The adaptive radiation of photosynthetic modes in the genus *Isoetes* (Isoetaceae)

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SUMMARY

1 Crassulacean Acid Metabolism (CAM) involves the capture of ambient CO_2 at night and the daytime release of that CO_2 within the mesophyll cell. Two very different ecological settings have selected for this ability: particular xeric environments where daytime stomatal closure limits CO_2 availability and particular aquatic habitats where ambient CO_2 levels are limiting to photosynthesis.

2 Amphibious species of *Isoetes* in seasonal pools have a well developed CAM pathway biochemically identical to that found in xeric adapted succulents. Studies of these pools show that free-CO_2 levels range from 0·25–0·70 mol m^{-3} at night and by noon on most days CO_2 is depleted in these waters. *Isoetes howellii* does not take up bicarbonate and therefore carbon uptake rates in this species are highly correlated with free-CO_2 levels in the water. Total daytime carbon uptake ranges from 40–70 μmol mg^{-1} Chl whereas night-time malic acid accumulation is commonly double that level; carbon uptake at night accounts for up to half of the malic acid accumulation and the remainder apparently is due to refixation of respiratory CO_2. Starch levels drop substantially overnight, but early in the season the diel fluctuation in starch is insufficient to account for all of the substrate for malic acid production. It is suggested that early in the season CAM is dependent upon the large starch stores in the corms.

3 Few of the other species with which amphibious *Isoetes* coexists in densely vegetated seasonal pools possess CAM. It is suggested that since night-time capture of carbon is of selective value as a means of avoiding daytime competition for carbon, this advantage would be greatly reduced if all plants in these pools were competing for the same night-time carbon pool.

4 As the water table drops and these amphibious *Isoetes* are exposed to the atmosphere they loose all capacity for CAM. This is consistent with the hypothesis that CAM was selected for by the carbon limitations of this particular aquatic environment.

5 These characteristics are contrasted with *Isoetes* species from other habitats. Many species in the genus are lacustrine and thus they are permanently submerged. These species also have a well developed CAM pathway. However, the soft water oligotrophic lakes to which these species are restricted are very different environments from the seasonal pool habitats of amphibious species. Carbon levels in the water do not fluctuate dielly and thus overnight levels are no higher than daytime levels. These environments are characterized by being at the extreme end

in total inorganic carbon for aquatic habitats. It is hypothesized that due to the low carbon conditions and high diffusive resistance of water, the macrophytes in these environments are carbon limited and that capturing carbon at night through CAM effectively doubles the total time available for carbon uptake. Studies of such species show that CAM accounts for half of the total carbon budget.

6 While lacustrine and amphibious *Isoetes* species are largely restricted to abiotically stressful carbon-limited environments, terrestrial species have radiated into seasonal drought environments. Two very different syndromes are evident: summer deciduous C_3 species of low-elevation temperate latitudes, and evergreen high-elevation tropical species which lack stomata and are hermetically sealed from the air and obtain the bulk of their carbon from the sediment. The former species have no capacity for CAM whereas the latter do, although quantitatively it does not appear to contribute substantially to the carbon budget of such species.

INTRODUCTION

Isoetes is a worldwide genus of more than 150 taxa most of which are either amphibious or lacustrine (Pfeiffer 1922; Tryon & Tryon 1982). A particularly intriguing aspect of these species is the presence of Crassulacean Acid Metabolism (CAM) (Keeley 1981a), a photosynthetic pathway commonly associated with terrestrial xerophytes. This phenomenon was initially discovered in the amphibious species *Isoetes howellii* Engelm, of western North America. Here I will focus on: (i) the evidence for CAM in this amphibious species; (ii) the adaptive significance of CAM in amphibious *Isoetes*; (iii) a survey of the photosynthetic characteristics of other species in the genus, distributed in a range of environments from aquatic to terrestrial habitats.

EVIDENCE FOR CRASSULACEAN ACID METABOLISM

The discovery of CAM in an aquatic species was unexpected and some investigators have been reluctant to accept it, often referring to it as CAM-like or coining new acronyms such as AAM for aquatic acid metabolism (Cockburn 1983). Much of the confusion is semantic and revolves around differing definitions as well as inherent problems in applying typological concepts to natural phenomenona.

Kluge & Ting (1978) suggested that for plants with 'true' CAM metabolism, stomatal opening occurs at night rather than during the day. While this definition is conceptually useful it does raise problems in classifying many species, not only aquatic macrophytes and some epiphytic orchids which lack stomata on photosynthetic tissues, but also many terrestrial species as well. Few terrestrial CAM plants show abrupt and complete stomatal closure throughout the day; normally species which obtain the bulk of their CO_2 during the night are considered 'good' CAM plants. However, across the range of terrestrial species with CAM, there is continuous variation with respect to the proportion of light CO_2 uptake versus dark

CO_2 uptake which contributes to the net carbon gain. Terms such as CAM-idling, CAM-C_3 intermediates, CAM-cycling, C_3-idling etc. have been coined to catagorize this variation.

