18 Aquatic CAM Photosynthesis

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18.1 Introduction

CAM photosynthesis in an aquatic plant was first suggested by the observation of marked diel (24 h) changes in malic acid in photosynthetic tissues, but not in nonphotosynthetic tissues, of *Isoetes howellii* (Lycophyta: Isoetaceae) (Keeley 1981). At the time everyone "knew" that CAM was an adaptation for increasing water-use efficiency in xeric adapted plants, and thus there was some reluctance to accepting the notion of an aquatic CAM plant. Phrases such as "CAM-like" or "aquatic acid metabolism (AAM)" were coined to avoid this apparent paradox (Cockburn 1983).

18.2 Evidence of CAM Photosynthesis

The evidence that crassulacean acid metabolism is present in the submerged aquatic *Isoetes howellii* is based on the following criteria.

1. CO_2 is assimilated in the dark and fixed into malic acid and remains in malic acid during the dark period (Fig 18.1).
2. Overnight acidification is followed by daytime deacidification resulting in a diel cycle of 100–300 mmol H^+ kg^{-1} fresh weight (Table 18.1).
3. Phospho*enol*pyruvate carboxylase (PEPC) activity is sufficient to account for the observed accumulation of malate (Table 18.2).
4. There is a 2:1 molar ratio between acidity and malate accumulation (Keeley 1981; Keeley and Busch 1984).
5. Dark fixation of CO_2 results in net CO_2 uptake in the dark (Keeley and Bowes 1982).
6. $^{14}CO_2$ fixed in the dark into malate is transferred in the light to phosphorylated compounds and accumulates in other soluble compounds such as sugars and insolubles such as starch (Fig. 18.2).
7. NADP-malic enzyme activity is sufficient to account for rates of decarboxylation (35 ± 8 mol kg^{-1} Chl h^{-1}, J.E. Keeley unpubl.).

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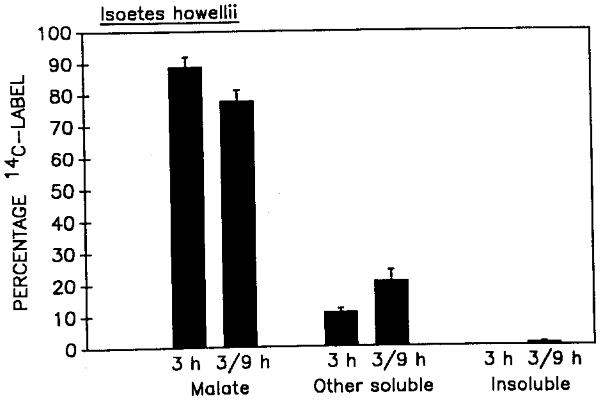

Fig. 18.1. Distribution of dark-fixed ^{14}C label after steady-state labelling for 3 h, and after labelling for 3 h followed by a chase of 9 h in ^{12}C medium in the dark for *Isoetes howellii*. (J.E. Keeley, unpubl.)

Table 18.1. Titratable acidity (TA) and malate (M) content in foliage of aquatic species. Values are means ± SD (number of samples in parentheses); FW = fresh weight

Species	(pH end-point	Titratable acidity (mmol H^+ kg^{-1} FW)		Malate (mmol kg^{-1} FW)		(n)	*t*-Test	
		Dusk	Dawn	Dusk	Dawn		TA	M
Slight acid accumulation:								
Ascophyllum nodosum	(7.0)	23 ± 3	44 ± 8	2 ± 2	6 ± 2	(5)	**	*
Eleocharis acicularis	(8.3)	16 ± 2	36 ± 12	6 ± 2	12 ± 5	(4)	*	ns
Orcuttia viscida	(7.0)	7 ± 5	23 ± 6	11 ± 6	11 ± 6	(10)	**	ns
Vallisneria americana	(7.0)	11 ± 7	42 ± 22	–	–	(5)	**	–
Substantial acid accumulation:								
Isoetes howellii	(6.4)	12 ± 1	229 ± 19	21 ± 9	123 ± 5	(3)	**	**
Sagittaria subulata	(7.0)	8 ± 3	83 ± 26	10 ± 2	45 ± 10	(6)	**	**
Crassula aquatica	(6.4)	0 ± 0	128 ± 13	15 ± 9	76 ± 5	(3)	**	**
Littorella uniflora	(6.4)	19 ± 22	112 ± 14	21 ± 14	66 ± 8	(4)	**	**

ns, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$.
Values are from J.E. Keeley (publ. and unpubl.) except *Ascophyllum* (Johnston and Raven 1986).

