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I. Abstract

Crassulacean acid metabolism (CAM) is a CO_2-concentrating mechanism selected in re-ıonse to aridity in terrestrial habitats, and, in aquatic environments, to ambient limitations of ırbon. Evidence is reviewed for its presence in five genera of aquatic vascular plants, includ-g *Isoëtes*, *Sagittaria*, *Vallisneria*, *Crassula*, and *Littorella*. Initially, aquatic CAM was con-dered by some to be an oxymoron, but some aquatic species have been studied in sufficient ɜtail to say definitively that they possess CAM photosynthesis. CO_2-concentrating mecha-sms in photosynthetic organs require a barrier to leakage; e.g., terrestrial C_4 plants have su-ɜrized bundle sheath cells and terrestrial CAM plants high stomatal resistance. In aquatic ᴀM plants the primary barrier to CO_2 leakage is the extremely high diffusional resistance of ater. This, coupled with the sink provided by extensive intercellular gas space, generates ıytime $CO_2(p_i)$ comparable to terrestrial CAM plants. CAM contributes to the carbon budget y both net carbon gain and carbon recycling, and the magnitude of each is environmentally ıfluenced. Aquatic CAM plants inhabit sites where photosynthesis is potentially limited by ırbon. Many occupy moderately fertile shallow temporary pools that experience extreme iel fluctuations in carbon availability. CAM plants are able to take advantage of elevated ighttime CO_2 levels in these habitats. This gives them a competitive advantage over non-AM species that are carbon starved during the day and an advantage over species that expend ıergy in membrane transport of bicarbonate. Some aquatic CAM plants are distributed in ighly infertile lakes, where extreme carbon limitation and light are important selective fac-ırs.

Compilation of reports on diel changes in titratable acidity and malate show 69 out of 180 ɔecies have significant overnight accumulation, although evidence is presented discounting AM in some. It is concluded that similar proportions of the aquatic and terrestrial floras have volved CAM photosynthesis. Aquatic *Isoëtes* (Lycophyta) represent the oldest lineage of AM plants and cladistic analysis supports an origin for CAM in seasonal wetlands, from hich it has radiated into oligotrophic lakes and into terrestrial habitats. Temperate Zone ter-ɜstrial species share many characteristics with amphibious ancestors, which in their tempo-ıry terrestrial stage, produce functional stomata and switch from CAM to C_3. Many lacustrine *soëtes* have retained the phenotypic plasticity of amphibious species and can adapt to an aer-ıl environment by development of stomata and switching to C_3. However, in some neotropi-al alpine species, adaptations to the lacustrine environment are genetically fixed and these onstitutive species fail to produce stomata or loose CAM when artificially maintained in an erial environment. It is hypothesized that neotropical lacustrine species may be more ancient n origin and have given rise to terrestrial species, which have retained most of the character-stics of their aquatic ancestry, including astomatous leaves, CAM and sediment-based carbon ıutrition.

Resumen

El metabolismo ácido Crasulacea (CAM) es un mecanismo concentrador de CO_2 seleccionado en respuesta a la aridez de hábitats terrestres, y, en ambientes acuáticos, a limitaciones de carbono en el medio. Se revisa la evidencia para su presencia en cinco géneros de plantas vasculares acuáticas, incluyendo *Isoëtes*, *Sagitteria*, *Vallisneria*, *Crassula* y *Littorella*. Inicialmente, el CAM acuático era considerado absurdo, pero algunas especies han sido estudiadas a detalle suficiente para determinar definitivamente que poseen fotosíntesis CAM. Los mecanismos concentradores de CO_2 en órganos fotosintéticos requieren de barreras contra la fuga del mismo; por ejemplo, plantas terrestres C_4 tienen células con una capa de cera y las plantas terrestres CAM poseen una alta resistencia en los estomas. En las plantas acuáticas la principal barrera para la fuga de CO_2 es la resistencia a la difusión extremadamente alta del agua. Esto, junto con el resumidero proporcionado por el amplio espacio gaseoso intercelular, genera $CO_2(p_i)$ diurno comparable a plantas terrestres CAM. CAM contribuye al presupuesto de carbono tanto por la ganancia neta de carbono como por su reciclaje, la magnitud de cada componente está influida por el ambiente. Las plantas CAM acuáticas habitan en sitios donde la fotosíntesis está potencialmente limitada por carbono. Muchas ocupan piscinas temporales poco profundas y moderadamente fértiles, que experimentan fluctuaciones diálicas extremas en la disponibilidad de carbono. Las plantas CAM son capaces de aprovechar los altos niveles nocturnos de CO_2 en estos hábitats, potencialmente adquiriendo una ventaja competitiva sobre las plantas no poseedoras de CAM, las cuales sufren la falta de carbono durante el día, o sobre las especies que utilizan energía en el transporte de bicarbonato a través de membranas. Otras plantas CAM acuáticas se encuentran distribuidas en lagos altamente infértiles, en los que la limitación extrema de carbono y luz son factores de selección importantes.

La compilación de reportes sobre cambios diálicos en ácido titulable y malato muestran que 69 de 180 especies tienen una acumulación nocturna significativa, aunque la evidencia es presentada descontando CAM en algunos casos. Se concluye que proporciones similares de las floras terrestres y acuáticas han evolucionado fotosíntesis CAM. *Isoëtes* acuática (Lycophyta) representa el linaje más antiguo de plantas CAM, y el análisis cladístico apoya la idea del origen de CAM en humedales estacionales, de donde radiaron a lagos oligotróficos y a hábitats terrestres. Las especies terrestres de zonas templadas comparten muchas características con sus ancestros anfibios, las cuales en su estado terrestre temporal producen estomas funcionales y cambian de CAM a C_3. Muchas *Isoëtes* lacustres han retenido la plasticidad fenotípica de especies anfibias y pueden adaptarse a una ambiente aéreo al desarrollar estomas y cambiar a C_3. Sin embrago, en algunas especies neotropicales alpinas, las adaptaciones al ambiente lacustre están determinadas géniticamente y estas especies fallan en producir estomas o perder CAM al mantenerlas artificialmente en un ambiente aéreo. Se presenta la hipótesis que éstas son de origen anterior y han dado lugar a las especies terrestres que retienen la mayorma de las características de su estado ancestral acuático, incluyendo hojas sin estomas, CAM y nutrición de carbono basado en sedimentos.

II. Introduction

Crassulacean acid metabolism—or CAM, as it is commonly known—is one of three recognized photosynthetic pathways. It involves nighttime fixation of carbon, largely into malic acid, which is temporarily stored, followed by daytime incorporation of CO_2—derived from decarboxylation of malate—into the Calvin cycle. The name derives from the substantial diel change in organic acid content of photosynthetic organs and the fact that the pathway was ginally studied in plants of the family Crassulaceae. In terrestrial species CAM is best repre- ted in arid land floras, a fact generally understood to result from the greater water-use effi- ıcy conferred upon plants with this photosynthetic pathway (Kluge & Ting, 1978). Thus, report of CAM in a submerged aquatic plant (Keeley, 1981) was initially met with some pticism.

The diel cycle of overnight acidification, followed by daytime deacidification (here de- ed ΔH^+) of photosynthetic tissues is considered an essential and defining feature of CAM ıtosynthesis (Fig. 1). While ^{14}C-labeling studies show that several dicarboxylic acids are duced during dark CO_2 fixation, malate(malic acid) is considered the primary acid in- ved in autotrophism (Lüttge, 1995). Therefore, I begin with a survey of ΔH^+ and Δmalate orts for aquatic algae and macrophytes. This will be followed by a review of evidence for M in aquatic species with diel acid fluxes and associated ecological and physiological char- ristics, and will conclude with a discussion of the distribution and evolution of aquatic M plants.

III. Diel Acid Changes (ΔH^+) in Submerged Aquatic Plants

The first suggestion of CAM in an aquatic macrophyte was the report of weak acid accumu- on and dark CO_2 fixation in *Hydrilla verticillata* (Holaday & Bowes, 1980), soon followed a report of substantial ΔH^+ and dark CO_2 fixation in *Isoëtes howellii* (Keeley, 1981) [John ven pointed out that Allsopp (1951) earlier reported high acid levels in *Isoëtes*, although sopp did not observe diel changes]. Over the past 15 years there has been a plethora of pub- ıed and unpublished reports on presence and absence of ΔH^+ in aquatic plants (Table I). To e, 180 aquatic species have been tested; 69 species, distributed in 14 genera, have signifi- t overnight accumulation of acids, ranging from 5 to 290 mmol H^+ kg^{-1} fresh mass (FM). comparison, terrestrial CAM plants commonly have ΔH^+ levels <100 and seldom >200 ıol H^+ kg^{-1} FM (Kluge & Ting, 1978; Winter & Smith, 1995a).

Aquatic species in five genera stand out as having acid accumulation that is substantially her than others and within the range of terrestrial CAM plants. These include the spore- ring *Isoëtes* (Lycophyta: Isoetaceae) and flowering plants (Anthophyta), both monocots, *gittaria* (Alismataceae) and *Vallisneria* (Hydrocharitaceae), and dicots, *Crassula* (Crassu- eae), and *Littorella* (Plantaginaceae). In these genera there is further evidence, beyond just ΔH^+ reports, that points to CAM photosynthesis (Section V). The extent to which CAM is plicated in aquatic species with more limited ΔH^+ (Table I), will be discussed in Section X.

Isoëtes (Fig. 2) is the largest genus of aquatic CAM plants, with all 38 aquatic species tested wing substantial ΔH^+ (Table I), with some species exhibiting ΔH^+ levels comparable to the hest levels for terrestrial CAM plants; $\Delta H^+ = 290$ mmol kg^{-1} FM or 62 mmol m^{-2} total leaf a. The *Isoëtes* tested represent a quarter of this worldwide genus (Tryon & Tryon, 1982) l include much of the geographical range and most all aquatic habitats occupied by the up (Section VII). These data suggest that all aquatic species in the genus may prove to be M; there are a few terrestrial species, some of which are not CAM (Section XII.A.1).

Sagittaria comprises about 20 species, largely in the Americas. All are aquatic and four of species tested have substantial ΔH^+ and other characteristics of CAM and two species have -level acid accumulation. *Vallisneria* is a genus of approximately six species, two of ich have significant, although not consistent, ΔH^+. *Crassula* is a genus of more than 200 cies. The vast majority are succulent terrestrial perennials with CAM, and are mostly en- nic to South Africa. A small number of *Crassula* are diminutive annuals, which are distrib- d worldwide and include both aquatics with CAM and terrestrials, which are not CAM

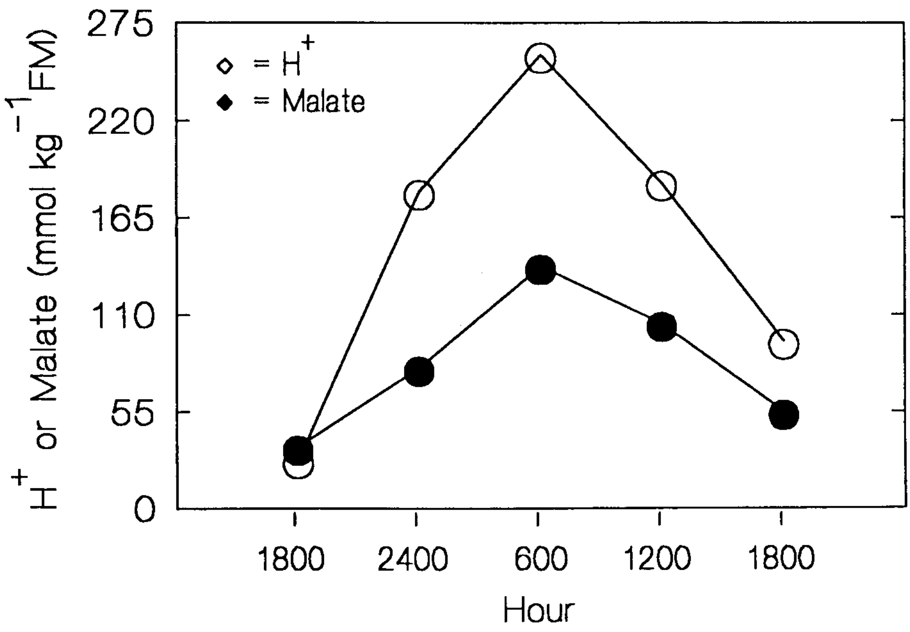

Fig. 1. Diel pattern of changes in H^+ and malate found only in plants with CAM photosynthesis; H^+ = titratable protons at pH 6.4 (a K_{diss} for malate) and FM = fresh mass (Keeley, unpubl. data on *Isoëtes howellii*).