In light of this inherent variation, Teeri (1982) has suggested that the minimum characteristics necessary to consider a plant CAM are that the 'photosynthetic cells have the ability to fix CO_2 in the dark via PEP carboxylase, forming malic acid which accumulates in the vacuole. During the following light period the malic acid is decarboxylated, and the CO_2 enters the PCR cycle in the same cell'.

For plants with CAM, as defined by Teeri, the primary question is: what does CAM contribute to the carbon budget of the plant?

Isoetes howellii

The conclusion that Crassulacean Acid Metabolism is present in *I. howellii* is based on the following: (i) dark CO_2-fixation occurs in photosynthetic tissues but not in corms; (ii) malic acid accumulates in these tissues overnight; (iii) there is a diel cycle of night-time acidification/daytime deacidification up to 300 μmol H^+ g^{-1} fresh weight; (iv) PEP carboxylase activity is sufficient to account for observed rates of acid accumulation; (v) PEP carboxykinase (ATP dependent) activities are sufficient to account for decarboxylation of malic acid (malate enzyme levels are low); (vi) carbon released from malic acid in light is incorporated in PGA and phosphorylated sugars (Keeley 1981a; Keeley & Bowes 1982; Keeley & Busch 1984; J. E. Keeley unpubl. data).

SIGNIFICANCE OF CAM TO THE AMPHIBIOUS *ISOETES HOWELLII*

In terrestrial CAM plants the contribution of dark versus light CO_2 uptake is regulated by changes in stomatal conductance. Although many amphibious species possess stomata they are typically non-functional while submerged (Sculthorpe 1967). *Isoetes howellii* leaves have stomata but on submerged leaves they remain closed and the aperture is lined with a wax. These leaves have a relatively thin cuticle and it is assumed that gas exchange occurs by diffusion across the epidermis. Consequently, stomatal behaviour does not regulate the contribution of dark versus light carbon uptake, rather it is largely a function of the ambient inorganic carbon conditions.

Isoetes howellii *and its habitat*

This species is distributed throughout western North America and is typically found in low elevational seasonal pools which fill during the winter rains and dry down in summer. Observations suggest that *I. howellii* requires an average time of

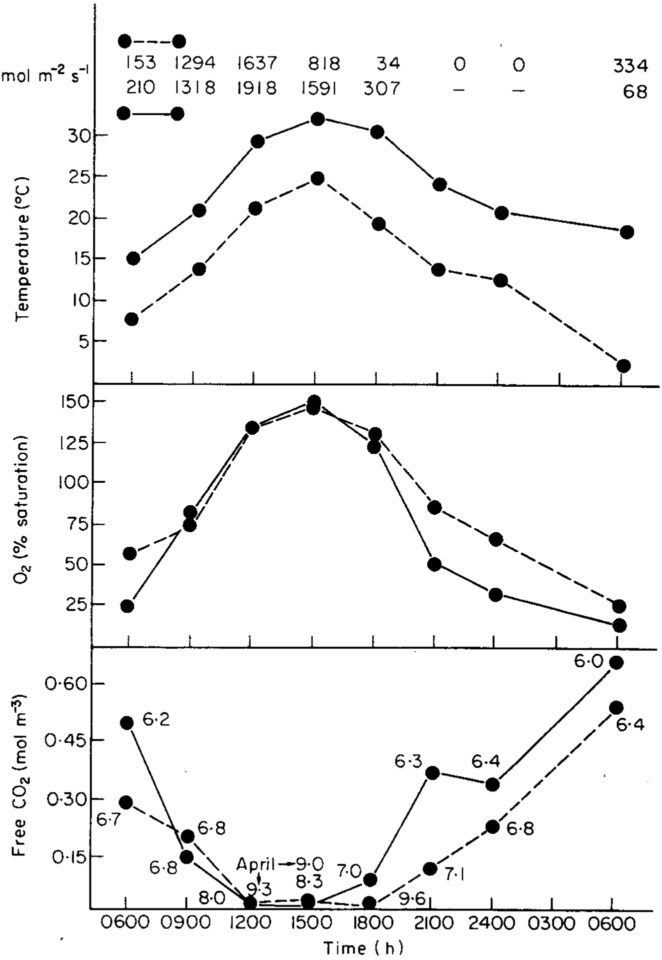

FIG. 1. Diel changes in physical characteristics of a seasonal pool inhabited by *Isoetes howellii* on Mesa de Colorado, Riverside County, California for 13–14 April (dashed line) and 24–25 May (solid line) 1983. pH of the water is indicated adjacent to the line in lower box. Specific conductance was 43 and 101 μmhos cm^{-1} respectively (methods are as described in Keeley (1983) and Keeley & Busch (1984)). Further details of this pool (Cl) and others associated with it are described in Lathrop & Thorne (1983).

inundation of approximately 2 months; it is uncommon in pools filled for a shorter time (Zedler 1984) or a longer time. Such pools are relatively shallow (20–40 cm depth) and densely vegetated.

