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Photosynthesis of *Littorella uniflora* grown under two PAR regimes: C_3 and CAM gas exchange and the regulation of internal CO_2 and O_2 concentrations

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Summary. The submersed aquatic macrophyte *Littorella uniflora* was grown under 50 and 300 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) (low and high PAR regimes) but identical sediment CO_2 supply (1.0 mol m^{-3}). The interactions between plant morphology, whole plant CO_2 and O_2 exchange, CAM activity, $[CO_2]_i$ and $[O_2]_i$ have been investigated in comparison with in vitro CO_2 and PAR response characteristics (using 1 mm leaf sections). In terms of morphology, high-PAR-grown plants were smaller and leaves contained less chlorophyll, although root growth was proportionally larger. Gas exchange fluxes over roots and shoots of intact plants were similar in direction under the two PAR regimes, with the majority of CO_2 uptake via the roots. Photosynthetic O_2 evolution from intact plants was greater in high-PAR-grown *L. uniflora* (2.18 compared with 1.49 μmol O_2 g^{-1} fresh weight h^{-1} for the low PAR regime). Although net daytime CO_2 uptake was similar for both PAR regimes (0.79 and 0.75 μmol g^{-1} fwt h^{-1}), net dark CO_2 uptake was at a higher rate (0.92 compared with 0.52 μmol CO_2 g^{-1} fwt h^{-1}), and dark fixation (as malic acid) was threefold greater in high PAR plants (ΔH^+ 117 compared with 42 μmol H^+ g^{-1} fwt). Comparison of dark CO_2 uptake with dark fixation suggested that much of the CO_2 fixed at night and regenerated during the day may be respiratory in origin (60% low PAR plants, 71% high PAR plants). Regeneration of CO_2 from CAM could account for 62% of daytime CO_2 supply in low PAR plants and 81% in high PAR plants. $[CO_2]_i$ values (ranging from 0.42 to 1.03 mol m^{-3}) were close to or above the concentration required to saturate photosynthesis in vitro (0.5 mol m^{-3}) under both PAR regimes, and combined with the low $[O_2]_i$ (2.6–4.3 mol m^{-3}) should have suppressed photorespiration. However, PAR inside leaves would have been well below the in vitro light saturation requirement (850–1000 μmol m^{-2} s^{-1} for both treatments). Thus PAR rather than CO_2 supply appeared to limit photosynthesis even in high PAR grown plants, and CAM appears to have an important role in the regulation of CO_2 supply for photosynthesis in response to variation in light regime.

Key words: *Littorella uniflora* – Gas exchange – Crassulacean acid metabolism – Lacunal CO_2 and O_2 concentrations – PAR acclimation

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Littorella uniflora is a submersed aquatic macrophyte with a rosette of small stiff leaves, a relatively large root area, and a system of intercellular gas channels (lacunae) continuous between shoots and roots (see Clapham et al. 1981; Raven et al. 1988). Such a life form has been defined by den Hartog and Segal (1964) as "isoetid", because of similarities in a number of families. The majority of CO_2 is acquired from CO_2-enriched sediments in which the plants are rooted. Free CO_2 diffuses into roots and, via the lacunae, into the leaves where fixation takes place in green cells lining the gas channels (Boston et al. 1987a, b; Richardson et al. 1984; Søndergaard and Sand-Jensen 1979a). An internal daytime CO_2 supply is also regenerated following dark fixation (Crassulacean acid metabolism, CAM: Robe and Griffiths 1988), resulting in high lacunal CO_2 concentrations ($[CO_2]_i$: Madsen 1987a, b; Robe and Griffiths 1988).

Several studies have investigated the effect of varying external CO_2 concentrations on CO_2 uptake, CAM and $[CO_2]_i$ in plants of the isoetid life form (Boston et al. 1987a, b; Madsen 1987a, b; Richardson et al. 1984; Roelofs et al. 1984; Søndergaard and Sand-Jensen 1979a). However the effects of variation in light intensity have been poorly characterised (see Farmer 1987; Sand-Jensen 1978). *L. uniflora* grown under low photosynthetically active radiation (PAR: 400–700 nm) showed a $[CO_2]_i$ sufficient to saturate daytime carboxylation and a relatively high *in vitro* light saturation requirement (Robe and Griffiths 1988) suggesting that light rather than CO_2 supply is the major factor limiting photosynthesis in deep water or shaded habitats. *L. uniflora* is also common in shallow water (Spence 1967) and is often found growing with water barely covering the leaf tips

or even fully exposed. Although under higher PAR it seems likely that CAM activity will increase (see Boston and Adams 1985; Madsen 1987c) as in terrestrial CAM species (Nobel 1982), resulting in increased CO_2 regeneration in the light, much of the CO_2 fixed at night and released during the day may be of respiratory origin (Keeley and Busch 1984; Madsen 1987b). The high $[CO_2]_i$ measured under low PAR (Robe and Griffiths, 1988) should suppress ribulose bisphosphate carboxylase (RUBISCO) oxygenase activity (Osmond 1981), but under higher light intensity internal O_2 concentrations may increase (see Hartman and Brown 1967; Sand-Jensen and Prahl 1982; Sorrell and Dromgoole 1986) although photosynthetically generated O_2 is released over both shoots and roots (Sand-Jensen et al. 1982). High rates of photorespiration, measured as CO_2 release, have been reported for *L. uniflora* (Søndergaard 1979), but there have been no measurements of $[O_2]_i$ or $[CO_2]_i/[O_2]_i$ ratio in these plants.

A complicating feature of isoetid studies to date is that measurements of CO_2 and O_2 exchange, CAM and $[CO_2]_i$ have generally been made in separate studies and under various experimental conditions which differ from those under which the plants had been growing (e.g. Boston et al. 1987a, b; Madsen 1987a, b, c). These experimental manipulations disrupt CO_2 and O_2 gradients between lacunae and incubating medium and the storage and recycling of lacunal gases (Sand-Jensen and Prahl 1982; Søndergaard 1979; Søndergaard and Sand-Jensen 1979b; Sorrell and Dromgoole 1986), and the measurements made may reflect a response to perturbation.

