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THE USE OF STABLE ISOTOPES IN THE STUDY OF PHOTOSYNTHESIS IN FRESHWATER PLANTS

JON E. KEELEY[1], LEONEL O. STERNBERG[2] and MICHAEL J. DENIRO[3]

[1] *Department of Biology, Occidental College, Los Angeles, CA 90041 (U.S.A.)*
[2] *Department of Biology, University of Miami, Coral Gables, FL 33124 (U.S.A.)*
[3] *Department of Earth and Space Sciences, University of California, Los Angeles, CA 90024 (U.S.A.)*

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ABSTRACT

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The ratio of $^{13}C/^{12}C$ for photosynthetic tissues of 22 aquatic species was unrelated to photosynthetic pathway. In three aquatic environments CAM and non-CAM species were shown to have similar $\delta^{13}C$ values. Although these CAM species derive up to half of their net carbon gain through dark fixation their $\delta^{13}C$ values are similar to associated non-CAM species in part because the carbon source for dark CO_2 uptake is CO_2 released, through respiration or decomposition, from organic carbon. Thus, the carbon source for CAM reflects previous isotope discrimination events. As carbon isotopes are not able to distinguish the photosynthetic pathway, there is good evidence that they may prove invaluable in the study of diffusional resistances to photosynthesis. Such evaluations require careful analysis of the photosynthetic pathway, carbon species utilized and $\delta^{13}C$ value of the source carbon. Although stable carbon isotope values do not allow differentiation between CAM and non-CAM aquatic species, there is evidence that hydrogen isotopes may be able to distinguish these two groups. Aquatic CAM species were shown to accumulate greater levels of deuterium than associated non-CAM species.

INTRODUCTION

In recent years there has been a noticeable increase in attention paid to photosynthetic pathways in submerged aquatic macrophytes. Work in this field has shown that the aquatic milieu has selected for a number of surprising photosynthetic characteristics. One example is the discovery of Crassulacean Acid Metabolism (CAM), a photosynthetic pathway once thought to be restricted to xerophytes, in submerged aquatic species of *Isoetes* (Keeley, 1981) and several other aquatic macrophytes (Keeley and Morton, 1982). Another noteworthy find is that by Bowes and co-workers of the unusual combination of C_3 and C_4 carboxylation reactions occurring within the same cells in leaves of *Hydrilla verticillata* (L.f.) Royle (Holaday and Bowes, 1980; Bowes and Salvucci, 1984).

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In terrestrial plant photosynthesis studies the stable isotopes of carbon and hydrogen have been shown to be useful indicators of the photosynthetic pathway. C_3 species are readily distinguished from C_4 species by the $\delta^{13}C$ ratio; it is typically in the range of $-28^0/_{00}$ or lower for the former group and -12 to $-14^0/_{00}$ for C_4 species. This technique, however, is not always capable of distinguishing Crassulacean Acid Metabolism in terrestrial plants. Species which obtain the bulk of their carbon by uptake and fixation at night have $\delta^{13}C$ ratios similar to C_4 plants. However, many species with a well developed CAM pathway will couple dark CO_2 uptake with CO_2 uptake in the light or, in some seasons, rely totally on light uptake and thus CAM plant $\delta^{13}C$ ratios will span the entire range from -12 to $-30^0/_{00}$ (Teeri, 1982).

Hydrogen isotopes have shown some promise for distinguishing CAM from non-CAM species due to the fact that CAM plants accumulate greater levels of deuterium than associated non-CAM species. This approach appears to work in both terrestrial and aquatic environments (Ziegler et al., 1976; Sternberg and DeNiro, 1983; Sternberg et al., 1984).

In this study, we explore the relationship of photosynthetic pathway, in particular the CAM and non-CAM modes, and the stable carbon and hydrogen isotope ratios of selected submerged aquatic macrophytes.

METHODS

All plants were collected, or maintained in cultivation, under submerged conditions. Field collections from various parts of California (U.S.A.) were made during the spring from two seasonal pools, Mesa de Colorado Pool (610 m), Riverside Co. and Mather Pool (1375 m), Tuolumne Co., and three lakes, Siesta Lake (2440 m), Tuolumne Co., Birch Lake (1375 m) Tuolumne Co. and Searsville Lake (110 m), San Mateo Co.

