mechanism (CCM), which improves diffusive supply of CO_2 underwater, is lost as leaves emerge into air (Keeley 1998a,b). *Littorella uniflora*, a small rosette plant, of isoetid life form, occupies a niche in shallow water along the margins of lakes, tarns, pools and reservoirs in northern Europe and North America (see Spence 1964; Tutin *et al*. 1964–1980; Boston, Adams & Pienkowski 1987a). Here, the plants are usually submersed and reproduce vegetatively, but water level fluctuations may be rapid and unpredictable and result in their emersion onto dry land for months during the summer (Arber 1920; Farmer & Spence 1986; Hostrup & Wiegleb 1991; Robe & Griffiths 1998). *L. uniflora* only flowers when emersed.

To investigate the extent of phenotypic plasticity shown by *L. uniflora* in response to emersion we studied this species at a reservoir, where in early summer 1995 the water level fell several centimetres a day, rapidly exposing the plants which remained on dry land for several months during an exceptionally warm, dry summer and early autumn. A previous paper (Robe & Griffiths 1998) describes the rapid and dramatic change from aquatic to terrestrial leaf morphology, and from vegetative reproduction to flowering, accompanied by an increase in growth rate; growth rate declined after flowering, and after three months out of water the plants had developed a small, prostrate growth form.

Submersed, aquatic *L. uniflora* shows both physical and biochemical CO_2-concentrating mechanisms. Thus, CO_2 uptake is predominantly *via* the roots from CO_2-enriched sediments; CO_2 diffuses in the gas phase through the lacunal system to the leaves (Söndergaard & Sand-Jensen 1979; Boston *et al.* 1987a,b; Robe & Griffiths 1990, 1998). In addition, daytime CO_2 supply is augmented by Crassulacean acid metabolism, a pathway traditionally associated with water conservation in plants in arid habitats (Winter & Smith 1996). CAM involves night-time fixation of CO_2 by phosphoenolpyruvate carboxylase (PEPC), storage as malic acid, and regeneration in the light for refixation by ribulose bisphosphate carboxylase oxygenase (Rubisco) and the C_3 pathway (Smith, Boston & Adams 1985; Madsen 1987a; Robe & Griffiths 1990). In the leaf lacunae, which are lined with photosynthetic cells (e.g. see Robe & Griffiths 1998), CO_2 concentrations range from 0·42 to 2·7 mol m^{-3} (1–6%; Madsen 1987a,b; Robe & Griffiths 1988, 1990, 1992), sufficient to saturate photosynthesis *in vitro* and suppress the oxygenase activity of Rubisco.

Emergent *L. uniflora* collected from close to the water's edge, with leaves similar to those of aquatic plants, and incubated with roots submersed, are photosynthetically very similar to aquatic plants; shoots perform CAM, and CO_2 uptake is *via* the roots (Farmer & Spence 1985; Nielsen, Gacia & Sand-Jensen 1991; Nielsen & Sand-Jensen 1997). However, the very different leaves of terrestrial *L. uniflora* growing on dry land do not exhibit CAM (very low overnight acidification and PEPC activity) provided relative humidity is low (Aulio 1986; Groenhof, Smirnoff & Bryant *et al.* 1988), and in the isoetid *Isoetes howellii* CAM is lost from the leaf tips as they emerge (Keeley & Busch 1984). There is also one report that terrestrial leaves of *L. uniflora* have a twofold higher Rubisco activity than aquatic leaves (Beer *et al.* 1991).

As there was no clear picture of how *L. uniflora* responds to emersion when growing *in situ* at a single location, and, in particular, no information on how photosynthetic characteristics change in relation to environmental conditions and leaf form, we monitored lacunal CO_2 concentrations, titratable acidity (a measure of CAM), and PEPC and Rubisco activities as part of our study at a reservoir (Robe & Griffiths 1998). The effects of emersion on water relations, and reserves of soluble carbohydrate and nitrogen which may buffer against changing environmental conditions (e.g. Millard 1988; Chapin, Schulze & Mooney 1990; Staswick 1994), were also investigated. Plant carbon isotope ratios ($\delta^{13}C$) were determined, as although they provide much insight into CO_2 sources and photosynthetic processes in terrestrial species (O'Leary 1988; Farquhar, Ehleringer & Hubick 1989), their application in aquatic macrophyte studies is still in its early stages (see Keeley & Sandquist 1992). Plant nitrogen isotope ratios ($\delta^{15}N$) which, with a better understanding of the complex underlying factors, have the potential to inform about N sources and metabolism (Handley & Raven 1992), were also determined as there were no data for an amphibious macrophyte. Our aim was to obtain an integrated picture of

physiological and photosynthetic responses of *L. uniflora* to rapid and prolonged emersion.

MATERIALS AND METHODS

Field site, plants and programme of sampling

This study was carried out during the warm and dry summer and early autumn of 1995 at Thirlmere Reservoir, Cumbria, UK (grid ref NY 323133). Environmental conditions and the appearance of the plants on the three sampling dates are illustrated in Table 1 and Fig. 1 of Robe & Griffiths (1998). Plants were sampled (i) in early June, when they were in very shallow water just before emersion (the water surface was a few centimetres above the leaf tips); these plants are referred to as 'aquatic' *L. uniflora*, (ii) in early July, 3–4 four weeks after emersion ('flowering' *L. uniflora*), and (iii) in early September after 3 months on dry land ('seed-bearing' plants). Particular care was taken to sample plants from areas of similar plant density and sediment type on each occasions, and to take plants of similar appearance within each area. For dawn and dusk measurements, adjacent clumps were sampled. The sampling areas were protected from grazing by wildfowl with a layer of fine, open-work plastic netting supported 0·4 m above the plants by wooden posts.

Sediment and plant water status

For determination of sediment water content, sediments taken from around the roots of the plants were weighed and dried to constant weight at 60 °C. For fresh weight (FW) to dry weight (DW) ratios and percentage water content of shoots, roots and stems, plant material was gently washed, blotted dry and dried to constant weight at 60 °C. For measurement of osmotic potential, shoots and roots were frozen in liquid nitrogen at midday, the cell sap was then expressed from the thawed material and centrifuged in a microfuge for 2 min. Determinations were made by the cryoscopic method using an Osmomat 030 (Gonotec, Berlin, Germany). For determination of water potential, plants were brought back to the laboratory at dusk, undisturbed, in large clumps of their own sediments and maintained under controlled conditions (temperature 18 °C, photosynthetic photon flux density in the 400–700 NM range (PPFD) 200 μmol m^{-2} s^{-1}, natural photoperiod) for 1 day. Whole shoots were cut from the stem at midday, quickly and carefully dried, sealed at the base with petroleum jelly, and then weighed and submersed in a graded series of mannitol concentrations in small test tubes. The water potential of the shoots was taken as that of the solution in which there was no change in fresh weight after 45 min (see Meidner & Sheriff 1976).

Leaf lacunal CO_2 concentrations, $[CO_2]_i$

Plants were brought back to the laboratory at dusk, undisturbed in large clumps of their own sediment, and main-

tained for 1 day under controlled conditions as described above. Leaf lacunal CO_2 concentrations, $[CO_2]_i$, were determined during a 4 h period around midday. Mature healthy leaves were carefully removed, sealed with a thin coating of petroleum jelly, and inserted into a short section of rubber tubing connected to an infra-red gas analyser (ADC 225.MK3, Hoddesdon, Hertfordshire, UK). The tubing was briefly flushed with CO_2-free air, then sealed and inter-lacunal gases expelled by pressure; the lacunal gases were then flushed through the infra-red gas analyser with CO_2-free air (see Robe & Griffiths 1988). Lacunal volume was determined by two methods: (a) as the difference in the volume of water displaced by the leaves before and after expelling lacunal gases, and (b) from a comparison of total and lacunal surface areas estimated from photographs of leaf transverse sections, using the technique of stereology (Steer 1981). Both methods gave very similar results.