8. Dark fixation contributes a substantial portion of the carbon gain (Keeley and Busch 1984).

In conclusion, it is apparent that *Isoetes howellii* is a submerged aquatic CAM plant.

Table 18.2. Phospho*enol*pyruvate carboxylase (PEPC) and ribulose 1,5-bisphosphate carboxylase (RUBISCO) activities in selected CAM and non-CAM aquatic species under submerged (Sub) and emergent (Emg) conditions (PEPC procedure after Jones et al. (1978) and RUBISCO after Lorimer et al. (1976)) compared with the terrestrial species *Spinacia oleracea* (C_3) and *Zea mays* (C_4). Data from J.E. Keeley (unpubl.)

		PEPC	RUBISCO	Ratio RUBISCO/PEPC
		(mol kg^{-1} Chl h^{-1})		
Isoetes howellii	(Sub)	36 ± 16	256 ± 68	7.1
	(Emg)	18 ± 10	553 ± 95	31.6
Crassula aquatica	(Sub)	178 ± 63	392 ± 161	2.2
	(Emg)	45 ± 5	854 ± 163	19.0
Eleocharis acicularis	(Sub)	84 ± 29	224 ± 941	2.7
	(Emg)	36 ± 7	675 ± 124	18.8
Orcuttia viscida	(Sub)	54 ± 25	285 ± 51	5.3
	(Emg)	168 ± 70	339 ± 58	2.0
Spinacia oleracea		54	865	16.0
Zea mays		842	462	0.5

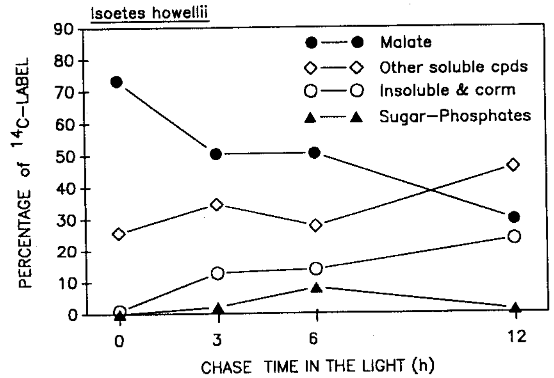

Fig. 18.2. Distribution of dark-fixed ^{14}C label after steady-state labelling in the dark for 3 h and a further 9 h incubation in the dark in ^{12}C medium, followed by a chase of 12 h in the light for *Isoetes howellii*. (J.E. Keeley, unpubl.)

18.3 Distribution of Aquatic CAM Plants

Detection of an overnight acidification/daytime deacidification cycle is the sine qua non of crassulacean acid metabolism and has been used routinely in surveys of aquatic plants for evidence of CAM. Detection of such an acid cycle is not conclusive evidence of CAM, as will be discussed below.

From the literature and unpublished data I can find reports of tests for overnight acid accumulation for 159 freshwater and marine species (Table 18.3).

Table 18.3. Distribution of detectable overnight acid accumulation in aquatic species. Substantial is > 50 mmol H^+ kg^{-1} fresh weight

	Overnight acid accumulation		
	None	Slight	Substantial
	(No. of species tested)		
[Cyanobacterial lichen]	1	0	0
Chlorophyta	13	0	0
Phaeophyta	11	8	0
Rhodophyta	9	0	0
Bryophyta	4	0	0
Lycophyta	0	0	33
Sphenophyta	1	0	0
Pterophyta	3	0	0
Anthophyta			
(Monocot)	28	8	1
(Dicot)	29	5	5
Total	99	21	39

Based on literature and unpublished data provided by myself, John Raven, Linda Handley, Barry Osmond, Jonathan Newman, Andrew Farmer and George Bowes.

Of these, 39 showed substantial (> 50 mmol H^+ kg^{-1} fresh weight) overnight accumulation of acidity, and another 21 have slight but statistically significant overnight acid accumulation (Table 18.3).