Physical characteristics for one such pool are shown in Fig. 1. Diel patterns typically show highest CO_2 levels early in the morning. As light and temperature increase through the morning the photosynthetic demand for carbon by the total pool flora, coupled with lower solubility, results in a rapid reduction in free-CO_2. Circumstantial evidence that the CO_2 depletion is a result of photosynthetic consumption is the observation of a highly significant negative correlation between

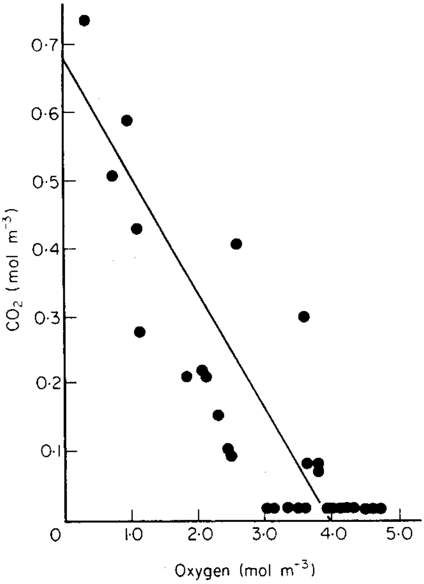

FIG. 2. Relationship between CO_2 and O_2 concentrations in the vernal pool described in Fig. 1, from 06.00 to 18.00 h on several days throughout the spring growing season of 1983. $r = -0{\cdot}89$; $r^2 = 0{\cdot}79$; $n = 32$.

CO_2 and O_2 levels in the water throughout the daylight hours (Fig. 2). During much of the day the water is supersaturated with O_2 and depleted of free-CO_2. Overnight conditions are reversed due largely to respiration by the pool flora and invertebrate fauna.

The magnitude of these diel changes is far greater than the seasonal changes, although some patterns are evident. In late spring, peak light levels and temperatures are greater and the rate at which CO_2 is depleted through the day is greater. The inorganic carbon chemistry also changes through the season (Table 1). Early in the spring a much greater proportion of the total carbon is as bicarbonate and carbonate. Later in the season, although the total pool of inorganic carbon remains unchanged, a much greater proportion is as free-CO_2. Since, throughout

TABLE 1. Comparison of carbon conditions in early April and late May of 1983 for seasonal pool shown in Fig. 1

	Hour	pH	Free CO_2 (mol m^{-3})	Alkalinity (mol m^{-3} as $CaCO_3$)
April 13				
	06.00	6·7	0·277	0·316
	12.00	9·3	0·000	0·272
May 24				
	06.00	6·2	0·508	0·184
	12.00	8·0	0·009	0·167

the season, free CO_2 is largely depleted by noon, the total inorganic carbon during the afternoon is much less in late spring than in early spring.

As a consequence of the diel patterns, ambient conditions for photosynthesis are poor throughout a good portion of the day; with very little free-CO_2 in the water, photosynthesis requires uptake of bicarbonate ions. Disadvantages to bicarbonate uptake are that it is an active uptake process and therefore is energetically costly, and that the affinity for bicarbonate ions is lower than for CO_2 (Wetzel & Grace 1983). Additionally the high ambient O_2 levels during much of the day suggest that elevated internal CO_2 levels will be required to overcome photorespiratory competition. Advantages to carbon uptake at night are centered around the fact that CO_2 levels at night are equal to or greater than the total inorganic carbon levels available during much of the day.

Carbon uptake rates for *Isoetes howellii* are closely tied to the changes in ambient CO_2 levels (Fig. 3). Peak rates occur early in the morning (through the C_3 cycle, Keeley 1981a) but are short-lived. Throughout the season of the study reported in Fig. 3 there was a highly significant correlation ($r = 0{\cdot}91$, $P < 0{\cdot}01$, $n = 29$) between carbon uptake rates in the light and free-CO_2 levels in the water. Dark CO_2 uptake rates are relatively low in the early evening but increase as free-CO_2 increases in the pool; the correlation between these two parameters is less than for the daytime ($r = 0{\cdot}58$, $P < 0{\cdot}01$, $n = 24$). Part of the reason for this may lie in the observation that dark CO_2 uptake rates always declined between midnight and 06.00; high internal acid levels near the end of the dark period may play a role in dampening dark CO_2 uptake as is the case in terrestrial CAM plants (Kluge & Ting 1978). An alternative explanation, however, may be that PEP substrate levels are limiting. This is suggested by the observation that although starch levels undergo a large drop overnight they are typically less than the moles of glucose equivalents required for the malic acid accumulation (Keeley 1983a). It is unknown whether other carbohydrates are involved or whether carbon is transported from the corms.