We have therefore undertaken an integrated study, with experimental manipulations similar to natural growing conditions, to investigate the effect of two PAR regimes on C_3 and CAM photosynthesis of *L. uniflora*. Plants were grown under 50 and 300 μmol m^{-2} s^{-1} with identical sediment and bulk water CO_2 supply. Morphology and whole plant CO_2 and O_2 exchange fluxes across roots and shoots were compared with CAM activity, $[CO_2]_i$ and $[O_2]_i$ in intact *L. uniflora*, and with CO_2 and PAR response characteristics of leaf sections (Robe and Griffiths 1988).

Materials and methods

Littorella uniflora growing in 0.25–0.4 m of water was collected from Esthwaite Water, Cumbria, U.K. (O.S. Grid Ref. SD 358969) in October 1986. Under common cloudy and breezy conditions PAR at the leaf tips was 100–200 μmol m^{-2} s^{-1} at midday, although the range of PAR measured was 20–740 μmol m^{-2} s^{-1}. Plants were transferred in blocks of their own sediment, 9 cm deep, into perspex tanks and maintained with lake water 2 cm above the leaf tips. Plants were grown on under two PAR regimes: 50 and 300 μmol m^{-2} s^{-1}, subsequently referred to as 'low PAR' and 'high PAR' respectively (temperature 19–20° C; natural and reverse 12/12 photoperiods). Light was provided by warm white fluorescent tubes (low PAR) and Wotan neutral white metal halide lamps (high PAR). Measurements were made during March–July 1987.

Sediment interstitial water sampling was carried out using *in situ* dialysis (Robe and Griffiths 1988). Determinations of O_2 and pH were carried out using a modified O_2 electrode chamber, and of total inorganic carbon and CO_2 using the headspace/IRGA method described in Robe and Griffiths (1988).

Light transmission through leaves of *L. uniflora* was measured by laying either leaves split down the centre or leaf epidermis over a quantum sensor, completely covering the surface, under illumination of 50 and 300 μmol m^{-2} s^{-1} (PAR).

For measurement of CO_2 and O_2 exchange low and high PAR grown *L. uniflora* of similar fresh weight were chosen. Plants were very carefully removed from the sediments so that roots and shoots were undamaged. Sediment was washed from the roots, and any epiphytes gently removed from the shoots. Plants were positioned in glass containers similar to those described in Raven et al. (1988) with shoots and roots in separate compartments (volume, 18–20 and 13–18 ml respectively) made water and gas tight with a seal of non toxic adhesive (Blu-Tack: Bostik Ltd., U.K.) covered with petroleum jelly. Shoot medium was filtered lake water buffered with 50 mol m^{-3} MOPS (pH adjusted to that of the lake water with NaOH). Root medium was filtered lake water buffered with 60 mol m^{-3} MES (pH adjusted with NaOH to that in sediment interstitial waters). The O_2 concentration was reduced to that of interstitial waters by sparging with nitrogen, followed by addition of an aliquot of HCO_3^- to bring the CO_2 concentration up to that in sediments. Root compartments were covered with aluminium foil. Control containers, without plants, were prepared in the same way. The proportion of control containers to those with plants was 3:1, 3:2, or 3:3.

Prior to gas exchange measurements plants were pre-incubated for 12 h. During this period the medium in the root containers was slowly circulated through a 500 ml reservoir, using a peristaltic pump, so that the CO_2 and O_2 concentrations surrounding the roots remained identical to those in the sediments. At the end of the pre-incubation period root and shoot containers were refilled with fresh media and the plants incubated for a further 7–9 h. Incubations were made at a temperature of 19–20° C and under a light intensity of 50 μmol m^{-2} s^{-1} (low PAR plants) and 300 μmol m^{-2} s^{-1} (high PAR plants), without stirring. Control containers were incubated under exactly the same conditions. At the end of the experimental period containers were gently inverted. Aliquots of 2.5 and 0.7 ml were taken from shoot and root media respectively, immediately placed into nitrogen-filled (CO_2-free), gas-tight, serum bottles and analysed for total inorganic carbon and CO_2 concentration using the headspace/IRGA method described in Robe and Griffiths (1988). An additional 10.0 ml was extracted into a glass syringe for O_2 and pH analysis, carried out using a modified O_2 electrode chamber as described in Robe and Griffiths (1988). There was no change in pH in plant or control containers during the incubations. Exchange of CO_2 and O_2 by plants was calculated as the difference between concentrations in control media and plant media. During the experimental incubations CO_2 concentration in the media round the plant roots was reduced by 11–13%. In the light, O_2 concentration in the shoot media increased by 40–50% and in the root media from 0.05 to 0.25 mol m^{-3}.

For determination of lacunal CO_2 and O_2 concentrations gas samples were collected during the 2 h before the end of dark and light periods. Plants were gently eased from the sediments taking care to keep shoots and roots undamaged. All healthy leaves were carefully removed from the plant. The ends previously attached to the stem, and the entire leaf surface, were immediately sealed with a thin coating of petroleum jelly and the leaves inserted into a short section of rubber tubing, which was flushed with helium and sealed. Lacunal gases were expelled by pressure sufficient to flatten the leaves. With half the tubing clamped, samples of 400 μl were collected in two gas-tight syringes reducing the pressure in the tubing to its original level. Control samples were extracted in the same way, from tubing without leaves.

Concentrations of CO_2 and O_2 were determined using a Shimadzu GC-8 APT gas chromatograph fitted with a thermal conductivity detector. For O_2 analysis samples of 200 μl were injected onto a column of molecular sieve (13 × 80–100 mesh) and for CO_2 analysis samples of 200 μl were injected into a column (12′ × 1/8″ OD) of 'Chromasorb' (102, 60/80 mesh). Both columns were fitted to the same instrument and maintained at 40° C with helium

as the carrier gas flowing at 15 cm^3 min^{-1}. Analysis was based on the integrated area under the peaks. Reference standards were 200 µl of outside air (0.04% CO_2; 21% O_2). The response of peak area was linear to injections of increasing volumes of air (up to 200 µl) and to injections of 200 µl of prepared concentrations of CO_2 and O_2 (up to 5% and 21% respectively). The GC was calibrated before and after measurements each day. Analysis of the control samples showed that the technique described above was completely successful in excluding atmospheric CO_2 (there was none present) and almost completely successful in excluding O_2. Correction was made for the O_2 in the controls when calculating the concentration in the plant samples, at ambient temperature. Lacunal volume was determined by displacement of water as described in Robe and Griffiths (1988).