18.3.1 Aquatic CAM Species

The 39 species with substantial overnight acid accumulation are distributed in four genera, *Isoetes*, *Crassula*, *Littorella*, and *Sagittaria*.

Isoetes is worldwide a genus of more than 150 taxa, most of which are either lacustrine or amphibious (Tryon and Tryon 1982). All have a nearly identical growth form of a rosette of leaves arising from a corm (Fig. 18.3), and all but one or two are perennial. Of these, 33 have been tested for an acidification/deacidification cycle (Keeley 1982, 1987, unpubl.) and all exhibit an overnight accumulation of titratable acidity and malate of a similar magnitude to that observed for *Isoetes howellii* (Table 18.1). In two other species, the lacustrine *I. bolanderi* and the amphibious *I. orcuttii*, further studies, such as those described in Section 18.2, indicate that these taxa are quite similar to *I. howellii*. In particular, the patterns observed in Figs. 18.1 and 18.2 are nearly identical between these three species. The 33 *Isoetes* species thus far tested represent taxa from the range of known aquatic habitats (e.g. seasonal pools, oligotrophic lakes, freshwater tidal creeks, irrigation canals) and from many parts of the world including North America, Central America, South America, Europe, Africa, and Australia. Thus, it seems warranted to suggest that all aquatic *Isoetes* species will eventually prove to be CAM.

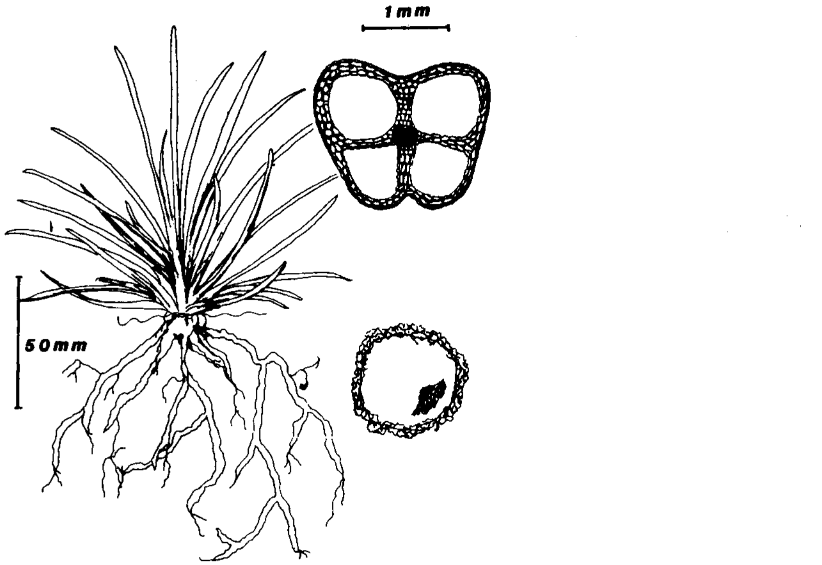

Fig. 18.3. Schematic illustration of *Isoetes* and cross-sections of a leaf and a root. (Keeley 1991)

Crassula (Crassulaceae) is a genus of 300 species, most of which are perennials. All aquatic species are annuals and very often co-occur with *Isoetes* in both oligotrophic lakes and seasonal pools. Four species (*C. aquatica*, *C. helmsii*, *C. natans*, *C. paludosa*) have been tested for CAM and all show an acidity cycle similar to that observed for *Crassula aquatica* (Table 18.1). Of the species tested, three were from seasonal pools in either North America, Africa, or Australia, and one was from oligotrophic lakes in the Andes of South America. *Crassula aquatica* has other features supporting the presence of CAM. Dark-fixed carbon is retained in malic acid throughout the night and is transferred during the day to phosphorylated compounds and accumulates in sugars and insoluble compounds. Additionally, PEPC activity is more than sufficient to account for dark fixation (Table 18.2).

The genus *Littorella* (Plantaginaceae) comprises two species, *L. americana* of North American high-latitude oligotrophic lakes, which has not been investigated for CAM, and the European *L. uniflora*, which is CAM; acidity and malate fluctuations are similar to other aquatic CAM species (Table 18.1) and the retention of dark-fixed carbon in malic acid during the night (such as shown in Fig. 18.1) and the transfer of dark fixed carbon in the light to phosphorylated sugars (such as shown in Fig. 18.2) are similar to *Isoetes* and *Crassula* taxa (J.E. Keeley; unpubl.). Also, the ratio of RUBISCO/PEPC (Farmer 1987) is quite similar to that reported for *Isoetes* (Table 18.2).