Total carbon uptake over the two 24-hour periods shown in Fig. 3 ranged from 64 μmol mg^{-1} Chl early in the season, to double that level late in the season (Table 2). In general, dark CO_2 uptake contributed between 1/3 and 1/2 of the total carbon gained, and dark uptake exceeded the contribution due to light uptake during the daylight period from 09.00 to 18.00 h. In short, there is a brief 'window' of time at the beginning of each day when ambient conditions lead to relatively high (C_3) photosynthetic rates. As ambient CO_2 levels become limiting to photosynthesis, malic acid stores are used up, thus maintaining elevated CO_2 levels in the cell. Evidence for environmental control of deacidification is provided by experimental manipulation of the ambient CO_2 levels (Table 2); lower ambient CO_2 levels lead to more rapid deacidification in the leaves.

Crassulacean Acid Metabolism plays a major role in the carbon economy of *Isoetes howellii*. Not only does it account for a large percentage of the gross carbon gain but apparently it is important in recapturing respiratory CO_2. Under CO_2 levels typically found at night in these pools, *I. howellii* shows no net CO_2 evolution

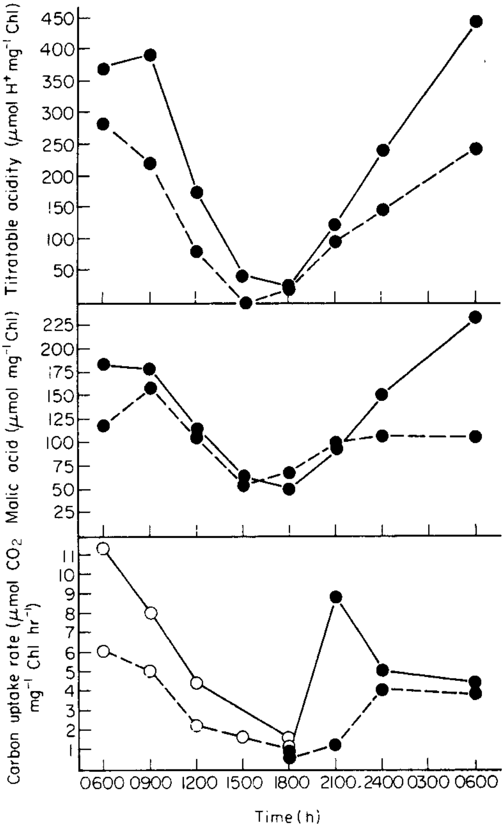

FIG. 3. Diel changes in titratable acidity (to pH 6·4), malic acid and carbon uptake measured by ^{14}C incorporation for leaves of *Isoetes howellii* in the pool described in Fig. 1 for 13–14 April (dashed line) and 24–25 May (solid line) (methods as described in Keeley & Busch 1984).

in the dark (Keeley & Bowes 1982). Additionally, malic acid accumulation is consistently larger than can be accounted for by overnight CO_2 uptake (Table 4). I interpret this to mean that a large portion of the malic acid production is derived from refixation of respiratory CO_2. Experimental manipulations support this interpretation (Table 5); leaves sparged overnight with CO_2-free gas still accumulated substantial levels of malic acid, about half of the level under the 1% CO_2 treatment or observed in the field. It appears that early in the season this

TABLE 2. Total 24 h carbon assimilation for *I. howellii* leaves in southern California seasonal pool estimated from carbon uptake curves in Fig. 3

	Total 24 h carbon uptake (μmol mg^{-1} Chl)	% due to dark uptake (18.00–06.00 h)	% due to light uptake (06.00–09.00 h)	(09.00–18.00 h)
13–14 April	64·1	39	26	35
24–25 May	126·5	47	26	27

TABLE 3. Daytime deacidification rates in *I. howellii* leaves sparged from 06.00 to 09.00 h with either 1% CO_2 or CO_2-free gas at two light intensities; high light = constant 1000 μmol m^{-2}s^{-1} and low light = a stepwise increase of 100 (06.00–07.00 h), 300 (07.00–08.00 h), and 500 (08.00–09.00 h) μmol m^{-2}s^{-1} (treatments were compared with the Student's *t*-test)

	Malic acid (μmol mg^{-1} Chl)	
	High light $\bar{X}$ ± SD (n)	Low light $\bar{X}$ ± SD (n)
06·00	147 ± 9 (3)	147 ± 33 (33)
09·00		
Sparged with 1% CO_2 (21% O_2)	112 ± 7 (3)	90 ± 11 (3)
Sparged with CO_2-free gas (21% O_2)	75 ± 13 (3)	59 ± 9 (3)
P	<0·01	<0·01