For titratable acidity determination samples were collected at dawn and then at four hourly intervals until dusk, with analysis carried out on freeze-thawed material as described in Robe and Griffiths (1988). Malate and citrate were determined enzymatically as described by Hohorst (1965) and Möllering (1985) respectively.

CO_2 and PAR response curves were determined using the tissue slice method described in Robe and Griffiths (1988). The numbers of sections used to make up the 50 mg batches were: 37, low PAR plants; 51, high PAR plants.

Results

Physico-chemical conditions and plant morphology

Concentrations of CO_2 and O_2, and pH in bulk water and sediment interstitial water were almost identical for the two PAR regimes (Table 1), and little changed from those in the field (in preparation). Concentrations of CO_2 in sediments were much higher than in bulk waters. Sediments were also relatively anaerobic compared with bulk waters, which were slightly supersaturated with oxygen.

Plants under both PAR regimes were healthy and producing new leaves and ramets. High PAR plants were smaller with shorter leaves but proportionally more root growth (Table 2). Sections through leaves of high PAR plants were smaller in diameter, with reduced lacunal transverse section area (Table 2). Expressed per unit fresh weight (fwt), leaf internal lacunal surface area and volume were lower (149 compared with 194 cm^2 g^{-1} and 0.75 compared with 1.09 cm^3 g^{-1}: data not shown). Shoot and root fresh:dry weight ratio in high PAR plants (10.4 and 7.3 respectively) were also reduced compared to the low PAR regime (15.3 and 13.6 respectively: data not shown).

Leaves of high-PAR-grown plants contained 38% less chlorophyll *a* (Table 2), although chlorophyll *a*/*b* ratios were identical under both treatments (1.54: data not shown). Average light intensity within leaves, illuminated from one side, was only 26% of incident PAR (Table 3). Morphology of high PAR leaves allowed slightly greater light penetration when illuminated under 300 µmol m^{-2} s^{-1}. It should be noted that leaves growing in their natural orientation would intercept fewer photons per unit area (Osborne and Raven 1986), although they would be more uniformly illuminated from all sides.

Whole plant gas exchange

The direction of whole-plant CO_2 and O_2 exchange fluxes were similar under the two PAR regimes (Fig. 1). Rates of exchange were generally increased under high PAR (Fig. 1), particularly the rate of O_2 loss from shoots in the light. However, CO_2 fluxes over shoots were reduced.

Table 1. Physico-chemical conditions for *Littorella uniflora* grown under two PAR regimes

	Low PAR[a]			High PAR		
	CO_2	O_2	pH	CO_2	O_2	pH
	(mol m^{-3})			(mol m^{-3})		
Bulk water	0.06±0.17	0.31±0.03	7.00±0.13	0.04±0.02[c]	0.30±0.00[c]	7.38±0.02[c]
Sediment interstitial water	1.17±0.11	0.05±0.03	5.90±0.27	0.97±0.13[c]	0.07±0.02[b]	5.81±0.09[b]

Sediments were 9 cm deep. [a] mean ±SD 5 determinations; [b] mean ±SD 4 determinations; [c] mean ±SD 3 determinations

Table 2. Morphology of *Littorella uniflora* grown under two PAR regimes

Growth regime	Fresh weight (g)[a]			Leaf section[b] diameter (mm)	Lacunal[b] TS area (mm^2)	Chlorophyll *a*[c] (mg g^{-1} fwt)
	Shoots	Roots	Shoot/root ratio			
Low PAR	0.36±0.12	0.07±0.005	5.1	1.93±0.16	1.47±0.39	0.95±0.09
High PAR	0.20±0.05	0.11±0.03	1.8	1.58±0.16	0.74±0.22	0.59±0.05

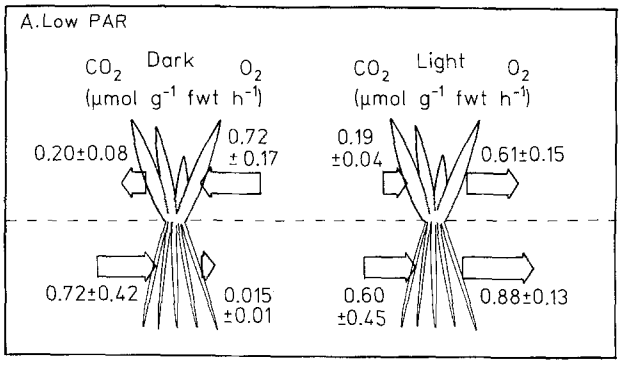
[a] mean ±SD for ten plants; [b] measurements were made on transverse sections of the middle portions of the two most recent mature leaves, values are mean ±SD of measurements made on five leaf sections; [c] mean ±SD for determinations on four batches of tissue sections

Table 3. Light transmission through epidermis and split leaves of *Littorella uniflora* grown under two PAR regimes. Leaves split down the centre or leaf epidermis only were laid over a quantum sensor completely covering the surface, under illumination of 50 and 300 μmol m^{-2} s^{-1} PAR. Values are mean ±SD of 5 determinations

Growth regime	Incident PAR (μmol m^{-2} s^{-1})					
	50			300		
	Leaf epidermis	Split leaf	Average internal PAR	Leaf epidermis	Split leaf	Average internal PAR
Low PAR	20.1±1.4	6.6±0.8	13	97.6±6.0	21.2±1.9	59
High PAR	23.6±1.5	8.9±1.7	16	126.0±8.2	33.2±2.7	80

CAM activity

Plants grown under the high PAR regime showed considerably greater CAM activity, as a result of a more prolonged and faster rate of acidification and deacidification (Fig. 2). Although dusk levels of titratable acidity were similar (26 and 27 μmol H^+ g^{-1} fwt), dawn minus dusk titratable acidity (ΔH^+) was almost three times greater under high PAR (117 compared with 42 μmol H^+ g^{-1} fwt for low PAR plants). The cell-sap of leaves from high PAR plants sampled at dawn contained similar pools of malate and citrate (Table 4). Malic and citric acids are known to fluctuate as part of CAM (see Borland and Griffiths 1989). However, at dusk only malate content was reduced to low levels (Table 4). A ΔMalic acid of 52.9 μmol g^{-1} fwt would require 105.8 μmol H^+ g^{-1} fwt, so accounting for virtually all the diurnal variation in titratable acidity in the leaves sampled (Table 4).