(Section XII.B). *Littorella* includes only three aquatic taxa distributed at high latitudes in Europe, North America, and South America. I agree with those who consider them to be subspecific varieties of *L. uniflora*, and in the remainder of this review I will refer to them simply as "*Littorella*."

IV. Criteria for CAM Photosynthesis

Biochemically, CAM requires nighttime fixation of inorganic carbon catalyzed by the cytoplasmic phosphoenolpyruvate carboxylase (PEPC). In order to be considered an autotrophic process this must be coupled with net uptake of CO_2. The first stable product, malate, is transported across the vacuolar tonoplast as malic acid. During the day it is transported out of the vacuole and CO_2 is released by cytoplasmic and/or mitochondrial decarboxylases, followed immediately by refixation of CO_2 with the chloroplastic ribulose 1,5-biphosphate carboxylase, oxygenase (RUBISCO). All reactions occur within a single photosynthetic cell (Winter, 1985). Criteria for CAM include:

1. Dark fixation of CO_2 via β-carboxylation with malate(malic acid) the first stable product.
2. Overnight storage of malic acid with little metabolism of this product in the dark.
3. Daytime decarboxylation of malic acid, resulting in substantial diel changes in both acidity and malate concentrations.
4. Opposite diel pattern of overnight starch (or sugar) depletion.

(Text continues on p. 127)

Table I

Diel changes (D) in titratable acidity and malate in submerged foliage of aquatic plants and other phototrophs. Duplicate reports on the same species from the same site are not included

						Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD)		Malate (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD)			t-test[k] (2-tailed)	
Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	PM	AM	PM	AM	n	T.A.	M.A.
[CYANOBACTERIAL LICHEN]												
Lichina pygmaeae	30	U.K.	56°N	0	Ml	17 ± 6	18 ± 4	—	—	4	ns	—
CHLOROPHYTA												
Caulerpaceae												
Caulerpa sp.	26	U.S.A.	25°N	0	Ml	5 ± 1	6 ± 2	—	—	4	ns	—
Characeae												
Chara contraria	17	U.S.A.	34°N	610	SP	0 ± 0	0 ± 0[f]	5 ± 4	4 ± 4	4	ns	ns
	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	7 ± 2	8 ± 4	2	ns	ns
Chara hispida	24	U.K.	57°N	—	L	9 ± 1	9 ± 3	—	—	4	ns	—
Codiaceae												
Codium australicum	25	Australia	36°S	0	Ml	3 ± 1	4 ± 1	—	—	4	ns	—
C. fragile	31	U.S.A.	35°N	0	Msl	2 ± 1	3 ± 1	—	—	4	ns	—
Cladophoraceae												
Chaetomorpha coliformis	25	Australia	36°S	0	Ml	4 ± 7	2 ± 2	—	—	4	ns	—
Cladophora glomerata	25	U.K.	56°N	—	R	5 ± 2	6 ± 2	—	—	4	ns	—
C. rupestris	25	U.K.	56°N	—	R	3 ± 2	2 ± 1	—	—	4	ns	—
Cladophoropsis membranacea	10	Bahamas	25°N	0	M	—	—	0	0[d]	1	—	—
Prasiolaceae												
Prasiola stipitata	29	U.K.	56°N	0	Mul	2 ± 1	3 ± 1	—	—	4	ns	—
Ulvaceae												
Enteromorpha linza	24	U.K.	56°N	—	R	2 ± 1	1 ± 2	—	—	4	ns	—
Ulva sp.	24	U.K.	56°N	—	R	14 ± 10	21 ± 11	—	—	4	ns	—
Zygbenataceae												
Spirogyra sp.	18	U.S.A.	34°N	610	SP	0 ± 0	0 ± 0[f]	10 ± 1	7 ± 3	2	ns	ns
PHAEOPHYTA												
Alariaceae												
Alaria esculenta	35	U.K.	56°N	0	Msl	5 ± 3	8 ± 4	—	—	7	ns	—
Ecklonia radiata	28	Australia	36°S	0	Msl	5 ± 1	6 ± 1	5 ± 2	7 ± 2	6	ns	ns

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
Cystoseiraceae												
Halidrys siliquosa	35	U.K.	57°N	0	Msl	5 ± 1	6 ± 1	5 ± 2	7 ± 2	6	ns	ns
Dictyotaceae												
Dictyota dicthtoma	31	U.S.A.	35°N	0	Msl	6 ± 3	6 ± 1	—	—	4	ns	—
Dilophus guineensis	10	Bahamas	25°N	0	M	—	—	0	0[d]	1	—	—
Padina vickersii	31	U.S.A.	35°N	0	Msl	12 ± 6	11 ± 5[g]	—	—	4	ns	—
Durvillaeacea												
Durvillaea potatorum	27	Australia	56°S	0	Ml	12 ± 6	11 ± 5[g]	—	—	4	ns	—
Fucaceae												
Ascophyllum nodosum	35	U.K.	57°N	0	Ml	21 ± 13	34 ± 16	—	—	10	ns	—
	12	U.K.	57°N	0	Ml	23 ± 3	44 ± 8	2 ± 2	6 ± 2	5	**	*
Fucus serratus	35	U.K.	57°N	0	Ml	9 ± 5	16 ± 3	—	—	7	**	—
F. spiralis	35	U.K.	57°N	0	Ml	11 ± 9	20 ± 7	—	—	9	*	—
F. vesiculosus	35	U.K.	57°N	0	Ml	11 ± 7	18 ± 7	—	—	6	ns	—
	32	U.K.	63°N	0	Ml	10 ± 2	21 ± 4	—	—	4	**	—
	31	U.S.A.	35°N	0	Ml	34 ± 4	46 ± 2	—	—	4	**	—
Pelvetia canaliculata	35	U.K.	56°N	0	Ml	4 ± 4	18 ± 7	—	—	9	**	—
Himanthaliaceae												
Himanthalia elongata	24	U.K.	56°N	0	Ml	12 ± 3	27 ± 16	—	—	8	*	—
Hormosiraceae												
Hormosira banksii	25	Australia	36°S	0	Ml	8 ± 2	15 ± 3	—	—	4	*	—
Laminariaceae												
Laminaria digitata	35	U.K.	57°N	0	Msl	5 ± 2	7 ± 2	—	—	6	ns	—
L. hyperborea	35	U.K.	56°N	0	Msl	5 ± 2	6 ± 4	—	—	9	ns	—
L. saccharina	35	U.K.	56°N	0	Msl	6 ± 4	7 ± 3	—	—	7	ns	—
Sargassaceae												
Sargassum filipendula	31	U.S.A.	35°N	0	Msl	6 ± 1	6 ± 1	—	—	4	ns	—
Turbinaria turbinata	10	Bahamas	25°N	0	Msl	—	—	80	63[d]	1	—	—
RHODOPHYTA												
Bangiaceae												
Porphyra purpurea	24	U.K.	56°N	—	R	5 ± 3	8 ± 1	—	—	4	ns	—
Champiaceae												
Lomentaria articulata	11	U.K.	56°N	—	R	1 ± 1	2 ± 1	—	—	4	ns	—

Table 1 (continued)

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	Titratable acidity AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	Malate AM	n	t-test[k] (2-tailed) T.A.	t-test M.A.
Delesseriaceae												
Delesseria sanguinea	11	U.K.	56°N	0	Ml	2 ± 0	2 ± 0	—	—	4	ns	—
Gigartinaceae												
Chondrus crispus	24	U.K.	56°N	—	R	1 ± 1	1 ± 1	—	—	4	ns	—
Lemaneaceae												
Lemanea mamillosa	25	U.K.	56°N	—	R	3 ± 1	3 ± 1	—	—	6	ns	—
Palmariaceae												
Palmaria palmata	11	U.K.	56°N	0	Ml	2 ± 0	2 ± 0	—	—	4	ns	—
Rhodomelaceae												
Laurencia papillosa	10	Bahamas	25°N	0	Ml	—	—	22	35[d]	1	—	—
L. pinnatifida	11	U.K.	56°N	0	Ml	34 ± 5	31 ± 4	—	—	4	ns	—
Polysiphonia lanosa	24	U.K.	56°N	—	R	68 ± 16	78 ± 15	—	—	4	ns	—
BRYOPHYTA												
Fontinalaceae												
Fontinalis antipyretica	18	U.S.A.	38°N	2440	L	0 ± 0	0 ± 0[f]	1 ± 1	1 ± 1	2	ns	ns
	25	U.K.	57°N	—	R	3 ± 1	4 ± 1	—	—	6	ns	—
Fontinalis sp.	13	Ecuador	1°S	4100	L	13 ± 3	13 ± 1	8 ± 3	12 ± 5	3	ns	ns
Hypnaceae												
Amblystegium riparium	18	U.S.A.	38°N	1375	L	0 ± 0	0 ± 0[f]	11 ± 0	14 ± 1	2	ns	ns
Drepanacladus exonnulatus	13	Colombia	4°N	3650	L	17 ± 2	19 ± 4	9 ± 9	12 ± 1	3	ns	ns
LYCOPHYTA												
Isoëtaceae												
Isoëtes australis	14	Australia	32°S	300	SP	16 ± 0	74 ± 0[f]	18 ± 2	46 ± 3	2	**	**
I. bolanderi	21	U.S.A.	38°N	2905	L	12 ± 1	229 ± 19[f]	21 ± 9	123 ± 5	3	**	**
I. boliviensis	13	Bolivia	17°S	4475	SP	14 ± 3	140 ± 25[f]	29 ± 7	75 ± 9	3	**	**
I. boyacensis	13	Colombia	6°N	3700	SP	20 ± 10	55 ± 8[f]	29 ± 5	45 ± 5	4	**	**
I. capensis	13	S. Africa	34°S	250	SP	12 ± 6	49 ± 8[f]	—	—	3	**	—
I. cleefii	13	Colombia	5°N	3700	L	7 ± 8	165 ± 22[f]	22 ± 2	110 ± 4	3	**	**
I. drummondii	14	Australia	35°S	300	SP	24 ± 1	106 ± 11[f]	10 ± 3	49 ± 3	2	**	**