TABLE 4. Seasonal comparison between carbon uptake, malic acid fluctuation and starch fluctuation (μmol mg^{-1} Chl) in *Isoetes howellii* leaves (calculated from data presented in Fig. 3 except starch levels are from Keeley (1983) and are from a different year)

	Carbon uptake in the light	Carbon uptake in the dark	Diurnal malic acid fluctuation	Diurnal fluctuation (as glucose equivalents)
April	39	25	112	32
May	68	59	122	144

TABLE 5. Overnight changes in titratable acidity (to pH 6·4) and malic acid in *I. howellii* leaves sparged from 18.00 to 06.00 h with either CO_2-free gas or 1% CO_2 gas (treatments were compared with the Student's *t*-test)

Hour	Titratable acidity (μmol H$^+$ mg^{-1} Chl) $\bar{X}$ ± SD (*n*)	Malic acid (μmol mg^{-1} Chl) $\bar{X}$ ± SD (*n*)
18.00	9 ± 3 (6)	45 ± 6 (6)
06.00		
Sparged overnight with 1% CO_2 gas (21% O_2)	334 ± 67 (6)	161 ± 34 (6)
Sparged overnight with CO_2-free gas (21% O_2)	162 ± 30 (6)	334 ± 70 (6)
P	<0·01	<0·01

overnight malic acid production is fed by starch reserves in the corm as the diel fluctuation in starch is insufficient to account for the level of acid accumulated (Table 4).

Photosynthetic characteristics of associated species

Throughout its range *Isoetes howellii* coexists with a variety of other species. One such community is shown in Table 6. Although vegetation zonation in seasonal pools has not been studied in great detail there are some clear differences between the centre and the periphery. Both sections had 70% total coverage but the number of species and composition varied; there were fifteen species in the centre portion whereas towards the periphery, due to the occurrence of a number of grassland elements, there were twenty-three species.

TABLE 6. Representative cover values for centre and periphery of southern California seasonal pool on Mesa de Colorado (Riverside County) and overnight changes in titratable acidity and malic acid

			Titratable acidity (μmol H^+ g^{-1} FW)		Malic acid (μmol g^{-1} FW)		
	Relative coverage		PM	AM	PM	AM	
Species (Family)	Periphery	Centre	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	(*n*)
Callitriche ssp.[*] (Callitrichicaeae)	2·5	12·8	0 ± 0	2 ± 1	2 ± 1	2 ± 1	(2)
Crassula aquatica (Crassulaceae)	12·4	0·0	9 ± 10	112 ± 9	17 ± 5	53 ± 9	(4)
Downingia cuspidata (Campanulaceae)	4·9	5·7	1 ± 1	0 ± 0	8 ± 4	4 ± 1	(2)
Elatine ssp.[†] (Elatinaceae)	6·2	11·4	1 ± 1	2 ± 1	4 ± 1	10 ± 2	(2)
Eleocharis acicularis (Cyperaceae)	8·6	5·7	3 ± 2	9 ± 4	5 ± 3	6 ± 1	(6)
E. macrostachya	8·6	11·4	(data not available)				
Isoetes ssp.[‡] (Isoetaceae)	2·5	21·4	26 ± 3	275 ± 18	34 ± 2	143 ± 10	(3)
Lilaea scilloides (Lilaeaceae)	2·5	2·9	1 ± 1	1 ± 1	1 ± 1	2 ± 1	(2)
Pilularia americana (Marsileaceae)	9·9	2·9	0 ± 0	0 ± 0	6 ± 3	9 ± 4	(2)
Plagiobothrys undulatus (Boraginaceae)	9·9	15·7	0 ± 0	0 ± 0	8 ± 3	10 ± 2	(4)
Ranunculus aquatilis (Ranunculaceae)	0·0	5·7	0 ± 0	3 ± 4	4 ± 1	10 ± 3	(2)
Other species	32·0	4·4					

[*] Cover data includes *C. longipedunculata* and *C. marginata*; only the former was assayed.

[†] Cover data includes *E. californica* and *E. chilensis*; only the latter was assayed.

[‡] Cover data includes *I. howellii* and *I. orcuttii*; data presented is for *I. howellii*, although the two species are indistinguishable in magnitude of acid metabolism (Keeley 1982).

Data for coverage from Kopecko & Lathrop (1975); data for acidity and malic acid from Keeley & Morton (1982); Keeley (1983), unpublished data; nomenclature is according to Munz (1974).