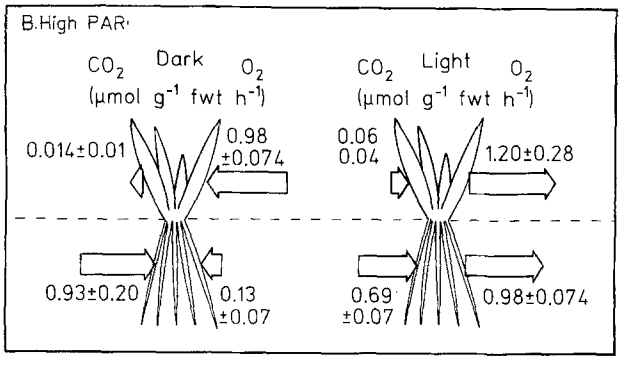


Fig. 1A, B. CO_2 and O_2 exchange over shoots and roots of *Littorella uniflora* expressed on a plant fwt basis. **A** Low PAR plants and **B** high PAR plants were incubated with shoots and roots in separate water- and gas-tight compartments with *in situ* CO_2 and O_2 concentrations and under 50 and 300 μmol m^{-2} s^{-1} PAR respectively. Values are mean ±SD for 6 plants

Lacunal CO_2 and O_2 concentrations

Despite the differences in morphology, gas exchange and CAM activity, $[CO_2]_i$ and $[O_2]_i$ were generally similar for plants under the two PAR regimes (Fig. 3). For low PAR plants dawn and dusk $[CO_2]_i$ were almost identical (0.96 and 1.03 mol m^{-3}). In high-PAR-grown plants $[CO_2]_i$ increased during the day from 0.42 to 0.86 mol m^{-3}. Expressed as a partial pressure and percentage, $[CO_2]_i$ ranged from 1.0 kPa, equivalent to 1.0% CO_2 in air (dawn, high PAR plants), to 2.3 kPa, equivalent to 2.3% (dusk, low PAR plants), or 25–57 times atmospheric CO_2 concentration.

$[O_2]_i$ increased by nearly 70% during the light period (Fig. 3). For low PAR plants $[O_2]_i$ increased from 2.9 to 4.3 mol m^{-3}, and for high PAR plants from 2.6 to 4.1 mol m^{-3}. Expressed as a partial pressure and percentage $[O_2]_i$ ranged from 6.9 and 6.4 kPa, equivalent

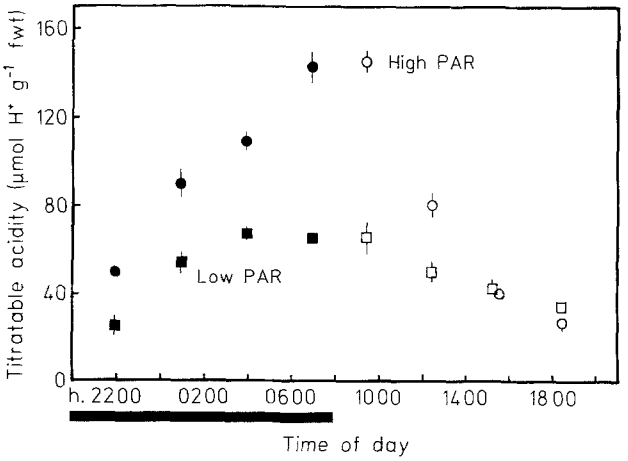


Fig. 2. Diurnal levels of cell-sap titratable acidity for *Littorella uniflora* grown under two PAR regimes. Values are mean ±SD of determinations on whole shoots of 3–6 plants

Table 4. Comparison of H^+, malate and citrate content of leaf cell-sap extract from *Littorella uniflora* grown under the high PAR regime. These results, obtained for the cell-sap of one plant at dawn and one plant at dusk are representative of those obtained for other plants

	Titratable acidity (μmol H^+ g^{-1} fwt)	Malate	Citrate
		(μmol g^{-1} fwt)	
Dawn	141.1	57.1	58.1
Dusk	29.5	4.2	50.4
Δ	111.6	52.9	7.7

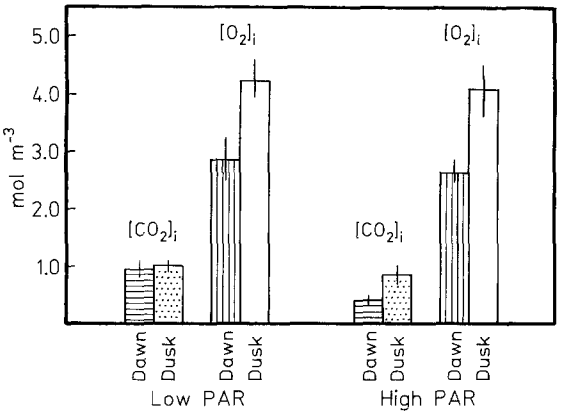


Fig. 3. Intercellular CO_2 and O_2 concentrations, $[CO_2]_i$ and $[O_2]_i$, for *Littorella uniflora* grown under two PAR regimes. Measurements were made during the 2 h before the end of dark (dawn) and light (dusk) periods, using all the healthy leaves from each plant sampled. Values are mean ±SD for 6 plants