Plants were tested for the presence of CAM by measuring the titratable acidity to pH 6.4 and malic acid content of photosynthetic tissues at 0.600—07.00 and 17.00—18.00 h. The techniques are as described in Keeley and Busch (1984).

The carbon isotope ($^{13}C/^{12}C$) ratios and hydrogen isotope (D/H) ratios were determined on plant and water samples as described by Sternberg et al. (1984). These ratios are expressed as $\delta(^0/_{00})$ = [(isotope ratio of sample/isotope ratio of standard) - 1] $\times$ 1000 relative to the common standards for these isotopes (see Sternberg et al., 1984).

The initial carboxylation products were determined for selected species with ^{14}C tracer. Leaves were incubated in 10 mM morpholino-ethane sulphonic acid-NaOH (pH 5.5) with 1 mM $NaH^{14}CO_3$ (25 μCi.l) with 1000 μmol m^{-2} s^{-1} photosynthetic photon flux density. After brief exposure tissues were killed in boiling methanol, homogenized and centrifuged. After drying, samples were resuspended in water, and products were separated with two-dimensional thin layer electrophoresis and chromatography followed by autoradiography as described in Schurmann (1969). Ribulose-1,5-bi-

phosphate (RuBP) carboxylase and phosphoenolpyruvate (PEP) carboxylase activities were assayed as described by Lorimer et al. (1976) and Waygood et al. (1969), respectively, and replicated 3 times.

RESULTS AND DISCUSSION

Across the spectrum of plant species tested only a few showed any evidence of CAM activity (Table I). The *Isoetes* species had high CAM activity as is true of all aquatic species in that genus (Keeley, 1982; unpublished data, 1983, 1984, 1985). *Littorella uniflora* (L.) Aschers. is also a CAM plant and this has been confirmed by Boston and Adams (1985), Aulio

TABLE I

Evidence of Crassulacean Acid Metabolism in selected aquatic plants

		Overnight increase (per g fresh weight)	
		μmol H^+ $\overline{X} \pm$ SD	μmol malic acid $\overline{X} \pm$ SD (*N*)
Chlorophyta			
Chara contraria Braun ex Kützing	(Characeae)	0 ± 0	1 ± 2 (2)
Chara sp.	(Characeae)	0 ± 0	0 ± 0 (2)
Spirogyra sp.	(Zygnemaceae)	0 ± 0	3 ± 2 (2)
Bryophyta			
Amblystegium riparium (Hedw.) BSG	(Hypnaceae)	0 ± 0	3 ± 1 (2)
Fontinalis antipyretica Hedw.	(Fontinalaceae)	0 ± 2	4 ± 4 (2)
Tracheophyta			
Lycopsida:			
Isoetes bolanderi Engelmann	(Isoetaceae)	206 ± 21	93 ± 11 (4)
I. howellii Engelmann	(Isoetaceae)	245 ± 9	109 ± 4 (3)
I. orcuttii A.A. Eaton	(Isoetaceae)	152 ± 5	70 ± 7 (2)
Spermopsida — Monocotyledoneae:			
Eleocharis acicularis (L.) R. & S.	(Cyperaceae)	6 ± 3	1 ± 2 (6)
E. macrostachya Britton in Small	(Cyperaceae)	0 ± 0	0 ± 0 (2)
Elodea canadensis Michx	(Hydrocharitaceae)	1 ± 3	7 ± 3 (2)
Potamogeton illinoensis Morong	(Potamogetonaceae)	0 ± 0	3 ± 2 (2)
P. pectinatus L.	(Potamogetonaceae)	0 ± 0	6 ± 4 (2)
Sagittaria cuneata Sheldon	(Alismaceae)	7 ± 1	5 ± 3 (2)
Spermopsida — Dicotyledoneae:			
Callitriche longipedunculata Morong	(Callitrichaceae)	1 ± 1	1 ± 1 (2)
Ceratophyllum demersum L.	(Ceratophyllaceae)	0 ± 1	−7 ± 3 (2)
Littorella uniflora (L.) Aschers.	(Plantaginaceae)	93 ± 11	45 ± 7 (4)
Lythrum hyssopifolium L.	(Lythraceae)	0 ± 0	0 ± 0 (2)
Mentha arvensis L.	(Lamiaceae)	0 ± 0	0 ± 0 (2)
Myriophyllum brasiliense Cambess.	(Haloragaceae)	0 ± 0	4 ± 2 (2)
Plagiobotrys undulatus (Piper) Jtn	(Boraginaceae)	0 ± 0	0 ± 0 (2)
Ranunculus aquatilis L.	(Ranunculaceae)	3 ± 4	6 ± 3 (2)
R. flammula L.	(Ranunculaceae)	0 ± 0	1 ± 1 (2)
Veronica comosa Richt.	(Scrophulariaceae)	0 ± 0	0 ± 0 (2)