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
I. echinospora ssp. *echinospora*	37	Spain	42°N	2120	L	11 ± 4	135 ± 10[h]	—	—	3	**	—
I. echinospora ssp. *maritima*	14	Canada	50°N	5	FTR	21 ± 7	136 ± 2[f]	19 ± 4	73 ± 3	2	**	**
I. engelmanni	14	U.S.A.	37°N	1160	SP	8 ± 4	52 ± 2[f]	14 ± 1	36 ± 1	2	**	**
I. flaccida	6	U.S.A.	29°N	5	FTR	8 ± 4	48 ± 11[f]	9 ± 2	35 ± 9	2	*	ns
I. glacialis	13	Bolivia	16°S	4450	L	19 ± 1	116 ± 3[f]	48 ± 6	90 ± 6	3	**	**
I. hergzogii	13	Bolivia	16°S	4750	L	32 ± 5	153 ± 17[f]	39 ± 8	82 ± 7	3	**	**
I. howellii	17	U.S.A.	34°N	610	SP	14 ± 11	161 ± 44[f]	44 ± 10	97 ± 26	24	**	**
	20	U.S.A.	38°N	1375	SP	49 ± 3	339 ± 0[f]	27 ± 2	180 ± 1	2	**	**
	20	U.S.A.	44°N	10	L	11 ± 3	114 ± 2[f]	26 ± 2	69 ± 13	2	**	**
I. karstenii	13	Venezuela	9°N	3450	L	22 ± 9	128 ± 25[f]	30 ± 11	77 ± 11	3	**	**
	13	Colombia	4°N	3650	L	16 ± 4	177 ± 8[f]	33 ± 6	115 ± 6	3	**	**
I. killipii	13	Ecuador	0°	3900	L	73 ± 27	231 ± 66[f]	24 ± 8	75 ± 16	3	**	**
I. kirkii	36	New Zealand	39°S	350	L		[79 ± 8][i]		[87][i]	4	—	—
I. lacustris	14	U.K.	53°N	180	L	24 ± 13	108 ± 23[f]	16 ± 8	55 ± 6	2	**	**
	33	U.K.	56°N	—	L	73	162[g]	—	—	2	—	—
	8	U.K.	58°N	100	L	38 ± 17	83 ± 26[f]	—	—	2	ns	—
	2	Finland	61°N	—	L	—	—	29 ± 6	71 ± 20	5	—	**
	22	Denmark	56°N	75	L	25 ± 2	40 ± 4[g]	—	—	4	**	—
	37	Spain	42°N	2120	L	10 ± 4	68 ± 2[g]	—	—	2	**	—
I. lithophila	14	U.S.A.	31°N	555	SP	40 ± 19	163 ± 23[f]	42 ± 17	90 ± 2	2	**	**
I. macrospora	4	U.S.A.	43°N	—	L	21 ± 1	164 ± 9[g]	—	—	3	**	—
I. malinverniana	13	Italy	45°N	300	C	0 ± 0	123 ± 6[f]	22 ± 1	65 ± 6	3	**	**
I. melanopoda	13	U.S.A.	33°N	500	SP	4 ± 2	97 ± 4[f]	17 ± 12	52 ± 2	2	**	**
I. melanospora	13	U.S.A.	33°N	180	SP	0 ± 0	160 ± 21[f]	18 ± 9	99 ± 15	2	**	**
I. mexicana	14	Guatemala	15°N	2850	SP	15 ± 8	8 ± 12[f]	11 ± 1	60 ± 3	2	**	**
I. occidentalis	14	Canada	50°N	200	L	38 ± 15	93 ± 9[f]	25 ± 8	54 ± 3	2	*	**
I. orcuttii	17	U.S.A.	34°N	610	SP	10 ± 6	155 ± 44[f]	27 ± 10	98 ± 25	3	**	**
I. palmeri	13	Colombia	4°N	3650	L	88 ± 13	156 ± 14[f]	38 ± 5	71 ± 17	3	**	**
I. peruvianum	13	Ecuador	0°	4050	SP	80 ± 16	265 ± 29[f]	33 ± 14	79 ± 12	3	**	**
I. piedmontana	13	U.S.A.	33°N	150	SP	0 ± 0	86 ± 11[f]	22 ± 1	58 ± 4	2	**	**
I. riparia	14	U.S.A.	39°N	5	FTR	33 ± 1	117 ± 59[f]	13 ± 3	78 ± 17	2	ns	*
I. savatieri	13	Chile	37°S	100	SP	63 ± 8	267 ± 75	—	—	4	**	—
I. setacea	39	Spain	42°N	2120	SP	19 ± 3	128 ± 12[f]	—	—	3	**	—
I. socia	13	Colombia	4°N	3650	SP	224 ± 12	382 ± 30	42 ± 6	121 ± 31	3	**	*

Table 1 (continued)

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
I. storkii	19	Costa Rica	10°N	2600	L	21 ± 5	171 ± 25[g]	9 ± 3	84 ± 6	4	**	**
I. tegetiformans	14	U.S.A.	34°N	110	SP	51	95[f]	18	58	1	—	—
I. ticlioensis [nom. nud.?]	13	Peru	11°S	4800	L	10 ± 3	59 ± 8[f]	41 ± 6	63 ± 6	3	**	**
I. sp. [unnamed species]	13	Chile	32°S	30	SP	65 ± 16	195 ± 19[f]	33 ± 11	89 ± 12	3	**	**
I. sp. [unnamed species]	13	Chile	37°S	500	SP	18 ± 4	263 ± 7	—	—	4	**	—
I. sp. [unknown species]	13	Venezuela	9°N	3450	SP	15 ± 4	116 ± 15[f]	34 ± 11	76 ± 21	2	**	**
SPHENOPHYTA												
Equisetaceae												
Equisetum bogotense	13	Ecuador	1°S	4100	L	26 ± 3	21 ± 4	20 ± 5	30 ± 2	3	ns	0
PTEROPHYTA												
Marsileaceae												
Marsilea vestita	17	U.S.A.	34°N	610	SP	9 ± 2	13 ± 3	11 ± 5	6 ± 6	6	ns	ns
Pilularia americana	17	U.S.A.	34°N	610	SP	0 ± 0	0 ± 0[f]	4 ± 4	9 ± 4	6	ns	ns
P. globulifera	8	U.K.	58°N	100	L	25 ± 1	12 ± 8[f]	—	—	2	ns	—
ANTHOPHYTA												
Monocotyledoneae												
Alismataceae												
Echinodorus berteroi	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	9 ± 3	3 ± 1	2	ns	ns
Sagittaria cuneata	18	U.S.A.	38°N	2440	L	3 ± 1	10 ± 1[f]	13 ± 4	18 ± 3	2	**	ns
S. graminea	13	U.S.A.	32°N	—	SP	5	10	—	—	1	—	—
S. isotiformis	13	U.S.A.	32°N	—	SP	6	42	—	—	1	—	—
S. teres	13	U.S.A.	42°N	—	SP	6	31	—	—	1	—	—
S. subulata	6	U.S.A.	29°N	5	FTR	6 ± 1	45 ± 1	9 ± 3	30 ± 14	2	**	ns
	13	U.S.A.	29°N	5	FTR	8 ± 3	83 ± 26	10 ± 2	45 ± 10	6	**	**
S. sp.	13	Chile	37°S	500	SP	17 ± 13	41 ± 9	—	—	4	*	—
Cymodoceaeae												
Amphibolis antarctica	28	Australia	36°S	0	M	7 ± 1	7 ± 1	5 ± 1	5 ± 1	6	ns	ns
Halodule wrightii	26	U.S.A.	26°N	0	MI	15 ± 3	17 ± 4	—	—	4	ns	—
Syrindodium filiforme	26	U.S.A.	26°N	0	MI	13 ± 3	10 ± 3	—	—	4	ns	—

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[c] (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
Cyperaceae												
Eleocharis acicularis	17	U.S.A.	34°N	610	SP	24 ± 19	37 ± 24	7 ± 4	13 ± 9	16	ns	ns
	13	U.S.A.	38°N	1375	SP	16 ± 2	36 ± 12[g]	6 ± 2	12 ± 5	4	*	ns
E. maculosa	13	Ecuador	2°S	4100	L	5 ± 5	12 ± 2	30 ± 1	25 ± 1	3	ns	**
E. schlechteri	13	S. Africa	34°S	250	SP	6 ± 2	9 ± 2	—	—	2	ns	—
E. sp.	13	Ecuador	0°	4050	SP	0 ± 0	0 ± 0	20 ± 8	42 ± 18	3	ns	ns
Scirpus setaceus	18	U.S.A.	44°N	10	L	0 ± 0	7 ± 1	10 ± 3	20 ± 3	4	**	**
S. subterminalis	3	U.S.A.	43°N	—	L	—	—	3	28	1	—	—
S. sp.	36	New Zealand	39°S	350	L	—	[2 ± 2][i]	—	—	5	—	—
Eriocaulaceae												
Eriocaulon septangulare	8	U.K.	57°N	100	L	5 ± 1	5 ± 1[f]	—	—	3	ns	—
E. decangulare	28	U.S.A.	27°N	50	L	13 ± 1	14 ± 1	12 ± 3	11 ± 2	8	ns	ns
Hydrocharitaceae												
Egeria densa	5	New Zealand	37°S	—	L	—	—	114	50[d]	1	—	—
	36	New Zealand	39°S	—	L	—	[2 ± 1][i]	—	—	5	—	—
Elodea canadensis	18	U.S.A.	38°N	1375	L	6 ± 3	5 ± 6[f]	9 ± 1	16 ± 3	2	ns	ns
	2	Finland	61°S	—	L	—	—	4 ± 1	2 ± 1	5	—	ns
	36	New Zealand	39°S	350	L	—	[6 ± 4][i]	—	—	5	—	—
Hydrilla verticillata	9	U.S.A.	30°N	—	L	30 ± 6	51 ± 13[c,g]	—	—	3	ns	—
Lagarosiphon major	36	New Zealand	39°S	350	L	—	[8 ± 5][i]	—	—	5	—	—
L. muscoides	13	S. Africa	34°S	250	SP	6 ± 5	13 ± 9	—	—	3	ns	—
Ottelia ovalifolia	36	New Zealand	39°S	—	L	—	[7 ± 5][i]	—	—	3	—	—
Thalassia testudinarum	26	U.S.A.	26°N	0	MI	7 ± 2	8 ± 2	—	—	4	ns	—
	13	Canada	45°N	75	L	0 ± 0	0 ± 0[f]	—	—	2	ns	ns
Vallisneria americana	13	Canada	45°N	75	L	11 ± 7	42 ± 12	8 ± 1	6 ± 1	5	**	ns
V. spirilis	28	U.K.	—	—	L	8 ± 1	9 ± 1	3 ± 1	3 ± 1	6	ns	ns
	36	New Zealand	39°S	—	L	—	[51 ± 1][i]	—	[54][i]	5	—	—
	13	Israel	32°N	75	L	6 ± 3	13 ± 6	6 ± 1	10 ± 4	5	*	ns
Lilaeaceae												
Lilaea scilloides	17	U.S.A.	38°N	1375	SP	1 ± 1	4 ± 2[f]	21 ± 18	18 ± 10	8	ns	ns

Table 1 (continued)