Andrew Farmer and George Bowes (unpubl.) have found that the freshwater tidal species *Sagittaria subulata* (Alismataceae) has significant overnight acid accumulation, and this has been verified (Table 18.1). Studies of the retention of dark-fixed carbon in malate during the night (such as in Fig. 18.1) and the transfer of dark fixed carbon in the light to phosphorylated sugars (such as in Fig. 18.2) are similar to *Isoetes* and *Crassula* taxa (J.E. Keeley, unpubl.). Unlike the previous three genera, there is documented evidence of lack of CAM in other species in this genus (Keeley and Morton 1982).

18.3.2 Ecological Distribution of Aquatic CAM Plants

In general, aquatic CAM plants are distributed in one of two habitats, oligotrophic lakes or mesotrophic shallow seasonal pools. The former habitats, being of low productivity, are widely distributed at high latitudes and at increasing latitudes with decreasing altitude. For example, *Isoetes* spp. are widely distributed in northern European lakes but are restricted to lakes above 3000 m at tropical and subtropical latitudes (J.E. Keeley, unpubl.). At low elevations in warm climates, permanent bodies of water are mesotrophic or eutrophic and CAM species are excluded from such habitats. Shallow seasonal pools at any altitude throughout the world are potential habitats for *Isoetes* and *Crassula* species.

This distribution is seemingly odd in the light of marked differences in the physical and chemical differences between such habitats (Fig. 18.4). Seasonal pools have a marked diurnal change in CO_2 availability due to the high photosynthetic demand resulting from the high temperatures, high light availability, and high plant biomass typical of such sites. Since these habitats are rain-fed, they are poorly buffered, and thus the pH is largely controlled by the inorganic carbon species; as CO_2 is consumed HCO_3^- predominates, and the pH may rise 2 to 4 units over a period of 4 to 6 h during the morning. The stress of such large pH changes may be tolerable due to the fact that the water is so poorly buffered relative to the cellular chemistry of the organisms that inhabit such environments.

Oligotrophic lakes, such as the Sierran lake illustrated in Fig. 18.4, show relatively little diurnal change in CO_2 availability. One characteristic they both share, however, is that during much of the day, CO_2 in the water is potentially limiting to photosynthesis. Although the level of CO_2 in almost all aquatic systems is higher than in air, the diffusional resistance of water is 4 orders of magnitude greater than in air, resulting in boundary-layer depletions around the plants (Raven et al. 1988). CAM-dominated lakes are more carbon limited than lakes lacking CAM species (Table 18.4).

There are a few exceptions to these distribution patterns. *Isoetes malinverniana* is restricted to drainage canals, often in the shade of trees, in northern Italy. *Sagittaria subulata*, and at least three *Isoetes* species, are restricted to freshwater tidal rivers where they alternate diurnally, according to the tides, between submergent and emergent conditions.

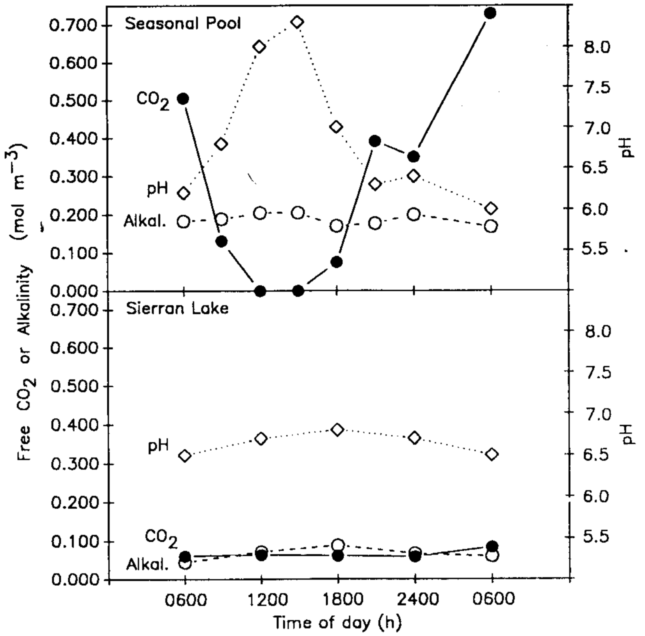

Fig. 18.4. Diel changes in water chemistry in a seasonal pool and a high-elevation oligotrophic Sierran lake (from Keeley et al. 1983; Keeley and Busch 1984; Alkal., Alkalinity (i.e. $HCO_3^- + CO_3^{2-}$)