(1985) and Farmer and Spence (1985). The only other known example of an aquatic species with overnight acid accumulation of the order of magnitude observed for these species is for *Crassula aquatica* (L.) Schönl. and other aquatic species of that genus (Keeley and Morton, 1982; J.E. Keeley, unpublished data, 1982, 1983, 1984, 1985). Evidence of CAM activity at much reduced levels, however, is known from several other submerged aquatic species, e.g. *Hydrilla verticillata* (Holaday and Bowes, 1980) and *Scirpus subterminalis* Torrey (Beer and Wetzel, 1981).

Previous studies have shown that in *Isoetes* species and *Crassula aquatica*, CAM activity is closely associated with the aquatic milieu and is lost under aerial conditions (Keeley and Morton, 1982; Keeley et al., 1983a). Aulio (1985) found this to be true for *Littorella uniflora*, but Farmer and Spence (1985) reported that terrestrial populations of this species retained CAM. Preliminary experiments suggest that the loss of CAM is cued by changes in leaf water potential as the leaves dry in the atmosphere (J.E. Keeley, unpublished data, 1985). Consequently, *Isoetes howellii* Engelm. will maintain high CAM activity out of water if maintained in an atmosphere > 95% relative humidity. The discrepancy between Aulio's and Farmer and Spence's findings may be tied to the fact that the latter investigators maintained their terrestrial population under conditions of very high humidity (A. Farmer, personal communication, 1985).

The photosynthetic characteristics of the non-CAM species shown in Table I have not been studied for all species, although some information is available. Based on ^{14}C incorporation studies it has been shown that some, e.g. *Potamogeton pectinatus* L. (Winter, 1978) and *Myriophyllum brasiliense* Cambess (Salvucci and Bowes, 1982, 1983), rely primarily on C_3 type fixation. A careful analysis of stable carbon isotope ratios by Osmond et al. (1981) strongly supports the classification of *Fontinalis antipyretica* Hedw. as a C_3 plant. None of the species shown in Table I have Krantz anatomy that would suggest C_4 photosynthesis (Hough and Wetzel, 1977; J.E. Keeley, unpublished data, 1982, 1984, 1985). However, Bowes and Salvucci (1984) have shown that Krantz anatomy is not a prerequisite for the C_4 metabolic pathway of photosynthesis. They demonstrated that a large portion of the initial carbon fixation products of *Hydrilla verticillata* were organic acids and these turned over rapidly as the label moved into products of the C_3 pathway. *Eleocharis acicularis* (L.) R.&S. seems to fit the *Hydrilla* pattern in that the initial products of CO_2 fixation in the light are about equally divided between phosphoglycerate and the organic acids malate plus aspartate (Morton, 1984). Studies of *Elodea canadensis* Michx suggest a similar C_4 pathway in certain populations of that species (DeGroote and Kennedy, 1977), but not in other populations (see below).

$\delta^{13}C$ ratios for a range of aquatic species collected during the spring and summer of 1983 from two seasonal pools (Mesa de Colorado and Mather) and three lakes in California are shown in Table II. Three of these habitats had CAM and non-CAM species and in all three cases there was no obvious difference in the $\delta^{13}C$ value between these groups.