Titratable acidity

Whole shoots were frozen in liquid nitrogen at dusk and dawn. At dusk, plants were sampled *in situ* at Thirlmere. For dawn sampling, large clumps of plants in their own sediment were taken from the site after dusk, transported to a garden, and kept in the open air until dawn. On removal from the liquid nitrogen, cell sap was expressed from the thawed material and titrated against NaOH with phenolphthalein as indicator (see Robe & Griffiths 1988 for details)

Rubisco and PEPC assays

Plants growing *in situ* were sampled at midday. Photon flux density, temperature and relative humidity around midday on the three sampling days are shown in Table 1 of Robe & Griffiths (1998). Youngest mature leaves were frozen in liquid nitrogen within 2–3 s of removal from the shoots. Both enzymes were assayed spectrophotometrically. The method used for extraction and assay of Rubisco was based on that described by Besford (1984) with adaptations from Lilley & Walker (1974), Ward & Keys (1989), Quick *et al.* (1991) and Sharkey, Savitch & Butz (1991). Only the distal halves of the leaves, minus the tips, were used for the assays (0·4 and 0·1 g of submersed and terrestrial leaf tissue, respectively). Leaves were taken from the liquid nitrogen, cut and weighed while still frozen and dropped into 2 cm^3 of ice-cold buffer (100 mol m^{-3} HEPES adjusted to pH 7·8 with KOH, 5 mol m^{-3} DTT, 0·2 mol m^{-3} EGTA and 0·06 g Polyclar AT, flushed with CO_2-free nitrogen) in a pre-frozen mortar and pestle standing on ice. The tissue was very quickly homogenized with a little acid-washed sand and centrifuged at 16 000 *g* (at 2–4 °C) for 30 s. The extract was then divided into two. Half was snap-frozen (in four 110 mm^3 aliquots contained in Eppendorf vials) and stored in liquid nitrogen for measurement of initial activity. The enzyme in the remaining half was activated on ice with 20 mol m^{-3} $KHCO_3^-$ and 25 mol m^{-3} $MgCl_2^-$. After 45 min, the activated extract was also snap-frozen, as above. The enzyme was assayed by the continuous measurement of 3-PGA-dependent NADH oxidation in a coupled enzyme system. The freshly prepared reaction mixture (890 mm^3 in cuvettes of 10 mm path length) contained 100 mol m^{-3} HEPES–KOH adjusted to pH 7·8, 20 mol m^{-3} $MgCl_2$, 5 mol m^{-3} DTT, 0·2 mol m^{-3} EGTA, 0·25 mol m^{-3} NADH, 3·5 mol m^{-3} ATP, 3·5 mol m^{-3} phosphocreatine, 10 units of 3-phosphoglycerate phosphokinase, 6 units of glyceraldehyde 3-phosphate dehydrogense, 16 units of creatine phosphokinase, 25 mol m^{-3} $KHCO_3^-$ and 0·4 mol m^{-3} ribulose bisphosphate (RuBP). The solution was allowed to stand for a few minutes to reach 20 °C and the reaction was started with the addition of 110 mm^3 of freshly thawed extract. Measurements were made at 340 nm using a Unicam 8700 series spectrophotometer (Unicam Ltd, Cambridge, UK), and the reaction was linear for at least 1·5–2 min, sometimes 3 min. Each extract was assayed three times for both initial and total (fully activated) activity. Control assays without RuBP gave a very low activity, and this was subtracted when calculating the rate of CO_2 fixation, using an absorption coefficient of 0·63 mol^{-1} mm^{-1}, according to Bergmeyer (1983).

The assay for phosphoenolpyruvate carboxylase (PEPC) activity was based on that of Borland & Griffiths (1992). Distal leaf tissue (0·2 g, weighed frozen as above) was quickly homogenized in 2 cm^3 of ice-cold extraction buffer identical to that used for the Rubisco assay, but with the addition of 5 mol m^{-3} $MgCl_2$. The crude homogenate was centrifuged at 16 000 *g* for 30 s and de-salted, to remove malate, by passage through a pre-packed column (bed volume 9·1 cm^3, bed height 5 cm^3) of Sephadex G25-M (Pharmacia Biotech Ltd, Milton Keynes, UK) held at a temperature of 2–4 °C, and the eluate used immediately for enzyme analysis. The reaction mixture (890 mm^3 in cuvettes of 10 mm path length) contained 100 mol m^{-3} Bicine, pH 8·2, 5 mol m^{-3} $MgCl_2$, 0·16 mol m^{-3} NADH, 10 units malic dehydrogensase, 10 mol m^{-3} $KHCO_3^-$ and 2 mol m^{-3} PEP. The mixture was allowed to warm to 20 °C and the reaction was initiated by the addition of 110 mm^3 of extract. Measurements were made at 340 nm and the reaction was linear for at least 6 min. Control assays without PEP showed a very low activity which was subtracted when calculating the rate of CO_2 fixation as described above. Soluble protein content of the extracts was determined by the method of Bradford (1976). All reagents and enzymes for both assays were purchased from Sigma.

Chlorophyll

Leaf tissue for chlorophyll determinations was also frozen *in situ* in liquid nitrogen. Chlorophyll was extracted from 50 mg of thawed tissue (distal half of leaves minus the tip) in 80% acetone, and the absorbance of centrifuged samples measured at 665 and 649 nm (Vernon & Seely 1966).

Carbohydrates

Whole plants (shoots, stem and as much of the root system as could be obtained) were frozen in liquid nitrogen at

dawn and dusk as described for titratable acidity above and stored at − 80 °C. Plants were taken from the same area of the site on each date and adjacent clumps were sampled for dawn and dusk determinations. Ethanol-soluble neutral sugars, warm water-soluble fructans, and starch were successively extracted using methods closely based on those described by Borland & Farrar (1985) and Farrar (1980). Whole shoots (minus any senescing leaves), roots and stems were thawed, weighed and homogenized in 95% ethanol. The extract was then incubated at 80 °C for 30 min, centrifuged at 20 000 *g* for 10 min and the supernatant removed and stored at 4 °C. The pellet was re-suspended and the extraction repeated twice more. The supernatants were combined and made up to a final volume of 25 cm³. The pellets were dried at 40 °C overnight and re-suspended in acetate buffer at pH 4·5. The extract was incubated at 30 °C for 8 h, centrifuged as described above and the supernatant removed and stored. The pellet was re-suspended and the extraction repeated twice more, the final volume of combined supernatants being made up to 25 cm³. The pellet was finally re-suspended in 6 cm³ acetate buffer pH 4·5 containing in excess of 100 units of amyloglucosidase (22 500 units g⁻¹ from *Rhizopus*; Sigma) and incubated at 45 °C for 24 h. The extract was centrifuged at 20 000 *g* and the supernatant removed for assay. Aliquots of the ethanol, acetate buffer and amyloglucosidase extracts were assayed for carbohydrate by the method of Dubois *et al*. (1956).

Soluble nitrogen

Whole plants, all from the same area of the site, were frozen in liquid nitrogen *in situ* at Thirlmere at midday. Shoots, roots and stems were stored individually at − 80 °C. Nitrate and free amino and soluble protein were extracted and determined spectrophotometrically as described by Robe & Griffiths (1994).