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm$ SD) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
Poaceae												
Alopecuris howellii	17	U.S.A.	34°N	610	SP	11 ± 1	12 ± 2	6 ± 3	4 ± 2	6	ns	ns
Orcuttia californica	17	U.S.A.	34°N	610	SP	1 ± 1	12 ± 1	4 ± 5	16 ± 3	6	**	**
O. viscida	13	U.S.A.	39°N	30	SP	7 ± 5	23 ± 6	11 ± 6	11 ± 6	10	**	ns
Tuctoria greenei	13	U.S.A.	40°N	60	SP	2 ± 3	3 ± 1	11 ± 2	6 ± 3	4	ns	ns
Neostapfia colusana	13	U.S.A.	38°N	20	SP	17 ± 7	16 ± 8	13 ± 6	8 ± 8	3	ns	ns
Potamogetonaceae												
Potamogeton crispus	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	10 ± 2	5 ± 1	2	ns	*
	36	New Zealand	39°S	—	L	—	[1 ± 0][i]	—	—	5	—	—
P. illinoensis	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	5 ± 3	2 ± 1	2	ns	ns
P. pectinatus	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	5 ± 3	2 ± 1	2	ns	ns
P. paramoanus	13	Ecuador	1°S	4100	L	16 ± 2	13 ± 4	4 ± 4	13 ± 3	3	ns	ns
Ruppiaceae												
Ruppia polycarpa	36	New Zealand	39°S	350	L	—	[0 ± 1][i]	—	—	5	—	—
Sparganiaceae												
Sparganium angustifolium	18	U.S.A.	37°N	110	L	7 ± 1	8 ± 1[f]	17 ± 4	16 ± 2	2	ns	ns
Zosteraceae												
Zostera angustifolia	25	U.K.	56°N	0	MI	9 ± 2	11 ± 2[f]	—	—	6	ns	—
Dicotyledoneae												
Apiaceae												
Eryngium aristulatum	17	U.S.A.	34°N	610	SP	3 ± 4	1 ± 1[f]	6 ± 6	6 ± 4	6	ns	ns
E. rostratum	13	Chile	36°S	400	SP	6 ± 1	4 ± 1	—	—	2	ns	—
E. pseudojunceum	13	Chile	38°S	200	SP	6 ± 2	6 ± 1	—	—	4	ns	—
Lilaeopsis attentuata	23	U.K.	52°N	—	L	9 ± 4	10 ± 4[f]	—	—	3	ns	—
L. lacustris	36	New Zealand	39°S	350	L	—	[41 ± 7][i]	—	[48][i]	5	—	—
L. schaffneriana	13	Colombia	4°N	3650	L	25 ± 15	21 ± 4	38 ± 13	29 ± 1	3	ns	ns
Asteraceae												
Lasthenia kunthii	13	Chile	36°S	125	SP	22 ± 3	21 ± 2	—	—	3	ns	—
Senecio zosterifolius	13	Chile	38°S	200	SP	6 ± 1	6 ± 3	—	—	4	ns	—
Boraginaceae												
Plagiobothrys undulatus	17	U.S.A.	34°N	610	SP	19 ± 6	21 ± 7	9 ± 2	6 ± 3	12	ns	ns
P. sp.	13	Chile	36°S	125	SP	19 ± 8	22 ± 1	—	—	4	ns	—
P. sp.	13	Chile	38°S	200	SP	5 ± 1	16 ± 2	—	—	4	ns	—

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm SD$) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm SD$) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
Brassicaceae												
Barbareae orthoceras	18	U.S.A.	38°N	1375	SP	3 ± 1	6 ± 1[f]	10 ± 6	12 ± 1	2	ns	ns
Cardamine sp.	13	Chile	37°S	100	SP	32 ± 9	17 ± 5	—	—	2	ns	—
Subularia aquatica	8	U.K.	58°N	—	L	12 ± 1	13 ± 1[f]	—	—	2	ns	—
Callitrichaceae												
Callitriche longipedunculata	17	U.S.A.	34°N	610	SP	9 ± 1	14 ± 2	11 ± 7	9 ± 6	6	**	ns
C. lechleri	13	Chile	36°S	400	SP	7 ± 2	6 ± 1	—	—	2	ns	—
C. nubigena	13	Colombia	4°N	3650	SP	20 ± 1	14 ± 3	17 ± 3	19 ± 1	3	*	ns
C. stagnalis	25	U.K.	56°N	—	R	12 ± 5	15 ± 5	—	—	6	ns	—
Campanulaceae												
Downingea bella	17	U.S.A.	34°N	610	SP	11 ± 1	9 ± 5	11 ± 3	17 ± 6	12	ns	**
D. cuspidata	18	U.S.A.	34°N	610	SP	1 ± 1	0 ± 0[f]	8 ± 4	4 ± 1	2	ns	ns
D. pusilla	13	Chile	37°S	100	SP	30 ± 1	24 ± 7	—	—	2	ns	—
Lobelia dortmanna	8	U.S.A.	56°N	—	L	21 ± 3	14 ± 2[f]	—	—	3	ns	—
	4	U.S.A.	43°N	—	L	20 ± 1	20 ± 1[g]	—	—	3	ns	—
	33	U.K.	56°N	—	L	21	21	—	—	2	—	—
	2	Finland	61°N	—	L	—	—	7 ± 3	11 ± 3	5	—	ns
	22	Denmark	56°N	75	L	12	12[g]	—	—	—	—	—
Ceratophyllaceae												
Ceratophyllum demersum	18	U.S.A.	37°N	110	L	2 ± 2	2 ± 3[f]	15 ± 1	8 ± 3	2	ns	ns
Crassulaceae												
Crassula aquatica	13,17	U.S.A.	34°N	610	SP	7 ± 10	129 ± 29[f]	14 ± 3	67 ± 21	6	**	**
C. helmsii	13	Australia	35°S	300	SP	5 ± 6	101 ± 19[f]	—	—	3	**	—
	23	U.K.	52°N	—	L	32 ± 3	108 ± 28	3 ± 1	35 ± 2	5,3	**	**
C. natans	13	S. Africa	34°S	250	SP	3	103	—	—	1	—	—
C. paludosa	13	Ecuador	0°	4050	L	0 ± 0	128 ± 13[f]	15 ± 9	76 ± 5	3	**	**
	13	Colombia	4°N	3650	L	28 ± 2	189 ± 44	25 ± 4	83 ± 17	3	**	**
C. peduncularis	13	Chile	36°S	125	SP	50 ± 28	219 ± 6	—	—	4	**	—
Elatinaceae												
Bergia glomerata	13	S. Africa	34°S	250	SP	3 ± 1	2 ± 0	—	—	2	ns	—
Elatine californica	17	U.S.A.	34°N	610	SP	0 ± 0	1 ± 1[f]	5 ± 1	10 ± 3	6	ns	**
E. chilensis	18	U.S.A.	34°N	610	SP	0 ± 0	0 ± 0[f]	4 ± 1	10 ± 2	2	ns	ns
E. minima	13	Colombia	4°N	3650	L	23 ± 10	31 ± 1	15 ± 2	17 ± 3	3	ns	ns

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x}$ ± SD) PM	AM	n	t-test[k](2-tailed) T.A.	M.A.
Haloragaceae												
Myriophyllum brasiliense	18	U.S.A.	37°N	110	L	0 ± 0	0 ± 0[f]	10 ± 2	6 ± 1	2	ns	ns
M. propinquum	36	New Zealand	39°S	350	L	—	[5 ± 2][i]	—	—	5	—	—
M. tenellum	4	U.S.A.	43°N	—	L	18 ± 1	18 ± 2	—	—	3	ns	—
M. triphyllum	36	New Zealand	39°S	350	L	—	[3 ± 4][i]	—	—	5	—	—
M. quitense	13	Ecuador	1°S	4100	L	30 ± 8	39 ± 2	2 ± 1	16 ± 5	3	ns	*
Lamiaceae												
Mentha arvensis	18	U.S.A.	38°N	1375	SP	0 ± 0	0 ± 0[f]	2 ± 1	3 ± 2	2	ns	ns
Pogogyne abramsii	13	U.S.A.	33°N	110	SP	0 ± 0	0 ± 0[f]	—	—	2	ns	—
Lyrthraceae												
Lythrium hyssopifolium	16	U.S.A.	34°N	610	SP	0 ± 0	0 ± 0	1 ± 1	1 ± 1	3	ns	ns
Nymphaeaceae												
Nuphar polysepalum	18	U.S.A.	38°N	2440	L	14 ± 1	10 ± 3[f]	5 ± 2	6 ± 2	2	ns	ns
Plantaginaceae												
Littorella uniflora	18	U.K.	53°N	180	L	19 ± 22	112 ± 14[f]	21 ± 14	66 ± 8	4	**	**
	1,2	Finland	61°N	—	L	24 ± 9	166 ± 13[f]	13 ± 3	81 ± 19	5	**	**
	8	U.K.	58°N	—	L	14 ± 1	66 ± 16[f]	—	—	3	**	—
	4	U.S.A.	43°N	—	L	21 ± 1	145 ± 66[g]	—	—	3	**	—
	34	U.K.	55°N	—	L	30	141[g]	4	57	1	—	—
	22	Denmark	56°N	75	L	30 ± 3	47 ± 46[g]	—	—	4	**	—
	38	Denmark	56°N	75	L	24	60[g]	12	30	4	—	—
Polemoniaceae												
Navarretia involucrata	13	Chile	37°S	500	SP	18 ± 1	20 ± 3	—	—	4	ns	—
Ranunculaceae												
Ranunculus aquatilis	17	U.S.A.	34°N	610	SP	8 ± 1	13 ± 5	6 ± 2	7 ± 4	6	ns	ns
R. bonariensis	13	Chile	36°S	125	SP	24	21	—	—	1	—	—
R. flagelliformis	13	Colombia	4°N	3650	SP	4 ± 3	2 ± 1	10 ± 10	8 ± 4	3	ns	ns
R. flammula	18	U.S.A.	38°N	1875	SP	0 ± 0	0 ± 0[f]	9 ± 1	8 ± 1	2	ns	ns
	8	U.K.			SP	24 ± 4	22 ± 10[f]	—	—	2	ns	—
R. fluitans	36	New Zealand	39°S	350	L	—	[1 ± 1][i]	—	—	5	—	—
R. penicillatus	25	U.K.	56°N	—	R	16 ± 4	14 ± 4[f]	—	—	6	ns	—

Table I *(continued)*

Taxa	Data	Country	Latitude	Elev. (m)	Habitat[a]	Titratable acidity[e] (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm SD$) PM	AM	Malate (mmol kg^{-1} F.M.)[b] ($\bar{x} \pm SD$) PM	AM	n	t-test[k] (2-tailed) T.A.	M.A.
Scrophulariaceae												
Limosella acaulis	18	U.S.A.	37°N	110	L	3 ± 1	0 ± 0[f]	6 ± 1	6 ± 3	2	ns	ns
L. capensis	13	S. Africa	34°S	250	SP	4 ± 0	4 ± 1	—	—	2	ns	—

[a] C, canal; FTR, freshwater tidal river; L ,lacustrine; Ml, marine-littoral; Msl, marine-sublittoral; Mul, marine-supralittoral; R, river; SP, seasonal pool.

[b] Expressed per kg fresh mass; —, data not available.

[c] Expressed per g chlorophyll.

[d] Expressed per g dry mass.

[e] Titratable acidity to pH 7.0.

[f] Titratable acidity to pH 6.4.

[g] Titratable acidity to pH 8.0 or 8.3.

[h] Titratable acidity to pH 7.6.

[i] Diel change between am and pm.

[k] ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; —, data not available.

Data sources:

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35 Surif & Raven, 1983
36 Webb et al., 1988
37 Gacia & Penuelas, 1991
38 Madsen, 1987a
39 Gacia & Ballesteros, 1993

Fig. 2. Typical "isoetid" growth form illustrated by *Isoëtes howellii*, a seasonal pool "quillwort" or "Merlin's grass," shown here growing in an aerial environment; height of tallest leaf is ~20 cm. (Photograph by J. Keeley.)