In our survey of plants for Crassulacean Acid Metabolism we have found two other species which show a marked ability for overnight acid accumulation: *Isoetes orcuttii* Engelm. and *Crassula aquatica*. The robust *Isoetes howellii* and diminutive *I. orcuttii* coexist in many pools throughout California and are indistinguishable in CAM characteristics; one study reported 69 and 70 μmol g^{-1} fresh weight, respectively, in overnight malic acid accumulation (Keeley 1981b). Typically *Isoetes* species occupy the deepest (central) parts of pools (Zedler 1984), as is the case for the pool shown in Table 6. In this portion of the pool, *Isoetes* species are the only ones which appear to obtain a major portion of their carbon from CAM. One explanation for the lack of CAM in other species may lie in phylogenetic constraints since CAM is not known in any of the families of species in the centre of the pool (other than *Isoetes*). I support an alternative explanation that community coexistence of a dense cover of all CAM plants would not be likely in seasonal pools, the reason being that the significance of CAM in *I. howellii* is that it avoids daytime competition for carbon by obtaining much of its carbon at night when the rest of the flora is evolving VO_2. The selective advantage for doing this would be greatly reduced if all plants were competing for the same night-time carbon pool. On the surface this sounds like 'group selection' but it need not be. One can imagine that as more species in the pool evolved CAM the selective coefficients for CAM would decrease, making the evolution of it less likely.

Towards the periphery of the pool *Isoetes* spp. are less abundant but the diminutive annual *Crassula aquatica* is the dominant CAM species. Evidence of CAM in this member of the Crassulaceae family is perhaps not suprising. If the aquatic habit was the derived condition in the family, CAM in *C. aquatica* could be a conservative trait which presently is of little adaptive value. Several observations argue against this interpretation. This species does not develop CAM if grown out of water. Also the closely related (nearly indistinguisable) terrestrial *C. erecta*, which grows (only metres away) on elevated ground in nearby rock outcrops, lacks CAM, even if artifically submerged (Keeley & Morton 1982). A few other species in Table 6 showed a very slight overnight increase in malic acid. Similar small diel fluctuations in acidity have been reported for a number of other aquatic macrophytes (Holaday & Bowes 1980; Beer & Wetzel 1981; Keeley & Morton 1982) suggesting the presence of low-level CAM activity; in these cases the contribution of CAM to the carbon economy of the plants in question is probably minor.

The terrestrial part of Isoetes howellii's *life cycle*

When CAM was originally reported in *I. howellii*, a number of colleagues suggested that it was undoubtedly of adaptive value as a means of conserving water after the pools have dried up; a notion which is clearly wrong as CAM is lost by leaves as they emerge (Table 7). This apparently happens on a cell-by-cell basis as the tops of leaves may be metabolically quite different from the bases (Table 7). This loss of

TABLE 7. Overnight changes in titratable acidity and malic acid in submerged and emergent leaves of *I. howellii* from seasonal pool on Mesa de Colorado, Riverside County, California (methods as in Keeley (1983), $n = 3$)

	Titratable acidity (μmol H^+ g^{-1} FW)		Malic acid (μmol g^{-1} FW)		Chlorophyll (mg g^{-1} FW)
	18.00 h	06.00 h	18.00 h	06.00 h	
Conditions	$\bar{X} \pm$ SD	$\bar{X} \pm$ SD	$\bar{X} \pm$ SD	$\bar{X} \pm$ SD	
Submerged	4 ± 1	141 ± 12	21 ± 4	79 ± 9	0·46
Partially submerged					
Submerged bases	3 ± 2	105 ± 28	9 ± 2	61 ± 28	0·16
Emergent tops	3 ± 2	7 ± 3	32 ± 7	39 ± 1	1·14
Emergent					
In moist soil	6 ± 2	12 ± 1	43 ± 4	44 ± 2	0·87
In dry soil	5 ± 2	10 ± 5	44 ± 4	50 ± 8	0·54

CAM upon emergence appears to be cued by changes in the water potential of the leaves (J. E. Keeley, unpubl. data).

Why does CAM not play a dual role, i.e. as a mechanism to alleviate CO_2 depletion in the water, and to alleviate water stress on land? Possibly it is because of the relatively short time for which it survives on land coupled with the very sharp changes in growing conditions. For approximately 2–3 weeks after the leaves are fully emergent the ground is quite moist but then drying and death of the *Isoetes* leaves occurs rapidly. Under these conditions it would be best to shift entirely to C_3 photosynthesis which is capable of much greater rates of carbon gain; emergent *I. howellii* leaves have substantially higher chlorophyll levels, RUBISCO activities and CO_2 uptake rates, than submerged leaves (Keeley & Busch 1984; J. E. Keeley, unpubl. data). This would far outweigh the advantages of remaining CAM and surviving for slightly longer into the summer drought. In all likelihood the same arguments apply to why annual plants, e.g., *Crassula erecta* described above, are seldom CAM.

ADAPTIVE RADIATION IN THE GENUS *ISOETES*

In addition to amphibious habitats, the genus *Isoetes* has radiated into lacustrine and terrestrial environments. A convenient means of dividing species within each of these different habitats is by their evergreen or deciduous habit (Table 8).