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), and total C and N analysis

Plant material, and sediments from around the roots, were dried at 60 °C and ground to a fine powder. Analysis of ^{13}C and ^{15}N natural abundance, and total C and N content, was carried out at Merlewood Research Station, Grange-over-Sands, Cumbria, using a Europa 20-20 isotope ratio mass spectrometer (Europa Scientific, Crewe, UK). ^{13}C and ^{15}N are stable isotopes of C and N which occur naturally in low abundance (1·1% in the case of ^{13}C and 0·366% in the case of N). $\delta^{13}C$ and $\delta^{15}N$ denote parts per thousand deviations, ‰, from the ratios $^{13}C:^{12}C$ and $^{15}N:^{14}N$ in a standard (a secondary standard referred to the original Pee Dee limestone in the case of C, and atmospheric N_2 in the case of N), and are calculated as: δ (‰) = $((R_{sample}/R_{standard}) - 1) \times 1000$, where *R* is the ratio $^{13}C:^{12}C$ or $^{15}N:^{14}N$. Small variations in natural abundance (fractionations) occur during physical, chemical and biological processes (Farquhar *et al*. 1989; Griffiths 1998).

Experimental partial emersion

Submersed *L. uniflora,* still rooted in large clumps of sediment, were removed from Thirlmere in May and maintained in tanks, with the water level at the tips of the original aquatic leaves (water surface 5 cm above the sediments). The tanks were kept outdoors in a semi-shaded position. New longer (10 cm), thinner leaves grew with their distal halves emersed and lower halves submersed. Measurements were made in September.

RESULTS

Water status of sediments and plants

Sediments dried out, particularly in the first 3–4 weeks after emersion (Fig. 1a). *L. uniflora* also lost water most rapidly just after emersion. Thus, fresh weight to dry weight ratios (Fig. 1b) and water content (Fig. 1c) declined. Osmotic potentials in shoots and roots (Fig. 1d) increased slightly just after emersion, but decreased substantially after flowering. Shoot water potential (Fig. 1d) declined slightly in the first 3–4 weeks after emersion, but more quickly after flowering. Shoot turgor pressure (calculated as water potential minus osmotic potential; Fig. 1d) fell most quickly just after emersion.

Leaf lacunal CO_2 concentration, CAM and activities of PEPC and Rubisco

Leaf lacunal CO_2 concentration, $[CO_2]_i$, increased after emersion (Fig. 2a). $[CO_2]_i$ was 1·7 mol m⁻³, equivalent to 3·8% in air, in the lacunae of aquatic *L. uniflora,* 3·1 mol m⁻³, equivalent to 6·9% CO_2 in air, in the lacunae of the flowering stage, and 7·0 mol m⁻³, equivalent to 15·7%, in the seed-bearing stage. In contrast, CAM activity was lost after emersion (Fig. 2b). In leaves of CAM species, the concentration of cell sap titratable acidity (H^+) reflects the overnight storage of CO_2 as malic acid (see Winter & Smith 1996). Shoots of submersed, aquatic *L. uniflora* showed a relatively high dawn H^+ and low dusk H^+ (Fig. 2b), and the ΔH^+ (dawn minus dusk titratable acidity) was 58 μmol H^+ g⁻¹ FW, signifying a moderate level of CAM (see Robe & Griffiths 1990, 1992). After only 3–4 days of emersion, CAM activity in aquatic leaves was reduced, by 70%, to 17 μmol H^+ g⁻¹ FW (not shown). The new terrestrial leaves of flowering *L. uniflora* showed no CAM, with a ΔH^+ of only 0·9 μmol H^+ g⁻¹ FW. In seed-bearing *L. uniflora*, the ΔH^+, although increased slightly to 6·1 μmol H^+ g⁻¹ FW, also indicated negligible CAM.

As shown in Fig. 2(c), the pattern of activity of maximum extractable PEPC mirrored ΔH^+, being relatively high in shoots of aquatic *L uniflora* and very low in leaves of the flowering and seed-bearing stages. Both initial activity of Rubisco (determined immediately after homogenization) and total activity (after full activation in the presence of activators CO_2 and Mg^{2+}) were determined. Initial and total activities of the Rubisco from *L. uniflora* increased 7–8-fold
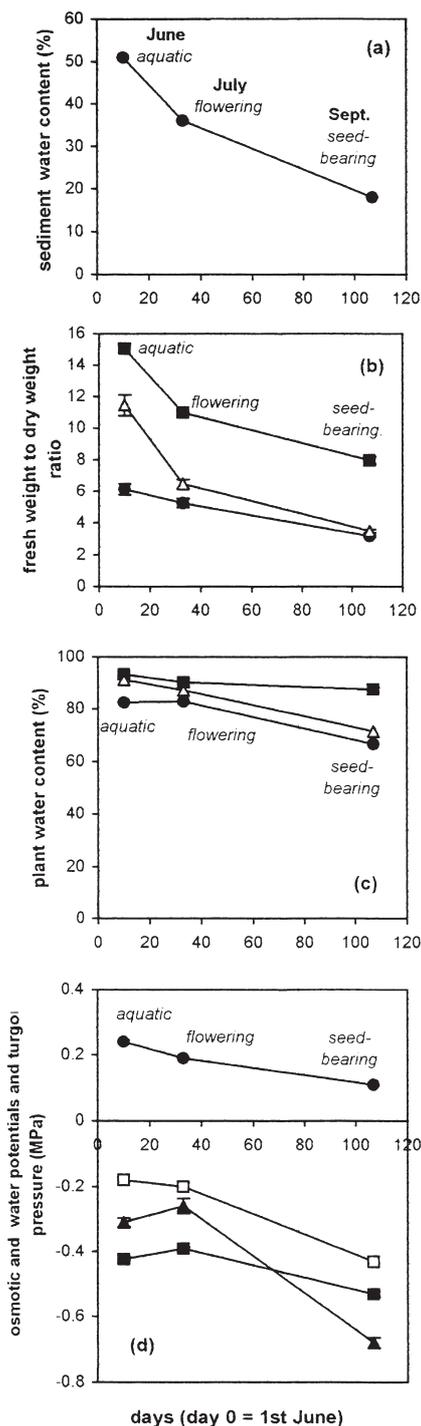
Figure 1. Sediment water content and plant water status for *Littorella uniflora* at Thirlmere Reservoir, Cumbria, at three sampling dates in 1995. (a) Water content of sediments around roots of *L. uniflora* expressed as a percentage calculated from fresh and dry weight measurements. (b) Fresh to dry weight ratios of shoots (■), roots (△) and stems (●) of *L. uniflora*. Mean for 10 plants with standard deviation (SD). There was a significant difference between fresh weight to dry weight ratios in June and July, and in July and September; $P < 0.001$. (c) Water content of shoots (■), roots (△) and stems (●) expressed as a percentage calculated from fresh weight to dry weight ratios. Mean for 10 plants. (d) Shoot osmotic potential (■), water potential (□), and turgor pressure (●) calculated as osmotic potential minus water potential, and osmotic potential of roots (▲). Mean for four plants at each sampling date, shown ± SD for osmotic potentials. The osmotic potential of shoots, but not roots, was significantly higher in flowering compared with aquatic *L. uniflora* ($P < 0.001$). In both shoots and roots, osmotic potentials in seed-bearing plants were significantly lower than in the flowering stage ($P < 0.001$). In some cases, the error bar is masked by the symbol.

aquatic, flowering and seed-bearing *L. uniflora*, respectively.

The chlorophyll (*a* and *b*) content of the distal halves of youngest mature leaves, minus the tip (the tissue used for PEPC and Rubisco assays) was 43% higher in flowering than in aquatic *L. uniflora* (Fig. 2d). Soluble protein concentration was also 3·5-fold higher, but declined after flowering (Fig. 2d). Activities of PEPC and Rubisco in *L. uniflora* expressed on chlorophyll and protein bases exhibited similar seasonal trends to those found when the data are expressed on a fresh weight basis, for example Rubisco total activity was 1·25, 5·31 and 2·38 μmol CO_2 mg^{-1} chlorophyll min^{-1} and 0·15, 0·37 and 0·35 μmol CO_2 mg^{-1} soluble protein min^{-1} in aquatic, flowering and seed-bearing stages, respectively.