Table 18.4. Comparison of lakes dominated by aquatic CAM species and lakes lacking CAM species. (Data gleaned from literature cited in text)

Lakes	pH	Total CO_2 (mol m^{-3})	Conductivity (μS m^{-1})
CAM-dominated	4 to 7	0.01 to 0.30	0.02 to 0.07
No CAM	7 to 10	2.5 to 5.0	2.0 to 7.0

18.3.3 Questionable Aquatic CAM Species

Slight but significant overnight acid accumulation has been observed in 21 species and examples of these taxa are shown in Table 18.1. It is questionable as to whether or not these acidity changes are related to CAM photosynthesis. Certainly in some species it is not. For example in *Orcuttia viscida* (Poaceae) it is apparent that carbon fixed in the dark does not remain in malate (Fig. 18.5), and the same is seen with the marine alga *Ascophyllum nodosum* (Fig 18.6), which was previously observed to have a CAM-like acid fluctuation (Johnston and Raven

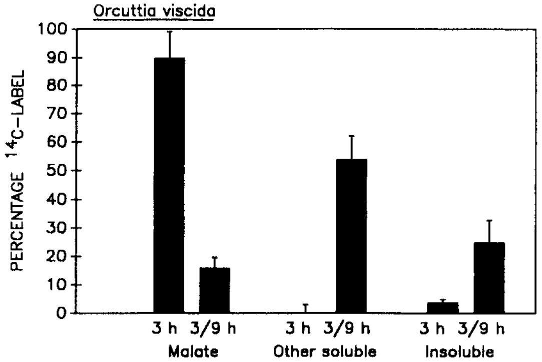

Fig. 18.5. Distribution of dark-fixed ^{14}C label after steady-state labeling for 3 h, and after labelling for 3 h followed by a chase of 9 h in ^{12}C medium in the dark for *Orcuttia viscida*. (J.E. Keeley, unpubl.)

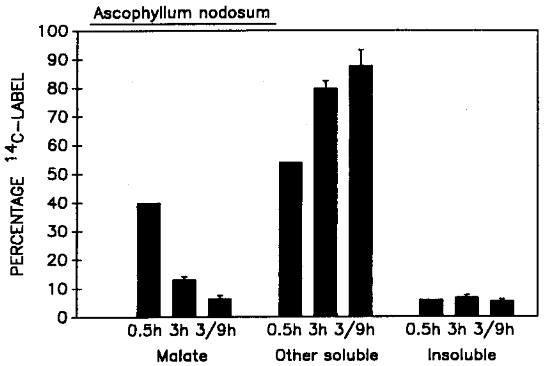

Fig. 18.6. Distribution of dark-fixed ^{14}C label after steady-state labelling for 0.5 h and 3 h, and after labelling for 3 h followed by a chase of 9 h in ^{12}C medium in the dark for *Ascophyllum nodosum*. (R. Montgomery and J.E. Keeley, unpubl.)

1986). The function of dark fixation and overnight acid accumulation in such species is unknown but several possibilities exist, e.g. it may play a role in nitrogen metabolism, as observed in some algae (Turpin et al. 1991).

18.4 Adaptive Significance of CAM in the Aquatic Environment

18.4.1 Seasonal Pool CAM Species

Under steady-state carbon conditions, *Isoetes howellii* shows net CO_2 uptake in the dark in excess of 20 mol kg^{-1} Chl h^{-1} at high CO_2 concentration, but uptake is markedly higher in the light (Fig. 18.7). However, under field conditions these