5. Refixation of the CO_2 resulting from decarboxylation of malate into products of the Calvin or PCR (photosynthetic carbon reduction) cycle.
6. Sufficient PEPC activity to account for overnight acidification.
7. Sufficient decarboxylase activity to account for daytime deacidification.
8. Net uptake of CO_2 in the dark.

Other characteristics often associated with CAM—such as preference for arid habitats, leaf succulence, diel pattern of high stomatal conductance at night and low daytime conductance, stoichiometry of (1:2:1) for (dark-CO_2 uptake:ΔH^+:Δmalate), the daytime suppression of β-carboxylation, pyruvate P_i dikinase activity, among others—are not strictly associated with the CAM pathway, in either terrestrial or aquatic floras.

V. Evidence of the CAM Pathway in Aquatic Plants

A. DARK FIXATION

Steady-state ^{14}C-labeling in the dark shows that all five of the genera *Isoëtes*, *Sagittaria*, *Vallisneria*, *Crassula*, and *Littorella* exhibit substantial dark fixation into malate (Table II). Presumably this is via β-carboxylation by the C_4 enzyme PEPC [as demonstrated for *Vallisneria spiralis* by Helder and van Harmelen (1982)], although detailed studies of C-atom position of the ^{14}C-label have not been done for other aquatics (as is true of most terrestrial CAM species).

In all of these aquatic species, malate produced by dark-fixation is stored overnight and largely not metabolized in the dark, as is evident from the pulse-chase studies in the dark (Table II). The bulk of the remaining dark-fixed label is in citrate (or isocitrate). Malate comprises the storage carbon utilized in CAM photosynthesis, a role apparently not ascribed to the other dicarboxylic acids, which apparently are labeled in the dark by transfer of ^{14}C-label from malate, and serve other metabolic functions (Lüttge, 1995). Seasonal changes in labeling patterns have been observed for *Vallisneria americana* (Table II), indicating greater CAM activity in the spring than in the autumn. This accounts for conflicting reports on acid accumulation in the related *V. spiralis* (Table I); significant ΔH^+ occurred in a summer study, whereas two other winter studies failed to find significant ΔH^+. Seasonal changes in level of CAM activity have been reported for several aquatic species and are discussed in Sections VIII and IX.

These labeling studies are incapable of distinguishing between malate and malic acid. However, consistent with the conclusion that dark-fixed label is transported in the protonated form malic acid is the highly significant correlation between ΔH^+ and Δmalate, evident across species of *Isoëtes* (Fig. 3). If malate were the only acid accumulating, a 2:1 stoichiometry for ΔH^+:Δmalate would give a regression line slope of 0.5. The observed deviation (Fig. 3) from that expectation is consistent with 10–20% dark-fixed label in citrate(citric acid) (Keeley, 1981, 1996), assuming a stoichiometry of $2H^+$ per malate and $3H^+$ per citrate. The slope of this regression line for *Isoëtes* is close to the slope of 0.42 reported for pineapple (Medina et al., 1993). *Littorella*, on the other hand did not deviate from a 2:1 stoichiometry for ΔH^+:Δmalate (Madsen, 1987a), indicating either that the ~20% citrate produced by dark fixation (Table II; Keeley, unpubl. data) is stored as the anion or that citric acid generation is variable between studies. Patterns similar to *Isoëtes* are evident in *Sagittaria subulata* and species of *Crassula*, where the molar ratio of ΔH^+:Δmalate ($\bar{x} \pm$ S.D.) = 2.3 ± 0.3 and 2.0 ± 0.2, respectively (Table I).

Table II
Dark fixation products following a 3 h $^{14}CO_2$-pulse and after a 9 h $^{14}CO_2$-free chase in the dark (from Keeley, unpubl. data)

	Percentage distribution of ^{14}C-label[a]					
	Malate		Other soluble		Insoluble	
Taxa	3 h	3 h + 9 h	3 h	3 h + 9 h	3 h	3 h + 9 h
Isoëtes bolanderi	80	72	20	26	0	2
I. howellii	89	78	11	22	0	0
I. orcuttii	88	82	12	17	0	1
Sagittaria subulata	66	70	29	27	5	3
Vallisneria americana						
Spring	61	66	36	29	3	5
Autumn	39	27	47	65	14	8
V. spiralis	54	53	43	42	3	5
Crassula aquatica	79	75	21	24	0	1
Littorella uniflora	83	79	15	20	2	1

[a] Average of 2 or more replicates.

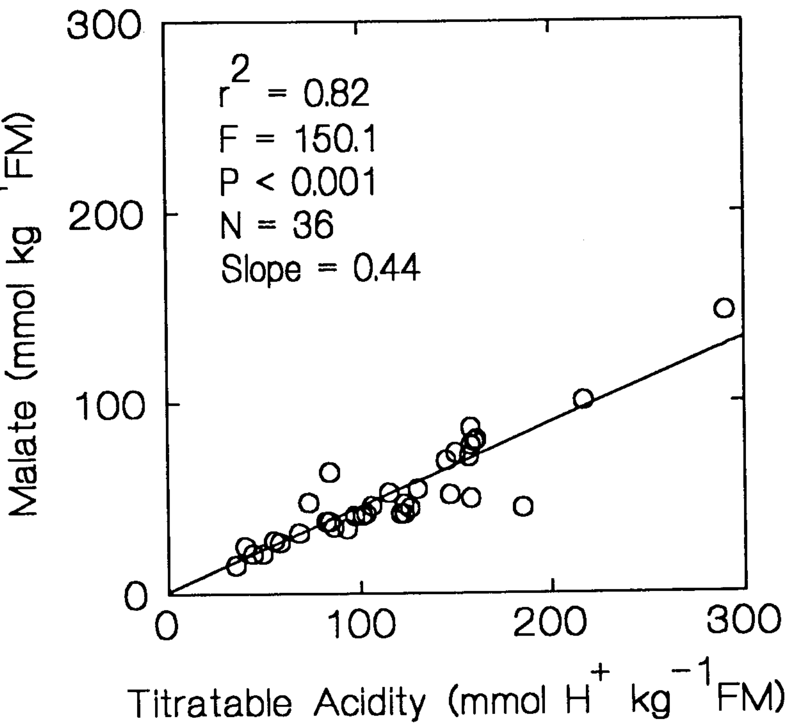

Fig. 3. Molar relationship of ΔH^+ and Δmalate in species of *Isoëtes* (from Table I).

These acid changes are restricted to photosynthetic organs and are absent from roots and corms of *I. howellii* (Keeley, 1981) and *I. setacea* (Gacia & Ballestros, 1993).

Consistent with glycolytic production of the CO_2-accepter molecule PEP, is the overnight depletion of starch observed in *I. bolanderi* (Keeley et al., 1983a) and *I. howellii* (Keeley, 1983a). In mid-season, diel changes in *I. howellii* leaf starch were 144 mol glucose-equivalents kg^{-1} Chl, comparable to the 122 mol malic acid kg^{-1} Chl (Keeley, 1987). Early in the season, however, diel changes in starch in the leaves were insufficient to account for levels of ΔH^+, suggesting either that there was a dependence upon starch stored in corms or that PEP was generated at this time from sugars (Black et al., 1995).

B. DAYTIME DEACIDIFICATION

During daytime deacidification (Fig. 1, Table I) there is substantial evidence that the released CO_2 is refixed via the C_3 pathway (Fig. 4). *Isoëtes orcuttii* and *Littorella* also show a turnover of ^{14}C-labeled malate, with label initially in phosphorylated compounds (not shown), followed by transfer of label to other soluble and insoluble compounds. Other aquatic CAM species demonstrate a similar pattern during the light deacidification phase (Keeley, unpubl. data).

C. CAM ENZYMES

Carboxylase activities (Table III) show that RUBISCO activities are similar between aquatic and terrestrial CAM plants, perhaps reflecting broadly similar photosynthetic rates (Section V.D). However, PEPC activities are substantially lower for aquatic CAM species than for terrestrial CAM plants (Dittrich et al., 1973), which is surprising since rates of acid production are similar. Nonetheless, PEPC activities in aquatic CAM plants are sufficient to account for the rates of nighttime malate production (10–20 mmol kg^{-1} FM hr^{-1}). Even though ratios of $^{RUBISCO}/_{PEPC}$ are higher in aquatic CAM plants, they nonetheless are still much lower than for a typical C_3 plant such as spinach (Table III). Also, when aquatic CAM plants are exposed to the atmosphere, the $^{RUBISCO}/_{PEPC}$ increases to levels comparable to terrestrial C_3 plants (Table III), which is consistent with the concomitant switch from CAM to C_3 (Section IX).

Thus, relative to terrestrial CAM plants, aquatic CAM species are capable of similar magnitudes of acid accumulation with a lower investment of energy and nutrients in PEPC. I hypothesize that the basis for this stems from differences in water and carbon availability. In aquatic CAM plants there is no obvious selective advantage to rapid dark fixation, whereas in terrestrial species higher PEPC activity may translate into a shorter duration of stomatal opening, and thus higher water use efficiency. Also, aquatic habitats have substantially higher CO_2 levels than air (Section VII). Under elevated carbon conditions, the naturally high substrate affinity of PEPC may result in vacuolar storage capacity for malic acid being a greater limiting factor to carbon gain, thus favoring reduced investment in PEPC. This explanation is supported by the increase in $^{RUBISCO}/_{PEPC}$ observed for terrestrial CAM plants in response to elevated CO_2, despite showing little change in ΔH^+ (Nobel et al., 1996). Also, the aquatic CAM *Littorella* exhibits a threefold drop in PEPC activity under elevated CO_2, without any drop in ΔH^+ (Hostrup & Wiegleb, 1991a).

Kinetic studies show many similarities between the PEPC from the aquatic CAM *Littorella* and terrestrial CAM plants (Groenhof et al., 1988); e.g., increased V_{max} and decreased K_m in the dark or in response to glucose-6-phosphate, and the opposite pattern in response to malate.