Figure 3 depicts ΔH^+, and the activities of PEPC and Rubisco, in the upper and lower halves of the partially emersed leaves of *L. uniflora* grown outdoors in tanks for 4 months (see Materials and Methods). The submersed portions showed moderate levels of CAM activity but low Rubisco activity. In contrast, the emersed portions, exposed to the air, showed little CAM activity but high levels of Rubisco activity. The activation state of Rubisco was 54% in the submersed halves and 74% in the emersed halves.

Carbohydrate

The concentrations of neutral sugars, fructans and starch, at dawn and dusk, increased after emersion, particularly in roots and stems, but at different rates and to different degrees (Fig. 4). In the first 3–4 weeks, neutral sugars (Fig. 4a) accumulated most rapidly, being 8- and 2·3-fold higher in roots and stems, respectively, at dawn in flowering *L. uniflora* (e.g. 60% of total C compared with 14% in

after emersion, but declined again after flowering. The activation state of the enzyme (the proportion of initial activity to total activity expressed as a percentage) was 62% in aquatic *L. uniflora*, 55% in the flowering stage and 66% in the seed-bearing stage. The ratios of (fully activated) Rubisco to PEPC activities were 0·45, 32·2 and 10·0 in
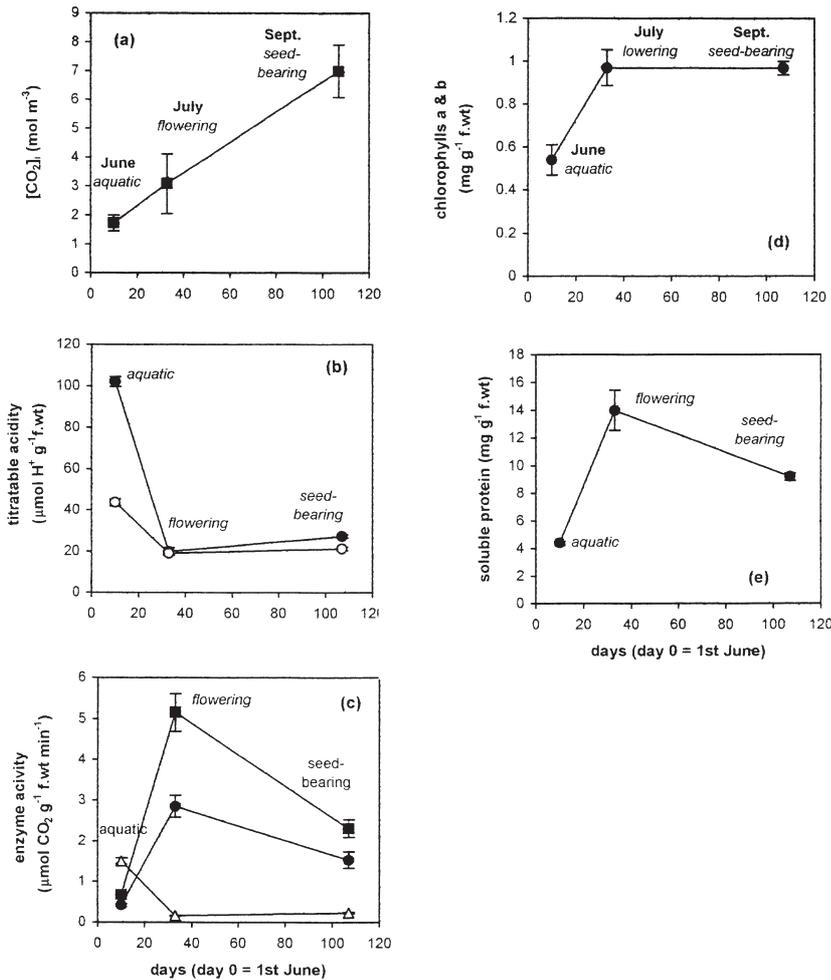
Figure 2. Lacunal CO_2 concentration, CAM, activities of PEPC and Rubisco, and chlorophyll and soluble protein content of shoots of *Littorella uniflora* at Thirlmere Reservoir, Cumbria, at three sampling dates in 1995. (a) Lacunal CO_2 concentration, $[CO_2]_i$, determined during a 4 h period around midday. Mean values for 20–25 leaves from 6–7 plants with standard deviations (SD). The $[CO_2]_i$ of flowering *L. uniflora* was significantly higher than that of the aquatic stage ($P = 0.003$), while that of seed-bearing *L. uniflora* was significantly higher than that of the flowering stage ($P < 0.001$). (b) Dawn (●) and dusk (○) titratable acidity of five shoots ± SD. Titratable acidity at dawn in seed-bearing *L. uniflora* was significantly higher than in the flowering stage ($P < 0.001$) (c) Activities of PEPC (△) and Rubisco enzymes. The data for Rubisco show both initial activity, immediately after extraction (●), and total activity, after full activation in the presence of Mg^{2+} and CO_2 (■). Results, shown ± SD, are for 4–6 extracts using the distal half (minus the tip) of 6–10 youngest mature leaves. Initial and total activities were significantly different at each sampling date ($P < 0.001$). (d) Chlorophyll ($a + b$) content, and (e) soluble protein concentration, of the distal halves (minus the tip) of youngest mature leaves. Mean ± SD of five extracts. In some cases, the error bar is masked by the symbol.

roots). In contrast, fructans (Fig. 4b) and starch (Fig. 4c) built up in concentration mainly after flowering. Thus, the starch concentrations of roots and stems were 4·3 and 6·5 times higher, respectively, at dawn in seed-bearing, compared with flowering *L. uniflora* (e.g. 52% of total C compared with 14% in roots). For June and July samplings, carbohydrate concentrations were generally higher at dusk than at dawn (Fig. 4a–c). In September, however, concentrations of carbohydrates in stems and roots were lower at dusk than at dawn.
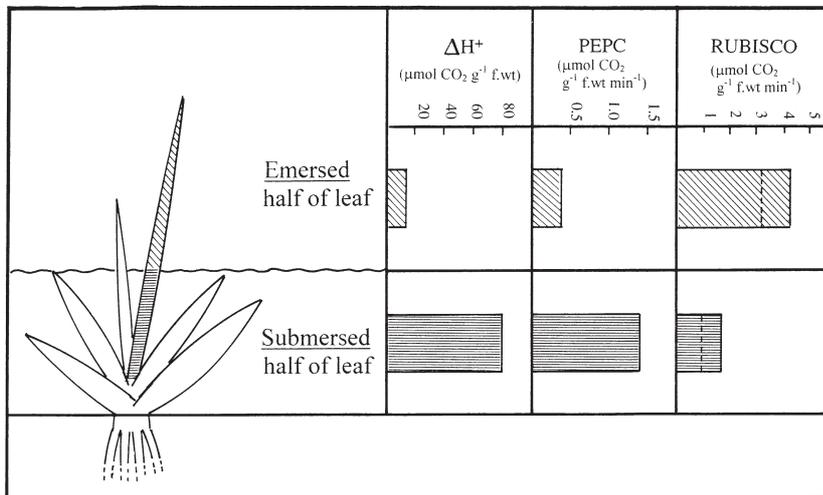