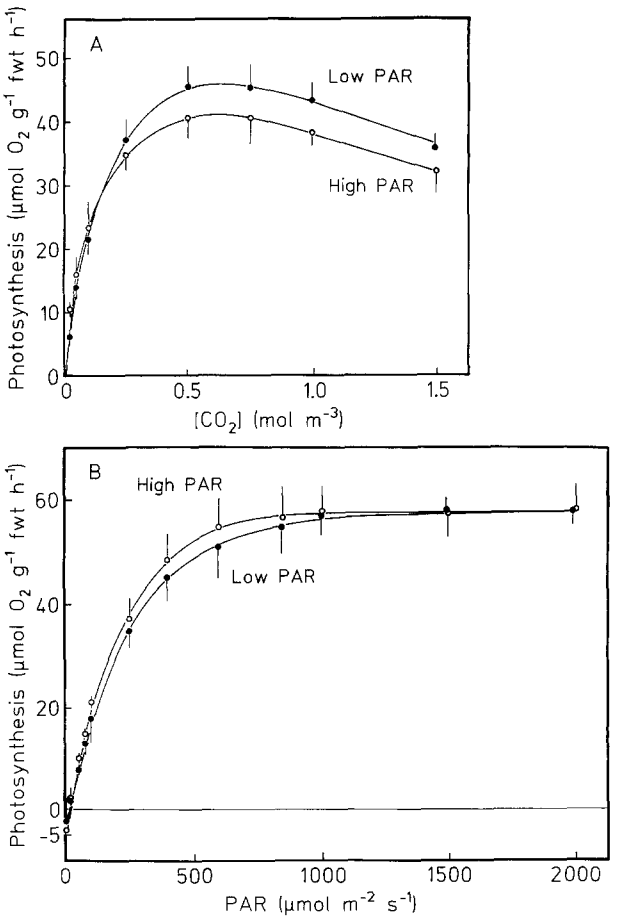


Fig. 4. Photosynthetic characteristics of *Littorella uniflora* grown under two PAR regimes determined as O_2 evolution. **A** CO_2 response, **B** PAR response. Values are mean ±SD for four 50-mg batches of 1-mm leaf sections

to 6.8 and 6.3% O_2 in air (dawn), to 10.3 and 10.0 kPa, equivalent to 10.2 and 9.9% (dusk). Dusk levels were only half atmospheric O_2 concentration (21 kPa; 21%).

CO_2 and PAR response curves

In vitro CO_2 and PAR response characteristics (determined as O_2 evolution using 1 mm leaf sections with rapid stirring) were also little affected by PAR regime (Fig. 4). The CO_2 concentration required to saturate photosynthesis was the same for both sets of plants; 0.5 mol m^{-3} (Fig. 4A). Photosynthetic capacity (at saturating CO_2 and PAR) expressed on a fresh weight or internal surface area basis was also virtually unchanged (45.5 μmol O_2 g^{-1} fwt h^{-1}, 0.53 μmol m^{-2} s^{-1} for low PAR plants compared with 40.7 μmol g^{-1} fwt h^{-1}, 0.58 μmol m^{-2} s^{-1} for high PAR plants). Although expressed as μmol O_2 mg^{-1} chlorophyll a h^{-1} maximum rates were greater in high PAR plants (66.8 compared with 47.2: data not shown) because of their lower chlorophyll content. Rate of dark respiration was 70% greater under high PAR (4.1 compared with 2.4 μmol g^{-1} fwt h^{-1}). However light compensation point (12 μmol m^{-2} s^{-1}) and saturation requirement (850–1000 μmol m^{-2} s^{-1}) were identical (Fig. 4B).

Discussion

The development under higher PAR of smaller leaves with a lower chlorophyll content (Table 2) is similar to the response of leaves of terrestrial plants (Bjorkman 1981; Boardman 1977) and has also been reported for *Lobelia dortmanna* (Farmer and Spence 1987; Szmeja 1987) and other submersed macrophytes (*Zostera* and *Potamogeton*: Dennison and Alberte 1982; Spence and Crystal 1970) growing in shallow as compared with deep water.

The direction of gas exchange fluxes (Fig. 1) were very similar to earlier findings for isoetids, the majority of CO_2 uptake being via the roots. At night 100% of CO_2 uptake occurred via the roots, although shoot uptake has been reported for *Isoetes lacustris* (Richardson et al. 1984). In the light 76% and 92% of exogenous CO_2 (low and high PAR plants respectively) was acquired from the root medium, similar to the 80–99% reported by Boston et al. (1987a, b). Roots were less important in O_2 exchange, although approximately 50% of O_2 lost went into the root medium, compared with an earlier figure of 28% for *Littorella uniflora* (Sand-Jensen et al. 1982).

Rates of gas exchange were at the low end of the range previously reported for isoetids. For example, for high PAR plants rate of dark CO_2 uptake by roots, equivalent to 8.2 μmol g^{-1} plant dry weight h^{-1}, compares with 30, 70 and 100 μmol g^{-1} plant dwt h^{-1} (Madsen 1987b). However, in Madsen's study *L. uniflora* was incubated with 0.7, 1.6 and 3.1 mol m^{-3} CO_2 in the root medium, after storage at a PAR of 500 μmol m^{-2} s^{-1} for 3–12 h in lake water prior to experimentation, which would have depleted lacunal CO_2 pools. The rate of O_2 loss from shoots in the light, equivalent to 10.7 μmol g^{-1} plant dwt h^{-1}, compares with 25 μmol g^{-1} plant dwt h^{-1} for *Eriocaulon decangulare* incubated with air-equilibrated water in the root medium, rather than water low in oxygen (Raven et al. 1988). However rate of daytime O_2 evolution via roots, equivalent to 8.6 μmol g^{-1} plant dwt h^{-1}, was only slightly lower than previous measurements for *L. uniflora* incubated under conditions more similar to those used in the present study; with roots in O_2-free water containing 2 mol m^{-3} CO_2, but PAR of 550 μmol m^{-2} s^{-1} (15.6 μmol O_2 g^{-1} plant dwt h^{-1}: Sand-Jensen et al. 1982). Our incubation of plants under near-natural conditions of CO_2, O_2 and PAR supply immediately after removal from the sediments in which they had been growing would have caused minimal disruption. The comparatively low rates of exchange we measured should therefore resemble those of rooted plants, and indeed rates of net carbon incorporation agree well with those found in the field (see below).

Carbon and oxygen balance over a 24-h period is shown in Table 5. For low and high PAR plants, 39% and 55% (respectively) of CO_2 uptake was acquired at night, which agrees with Boston and Adams' (1986) estimate of the contribution of CAM to annual carbon gain (40–50%). Over light and dark periods CO_2 uptake exceeded O_2 efflux, although in the light O_2 loss was greater than CO_2 uptake. To explain this discrepancy, CAM activity and natural recycling of CO_2 and O_2 within the lacunae (Griffiths 1988; Sand-Jensen and Prahl 1982; Søndergaard 1979; Søndergaard and Sand-Jensen 1979b; Sorrell and Dromgoole 1986) need to be taken into account. Gross photosynthetic evolution (net plus dark respiration: 26 and 39 μmol g^{-1} fwt for low and high PAR plants respectively) and gross CO_2 fixation (net plus CO_2 regenerated from dark fixation: 27 and 46 μmol g^{-1}) agree almost exactly for low PAR plants; the excess CO_2 fixation over O_2 evolution for high PAR plants may reflect greater lacunal storage.