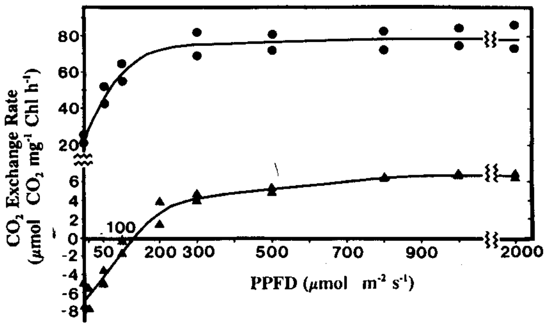

Fig. 18.7. Light response curves for *Isoetes howellii* leaves at 86.7 (circles) and 11.8 (triangles) µmol l^{-1} CO_2. (Keeley and Bowes 1982)

plants are not exposed to steady-state carbon conditions (Fig 18.4), and thus carbon uptake during the day is restricted to a brief window of time in the early morning (Fig. 18.8) In these environments, due to lower temperatures and respiration by the pool flora and invertebrate fauna, CO_2 builds up in the water overnight. For *Isoetes howellii*, dark CO_2 uptake under field conditions represents about 40% of the total 24-h carbon uptake (Keeley and Busch 1984). However, assimilation of ambient carbon during the night accounts for only about one-third of the total dark fixation (Keeley and Busch 1984). It would appear that much of the dark fixation and malic acid accumulation arises from re-fixation of respiratory CO_2 or CO_2 supplied by the roots. The latter is less likely in *Isoetes howellii* than in other *Isoetes* where it has been documented (Richardson et al. 1984). This is because the compact clay substrate would not lend itself to diffusion

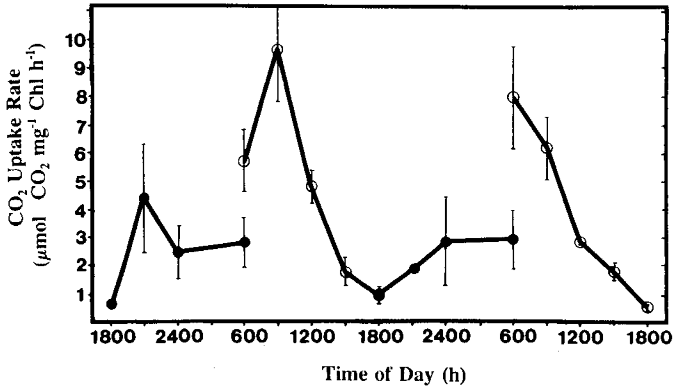

Fig. 18.8 Carbon-assimilation rates for submerged *Isoetes howellii* leaves in the field in southern California; *open circles* light uptake; *filled circles* dark uptake. (Keeley and Busch 1984)

of gases and the roots of this species are thin with dense cortex, distinctly unlike *Isoetes* from lacustrine environments where root uptake of carbon seems to be commonplace. Additionally, experiments with detached leaves sparged overnight with CO_2-free gas are still capable of overnight acid accumulation of about 150 mol H^+ kg^{-1} Chl (Keeley and Busch 1984).

Daytime depletion of acids in *Isoetes* foliage follows a pattern similar to the daytime depletion of CO_2 in the water, although it lags behind by about 3 h. It is also responsive to different ambient carbon conditions, being most rapid and complete under conditions of rapid ambient CO_2 depletion (Keeley and Busch 1984). These patterns are remarkably similar in *Isoetes orcuttii* and *Crassula aquatica*, which co-occur with *I. howellii* (J.E. Keeley, unpubl.).

The significance of CAM in seasonal pool plants is that it plays a major role in the carbon budget by allowing for the assimilation of carbon at night when it is abundant and by recapturing respiratory carbon that would otherwise be lost. Another way of looking at it is that these CAM plants compete for carbon at a time when other plants are not assimilating carbon. Ecologically, this may play an important role in promoting coexistence of these relatively diverse communities (Keeley and Sandquist 1991).

18.4.2 Lacustrine CAM Species

Like seasonal-pool CAM plants, Madsen (1987) has shown that *Isoetes lacustris* and *Littorella uniflora* are likewise capable of daytime CO_2 uptake; however, by extracting gas samples from the lacunae he has shown that under ambient CO_2 concentrations of 30 to 200 mmol m^{-3} the contribution of internal sources of carbon exceed external sources. It was suggested that decarboxylation of malic acid during the day was the source of internal CO_2, and the rate of decarboxylation was dependent upon ambient CO_2 levels (Fig. 18.9). It is also clear from these experiments, and others (Richardson et al. 1984; Raven et al. 1988), that substantial carbon is taken up from the roots. Similar patterns of dark and light fixation were also observed for the lacustrine *Isoetes bolanderii* (Sandquist and Keeley 1990) and the lacustrine *I. macrospora* (Boston and Adams 1986). In these species it was estimated that CAM contributed 45 to 55% of the annual carbon gain.