Figure 3. CAM activity (as ΔH^+; dawn minus dusk titratable acidity) and activities of PEPC and Rubisco in partially emersed leaves of *Littorella uniflora* from Thirlmere, grown in tanks in a garden. Submersed *L. uniflora,* still rooted in large clumps of sediment, were removed from Thirlmere in May, and maintained in tanks with the water level at the tips of the original aquatic leaves (water surface 5 cm above the sediment). The tanks were kept outdoors in a semi-shaded position. New longer (10 cm), thinner leaves grew with their distal halves emersed and lower halves submersed. Both halves of the leaves were green and healthy. Measurements for upper and lower portions of six leaves combined were made in September.
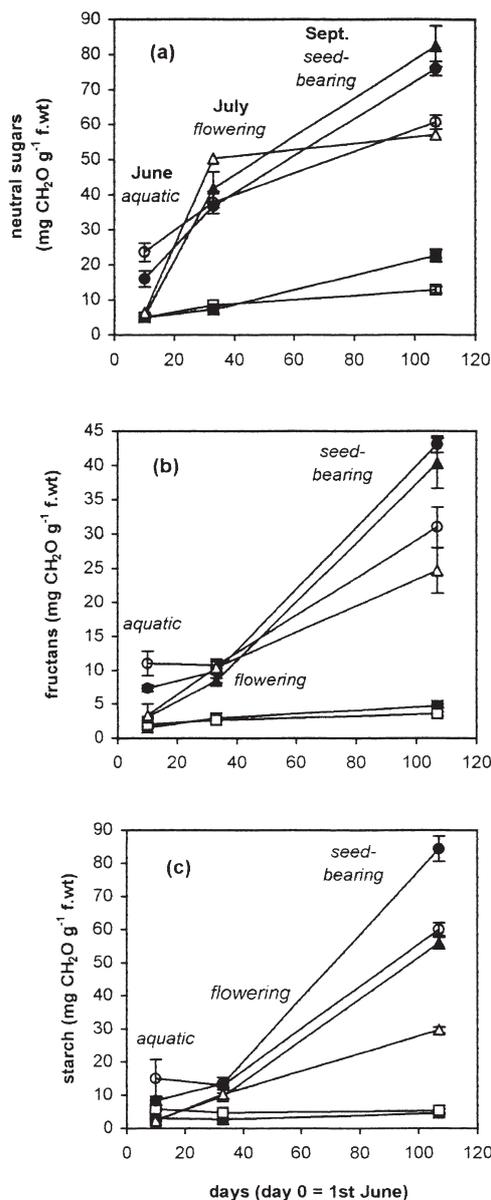
Figure 4. Soluble carbohydrate content of shoots, roots and stems of *Littorella uniflora* at dawn and dusk at Thirlmere Reservoir, Cumbria, at three sampling dates in 1995. (a) Ethanol (95%)-soluble neutral sugars, i.e. sucrose, fructose, glucose and fructans with a low degree of polymerization. (b) Water (i.e. buffer)-soluble fructans. (c) Starch. Means for whole shoots (dawn ■ and dusk □), roots (dawn ▲ and dusk △) and stems (dawn ● and dusk ○) of three plants are shown with standard deviation. Data for shoots of seed-bearing *L. uniflora* do not include the seeds. Only starch in shoots was significantly higher at dusk than at dawn in flowering compared with aquatic *L. uniflora* ($P = 0.04$). Neutral sugars in shoots and all carbohydrates in roots and stems were significantly lower ($P = 0.05 – 0.001$) at dusk than at dawn in seed-bearing compared with flowering *L. uniflora*. In some cases, the error bar is masked by the symbol.

Soluble nitrogen

Nitrate concentrations in shoots, roots and stems (Fig. 5a) declined sharply in the first 3–4 weeks after emersion, and were at almost undetectable levels in these tissues of seed-bearing *L. uniflora*. Free amino acid concentrations also declined most rapidly in the first 3–4 weeks (Fig. 5b). In aquatic *L. uniflora*, nitrate and free amino acid nitrogen comprised 7·4 and 4%, respectively, of total nitrogen in shoots, 3 and 4% in stems, and 12·5 and 8% in roots.

Carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), and percentage C and N

Figure 6 shows $\delta^{13}C$ and total C, and $\delta^{15}N$ and total N in sediments and *L. uniflora*. The $\delta^{13}C$ of plant tissues ranged from $-23\cdot9$ to $-29\cdot1$‰ and declined (became more nega-
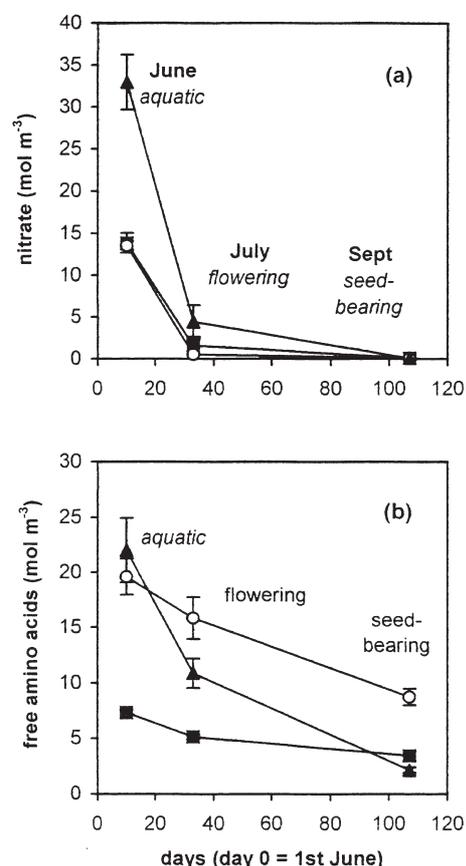