TABLE II

$\delta^{13}C_{PDB}$ values of total organic matter for selected plant species from seasonal pools or permanent lakes in California and summary of water characteristics during the growing season

	Mesa de Colorado		Mather	Siesta	Birch		Searsville
	April	May			June	August	
Chlorophyta							
Chara contraria	−15.8	−25.1					
Chara sp.				−27.1		−30.6	
Bryophyta							
Amblystegium riparium					−34.7	−34.3	
Fontinalis antipyretica				−26.9			
Tracheophyta							
Lycopsida:							
Isoetes bolanderi				−25.1			
L. howellii	−29.1	−28.4	−26.2				
L. orcuttii	−24.0	−27.6					
Spermopsida — Monocotyledoneae:							
Eleocharis acicularis	−25.0	−28.9	−30.9				
E. macrostachya		−28.6					
Elodea canadensis					−19.1	−18.2	
Potamogeton illinoensis							−25.3
P. pectinatus							−22.1
Sagittaria cuneata				−22.7			
Spermopsida — Dicotyledoneae:							
Callitriche longipedunculata				−27.1			
Ceratophyllum demersum							−29.8
Lythrum hyssopifolium		−30.7					
Mentha arvensis			−25.3				
Myriophyllum brasiliense							−28.4
Plagiobotrys undulatus		−27.4					
Ranunculus aquatilis	−14.5	−20.7	−24.0				
R. flammula			−27.7				
Veronica comosa			−26.4				
$\delta^{13}C$ of inorganic carbon (‰)							
AM		−20.4					
PM	—	−21.2	−16.3	−11.5	—	−12.9	−11.5
Water characteristics*							
Total carbon (mol m^{-3})	0.5—0.8		1.0—1.7	0.2—0.3	1.4—1.5		4.6—5.1
pH AM	6.6—7.0		6.6	6.3	7.0		7.5
PM	8.5—10.10		7.6	6.7	7.4		7.8
Temperature (C) Min	10		15	15	15		20
Max	30		28	28	25		30

*From Keeley and Morton (1982), Keeley et al. (1983a, b), Keeley and Busch (1984) and J.E. Keeley (unpublished data, 1984, 1985).

One interesting observation is that the $\delta^{13}C$ value of the water inorganic carbon was markedly more negative in the two seasonal pools than in the lakes. In addition, comparison of these numbers with values from earlier studies at Mesa de Colorado suggests that the $\delta^{13}C$ value of the water becomes progressively more negative from early- to late-spring; $\delta^{13}C$ water (‰) = - 16.5, - 18.5 and - 20.3, respectively, for 4 April 1981, 3 May 1981 (Keeley and Busch, 1984) and 25 May 1983 (Table II). The very negative $\delta^{13}C$ values for the Mesa de Colorado pool, and to a lesser extent the Mather pool, can be accounted for by heterotrophic release of previously fractionated carbon. This could come about through decomposition of organic material and respiration by the pool biota; both pools exhibit marked diurnal changes in CO_2 content resulting from daytime photosynthetic depletion

and overnight respiratory input (Keeley et al., 1983a; Keeley and Busch, 1984). The use of these ^{13}C-depleted CO_2 sources would account for the similar $\delta^{13}C$ values for CAM and non-CAM species in these pools, despite the fact that half of the carbon uptake in *Isoetes* species is initially fixed in the dark via PEP carboxylase (Keeley and Busch, 1984).

Isoetes bolanderi Engelm. in Siesta Lake is also indistinguishable from associated non-CAM species in $\delta^{13}C$ values, despite the fact that a substantial portion of its carbon uptake is via CAM (Keeley et al., 1983b; Sandquist and J.E. Keeley, unpublished data, 1985, 1986). This species, however, is likely to be fixing carbon which has already undergone fractionation since it depends to a large extent on CO_2 uptake from the decomposition of organic matter in the sediment (J.E. Keeley, unpublished data, 1983, 1984) as is true of other *Isoetes* species from oligotrophic lakes (Richardson et al., 1984; Boston and Adams, 1985; Farmer and Spence, 1985).