Expressed as carbon incorporation per unit dry weight, CO_2 uptake over 24 h was equivalent to 3.4 μgC mg^{-1} dwt day^{-1} (low PAR plants) and 2.2 μgC mg^{-1} dwt day^{-1} (high PAR plants). These figures compare very well with those for leaves of *L. uniflora* at Esthwaite Water, Cumbria. Seasonal rates of net carbon incorporation (μg C mg^{-1} dwt day^{-1}) were: May 0.8; June 4.4; July 3.9; August 3.1; September 2.2; November 1.4, although these rates would be higher if root growth were taken into account Robe and Griffiths, in preparation.

The diurnal change in titratable acidity (Fig. 2) was similar to that found in earlier studies with *L. uniflora* and *Isoetes* spp. (Boston and Adams 1985; Keeley and Busch 1984; Madsen 1985). When compared with ΔH^+ under 850 μmol m^{-2} s^{-1} (140–160 μmol g^{-1} fwt: W.E. Robe and H. Griffiths, unpublished data), the dependence of overnight acidification on PAR corresponds with that found in terrestrial CAM plants (Nobel 1982, Fig. 1; Nobel and Hartsock 1983) and would explain the seasonal variations in CAM activity of isoetids in the field (Boston and Adams 1985; Keeley and Busch 1984; Robe and Griffiths, in preparation).

Malic acid appeared to be the only acid participating in the diurnal cycle (Table 4), as also indicated by earler studies (Farmer et al. 1986; Keeley and Busch 1984; Keeley et al. 1981; Groenhof et al. 1988). Therefore, using the stoichiometry $2H^+$:1mal:1CO_2 (see Griffiths 1988), 21 and 58 μmol CO_2 g^{-1} (shoot) fwt was fixed at night and released during day within the leaves of low- and high-PAR-grown plants respectively.

However, net CO_2 uptake (ΔCO_2: Table 6) was less than dark CO_2 fixation (see above). The difference ($0.5 \times \Delta H^+ - \Delta CO_2$), 12.6 and 41.6 μmol g^{-1} shoot fwt, may be accounted for by fixation of respiratory CO_2 as in terrestrial CAM plants (Griffiths 1988). We note that dark respiration both *in vivo* and *in vitro* (Figs. 1, 4B) was almost twice the rate in high PAR compared with low PAR plants. Expressed as a percentage, respiratory CO_2 fixation comprised 60% and 71% of total dark CO_2 fixation for low and high PAR plants respectively, at the upper end of the wide range previously determined

Table 5. 24-h carbon and oxygen balance sheet for *Littorella uniflora* using the results in Fig. 1. (+) indicates uptake by shoots or roots and (−) loss from shoots or roots.

		Low PAR		High PAR	
		CO_2	O_2	CO_2	O_2
		(μmol g^{-1} plant fwt)		(μmol g^{-1} plant fwt)	
12 h Light	Net exchange	+ 9.5	−17.8	+ 9.0	−26.2
12 h Dark	Net exchange	+ 6.2	+ 8.6	+11.0	+13.3
24 h	Total	+15.7	− 9.2	+20.0	−12.9

Table 6. ΔH^+, ΔCO_2 and recycling of respiratory CO_2 in *Littorella uniflora* grown under two PAR regimes

Growth regime	ΔH^+ (μmol H^+ g^{-1} fwt)	ΔCO_2 (μmol CO_2 g^{-1} fwt)	% Recycling	Absolute recycling (δH^+)
Low PAR	42	+ 8.4	60	26
High PAR	117	+16.9	71	83

ΔH^+ is the difference between dawn and dusk titratable acidity. ΔCO_2 is net dark CO_2 uptake expressed on a shoot fwt basis, % recycling is calculated as $\frac{(0.5 \times \Delta H^+) - \Delta CO_2}{0.5 \times \Delta H^+} \times 100$ and absolute recycling (δH^+) is $\Delta H^+ - (2 \times \Delta CO_2)$, assuming stoichiometry of $2H^+$: 1 malate: 1CO_2

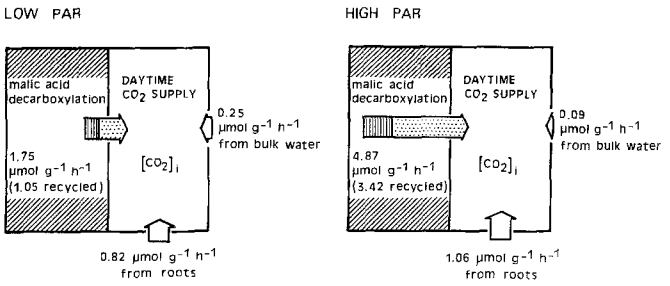


Fig. 5A, B. Potential components of daytime CO_2 supply and $[CO_2]_i$ in low- and high-PAR-grown *Littorella uniflora*. Rates of influx are expressed in μmol CO_2 g^{-1} (shoot fwt) h^{-1}, using the data in Fig. 1. (▥) exogenous CO_2 fixed at night, (▤) respiratory CO_2 fixed at night

for isoetids by the more direct method of measuring overnight acid accumulation for leaves incubated in CO_2-free water (30–99%: Keeley and Busch 1984; Madsen 1987b; Smith et al. 1985).

Lacunal CO_2 concentrations, $[CO_2]_i$, (0.42–1.03 mol m^{-3}: Fig. 3) were higher than recorded in a previous studie with *L. uniflora* (Madsen 1987a). In Madsen's study $[CO_2]_i$ in detached leaves declined from 0.45 to approximately 0.03 mol m^{-3} during a 10-h light incubation following 12 h storage in lake water. The $[CO_2]_i$ values for both low and high PAR grown plants in our study were close to or above the concentration required to saturate carboxylation *in vitro* (0.5 mol m^{-3}: Fig. 4A) as also found for *L. uniflora* grown with a range of sediment CO_2 concentrations (Robe and Griffiths 1988). With PAR within the leaves (Table 3) being much lower than required for saturation of photosynthesis (850–1000 μmol m^{-2} s^{-1}: Fig. 4B), light rather than CO_2 supply seems to be the major factor limiting rates of carbon fixation.