Figure 5. Soluble nitrogen content of shoots, roots and stems of *Littorella uniflora* at Thirlmere Reservoir, Cumbria, at three sampling dates in 1995. (a) Nitrate concentration in shoots (■), roots (▲) and stems (○). (b) Free amino acid concentration in shoots (■), roots (▲) and stems (○). Results, shown with standard deviation, are mean values for whole shoots, stems and roots of five plants sampled at midday. The differences between amino acids concentrations in aquatic and flowering *L. uniflora* and in flowering and seed-bearing plants were significant ($P < 0.001$) for shoots, roots and stems. Data for shoots of seed-bearing *L. uniflora* do not include the seeds. In some cases, the error bar is masked by the symbol.
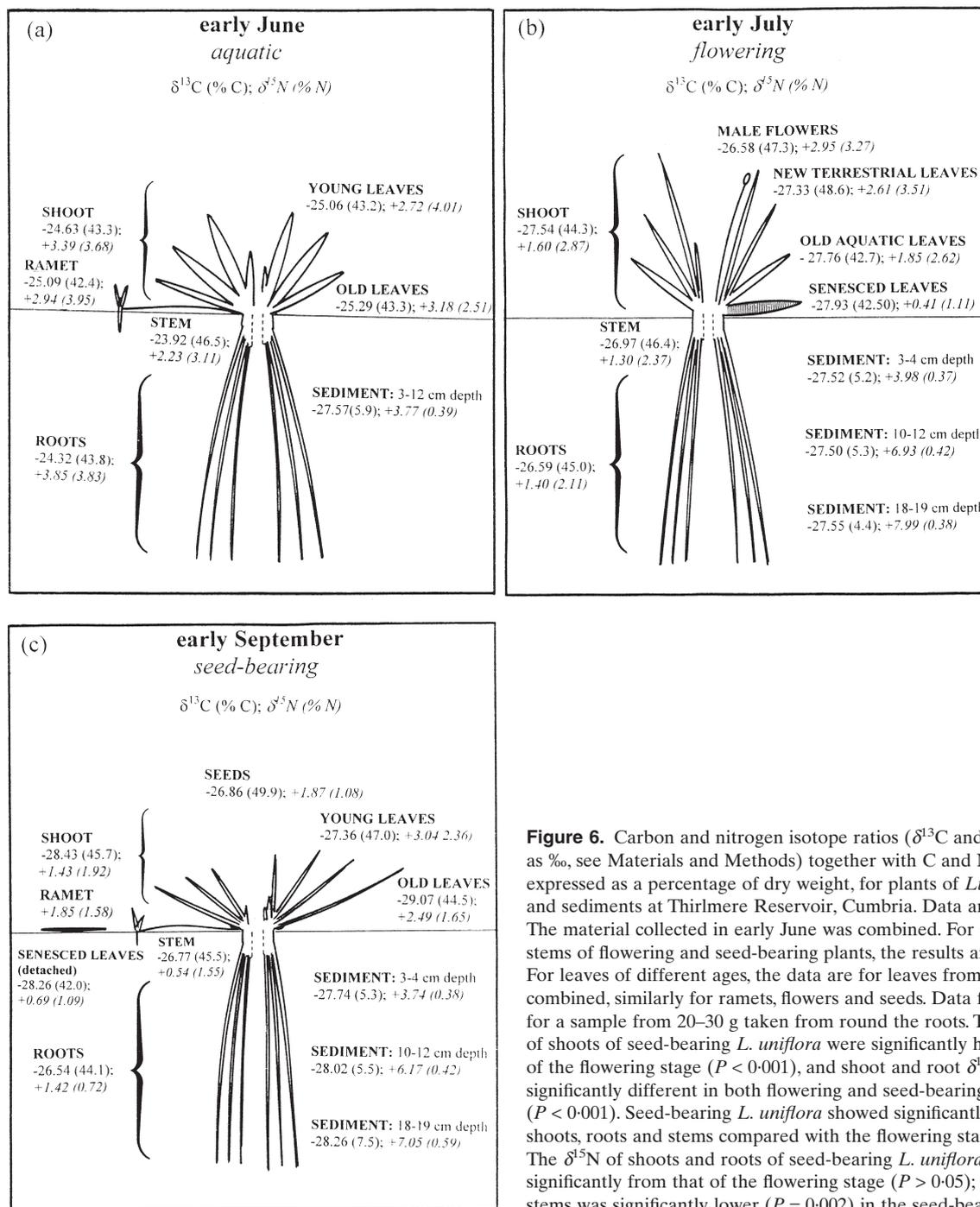
(a)
early June
aquatic

$\delta^{13}C$ (% C); $\delta^{15}N$ (% N)

YOUNG LEAVES
-25.06 (43.2); +2.72 (4.01)

SHOOT
-24.63 (43.3);
+3.39 (3.68)

RAMET
-25.09 (42.4);
+2.94 (3.95)

OLD LEAVES
-25.29 (43.3); +3.18 (2.51)

STEM
-23.92 (46.5);
+2.23 (3.11)

SEDIMENT: 3-12 cm depth
-27.57(5.9); +3.77 (0.39)

ROOTS
-24.32 (43.8);
+3.85 (3.83)

(b)
early July
flowering

$\delta^{13}C$ (% C); $\delta^{15}N$ (% N)

MALE FLOWERS
-26.58 (47.3); +2.95 (3.27)

NEW TERRESTRIAL LEAVES
-27.33 (48.6); +2.61 (3.51)

SHOOT
-27.54 (44.3);
+1.60 (2.87)

OLD AQUATIC LEAVES
- 27.76 (42.7); +1.85 (2.62)

SENESCED LEAVES
-27.93 (42.50); +0.41 (1.11)

STEM
-26.97 (46.4);
+1.30 (2.37)

SEDIMENT: 3-4 cm depth
-27.52 (5.2); +3.98 (0.37)

SEDIMENT: 10-12 cm depth
-27.50 (5.3); +6.93 (0.42)

ROOTS
-26.59 (45.0);
+1.40 (2.11)

SEDIMENT: 18-19 cm depth
-27.55 (4.4); +7.99 (0.38)

(c)
early September
seed-bearing

$\delta^{13}C$ (% C); $\delta^{15}N$ (% N)

SEEDS
-26.86 (49.9); +1.87 (1.08)

YOUNG LEAVES
-27.36 (47.0); +3.04 2.36)

SHOOT
-28.43 (45.7);
+1.43 (1.92)

RAMET
+1.85 (1.58)

OLD LEAVES
-29.07 (44.5);
+2.49 (1.65)

STEM
-26.77 (45.5);
+0.54 (1.55)

SENESCED LEAVES
(detached)
-28.26 (42.0);
+0.69 (1.09)

SEDIMENT: 3-4 cm depth
-27.74 (5.3); +3.74 (0.38)

ROOTS
-26.54 (44.1);
+1.42 (0.72)

SEDIMENT: 10-12 cm depth
-28.02 (5.5); +6.17 (0.42)

SEDIMENT: 18-19 cm depth
-28.26 (7.5); +7.05 (0.59)

Figure 6. Carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ expressed as ‰, see Materials and Methods) together with C and N contents expressed as a percentage of dry weight, for plants of *Littorella uniflora* and sediments at Thirlmere Reservoir, Cumbria. Data are for 6–8 plants. The material collected in early June was combined. For shoots, roots and stems of flowering and seed-bearing plants, the results are mean values. For leaves of different ages, the data are for leaves from several plants combined, similarly for ramets, flowers and seeds. Data for sediments are for a sample from 20–30 g taken from round the roots. The $\delta^{13}C$ and %C of shoots of seed-bearing *L. uniflora* were significantly higher than those of the flowering stage ($P < 0.001$), and shoot and root $\delta^{13}C$ were significantly different in both flowering and seed-bearing plants ($P < 0.001$). Seed-bearing *L. uniflora* showed significantly lower %N in shoots, roots and stems compared with the flowering stage ($P < 0.001$). The $\delta^{15}N$ of shoots and roots of seed-bearing *L. uniflora* did not differ significantly from that of the flowering stage ($P > 0.05$); however, $\delta^{15}N$ of stems was significantly lower ($P = 0.002$) in the seed-bearing stage.

tive) after emersion. Young leaves showed higher values than old or senesced leaves, particularly in September. Root $\delta^{13}C$ was 1–2‰ less negative than that of shoots at all sampling dates. Sediment $\delta^{13}C$ was in the range − 27·5 to − 28·2‰. Plant C content increased after emersion and varied slightly between tissues (e.g. 42·5% in senesced leaves and 48·6% in new leaves of flowering plants).

The $\delta^{15}N$ of plant tissues was in the range + 0·41 to + 3·85‰. The $\delta^{15}N$ of shoots, stems and roots declined after emersion. At all sampling dates, $\delta^{15}N$ of shoots, stems and

roots differed, with stems having the lowest (least positive) $\delta^{15}N$. In leaves, $\delta^{15}N$ varied with leaf age (e.g. in flowering *L. uniflora*, the $\delta^{15}N$ of senesced, old aquatic, and new terrestrial leaves were + 0·41, + 1·85 and + 2·61‰, respectively). The $\delta^{15}N$ of sediments increased with depth. Plant N, expressed as a percentage of dry weight, ranged from 4·01 to 0·72%, declined after emersion, and varied markedly between leaves of different ages (e.g. for flowering *L. uniflora*; 1·11% in senesced leaves and 3·51% in new terrestrial leaves).