Amphibious Isoetes

Amphibious species are defined as ones which occur in environments which alternate between submerged and emergent conditions. This may occur seasonally as is the case for vernal pools or it may occur dielly as in the case of tidal creeks.

Isoetes species which occur in seasonal pools require a period of several months submergence followed by a period of drying, during which they survive as dormant underground corms or spores. Commonly such species are collected after the

TABLE 8. Habit and habitat characteristics for species of *Isoetes* (including stylites)

Habit	Lacustrine		Amphibious species		Terrestrial species	
	Winter deciduous (>5 taxa)	Evergreen (>20 taxa)	Summer deciduous (>25 taxa)	Evergreen (>3 taxa)	Summer deciduous (>5 taxa)	Evergreen (>4 taxa)
Distribution						
Latitude	Temperate and boreal	All latitudes	Temperate and tropical	Temperate and tropical	Temperate	Tropical
Elevation	>3000 m	Sea-level to 5000 m	Sea-level to 3000 m	Sea-level	<1000 m	>3600 m
Habitat	Soft water oligotrophic lakes	Soft water oligotrophic lakes	Seasonal pools	Freshwater tidal creeks	Borders of rock outcrops	Seasonally wet bogs
Abiotic stress	High due to very low inorganic carbon levels	High due to very low inorganic carbon levels	High due to summer drought	High (?) due to diurnal submergence	High due to summer drought	High due to 'summer' drought and 'winter' flooding
Biotic stress	Low (usually sparsely vegetated)	Low (usually sparsely vegetated)	High (dense vegetation produces CO_2 limitation)	Low (sparsely vegetated)	Low (sparsely vegetated	High (densely vegetated)
Functional attributes						
Photosynthesis	CAM contribution substantial	CAM contribution substantial	CAM contribution substantial	CAM contribution may be substantial	CAM not detectable	CAM present
Source of ambient carbon uptake	Largely leaves, perhaps 1/3 root contribution	Largely leaves, perhaps 1/3 root contribution	Largely leaves, some root contribution	?	Largely (if not entirely) leaves	Largely (if not entirely) roots
Structural attributes						
Stomata	Absent but inducible under aerial conditions	Absent but inducible under aerial conditions	Present but non-functional when submerged	Present but behaviour unknown	Present (presumably functional)	Absent
Cuticle	Present but thin	Present but thin	Present but thin	Present but thin	Present	Present and thick
Leaf cross-sectional shape	Terete to quadrangular	Terete to quadrangular	Terete to quadrangular	Terete to quadrangular	Quadrangular	Triquetrous
Four lacunal chambers	Present	Present	Present	Present	Present	Present
Root:shoot ratio	≥1	≥1	≤1	?	<1 ?	>>>1

water-table has dropped, when they are more conspicuous, and thus they are commonly described in the literature as 'terrestrial' (cf. Keeley 1984). However, submergence is a critical part of their life cycle in that it provides a form of refuge from non-aquatic competitors; likewise the drying-down of the pool provides a refuge from true-aquatic competitors which dominate permanent bodies of water. Seasonal pools occur wherever shallow basins form over a relatively impervious substrate, in regions of seasonal rainfall. Consequently, amphibious *Isoetes* occur throughout the world: e.g., *I. lithophila* Pfeiffer in seasonal pools on granite outcrops in Texas, U.S.A. or *I. australis* Williams in similar habitats in Western Australia, *I. abyssinica* Chiov. in shallow pools of central Africa, *I. panamensis* Max. & Mort. in lowland Neotropics or *I. hystrix* Bory & Dur. in southern Europe. The first two are known to be CAM species (Keeley 1982, 1983c) and I presume all such species will prove to be similar.

Tidal creek species are submerged at high tide and emergent at low tide. They do not have a dormant season and thus are evergreen. Species such as *I. echinospora* ssp. *maritima* (Underw.) Love in British Columbia or *I. riparia* Engelm. on the eastern seaboard of the U.S.A. occur far enough upstream in tidal creeks to be considered freshwater species and they possess a well developed CAM pathway when submerged (Keeley 1982). The South American tidal species *I. clavata* U. Weber is described as occurring in salt water (Hickey 1985), a report which needs confirmation since such conditions would most likely preclude CAM photosynthesis.

Lacustrine Isoetes

Lacustrine species are those that normally spend their entire lifecycle submerged, and by and large are restricted to softwater oligotrophic lakes. The distribution of these species, as well as the conditions which promote low productivity lakes, follows a pattern in which elevational distribution is inversely related to latitude. Lacustrine *Isoetes* may be at sea-level in boreal latitudes but in the tropics they are normally restricted to >3000 m.