The adaptive significance of CAM for lacustrine species lies in the enhanced capacity for carbon assimilation by extending the period for carbon assimilation to 24 h. The general absence of CAM from eutrophic environments is related to the inefficiency of this pathway under high-carbon conditions. For example, Madsen (1987) demonstrated that, for both *Littorella uniflora* and *Isoetes lacustris*, the relative contribution of CAM to the total CO_2 uptake over 24 h declined from 95% at an external concentration of 30 mmol m^{-3} CO_2 to about 36% at 200 mmol m^{-3} CO_2. At 1.4 mol m^{-3} CAM ceased in *Littorella uniflora*, and this was tied to a decrease in PEPC activity (Hostrup and Wiegleb 1991). The more rapid growth rates of C_3 species under high inorganic nutrient eutrophic conditions undoubtedly play a role in the competitive exclusion of CAM plants from such sites, since CAM is suppressed under low PPFD (Robe and Griffiths 1990). Occasional reports of *Isoetes* from such eutrophic habitats (Seddon 1965) are often related to disturbance, which maintains the system in disequilibrium.

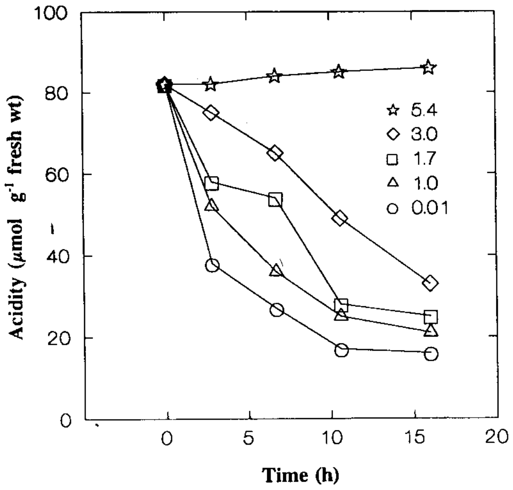

Fig. 18.9. Changes in titratable acidity in the light in leaves of *Littorella uniflora* incubated at 5.4, 3.0, 1.7, 1.0 and 0.01 mol m^{-3} free CO_2 in the water around the roots and with 0.01 mol m^{-3} free CO_2 in the water surrounding the leaves. (Madsen 1987)

18.5 Aquatic CAM Plants in an Aerial Environment

All plants in seasonal pools become aerial at some time during their life cycle. *Isoetes howellii* loses CAM in aerial foliage, and this happens on a cell-by-cell basis while the bases of partially submerged plants retain CAM (Fig. 18.10). The same has been noted in CAM species from lacustrine habitats which become emergent at irregular intervals (Keeley et al. 1983; Aulio 1985).

The precise factors responsible for loss of CAM are unknown. The proximate answer seems to be that decreases in water potential at the leaf surface trigger the loss of CAM. This is supported by the observation that CAM is retained in *Isoetes howellii* under aerial conditions if maintained under high humidity, and is greatly reduced in submerged plants incubated in mannitol solutions with osmotic pressures > 0.1 MPa (equivalent to about 41 mol m^{-3} mannitol) (Table 18.5).

The ultimate answer for the loss of CAM is that in the aerial atmosphere diffusional resistances no longer limit CO_2 to photosynthetic organs, and a competitive advantage is likely gained by switching to strictly C_3 photosynthesis. The hypothesis is also supported by the absence of CAM in many terrestrial *Isoetes* species, which are likely to have derived from aquatic ancestors (Keeley 1983). The exception to this rule is the retention of CAM in the terrestrial *I. andicola* (= *Stylites andicola*) (Keeley et al. 1984) and other related terrestrials