DISCUSSION

Plant water relations

Emersion had little immediate effect on plant water relations. Water and osmotic potentials remained at the high end of the range for aquatic and succulent terrestrial species (Milburn 1979; Larcher 1983), and there was only a small drop in turgor. The fact that *L. uniflora* has a well developed vascular system (Hostrup & Wiegleb 1991) and that water was probably still freely available in the sediments perhaps buffered against the full dehydrating effects of emersion; there was a strong positive correlation between turgor pressure and soil water content ($r = 0.996$, $P = 0.01$). However, there may also have been changes in cuticle thickness and/or permeability, and control of water loss at the membrane level (e.g. Chrispeels & Maurel 1994). It seems that water stress was not experienced until after flowering, when leaf water potential decreased more quickly (Fig. 1d). Osmotic adjustment in leaves and particularly roots, suggested by the decrease in osmotic potentials (Morgan 1984), did not prevent loss of water and turgor. Water stress may have been a cause of the abrupt drop in growth after flowering (Fig. 5a; Robe & Griffiths 1998).

Lacunal CO$_2$ concentrations

The increase in [CO$_2$]$_i$ after emersion (Fig. 2a) suggests that sediments may have been an important source of CO$_2$ for the fully terrestrial form of *L. uniflora* growing on dry land at Thirlmere (see also Nielsen *et al*. 1991). As the sediments became aerobic, carbon breakdown may have increased, and CO$_2$ could continue to diffuse into the root lacunae and *via* the stems to the reduced lacunal system in the leaves, eventually escaping through stomates or cuticle. The relative importance of sediment and atmospheric CO$_2$ supply for terrestrial *L. uniflora* might depend on sediment type; sediments with a higher proportion of gravel probably contain less CO$_2$ (e.g. Robe & Griffiths 1988). The residual lacunal system could allow gas exchange with the sediments to continue on re-submersion, so that a period of reliance on bulk water CO$_2$ uptake and anaerobic metabolism in the roots (e.g. ap Rees *et al*. 1987; see also Jackson & Armstrong 1999) would be avoided.

CAM and C$_3$ photosynthesis: PEPC and Rubisco activity

In aquatic *L. uniflora* carrying out moderate levels of CAM, PEPC activity, assayed in the absence of malate, exceeded Rubisco activity (Fig. 2c), and the ratio of fully activated Rubisco activity to PEPC activity was 0.45, similar to that in terrestrial CAM species (see Dittrich, Campbell & Black 1973), although lower than previously reported for *L. uniflora* (Farmer, Maberly & Bowes 1986; Baattrup-Pedersen & Madsen 1999) and other aquatic CAM species (Keeley 1999). PEPC activity was in excess of that predicted from the rate of overnight CO$_2$ fixation calculated from ΔH$^+$ (by 22-fold; 1.5 compared with 0.069 μmol CO$_2$ g^{-1} FW min^{-1})

as also reported for terrestrial CAM species (see Borland & Griffiths 1992). In aquatic *L. uniflora*, Rubisco total activity (0.677 μmol CO$_2$ g^{-1} FW min^{-1} or 40.6 μmol CO$_2$ g^{-1} FW h^{-1}) closely matched maximum rates of photosynthesis in thin tissue slices incubated with rapid stirring and saturating CO$_2$ and PPFD (35–40 μmol O$_2$ g^{-1} FW h^{-1}; see Robe & Griffiths 1992). Rates of photosynthesis in intact plants incubated under near-natural conditions (50–300 μmol m^{-2} s^{-1} incident PPFD) are lower (4- to 12-fold: Robe & Griffiths 1990), probably due to a combination of low incident and within-leaf light intensities (Robe & Griffiths 1990).

The plasticity of photosynthesis in *L. uniflora* is illustrated by the rapid loss of CAM from newly emersed, non-senescent leaves, its absence from new terrestrial leaves which show several-fold higher activity of Rubisco, and the contrasting activities of PEPC and Rubisco in the two halves of partially emersed leaves. In contrast, permanently submersed *L. uniflora* show gradual seasonal changes in CAM activity and photosynthetic capacity (Robe & Griffiths 1992). For the plants growing at Thirlmere, the switch from CAM to C$_3$ underlay rapid new leaf production culminating in flowering only 3–4 weeks after emersion (Robe & Griffiths 1998), so could be important for seed output in a habitat where re-submersion is unpredictable. Photosynthetic pathway plasticity has been discovered in several other amphibious species, thought to be recently evolved from terrestrial ancestors, which are CAM or C$_3$ + C$_4$ when submersed and C$_3$ when emersed (Keeley 1998a, 1999), or C$_3$ when submersed and C$_4$ when emersed (Ueno 1996), or which show several-fold higher Rubisco activity in emergent tissues (Maberly & Spence 1989).

The loss of CCMs and switches to C$_3$ in amphibious species on emergence into the aerial environment have been related to the disappearance of diffusional limitation of CO$_2$ (e.g. Aulio 1986; Raven *et al*. 1988; Nielsen *et al*. 1991; Keeley 1996; Raven & Spicer 1996; Keeley 1999). However, there is no information on how emersion was sensed by *L. uniflora* or on how photosynthetic pathway changes were triggered. One possibility is that localized decreases in intercellular CO$_2$, for example around cells near the epidermis, were sensed in some way (see e.g. Assman 1999; Matsuda, Bozzo & Colman 1997; Kaplan & Reinhold 1999). Alternatively, water loss might have been detected in the small whole-leaf reductions in turgor (Fig. 1d) or as reductions in cell volume, or increased osmotic strength (e.g. Bray 1993; Shinozaki & Yamaguchi-Shinozaki 1997), particularly in epidermal cells. Loss of water as a signal of emersion could perhaps most easily explain the absence of CAM in the upper halves of partially emersed leaves (Fig. 3), the rapid loss of CAM from emersed aquatic leaves of *L. uniflora* (see Results), and also *Isoetes howellii* (see Keeley 1996), and the presence of CAM in terrestrial *L. uniflora* under conditions of high humidity (Farmer & Spence 1985; Aulio 1986). Signal transduction may have involved plant growth substances such as ABA, and photosynthetic enzyme expression could be modulated by other environmental and internal factors,

such as photoperiod, temperature, light quality, ethylene, O_2, and nutrient status (see Cushman & Bohnert 1997; Trewavas & Malhó 1997). In terrestrial CAM species, water stress induces CAM by initiating transcription of PEPC (Cushman & Bohnert 1997) and fluctuations in the content of Rubisco small subunit mRNA (Michalowski *et al.* 1989), raising intriguing questions about how signal perception and transduction, and control of gene expression could differ in *L. uniflora*.

The Rubiscos of the aquatic macrophytes examined so far have a relatively high K_m for CO_2 (low affinity for CO_2; Yeoh, Badger & Watson 1981), and are optimized to operate at high CO_2 concentrations (see Raven, Osborne & Johnston 1985). Preliminary measurements indicate that the Rubisco of submersed *L. uniflora* may have a $K_m(CO_2)$ up to fivefold higher than that of *Plantago major* (unpublished results). However, a high-$K_m(CO_2)$ Rubisco would be a disadvantage in the aerial environment because of the higher levels of O_2 fixation and photorespiration. Perhaps in amphibious plants such as *L. uniflora,* regulation of the activation state of Rubisco (see Fig. 2c, Fig. 3), could compensate for variations in CO_2 supply and also allow acclimatization to changes in light intensity or content of Rubisco protein (see Mott, Snyder & Woodrow 1997; Hammond *et al.* 1998).

Carbohydrate storage

In aquatic *L. uniflora*, starch seemed to be the main overnight store of C and source of energy and C for biosynthesis. After emersion, considerably more excess photosynthate was available, and this generally accumulated as sugars. Sugars stored in the vacuole may have been associated with osmotic adjustment (see Stewart & Larher 1980; Morgan 1984) as water stress began to develop after flowering. After flowering, accumulation of fructans and starch in stems and roots increased. Fructans, polymers of fructose, stored in the vacuole, seem to be involved in water uptake and retention, and cell expansion (Hendry 1993; Albrecht, Biemelt & Baumgartner 1997; Vijn & Smeekens 1999), and may have physiological importance for an amphibious species such as *L. uniflora* during water deficit and re-submersion. Storage carbohydrates could also provide interim energy and CO_2 supplies (see ap Rees *et al.* 1987) during the rapid re-growth of aquatic leaves which can follow re-submersion (unpublished results).