The only instance in which there was a very marked and consistent difference in $\delta^{13}C$ ratio between species was at Birch Lake. Throughout the season there was a $16^{0}/_{00}$ difference between the moss *Amblystegium riparium* (Hedw.) BSG and *Elodea canadensis*. Short-term ^{14}C labeling experiments and carboxylase activities showed that these two species are largely dependent on C_3-type carboxylation (Table III), despite previous reports of substantial C_4 activity in *Elodea canadensis* from the midwest region of the U.S.A. (DeGroote and Kennedy, 1977). The difference in labeling pattern between their report and the present study suggests that there may be population differences in *Elodea canadensis* from different parts of the country.

TABLE III

Light fixation products with 15 second steady-state ^{14}C labeling and enzyme activities for non-CAM submerged aquatic species from Birch Lake

	Percentage of label				Carboxylase activity (μmol mg^{-1} Chl h^{-1})	
	PGA[1]	Malate	Aspartate	Other	RuBPcase	PEPcase
Amblystegium riparium						
(10 h day 15° C/10° C)	82	9	1	8	11.8	0.7
Elodea canadensis						
(10 h day 15° C/10° C)	79	16	5	0	6.0	0.3
(14 h day 27° C/20° C)	70	27	2	1		

[1] PGA = phosphoglycerate.

Clearly, at Birch Lake, factors other than the carboxylation pathway are determining the $\delta^{13}C$ values of *Amblystegium riparium* and *Elodea canadensis*. Factors other than PEP carboxylase activity that are known to reduce carbon isotope discrimination (and thus produce less negative $\delta^{13}C$ values) in aquatic species include phenomena affecting the diffusional resistance of carbon and the extent of HCO_3^- utilization (Smith and Walker, 1980;

Osmond et al., 1981; Raven et al., 1982). Diffusional resistances have not been quantified for these two species. However, they are not likely to account for the fractionation differences. At Birch Lake *Amblystegium riparium* and *Elodea canadensis* grow intermixed in the relatively stagnant water and thus there is surely little or no difference in the degree of water turbulence each is exposed to. Both species have similar leaf shapes and leaf sizes. Structurally both leaf types are remarkably similar in being a double cell layer with no lacunae and very little internal air space. Differential bicarbonate use may, however, account for the different $\delta^{13}C$ values. Raven et al. (1982) provided evidence that a 16‰ difference in $\delta^{13}C$ between submerged aquatic species of *Ranunculus* and *Lemanea* was attributable in part to differential HCO_3^- utilization. *Amblystegium riparium* very likely does not utilize bicarbonate since this ability, although not unknown (Penuelas, 1985), is rare in bryophytes (Bain and Proctor, 1980). *Elodea canadensis* on the other hand, has been reported by several authors to utilize HCO_3^- freely (Simpson et al., 1980; Prins et al., 1982).

Some of the $\delta^{13}C$ values reported here contrast markedly with previously published values for the same species. For example, Osmond et al. (1981) reported $\delta^{13}C$ values of -33.4 to -49.4 for *Fontinalis antipyretica* from Britain and Finland. They found that much of the variation could be accounted for by differences in $\delta^{13}C$ of the different water carbonate sources since $\Delta\delta^{13}C$ (= $\delta^{13}C$ plant - $\delta^{13}C$ water) values tended to center around -33‰ for their populations of *F. antipyretica*. In our study *F. antipyretica* had an isotope value of -26.9 and the $\Delta\delta^{13}C$ value was -15.4. Thus, taking source carbon into account, our $\delta^{13}C$ value was much less negative than any observed by Osmond et al. (1981) for *F. antypyretica* populations. PEP carboxylase activity is unlikely to be a factor since it has not been found to play an important role in photosynthesis for any bryophyte. Also Osmond et al. (1981) noted that *F. antipyretica* from fast-moving streams, where external CO_2 diffusional resistances are minimized, had $\Delta\delta^{13}C$ values consistent with expectation for plants which depended exclusively upon RuBP carboxylase (assuming no HCO_3^- use); i.e. $\Delta\delta^{13}C$ (expected) = -8‰ (correction for equilibrium fractionation between HCO_3^- and CO_2) + -27‰ (RuBPcase fractionation; this is possibly more negative, see Raven et al., 1982) = -35‰. Very likely the less negative $\Delta\delta^{13}C$ value reported in our study is due to much greater diffusional resistances encountered in the relatively stagnant Siesta Lake. Bicarbonate uptake, however, cannot be ruled out. Although it has been reported for some populations of *Fontinalis antipyretica* (Penuelas, 1985), it is absent in other populations (James, 1928) and it is not likely to be important under the relatively low pH conditions in Siesta Lake.