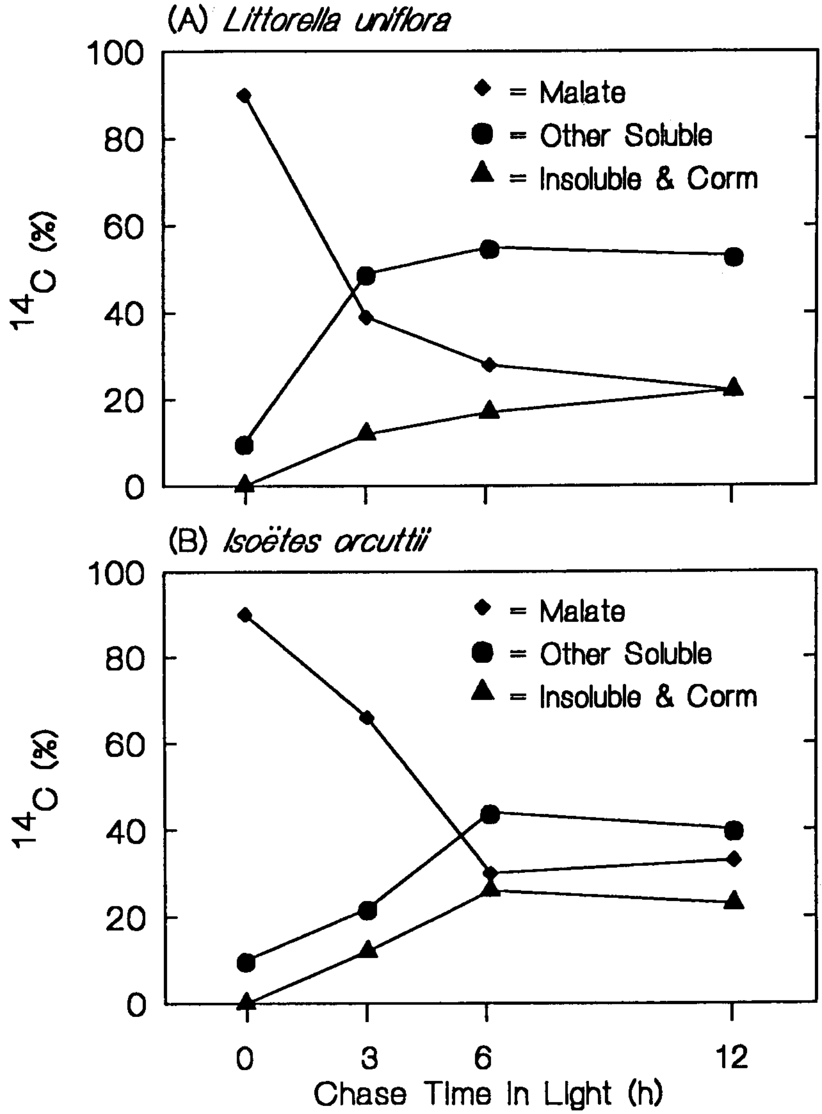

Fig. 4. Distribution of dark-labeled products during a 12 h chase in the light for two aquatic CAM species, (A) *Littorella uniflora* and (B) *Isoëtes orcuttii*; ~20°C, 10 mol m^{-3} MES buffer, pH 6.0 (Keeley, unpubl. data).

Decarboxylase activities are sufficient to account for rates of daytime deacidification and in three species studied, NADP malic enzyme is the primary decarboxylase (Table III). Another potential decarboxylase, PEP carboxykinase, has not been detected in *I. howellii* or *C. aquatica* (Keeley, 1998b), and, like terrestrial CAM plants lacking this enzyme (Kelly et al., 1989; Black et al., 1995), these two aquatics have significant pyruvate, P_i dikinase activity.

Table III

Activity of carboxylating enzymes, RUBISCO and PEPC, and other photosynthetic enzymes in submerged aquatic foliage or emergent aerial leaves and selected terrestrial species included for comparison[a]

Taxa		Data source[b]	RUBISCO	PEPC	RUBISCO/PEPC	ME-NAD$^+$	ME-NADP	PEPCK	Pyruvate P-dikinase
Aquatic CAM species									
Isoëtes howellii	Submerged	8	256	36	7.1	2	37	nd	110
	Aerial	8	553	18	30.7	nd	42	nd	186
I. lacustris	Submerged	4	75	22	3.4	—	—	—	—
	Aerial	1	141	—	—	—	—	—	—
I. orcutii	Submerged	8	225	46	4.9	—	—	—	—
	Aerial	8	480	15	32.0	—	—	—	—
Crassula aquatica	Submerged	8	392	178	2.2	2	78	nd	208
	Aerial	8	854	45	19.0	4	156	nd	nd
Littorella uniflora	Submerged	4	187	95	2.0	—	—	—	—
	Submerged	7	—	165	—	—	—	—	—
	Submerged	5	—	819	—	nd	42	—	—
	Aerial	5	—	65	—	nd	nd	—	—
Terrestrial CAM species									
Ananas comosus		8	—	—	—	—	—	83	908
Crassula argenta		2	59	270	0.2	192	—	—	—
Kalanchoe daigremontiana		8	—	—	—	96	73	—	—
Mesembryanthemum crystallinum									
CAM mode		6	306	1074	0.3	—	—	—	—
C_3 mode		6	438	24	183	—	—	—	—
Other terrestrial species									
Spinacea oleracea C_3		8	865	54	16.0	—	—	—	nd
Zea mays C_4		8	462	842	0.5	—	—	—	289
Zea mays C_4		1	184	—	—	—	—	—	—

[a] nd, not detectable; —, not assayed.

[b] 1, Beer et al., 1991; 2, Dittrich et al., 1973; 3, Farmer, 1987; 4, Farmer et al., 1986; 5, Groenhof et al., 1988; 6, Holtum & Winter, 1982; 7, Hostrup & Wiegleb, 1991a; 8, Keeley, 1997a, and unpubl. data.

lso, consistent with lack of PEP carboxykinase (Winter & Smith, 1995a; cf. Christopher & ɔltum, 1996), *I. howellii* utilizes starch as the source of the CO_2 acceptor PEP (Keeley, ›83a).

D. GAS EXCHANGE

Gas exchange patterns for aquatic CAM plants are more complex than for terrestrial CAM ants due to multiple carbon sources and dynamic diel changes in availability. In this section, ıs exchange characteristics under steady-state conditions (pH 5.5 with vigorous agitation) ill be described, and in Section VIII these patterns will be contrasted with patterns under ɘld conditions.

For solutions equilibrated near atmospheric levels of CO_2 (~0.011 mol m^{-3}), *Isoëtes howel-* exhibits no net CO_2 uptake in the dark (Keeley & Bowes, 1982), but at higher CO_2 levels, ore typical of its natural environment, dark uptake rates were ~27 mol kg^{-1} Chl hr^{-1} (Fig. 5), , based on allometric values in Keeley & Sandquist, 1991, 210 mmol kg^{-1} dry mass hr^{-1} or 8 mmol m^{-2} total leaf area hr^{-1}. These rates are comparable to dark CO_2 uptake in terrestrial ᴀM plants (Kluge & Ting, 1978)—a surprising conclusion since, collectively, aquatic plants ıve substantially lower photosynthetic rates than terrestrial plants (Bowes & Salvucci, ›89). This seeming paradox may be explained as follows. Differences in daytime photosyn-etic rate between aquatic and terrestrial plants are largely a function of transport processes, hich are very different between land and water (Raven, 1984). Dark fixation, on the other ınd, is more a function of vacuolar storage capacity (Kluge & Ting, 1978), which is more eq-tably distributed between aquatic and terrestrial CAM plants.

In contrast to many, but not all, terrestrial CAM plants, under steady-state CO_2 conditions, e aquatic CAM *I. howellii* shows no daytime suppression of CO_2 uptake (Keeley & Bowes, ›82). In terrestrial CAM plants, suppression results from stomatal closure but does not occur aquatic plants under steady-state conditions because they lack functional stomata (Section [.A). In these aquatics, CO_2 uptake is controlled by ambient CO_2 concentration and diffu-ve resistances, factors that, under field conditions (Section VIII), produce more dynamic ıtterns of CO_2 uptake than observed in steady-state (Fig. 5). This explanation is supported by e fact that terrestrial CAM plants exhibit CO_2 uptake in the light if stomatal resistance is ʼercome, either by removal of the epidermis or with isolated protoplasts (Chellappan et al., ›80; Winter & Smith, 1995a).

Under steady-state conditions (Fig. 5), CO_2 uptake in the light may be 2–3 times greater an uptake in the dark, across a wide range of naturally occurring CO_2 concentrations. As ith terrestrial CAM plants, CO_2 uptake in the light is assimilated directly through the C_3 path-ay—as demonstrated (for *Crassula aquatica* and *Isoëtes* spp.) by the initial fixation of ^{14}C-bel in PGA and transfer to other phosphorylated compounds, coupled with lack of label in carboxylic acids (Keeley, 1998b).

VI. Other Attributes of Aquatic CAM Plants

A. STRUCTURAL CHARACTERISTICS

Three of the five genera with CAM have the "isoetid" growth form, so named because of e resemblance to *Isoëtes* (e.g., Fig. 2), although not all isoetids have CAM (Richardson et al., ›84).

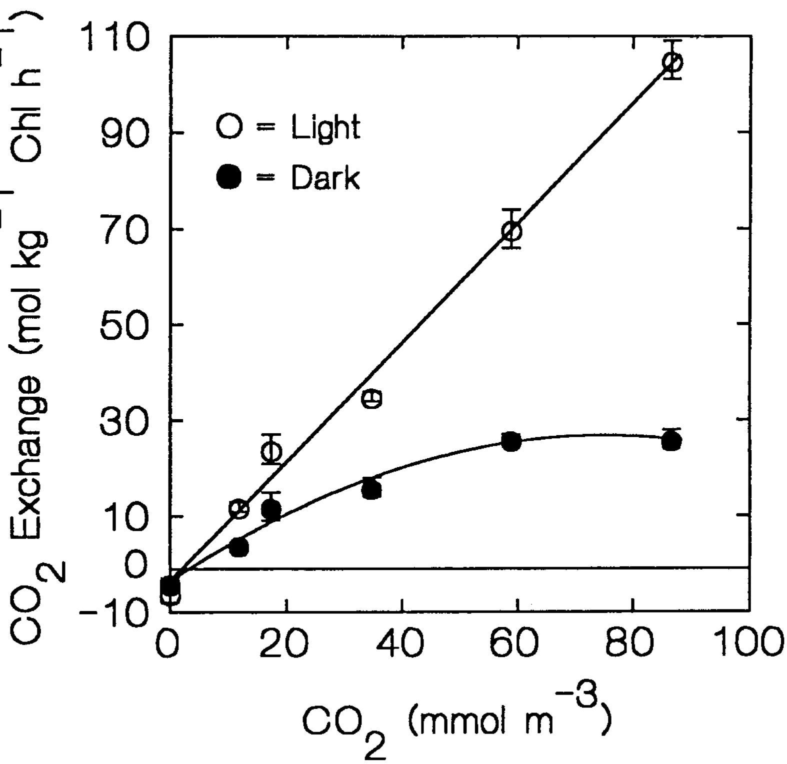

Fig. 5. CO_2 uptake rate in response to ambient CO_2 concentration for *Isoëtes howellii* leaves with vigorous agitation, @ 1000 μmol m^{-2} s^{-1} PAR and 25°C in the dark or light, 10 mol m^{-3} MES, pH 5.5. (Redrawn from Keeley & Bowes, 1982.)

1. Morphological Variation in Isoëtes

Despite the rather large number of *Isoëtes*, there is remarkable morphological similarity. All but three species (Hickey, 1990) have the isoetid rosette of stiff terete leaves attached to a small rounded corm. Isoetids have a relatively low surface:volume ratio (1–2 vs. 10–20 for other aquatic macrophytes) and high root:shoot ratio (>1 vs. <0.2 for other macrophytes) (Raven et al., 1988; Boston et al., 1989; Keeley, 1991; Madsen et al., 1993). All isoetids have lacunal air chambers, and in *Isoëtes* species, both aquatic and terrestrial, there are always four lacunae, which, depending on species and habitat, represent 20–90% cross-sectional airspace. A common feature is the concentration of chloroplasts in mesophyll cells surrounding lacunae and, unlike other aquatic macrophytes, few if any epidermal chloroplasts. Both aquatic and terrestrial species have a relatively substantial-appearing cuticle, although little is known about permeability characteristics (but see Keeley et al., 1984). Leaves are attached to a modified stem-rhizophore with traces from the central vascular core connecting leaves and roots (Sculthorpe, 1967).

The most obvious variation in the genus lies in size, which ranges from a centimeter in some rock-outcrop seasonal pool species to large robust species with leaves nearly half a me-r long and roots several times longer in some tropical alpine lacustrine species. On rich ›odplain sites in the eastern United States, specimens up to 90 cm have been reported (Muslman & Knepper, 1994).