In temperate latitudes the growing conditions for these lacustrine *Isoetes* are normally cut short during the winter. Most species remain evergreen and some, such as *I. macrospora* Duriev., even remain photosynthetically active under winter ice cover (Boylen & Sheldon 1976). The exact number of taxa which are deciduous is unknown but *I. bolanderi* Engelman from high elevations in California has been reported to be deciduous in winter (Keeley, Walker & Mathews 1983), probably due to the very high winter snow pack covering lakes in that region.

Oligotrophic lakes occupied by *Isoetes* species are typically sparsely vegetated compared to habitats occupied by amphibious species. Also the physical conditions are much different than conditions in low elevation seasonal pools (Table 9). There is no large diel fluctuation in free-CO_2 level, and therefore it is initially suprising that lacustrine *Isoetes* species have Crassulacean Acid Metabolism of the same order of magnitude as observed for amphibious species such as *I. howellii* (Keeley,

TABLE 9. Seasonal pattern of pH, free-CO_2 and alkalinity of Siesta Lake in the Sierra Nevada, California (elevation 2440 m) inhabited by *Isoetes bolanderi* (data from Keeley, Walker & Mathews 1983)

	pH		Free CO_2 (mol m^{-3})		Alkalinity (mol m^{-3} as $CaCO_3$)	
	06.00 h	12.00 h	06.00 h	12.00 h	06.00 h	12.00 h
16 June	6·2	5·7	0·116	0·150	0·078	0·040
1 July	6·8	6·6	0·114	0·086	0·156	0·098
27 July	6·6	6·2	0·099	0·052	0·122	0·136
18 August	6·2	6·6	0·100	0·109	0·130	0·136
5 September	6·6	6·6	0·111	0·073	0·100	0·100

Walker & Mathews 1983; Boston & Adams 1983; Richardson *et al.* 1983). For aquatic habitats, however, these oligotrophic lakes are at the extreme end in terms of total inorganic carbon. I suggest that due to low carbon levels and the high diffusive resistance of water, the macrophytes in these environments are carbon limited and that capturing carbon at night through CAM effectively doubles the total time available for carbon uptake. Detailed studies on *I. macrospora* from Wisconsin lakes indicate that CAM contributes 45–50% of the annual carbon gain (Boston & Adams 1985).

Lacustrine *Isoetes* species will frequently form nearly pure stands or coexist with another CAM species such as *Littorella* species (Plantaginaceae) (Keeley & Morton 1982; Boston & Adams 1985) in temperate latitudes or with *Crassula (Tillaea)* species in tropical latitudes (J. E. Keeley, unpubl. data). This is quite different from the pattern observed in seasonal pools where CAM species do not normally coexist in close association with other CAM species. This pattern, however, is consistent with the hypotheses proposed above for CAM in these two environments. In oligotrophic lakes the major advantage to CAM is not that there is a large biogenic source of CO_2 at night but that CAM doubles the time available for uptake. This, coupled with the dependence upon sediment CO_2 uptake through the roots (Richardson *et al.* 1983), suggests that night-time competition for carbon by other CAM species is likely to be less intense than in seasonal pools.

Terrestrial Isoetes

All terrestrial *Isoetes* species possess four lacunal chambers which run the length of the leaf, a feature uncommon in terrestrial plants and one which suggests an aquatic ancestry.

At low elevations in temperate latitudes these are several species of *Isoetes* which are strictly terrestrial. These species are restricted to seasonally moist sites and all are deciduous during the summer drought; examples include *I. nuttallii* A. Br. ex. Engelm. from western North America, *I. butleri* Engelm. from southeastern North America and *I. durieui* Bory from Europe. These species are not CAM and CAM can not be induced even if they are artificially submerged (Keeley 1983b).

At extremely high elevations in tropical latitudes there are several terrestrial *Isoetes* species restricted to seasonally wet bogs. These species include *I. andicola*

Amstutz (formerly *Stylites a.*) of the central Andes of South America, *I. andina* Hook and *I. novo-granadensis* Fuchs of the northern Andes and *I. hopei* Croft from Papua New Guinea. All are characterized by having fibrous, astomatous leaves with a thick cuticle, and most of the leaf length occurs below ground level, only the tips (which often have spines) being green and aerial. These photosynthetic surfaces however are hermetically sealed from the atmosphere and, as shown for stylites, the bulk of the carbon for photosynthesis is derived from the sediment (Keeley, Osmond & Raven 1984). This species has some capacity for CAM but it does not appear to contribute substantially to the carbon economy of the plant.

The explanation for this unusual mode of carbon nutrition is unknown. These species may represent a line of early land plants which failed to evolve stomata and thus gas exchange with the sediment (with its much greater diffusional resistances) being the price for avoiding dessication on periodically dry terrestrial sites. This hypothesis is not supported by cladistic analysis which suggest that the evolution of this syndrome in the above species was polyphyletic (Hickey 1985). A more likely hypothesis is that there are unique features of these tropical seasonal bogs at high elevations which have selected for this strategy. This problem is the major focus of my current research.

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