In Fig. 5 we have compared exogenous CO_2 uptake and CO_2 regenerated from dark fixation as components of CO_2 supply for photosynthesis and also of $[CO_2]_i$. We have not included photorespiratory CO_2 (see below) or any daytime respiratory CO_2 component. CO_2 regenerated from CAM formed 62% of daytime CO_2 supply under low PAR and 81% under high PAR; the additional dark fixation in high-PAR-grown plants being predominantly of respiratory CO_2 (see also Table 6). The relatively small amount of CO_2 uptake as compared to respiratory CO_2 fixation may be a factor in the slow growth of these plants.

Values of $[O_2]_i$ (5–10% in air: Fig. 3) were low compared with previous measurements; 23–30% O_2 in air for *Lobelia dortmanna* (but with roots in air equilibrated water), *Elodea* and *Myrophyllum* (but with a lacunal system which does not extend to the roots, or a smaller root system: Hartman and Brown 1967; Sand-Jensen and Prahl 1982; Westlake 1978), and up to 40% for terrestrial CAM plants with stomata closed (Spalding et al. 1979). For *L. uniflora* it seems that loss of O_2 from relatively permeable leaves, and also via the lacunal system into roots and then into anaerobic sediments, could account for the low $[O_2]_i$ values.

With high $[CO_2]_i$ and low $[O_2]_i$ (e.g. 0.86 and 4.1 mol m^{-3} respectively for PAR plants: Fig. 3) photorespiration should be suppressed. There is no data on the kinetic parameters of RUBISCO in *L. uniflora*. However using the equation of Segal (1975) and with a K_m (O_2) of 535 mmol m^{-3} and a K_m (CO_2) of 18 mmol m^{-3} (at 15° C: MacFarlane and Raven 1989; Smith and Walker 1980) inhibition would be 14%; or with a lower value of 4.38 mmol m^{-3} for K_m (CO_2), 3.75%. The previous estimate of high levels of photorespiration in *L. uniflora* may have been due to the release of CO_2 from malic acid in the light (see Søndergaard 1979).

Since $[CO_2]_i$ and $[O_2]_i$ are known it is possible to compare the rates of CO_2 and O_2 exchange with the concentration gradients between lacunae and external medium. For example for low PAR plants incubated in the light the difference in CO_2 concentration is (0.04 to 0.21 mol m^{-3}). Applying Fick's equation for diffusion, with a boundary layer one-eighth the width of the container and with a root fresh weight to surface area relationship of 1 g = 78.2 cm^2 (15 roots), gives a rate of influx of 0.14 to 0.76 μmol CO_2 g^{-1} plant fwt h^{-1} compared with the measured rate of 0.60 μmol g^{-1} fwt h^{-1}. However the diffusion resistance (permeability) of roots would also need to be taken into account (see Madsen 1987b; Sand-Jensen and Prahl 1982). CO_2 loss from shoots at night and O_2 efflux from roots also takes place down a concentration gradient. Both CO_2 uptake in light by shoots (also found by Boston et al. 1987a, b; Madsen 1987a) and O_2 efflux by shoots, were 'uphill'. This may simply be due to concentration gradients across the two or three cells between lacunae and external medium. However the possibility of some interaction between an active inorganic carbon uptake mechanism and leaf permeability needs further investigation (cf. Madsen 1987a).

To summarise, acclimation to growth under higher PAR involved a reduction in plant size and in leaf chlorophyll content, but almost no change in *in vitro* CO_2 and PAR response characteristics. We found a small increase in rate of whole-plant photosynthesis (measured as O_2 evolution), with no accompanying increase in net daytime CO_2 uptake, but three-fold greater dark fixation mainly due to increased respiratory CO_2 fixation. With $[CO_2]_i$ maintained close to or above the concentration required to saturate carboxylation, but a low internal PAR compared with the light saturation requirement, it seems that PAR rather than CO_2 supply was the major factor limiting carbon fixation. CAM appears to have an important role in the regulation of CO_2 supply for photosynthesis in response to variation in light regime.

It is difficult to simulate exactly *in situ* CO_2 and O_2 concentrations in the gas exchange experiments. Changes in concentration occur during incubations and although use of a flow-through system with stirring would avoid this, fluxes would be overestimates since plants were grown under unstirred conditions. However, this is the first integrated study which has attempted to investigate interactions between C_3 and CAM photosynthesis in an isoetid. We now need to study performance of these plants under the higher light intensity

and fluctuating PAR found under natural conditions, where CAM activity may also be modulated by CO_2 and nitrogen supply (see Borland and Griffiths 1989; Robe and Griffiths 1988).

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References

Bjorkman O (1981) Responses to different quantum flux densities. In Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology I. (Encyclopedia of plant physiology, NS, vol 12A). Springer-Verlag, Berlin Heidelberg New York, pp 57–107

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Ann Rev Plant Physiol 28:355–377

Borland AM, Griffiths H (1989) The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. J Exp Bot 40 (210):53–60

Boston HL, Adams MS (1985) Seasonal diurnal acid rhythms in two aquatic crassulacean acid metabolism plants. Oecologia 65:573–579

Boston HL, Adams MS (1986) The contribution of crassulacean acid metabolism to the annual productivity of two aquatic vascular plants. Oecologia 68:615–622

Boston HL, Adams MS, Pienkowski TP (1987a) Utilization of sediment CO_2 by selected North American isoetids. Ann Bot 60:485–494

Boston HL, Adams MS, Pienkowski TP (1987b) Model of the use of root-zone CO_2 by selected North American isoetids. Ann Bot 60:495–503

Clapham AR, Tutin TG, Warburg GF (1981) Excursion flora of the British Isles. Cambridge University Press

Dennison WC, Alberte RS (1982) Photosynthetic responses of *Zostera marina* L. (Eelgrass) to *in situ* manipulations of light intensity. Oecologia 55:137–144