Carbon isotope ratios

The $\delta^{13}C$ values for *L. uniflora* were similar to previous reports for this species (− 25‰ for submersed plants; Keeley & Sandquist 1992) and other isoetids (− 21 to − 33‰; Osmond *et al.* 1981), and were within the wide range recorded for aquatic and amphibious macrophytes (− 13·4 to − 50·7‰; Keeley & Sandquist 1992). The $\delta^{13}C$ of sediment C was also similar to that previously recorded for terrestrial soils (Schleser & Jayasekera 1985).

Plant $\delta^{13}C$ reflects the $\delta^{13}C$ of the source CO_2, the fractionation of isotopes which occurs during Rubisco and PEPC carboxylation, and processes of diffusion, dissolution and hydration, and is mainly determined by the rate-limiting step (O'Leary 1988; Farquhar *et al.* 1989). In terrestrial species using atmospheric CO_2 ($\delta^{13}C$ − 7 to − 9‰), a switch from CAM (in which fixation by Rubisco occurs in a relatively closed system) to C_3 (fixation of CO_2 taken up *via* stomata) can result in a large shift to more negative $\delta^{13}C$ values (e.g. − 14 to − 30‰) as discrimination by Rubisco against the heavier isotope of C is expressed. However, in *L. uniflora*, emersion and loss of CAM resulted in a very small (3–4‰) decline in $\delta^{13}C$. A similar small difference between the $\delta^{13}C$ of submersed and emersed *Isoetes howellii* (− 26 and − 29‰, respectively) was found by Keeley & Sandquist (1992). In *I. howelli*, a seasonal pool species which seems to take up little, if any, sediment CO_2 (Keeley 1998b), a change in the $\delta^{13}C$ of source CO_2, from − 15 to − 20‰ for submersed plants (lacunal CO_2 from respiration and malate decarboxylation), to − 7‰ for emergent plants (atmospheric CO_2), combined with substantially increased discrimination by Rubisco, could explain the slightly more negative leaf $\delta^{13}C$. However, *L. uniflora* at Thirlmere seems to have continued to take up sediment CO_2 with a $\delta^{13}C$ of around − 23·5‰ (CO_2 in the interstitial water of sediments; unpublished results). The more negative $\delta^{13}C$ of emersed plants may therefore indicate changes in the $\delta^{13}C$ of lacunal CO_2 due to cessation of PEPC fixation and malate decarboxylation, increased rates of daytime C fixation and a small increase in discrimination by Rubisco in a slightly more open system.

Nitrogen storage

Aquatic *L. uniflora* contained high concentrations of nitrate and free amino acids, similar to those found in spring in permanently submersed plants in natural habitats (see Robe & Griffiths 1994). The disappearance of a large part of these soluble N reserves in the first 3–4 weeks of emersion suggests that they may have been used for new growth. The low N content of senesced and old leaves compared with young leaves at all three sampling dates (72% lower; Fig. 6) also suggests recycling of functional and structural protein (see Stoddart & Thomas 1982; Millard 1988), although volatilization and/or leaching may account for some of the loss (Feller & Fischer 1994). The Rubisco protein which was not activated *in vivo* (34–45%; Fig. 2c) may also constitute a store of N (e.g. see Stitt & Schulze 1994). Although plant N content declined after emersion, %N was above the 1·3% critical value (minimum N for maximum growth) determined for aquatic macrophytes by Gerloff & Krombholz (1966), and well within the range for terrestrial macrophytes growing with a wide range of N supply (0·68–4·0%; Gebauer, Rehder & Wollenweber 1988) in all non-senesced tissues except roots of seed-bearing *L. uniflora*.

Nitrogen isotope ratios

The $\delta^{15}N$ of *L. uniflora* at Thirlmere ($+ 0.41$ to $+ 3.85$‰) was within the range for field-grown species ($- 9$ to $+ 12$‰; Shearer *et al.* 1983; Pate, Stewart & Unkovich 1993; Handley & Scrimgeour 1997; Michelsen *et al.* 1998; Neilson *et al.* 1998). Values for sediment total N ($+ 3.77$ to $+ 7.99$‰) and the increase with depth were also characteristic of undisturbed soils ($- 6$ to $+ 10$‰; Ledgard, Freney & Simpson 1984; Gebauer & Schulze 1991; Koopmans *et al.* 1997; Neilson *et al.* 1998). A lower $\delta^{15}N$ in plants compared with soils has been found previously in undisturbed habitats (Handley & Scrimgeour 1997; Neilson *et al.* 1998; Michelson *et al.* 1998). A positive correlation between $\delta^{15}N$ and %N ($r = 0.817$, $P = 0.001$ for whole shoots, roots and stems of *L. uniflora*, at all sampling dates, combined) has also been reported previously (e.g. Vitousek *et al.* 1989; Schulze *et al.* 1991; Stewart *et al.* 1995), as have seasonal and intra-plant differences in $\delta^{15}N$ (e.g. see Shearer *et al.* 1983; Gebauer & Schulze 1991; Handley & Scrimgeour 1997; Neilson *et al.* 1998), but there are no comparable data for amphibious macrophytes.

The $\delta^{15}N$ signals found in *L. uniflora* will be a function of both the $\delta^{15}N$ of the source N, and the fractionation which has occurred during acquisition and metabolism (Robinson, Handley & Scrimgeour 1998). The $\delta^{15}N$ of soil total N is probably not a good approximation of plant available pools (aquatic *L. uniflora* is known to utilize both NO_3^- and NH_4^+; Schuurkes, Kok & Den Hartog 1986; Robe & Griffiths 1994), which are thought to turn over quickly and have a rapidly changing $\delta^{15}N$ (Hogberg 1997). However, methods to accurately extract and analyse the $\delta^{15}N$ of these pools are not available (Handley & Scrimgeour 1997). The varying $\delta^{15}N$ of *L. uniflora* could partly reflect changes of source and fractionation during uptake by mycorrhizal fungi (Hogberg 1987); mycorrhiza have been reported in roots of *L. uniflora* (Söndergaard & Laegaard 1977). Intra-plant $\delta^{15}N$ differences could be the result of changes in the proportion of root and shoot reduction (Evans *et al.* 1996), which in *L. uniflora* do occur in response to NO_3^- supply and light intensity (Robe & Griffiths 1994). Fractionation during N incorporation into amino acids and proteins, mixing of different pools (as a result of translocation), and losses during senescence (e.g. of NH_3) may also have affected intra-plant $\delta^{15}N$ (see Hogberg 1997; Robinson, Handley & Scrimgeour 1998).

CONCLUSION

In *L. uniflora*, marked physiological and photosynthetic changes accompanied emersion onto dry land at a reservoir, and these findings provide a clearer picture of the extent of phenotypic plasticity in this amphibious species. The switch from CAM to C_3 photosynthesis seemed an important component of the rapid new leaf growth which preceded flowering only 3–4 weeks after emersion (Robe & Griffiths 1998). At the same time, the accumulation of carbohydrate reserves, low water and osmotic potentials and possibly continued uptake of CO_2 from sediments suggested a plant poised for re-submersion. These characteristics seem to be adaptive in a habitat where water level fluctuations are large and unpredictable. Since plasticity has a genetic basis and can evolve rapidly (Bradshaw 1965; Pigliucci, Cammell & Schmitt 1999), the population of *L. uniflora* at Thirlmere may be more responsive to emersion than those in surrounding tarns which are almost permanently submersed.

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