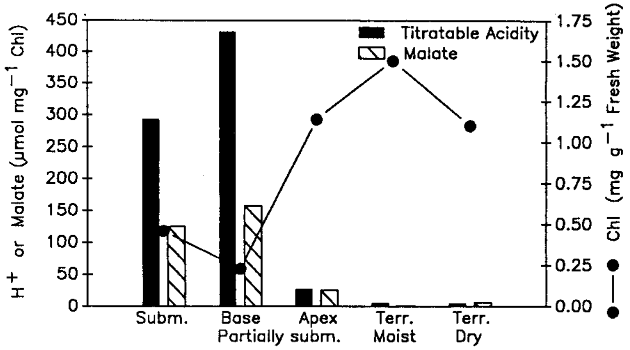

Fig. 18.10. Overnight accumulation of acidity and malate in *Isoetes howellii* foliage submerged (*Subm.*), partially submerged, and fully emergent (*Terr.*) under moist and dry soil conditions. (Keeley and Busch 1984)

Table 18.5. Titratable acidity and malate content in foliage of the aquatic CAM plant *Isoetes howellii* under submerged conditions for 1 month with mannitol or abscisic acid and under emergent conditions of differing humidities (from J.E. Keeley, unpubl.). Values are means ± SD (number of samples in parentheses)

	Titratable acidity (mmol H$^+$ kg^{-1} FW)		Malate (mmol kg^{-1} FW)		
	Dusk	Dawn	Dusk	Dawn	
Submerged:					
Control	16±11	143±25	14±5	71±17	(4)
ABA (0.01 mol m^{-3})	10±3	201±48	9±8	97±9	(4)
Mannitol					
80 mol m^{-3}	18±16	38±13	29±22	45±19	(3)
120 mol m^{-3}	3±1	6±5	1±1	12±12	(3)
240 mol m^{-3}	7±2	7±3	3±2	15±8	(3)
Emergent:					
RH < 65%	6+11	43±15	2±2	29±11	(4)
RH > 90%	16±11	277±25	5±3	128±13	(4)

above 4000 m in the Andes of South America (Keeley et al. 1994). These species are unique in that they lack stomata, and thus CAM seems to play a role in the recycling of CO_2 in these species hermetically sealed from the atmosphere.

18.6 Carbon-Isotope Discrimination

Carbon-isotope discrimination by aquatic CAM species is indistinguishable from co-occurring species of other photosynthetic pathways (Table 18.6). In general CAM species are more likely to be similar to C_3 species in the same habitat than to CAM species in other habitats. The factors accounting for this pattern are described more fully in Keeley and Sandquist (1992), but are mainly related to the fact that isotope ratios are markedly affected by the signature of the source carbon and by the fact that discrimination by carboxylases may be eliminated by diffusional resistances in the aquatic environment.

Table 18.6. Carbon-isotope ratios ($\delta^{13}C$) and discrimination (Δ) in aquatic plants (assumes free CO_2 is the only source of inorganic carbon) and emergent terrestrial (terr.) forms of two species (data from Keeley and Sandquist 1992). Values of Δ were calculated as described in Chapter 15 [Eq. (15.1), p. 239]

		$\delta^{13}C$ (‰)		
		Plant	Source	Δ (‰)
Fontinalis antipyretica	C_3	−26.9	−11.5	15.8
Isoetes bolanderi	CAM	−25.1	−11.5	14.0
Plagiobothrys undulatus	C_3	−27.4	−20.4	7.2
Isoetes howellii	CAM	−28.4	−20.4	8.2
	C_3 (terr.)	−29.4	−8.0	21.5
Orcuttia viscida[a]	C_4	−19.0	−11.0	8.2
	C_4 (terr.)	−12.9	−8.0	4.9

[a] The species shows a ^{14}C-labelling pattern in the light suggestive of C_4 photosynthesis but lacks Kranz anatomy (J.E. Keeley, unpubl.).

18.7 Conclusions

CAM photosynthesis is one of the more remarkable examples of evolutionary convergence in the plant kingdom. Crassulacean acid metabolism is an evolutionary response to the stress of daytime CO_2 limitation. This comes about in arid habitats by daytime stomatal closure (selected to reduce water loss), whereas in aquatic habitats it comes about because ambient factors such as low carbon and high diffusional resistances limit the plant's access to CO_2. Terrestrial and aquatic CAM plants share the feature that both are largely restricted to relatively oligotrophic sites where there is a premium on surviving abiotic stresses. These oligotrophic sites limit plant growth, and thus there is little likelihood that the restricted growth rates of CAM plants will make them susceptible to competitive exclusion by neighbouring plants.

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