Osmond et al. (1981) argue that, for aquatic plants which use CO_2 and rely exclusively on RuBP carboxylase, as the resistances (both internal and external) to CO_2 diffusion increase, the $\delta^{13}C$ value of the biomass should approach that of the source carbon. Such analysis suggests that very large dif-

fusional resistances are encountered by *Potamogeton pectinatus* in Searsville Lake. This species, according to Winter (1978), is not a bicarbonate user (cf. Sand-Jensen, 1983) and fixes carbon via the C_3 pathway. If these characteristics are true for the Searsville population then the $\delta^{13}C$ of the source carbon for *P. pectinatus* would equal $-11^0/_{00} + -8^0/_{00}$ (correcting for equilibrium fractionation between HCO_3^- and CO_2, Mook et al., 1974) = $-19^0/_{00}$. This value is close to the $-22.1^0/_{00}$ noted for *P. pectinatus* (Table II) and indicates that photosynthesis is largely limited by diffusion of CO_2. Much of this resistance is apparently due to boundary layer effects around the leaf, since internal resistance accounts for only about 5% of the total resistance to CO_2 assimilation in species of *Potamogeton* (Wetzel and Grace, 1983). Data presented by LaZerte and Szalados (1982) show a similar pattern for *P. pectinatus* from Canadian lakes.

Diffusional resistances would also seem to play an important role in the Mesa de Colorado pool. For species in the pool that rely entirely on CO_2, the source carbon would have a $\delta^{13}C$ value of $-23^0/_{00}$ early in the season and $-28^0/_{00}$ late in the season (assuming a $-8^0/_{00}$ correction, an approximate estimate in light of the marked diurnal temperature and pH changes, Table II). These values are very similar to the $\delta^{13}C$ values for plant material of most species. The fact that throughout the season *Ranunculus aquatilis* L. had $\delta^{13}C$ values $8^0/_{00}$ less negative than these values suggests dependence on bicarbonate uptake.

In summary, it is clear that species in the same aquatic environment, but with very different photosynthetic pathways, may have very similar $\delta^{13}C$ ratios. Additionally, co-existing species with similar photosynthetic pathways, may have distinctly different $\delta^{13}C$ values. Although carbon isotopes are not able to distinguish photosynthetic pathways, they may prove valuable for studying other aspects of aquatic plant photosynthesis.

Although stable carbon isotope values do not allow differentiation between CAM and non-CAM aquatic species, there is evidence that hydrogen isotopes may play an important role in this regard. Table IV shows the δD

TABLE IV

δD values of cellulose nitrate, relative to the source water, for aquatic species grown submerged in aquaria

CAM species:	
Isoetes howellii	+58
Littorella uniflora	+52
Non-CAM species:	
Chara contraria	-14
Eleocharis acicularis	+18
Potamogeton pectinatus	-26
Ranunculus aquatilis	0
Spirogyra sp.	-139

values for a number of aquatic species grown in cultivation. In general CAM species have distinctly heavier δD values than associated non-CAM species. Of the 'non-CAM' species, *Eleocharis acicularis* has the heaviest δD value and this may stem in part from a slight tendency for some CAM activity (Table I). Although only a small overnight increase in acidity is detectable, this species is capable of some PEP carboxylase-mediated dark uptake of CO_2 (Morton, 1984). Similar δD patterns have been observed for aquatic CAM and non-CAM species from field situations (Sternberg et al., 1984). The fact that this technique distinguishes aquatic CAM species from non-CAM ones argues strongly for a fractionation based on the cycling of internal metabolic pools rather than transpirational differences as suggested by some investigators (Ziegler et al., 1976). In conclusion, this technique deserves greater attention as an important tool for aquatic plant photosynthesis studies.