Variation in vegetative structure is apparent in stomatal distribution and root architecture ıd is closely tied to habitat. Amphibious or seasonal pool species are all drought deciduous ıd have nonfunctional stomata on submerged foliage. Upon exposure to the atmosphere, stoata become functional and there is a greater density on leaves produced under aerial condi›ns. Lacustrine species are largely evergreen, although those in lakes subject to thick .owpack are winter deciduous (Keeley, 1987). These lake species exhibit two patterns, apırently tied to latitude. In the Temperate Zone, species such as *Isoëtes bolanderi*, *I. macro-'ora*, and *I. lacustris* produce astomatous leaves underwater but, if exposed, will initiate aves with functional stomata (Keeley, unpubl. data). In tropical alpine species such as *I. ılmeri*, *I. lechleri*, and *I. karsteni*, submerged leaves are astomatous, and stomata are rarely 'oduced under aerial conditions (Keeley, unpubl. data). Terrestrial species, comprising ›out 10% of the genus, exhibit a similar latitudinal pattern; Temperate Zone species are lowevation, summer-deciduous plants with functional stomata whereas tropical alpine *Isoëtes* e evergreen plants lacking stomata.

Roots are remarkably variable. Amphibious species from seasonal pools, commonly on ne clay sediments, have relatively thin, highly branched roots with extensive root-hair de›lopment. In contrast, many lacustrine species, particularly in tropical alpine lakes with .ndy substrates, have thick, unbranched roots, lacking root hairs (Keeley, unpubl. data). In at ast some *Isoëtes* these differences are plastic responses to sediment (Karrfalt, 1984). All *oëtes* have a single large lacunal chamber that fills the center of the root and varies in cross-:ctional area. Also, all species have a mechanism for burying corms that is analogous to :ontractile roots" (Karrfalt, 1977).

2. Other Aquatic CAM Plants

Littorella resembles *Isoëtes* in the isoetid growth form, although the corm is replaced by a olon or rhizome. *Littorella* leaves have extensive lacunal airspace, lack of epidermal chloro-.asts and concentration of chloroplasts in cells surrounding lacunae (Hostrup & Wiegleb, ›91b). This species can alter the extent of lacunal surface area in response to sediment char-:teristics (Robe & Griffiths, 1988) or upon emergence (Hostrup & Wiegleb, 1991b). Leaf :ientation varies from stiffly erect terete leaves in submerged plants to reflexed flattened aves in terrestrial plants, a character shared with *Isoëtes*.

Some *Sagittaria* are also isoetids, with rosettes of stiff semi-terete to subulate phyllodes in ıe aquatic stage. Depending on environmental conditions, these cylindrical leaves are re-.aced by elongated ribbon-shaped submerged leaves (pseudo-lamina) or broadened sagittate ›mi-floating leaves (Sculthorpe, 1967). Some, e.g., *S. cuneatus* and *S. graminea* (with limэd ΔH^+, Table I) apparently lack the isoetid stage.

Two aquatic CAM genera are not isoetids: *Vallisneria* spp. have ribbon-shaped leaves and *rassula* spp. are diminutive caulescent annuals, with short semi-cylindrical leaves and often :ostrate stems, which constitute much of the photosynthetic surface area.

Succulence is a characteristic typical of a great many terrestrial CAM plants but is not char-:teristic of aquatic CAM plants. For terrestrial species, mesophyll succulence (kg H_2O g^{-1} hl) is <1 for non-CAM plants but up to an order of magnitude higher for most terrestrial CAM lants (Kluge & Ting, 1978). Aquatic CAM plants commonly have mesophyll succulence raos >1, but as a group are indistinguishable in this character from non-CAM aquatic plants

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B. INORGANIC CARBON SOURCE

Aquatic plants have access to carbon sources not available to terrestrial plants. Bathed in solution, these plants are exposed to dissolved CO_2, HCO_3^-, or CO_3^{2-}, with CO_2 predominating at acidic pH but nil above pH 8. Aquatic plants are often described as "preferring" CO_2, meaning the apparent K_m is substantially lower for CO_2 uptake, even in species with the capacity for HCO_3^- uptake. Despite the fact that bicarbonate is the active form assimilated by PEPC, aquatic CAM species lack the capacity for bicarbonate uptake. In *Isoëtes* spp., at constant CO_2 concentration, photosynthetic rates at pH 5 are higher than rates at pH 8, despite the substantially higher inorganic carbon present at the higher pH (Keeley, unpubl. data). Of course, this could reflect inhibition due to high pH or alkalinity.

The pH-drift technique, where final pH is a function of alkalinity plus carbon-extracting ability of the plant (Allen & Spence, 1981), shows *Elodea canadensis* (a known bicarbonate user) has much greater carbon extracting ability than the non–bicarbonate user *Isoëtes howellii* (Fig. 6). While species such as *E. canadensis* may drive up the pH during such experiments to above pH 10, non–bicarbonate users such as *I. howellii* seldom raise the pH much beyond 8. A useful comparative parameter is the final total carbon (C_t):alkalinity ratio, which is 0.73–0.79 for *E. canadensis* and 0.97–1.00 for *I. howellii* (Gearhart & Keeley, unpubl. data), values characteristic of bicarbonate and non–bicarbonate users, respectively. Using similar techniques, Sand-Jensen (1987) demonstrated a lack of bicarbonate uptake also for the CAM species *Isoëtes lacustris*, and also for *I. macrospora* and *Littorella* (Boston et al., 1987; Maberly & Spence, 1983, 1989), *Crassula aquatica* (Keeley, unpubl. data) and *C. helmsii* (Newman & Raven, 1995). Capacity for bicarbonate uptake is widespread in aquatic plants but is likely missing from many species because ions such as HCO_3^- must be actively transported across the epidermal membrane, which makes it energetically more expensive than passive uptake of CO_2. Bicarbonate uptake is a CO_2-concentrating mechanism best viewed as an alternative to CAM.

C. ISOTOPE FRACTIONATION

Keeley and Sandquist's (1992) review of ^{13}C:^{12}C ratios in aquatic species can be summarized as follows. Consistent with the pattern in terrestrial CAM plants, $\Delta^{13}C$ values for *Isoëtes* species are substantially lower for submerged leaves in the CAM mode than for aerial leaves in the C_3 mode (see Section IX). Also, in *Isoëtes*, $\Delta^{13}C$ is lower for aquatic CAM species than for terrestrial C_3 species (Richardson et al., 1984; Keeley & Sandquist, 1992). However, aquatic CAM species often have ratios indistinguishable from aquatic C_3 species (Keeley & Sandquist, 1992; cf. Richardson et al., 1984). This derives from additional factors that determine ratios in

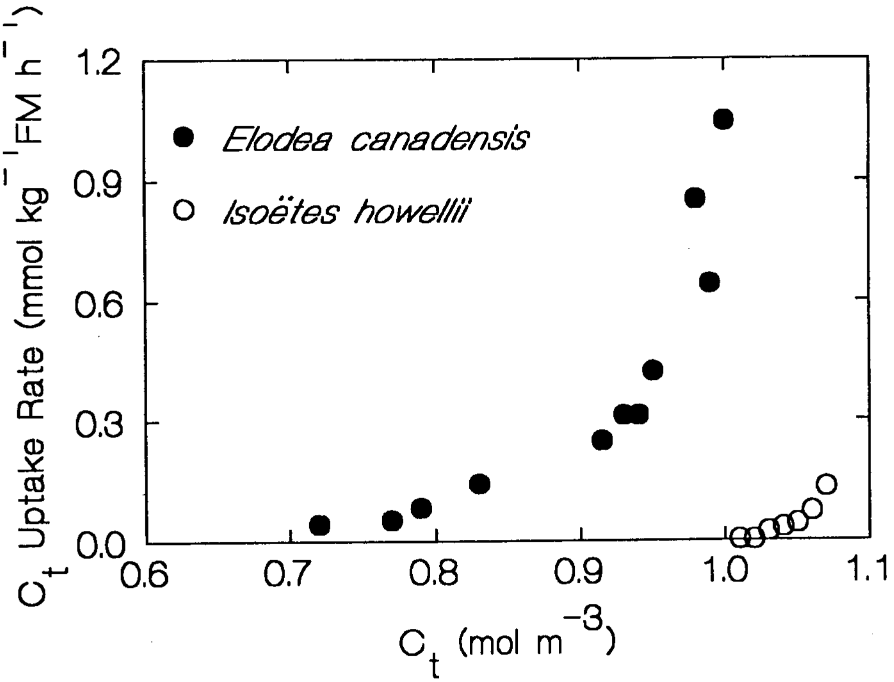

Fig. 6. Rate of photosynthetic carbon assimilation as a function of total carbon (C_t) in a closed system with constant alkalinity of 1.0 mole m^{-3} @ 25°C and ~500 μmol m^{-2} s^{-1} PAR, according to the technique of Allen & Spence, 1981. The two-phase curve for *Elodea canadensis* represents overlapping of the kinetic curves for CO_2 and HCO_3^- uptake. In contrast, the linear curve for *Isoëtes howellii* demonstrates lack of carbon-extracting ability at lower C_t and assimilation restricted to CO_2 uptake (A. Gearhart & Keeley, unpubl. data).

VII. Habitat Distribution

Enhanced water use efficiency is an important selective force in the evolution and maintenance of CAM in terrestrial plants and is reflected in the abundance of CAM in many arid land floras (Kluge & Ting, 1978). Even in tropical rain forest CAM epiphytes, water use efficiency is considered an important selective factor (Griffiths, 1989). Clearly, such is not the case with aquatic CAM plants; rather, this pathway is strongly correlated with habitats imposing severe carbon-limitation. These habitats include shallow rain-fed seasonal pools and oligotrophic lacustrine habitats.

A. SEASONAL POOLS

Shallow seasonal pools form in many parts of the world and commonly have species of *Isoëtes* and/or *Crassula* (Keeley & Zedler, 1998). Many fill during winter and spring, when precipitation exceeds evapotranspiration, and because they are rain-fed, such "vernal pools" typically have low conductance, with pH controlled by the weak buffer system of CO_2/HCO_3^- CO_3^{2-}. They are generally shallow with high levels of photosynthetically active radiation (PAR) (Keeley et al., 1983b). Plant biomass is high, and thus early morning photosynthetic consumption of CO_2 drives pH up and by mid-day free-CO_2 in the bulk water is nil (Fig. 7A). This leaves bicarbonate as the primary source of carbon, and most communities have some species capable of utilizing this source and thus driving up the pH to 9–10 (Keeley & Busch, 1984). Since these pools are densely vegetated and relatively stagnant, CO_2 depletion in the leaf boundary layer is likely to occur rapidly (Smith & Walker, 1980), suggesting that plants are subject to a considerably longer period of CO_2 starvation than is evident in the bulk water (Fig. 7A). At night, release of respiratory carbon drives up the ambient CO_2 levels, resulting in a largely biogenically driven diel pattern of CO_2 availability, or what Raven and Spicer (1995) refer to as a landscape-level "CO_2 pump." Dynamic fluctuations in pool chemistry, similar to those illustrated for California (Fig. 7A), have been demonstrated for seasonal pools in Spain (Gacia & Ballestros, 1993), Chile, and South Africa (Keeley, unpubl. data). As a matter of speculation, forest understories exhibit similar diel changes in CO_2 availability (Broadmeadow & Griffiths, 1993), which may account for the odd occurrence of terrestrial CAM plants in these habitats.