Den Hartog G, Segal C (1964) A new classification of the water-plant communities. Act Bot Neerl 13:367–393

Farmer AM (1987) Photosynthetic adaptation in *Lobelia dortmanna* and other isoetids. Photosynthetica 21(1):51–55

Farmer AM, Spence DHN (1987) Environmental control of the seasonal growth of the the submersed aquatic macrophyte *Lobelia dortmanna* New Phytol 106:289–299

Farmer AM, Maberley SC, Bowes G (1986) Activities of carboxylation enzymes in freshwater macrophytes. J Exp Bot 37 (183):1568–1573

Griffiths H (1988) Crassulacean acid metabolism. A re-appraisal of physiological plasticity in form and function. Adv Bot Res 15:347–50

Groenhof AC, Smirnoff N, Bryant JA (1988) Enzymic activities associated with the ability of aerial and submerged forms of *Littorella uniflora* (L.) Aschers to perform CAM. J Exp Bot 39 (200):353–361

Hartman RT, Brown DL (1967) Changes in internal atmosphere of submersed vascular hydrophytes in relation to photosynthesis. Ecology 48(2):252–258

Hohorst HJ (1965) L-(−)-Malate. Determination with malic dehydrogenase and DNP. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York London, pp 328–332

Keeley JE, Busch G (1984) Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. Plant Physiol 76:525–530

Keeley J, Morton B, Babcock B, Castillo P, Fish B, Jerauld E, Johnson B, Landre 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

MacFarlane JJ, Raven JA (1989) Quantitative determination of the unstirred layer permeability and kinetic parameters of RUBISCO in *Lemanea mamillosa*. J Exp Bot 40 (212):321–327

Madsen TV (1985) A community of submerged aquatic CAM plants in Lake Kalgaard, Denmark. Aquat Bot 23:97–108

Madsen TV (1987a) 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 Phytol 106:35–50

Madsen TV (1987b) Sources of inorganic carbon acquired through CAM in *Littorella uniflora* (L.) Aschers. J Exp Bot 38:367–377

Madsen TV (1987c) The effect of different growth conditions on dark and light carbon assimilation in *Littorella uniflora*. Physiol Plant 70:183–188

Möllering H (1985) Citrate. Determination with citrate lyase, MDH and LDH In: Bergmeyer HU (ed) Methods in enzymatic analysis, 3rd edition, vol VII. Academic Press, New York London, pp 2–12

Nobel PS (1982) Interaction between morphology, PAR interception, and nocturnal acid accumulation in cacti. In: Ting IP, Gibbs M (eds) Crassulacean acid metabolism, American Society of Plant Physiologists, Baltimore Maryland USA, pp 260–277

Nobel PS, Hartsock TL (1983) Relationship between photosynthetically active radiation, nocturnal acid accumulation, and CO_2 uptake for a crassulacean acid metabolism plant, *Opuntia ficus-indica*. Plant Physiol 71:71–75

Osborne BA, Raven JA (1986) Light absorption by plants and its implications for photosynthesis. Biol Rev 61:1–61

Osmond CB (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. Biochim Biophys Acta 639:77–98

Raven JA, Handley LL, Macfarlane JJ, McInroy S, McKenzie L, Richards JH, Samuelsson G (1988) The role of CO_2 uptake by roots and CAM in acqusition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. New Phytol 108:125–148

Richardson K, Griffiths H, Reed ML, Raven JA, Griffiths NA (1984) Inorganic carbon assimilation in the isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L. Oecologia 61:115–121

Robe WE, Griffiths H (1988) C_3 and CAM photosynthetic characteristics of the submerged aquatic macrophyte *Littorella uniflora*: Regulation of leaf internal CO_2 supply in response to variation in rooting substrate inorganic carbon concentration J Exp Bot 39:1397–1410

Roelofs JGM, Schuurkes JAAR, Smits AJM (1984) Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. Aquat Bot 18:389–411

Sand-Jensen K (1978) Metabolic adaptation and vertical zonation of *Littorella uniflora* (L.) Aschers. and *Isoetes lacustris* L. Aquat Bot 4:1–10

Sand-Jensen K, Prahl C (1982) Oxygen exchange with the lacunae and across leaves and roots of the submerged macrophyte, *Lobelia dortmanna* L. New Phytol 91:103–120

Sand-Jensen K, Prahl C, Stokholm H (1982) Oxygen release from roots of submerged aquatic macrophytes. Oikos 38:349–354

Segal IH (1975) Enzyme Kinetics. John Wiley & Sons, New York

Smith FA, Walker NA (1980) Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotope discrimination. New Phytol 86:245–259

Smith WG, Boston HL, Adams MS (1985) A preliminary study of the source and fate of carbon acquired via CAM in *Littorella uniflora* var. americana (Fern.) G1. J Freshwater Ecol 3(2):203–209

Søndergaard M (1979) Light and dark respiration and the effect of the lacunal system on refixation of CO_2 in submerged aquatic plants. Aquat Bot 6:269–283

Søndergaard M, Sand-Jensen K (1979a) Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. Aquat Bot 6:1–12

Søndergaard M, Sand-Jensen K (1979b) The delay in ^{14}C fixation

rates by three submerged macrophytes. A source of error in the ^{14}C technique. Aquat Bot 6:111–119

Sorrell BK, Dromgoole FI (1986) Errors in measurements of aquatic macrophyte gas exchange due to oxygen storage in internal airspaces. Aquat Bot 24:103–114

Spalding MH, Schmitt MR, Ku JB, Edwards GE (1979) Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2 concentrations in *Sedum praealtum* DC. Plant Physiol 63:738–743

Spence DHN (1967) Factors controlling the distribution of freshwater macrophytes with particular reference to the lochs of Scotland. J Ecol 55:147–170

Spence DHN, Crystal J (1970) Photosynthesis and zonation of freshwater macrophytes. I. Depth distribution and shade tolerance. New Phytol 69:205–215

Szmeja J (1987) The ecology of *Lobelia dortmanna* L. III. The plasticity of individuals along a gradient of increasing depth in oligotrophic lakes. Ekol Polska 35 (3–4):545–558

Westlake DF (1978) Rapid exchange of oxygen between plant and water. Verh Int Ver theor ang Limnol 20:2363–2367