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REFERENCES

Aulio, K., 1985. Differential expression of diel acid metabolism in two life forms of *Littorella uniflora* (L.) Aschers. New Phytol., 100: 533—536.

Bain, J.T. and Proctor, M.C.F., 1980. The requirement of aquatic bryophytes for free CO_2 as an inorganic carbon source: some experimental evidence. New Phytol., 87: 269—283.

Beer, S. and Wetzel, R.G., 1981. Photosynthetic carbon metabolism in the submerged aquatic angiosperm *Scirpus subterminalis*. Plant Sci. Lett., 21: 199—207.

Boston, H.L. and Adams, M.S., 1985. Seasonal diurnal acid rhythms in two aquatic crassulacean acid metabolism plants. Oecologia, 65: 573—579.

Bowes, G. and Salvucci, M.E., 1984. *Hydrilla*: Inducible C_4-type photosynthesis without kranz anatomy. In: C. Sybesma (Editor), Advances in Photosynthesis Research, Vol. 3, Junk, The Hague, pp. 829—832.

DeGroote, D. and Kennedy, R.A., 1977. Photosynthesis in *Elodea canadensis* Michx. Four-carbon acid synthesis. Plant Physiol., 59: 1133—1135.

Farmer, A.M. and Spence, D.H.N., 1985. Studies of diurnal acid fluctuations in British isoetid-type submerged aquatic macrophytes. Ann. Bot., 56: 347—350.

Holaday, A.S. and Bowes, G., 1980. C_4 acid metabolism and dark CO_2 fixation in a submerged aquatic macrophyte (*Hydrilla verticillata*). Plant Physiol., 65: 331—335.

Hough, R.A. and Wetzel, R.G., 1977. Photosynthetic pathways of some aquatic plants. Aquat. Bot., 3: 297—313.

James, W.O., 1928. Experimental researches on vegetable assimilation and respiration. XIX. The effect of variations of carbon dioxide supply upon the rate of assimilation of submerged water plants. Proc. R. Soc. Br., 103: 1—42.

Keeley, J.E., 1981. *Isoetes howellii*: A submerged aquatic CAM plant? Am. J. Bot., 68: 420—424.

Keeley, J.E., 1982. Distribution of diurnal acid metabolism in the genus *Isoetes*. Am. J. Bot., 69: 254—257.

Keeley, J.E. and Busch, G., 1984. Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. Plant Physiol., 76: 525—530.

Keeley, J.E. and Morton, B.A., 1982. Distribution of diurnal acid metabolism in submerged aquatic plants outside the genus *Isoetes*. Photosynthetica, 16: 546—553.

Keeley, J.E., Mathews, R.P. and Walker, C.M., 1983a. Diurnal acid metabolism in *Isoetes howellii* from a temporary pool and a permanent lake. Am. J. Bot., 70: 854—857.

Keeley, J.E., Walker, C.M. and Mathews, R.P., 1983b. Crassulacean acid metabolism in *Isoetes bolanderi* in high elevation oligotrophic lakes. Oecologia, 58: 63—69.

LaZerte, G.D. and Szalados, J.E., 1982. Stable carbon isotope ratio of submerged freshwater macrophytes. Limnol. Oceanogr., 27: 413—418.

Lorimer, G.H., Badger, M.R. and Andrews, T.J., 1976. The activation of Ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions. Equilibria, kinetics, a suggested mechanism, and physiological implications. Biochemistry, 15: 529—536.

Mook, W.G., Bommerson, J.C. and Staverman, W.H., 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Plant. Sci. Lett., 22: 169—176.

Morton, B.A., 1984. Photosynthesis in the seasonally submerged vernal pool sedge *Eleocharis acicularis*. M.A. Thesis, Occidental College, Los Angeles, CA, 71 pp.

Osmond, C.B., Valaane, N., Haslam, S.M., Uotila, P. and Roksandic, Z., 1981. Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. Oecologia, 50: 117—124.

Penuelas, J., 1985. HCO_3^- as an exogenous carbon source for aquatic bryophytes *Fontinalis antipyretica* and *Fissidens grandifrons*. J. Exp. Bot., 36: 441—448.

Prins, H.B.A., Snel, J.F.H., Zanstra, P.E. and Helder, R.J., 1982. The mechanism of bicarbonate assimilation by the polar leaves of *Potamogeton* and *Elodea*. CO_2 concentrations at the leaf surface. Plant, Cell Environ., 5: 207—214.

Raven, J., Beardall, J. and Griffiths, H., 1982. Inorganic C-sources for *Lemanea, Cladophora* and *Ranunculus* in a fast-flowing stream: measurements of gas exchange and of carbon isotope ratio and their ecological implications. Oecologia, 53: 68—78.

Richardson, K., Griffiths, H., Reed, M.L., Raven, J.A. and Griffiths, N.M., 1984. Inorganic carbon assimilation in the isoetids, *Isoetes lacustris* L. and *Lobelia dortmanna* L. Oecologia, 61: 115—121.

Salvucci, M.E. and Bowes, G., 1982. Photosynthetic and photorespiratory responses of the aerial and submerged leaves of *Myriophyllum brasiliense*. Aquat. Bot., 13: 147—164.

Salvucci, M.E. and Bowes, G., 1983. Two photosynthetic mechanisms mediating the low photorespiratory state in submerged aquatic angiosperms. Plant Physiol., 73: 488—496.

Sand-Jensen, K., 1983. Photosynthetic carbon sources of stream macrophytes. J. Exp. Bot., 34: 198—210.

Schurmann, P., 1969. Separation of phosphate esters and algal extracts by thin-layer electrophoresis and chromatography. J. Chromatogr., 39: 507—509.

Simpson, P.S., Eaton, J.W. and Hardwick, K., 1980. The influence of environmental factors on the apparent photosynthesis and respiration of the submerged macrophyte *Elodea canadensis*. Plant, Cell Environ., 3: 415—423.

Smith, F.A. and Walker, N.A., 1980. Photosynthesis by aquatic plants: Effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. New Phytol., 86: 245—259.

Sternberg, L. and DeNiro, M.J., 1983. Isotopic composition of cellulose from C_3, C_4 and CAM plants growing in the vicinity of one another. Science, 220: 947—948.

Sternberg, L., DeNiro, M.J. and Keeley, J.E., 1984. Hydrogen, oxygen and carbon isotope ratios of cellulose from submerged aquatic crassulacean acid metabolism and non-crassulacean acid metabolism plants. Plant Physiol., 76: 69—70.

Teeri, J., 1982. Photosynthetic variation in the Crassulaceae. In: I.P. Ting and M. Gibbs (Editors), Crassulacean Acid Metabolism. American Society of Plant Physiologists, Rockville, MD, pp. 244—259.

Waygood, E.R., Mache, R. and Tan, C.K., 1969. Carbon dioxide, the substrate for phosphoenolpyruvate carboxylase from leaves of maize. Can. J. Bot., 47: 1455—1458.

Wetzel, R.G. and Grace, J.B., 1983. Aquatic plant communities. In: E.R. Lemon (Editor), CO_2 and Plants, The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. Westview Press, Boulder, CO, pp. 223—280.

Winter, K., 1978. Short-term fixation of ^{14}Carbon by the submerged aquatic angiosperm *Potamogeton pectinatus*. J. Exp. Bot., 112: 1169—1172.

Ziegler, H., Osmond, C.B., Stickler, W. and Trimborn, D., 1976. Hydrogen isotope discrimination in higher plants: Correlation with photosynthetic pathway and environment. Planta, 128: 85—92.