Seasonal pools develop under many circumstances, but not all are suitable CAM plant habitats (Keeley & Zedler, 1998). Alkaline pools generally lack CAM species, as the high pH results in little diel change in pH and CO_2 availability. Pools that develop along temporary stream courses or within large drainage basins also seldom are dominated by CAM plants. This is because the enriched nutrient content, due to allochthonous input of inorganic and organic nutrients (Wetzel, 1975), buffer the water against sharp diel changes in carbon as well as favoring faster-growing competitors.

B. LACUSTRINE

Lacustrine habitats dominated by CAM plants are generally softwater oligotrophic lakes, which are common at high latitudes or, in lower latitudes, only at high elevations. Oftentimes such lakes are completely dominated by CAM plants. For example, in Lake Kalgaard (Table IV) 99% of the biomass is contributed by two CAM species, *Littorella* in a zone 0–2 m deep and *Isoëtes lacustris* at 2–4.5 m (Sand-Jensen & Søndergaard, 1979)—a pattern repeated elsewhere in Europe (Szmeja, 1994). In North America, CAM species such as *I. macrospora* reach peak biomass at depths below 7 m (Collins et al., 1987). Depth distribution patterns in general vary in accordance with water transparency (Middelboe & Markager, 1997). In shallow neotropical alpine lakes, *Isoëtes* and *Crassula* often cover three-fourths or more of the lake bottom, with few other species present (Keeley, pers. obs.). Although *Isoëtes* are commonly distributed in lakes with circumneutral pH (Jackson & Charles, 1988; Gacia et al., 1994), they often dominate under more acidic conditions (Moyle, 1945; Pietsch, 1991; Vöge, 1997).

Diel changes in CO_2 and O_2 are a function of metabolic and physical processes and in poorly buffered water are controlled by the ratio of biomass:water-volume. Because this ratio is very low in oligotrophic lakes, these habitats do not exhibit predictable diel patterns of CO_2 availability (Sand-Jensen et al., 1982; Keeley et al., 1983a; Sand-Jensen, 1989; Sandquist & Keeley, 1990). These habitats, however, have inorganic carbon levels one to two orders of magnitude lower than for seasonal pools or for mesotrophic lakes dominated by non-CAM plants (e.g., Searsville Lake, Table IV). Although CO_2 levels in oligotrophic lakes are still greater than the levels expected from equilibrium with the atmosphere (~0.01 mol m^{-3}), the diffusive resistance of water (10^4 times greater than air) limits the availability of CO_2 in unstirred layers around leaves. These infertile habitats are also low in other inorganic nutrients, in particular nitrate and phosphate (Søndergaard & Sand-Jensen, 1979b; Pietsch, 1991). Irra-

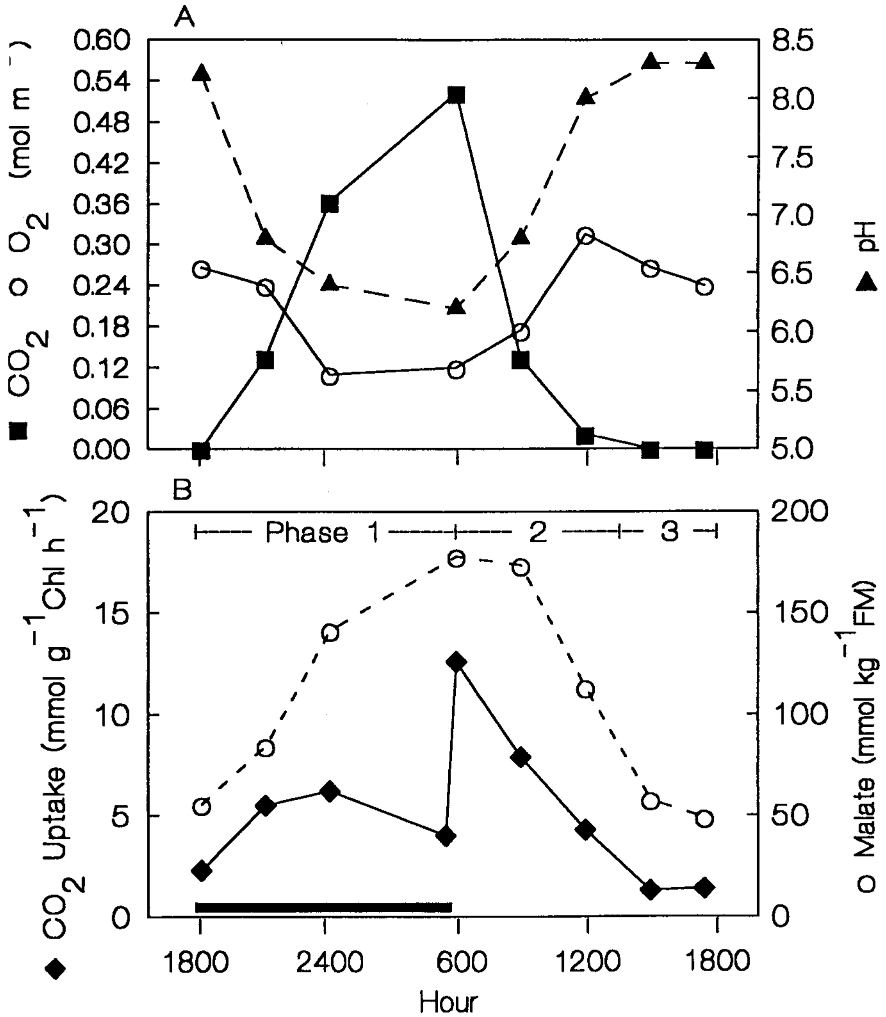

Fig. 7. Seasonal pool in southern California, in mid-spring. **A.** Pool chemistry, CO_2, O_2, and pH. **B.** CO_2 uptake rate and malate levels in leaves of *Isoëtes howellii*. (Redrawn from Keeley & Busch, 1984.)

iance levels are higher than in mesotrophic lakes (due to low phytoplankton biomass) but ubstantially lower than in shallow seasonal pools (Kirk, 1983). In addition to the irradiance ttenuation with depth, some high-elevation lakes experience abbreviated day length due to hading by adjacent forests and rugged terrain (Sandquist & Keeley, 1990).

One noteworthy characteristic of lacustrine habitats dominated by CAM species is the subtantially higher sediment CO_2 level (Table IV), an important factor in the carbon balance of soetids (Section VIII.B.1). It is of some interest that *Isoëtes* distributed in acidic infertile akes in tropical Andean sites have a tendency to grow in extremely dense clumps of 10^3–10^4 ılants m^{-2}, due in part to vegetative reproduction by axillary gemmae (Hickey, 1986; Keeley,

Table IV
Comparison of typical water and sediment chemistry characteristics of selected lakes dominated by CAM macrophytes and lakes dominated by non-CAM macrophytes[a]

						Water column			Sediment water	
Lake (country)	Latitude	Elev. (m)	Dominant macrophytes	Photosynthetic pathway	Data source[b]	pH	Free-CO_2 (mol m^{-3})	Conductivity (μS cm^{-3})	pH	Free-CO_2 (mol m^{-3})
Kalgaard (Denmark)	56°N	75	*Isoëtes lacustris*	CAM	1	7.4	0.03	66	5.5	3.00
			Littorella uniflora	CAM						
Esthwaite (Denmark)	56°N	—	*Isoëtes lacustris*	CAM	2	6.0	0.06	—	6.5	1.01
			Littorella uniflora	CAM						
Weber (U.S.A.)	43°N	—	*Isoëtes macrospora*	CAM	3	6.1	0.10	—	5.8	0.80
			Littorella uniflora	CAM						
Ellery (U.S.A.)	38°N	2900	*Isoëtes bolanderi*	CAM	4	6.8	0.12	22	6.5	1.70
			Eleocharis acicularis	non–CAM						
"Km 31" (Colombia)	4°N	3650	*Isoëtes karstenii*	CAM	5	5.1	0.23	10	4.8	1.51
			Crassula paludosa	CAM						
"Larga" (Colombia)	4°N	3650	*Isoëtes palmeri*	CAM	5	5.3	0.12	15	4.9	1.79
			Crassula paludosa	CAM						
"Temprano" (Ecuador)	0°	4050	*Isoëtes peruvianum*	CAM	5	6.3	0.14	—	5.9	0.75
			Crassula paludosa	CAM						
Searsville (U.S.A.)	37°N	110	*Myriophyllum brasiliense*	non–CAM	5	7.7	4.86	750	—	—
			Potamogeton spp.	non–CAM						
			Ceratophyllum demersum	non–CAM						

[a] —, data not available.
[b] 1, Sand-Jensen & Søndergaard, 1979b; 2, Robe & Griffiths, 1988; 3, Boston & Adams, 1985; 4, Keeley et al., 1983a, and Sandquist & Keeley, 1990; 5, Keeley, unpubl. data.

:rs. obs.). As a consequence, organic matter is concentrated beneath the clumps and thus :diment CO_2 levels are substantially greater than in the interstitial spaces between clumps ꞔeeley, unpubl. data), perhaps facilitating CO_2 uptake from the sediment.

In general, CAM species are poorly represented in mesotrophic lakes and are seldom found ıder eutrophic conditions (Seddon, 1965, 1972; Rørslett & Brettum, 1989; Gacia et al.,)94). Eutrophication often leads to the disappearance of CAM species (Kurimo & Kurimo,)81; Farmer & Spence, 1986). Numerous authors have suggested that the restriction of isoids to infertile sites is because they are competitively displaced in more fertile habitats—a ʀpothesis with some experimental support (Lee & Belknap, 1970). Preference for oligoophic conditions by aquatic CAM plants is similar to the pattern observed for terrestrial CAM ants.

C. OTHER HABITATS

There is some overlap between oligotrophic lake and seasonal pool habitats—e.g., *Litrella* often is distributed in the eulittoral zone that periodically dries. These habitats are ıallow enough to potentially experience diel changes similar to seasonal pools, and populaons persist in this amphibious state (Nielsen et al., 1991). *Isoëtes asiatica* is a species of ıallow lakes where only a portion of the population is amphibious (Pietsch, 1991). Also, ›me tropical alpine ephemeral pools dominated by CAM species (*Isoëtes* and *Crassula*) are :ry oligotrophic and, because of this state and the low temperatures, fail to generate signifiınt diel changes in CO_2 (Keeley, unpubl. data).

Other CAM habitats include slow-moving shallow streams (*Isoëtes flaccida*), shaded secons of relatively fast-moving irrigation canals (*I. malinverniana*), and the eulittoral zone of eshwater tidal rivers (*I. riparia* and *Sagittaria subulata*) (Keeley, 1987). These require furıer study to elucidate the relevant selective factors favoring CAM.

In summary, aquatic CAM distribution is a function of two factors: inorganic carbon and irıdiance. CAM plants dominate under carbon-limited conditions, and as trophic conditions nprove and free CO_2 levels go up, CAM plants dominate only under conditions that generate ıarked diel patterns of availability. Within oligotrophic habitats, irradiance may play a role ʏ limiting the length of time available for light-requiring reactions, and here CAM may play a ›le in extending the depth to which certain *Isoëtes* can colonize.

VIII. CAM and the Carbon Budget

Although enhanced water use efficiency is the ultimate selective force in terrestrial CAM lant evolution, the proximal selective factor is enhanced daytime intercellular CO_2 partial ressure (p_i). High $CO_2(p_i)$ on the order of 40 mPa Pa^{-1} or 4% v/v results from high stomatal :sistance, coupled with decarboxylation of malate stores (Winter & Smith, 1995a). In effect, AM is a CO_2-concentrating mechanism and thus requires a physical setting in which a diseuilibrium is created between exogenous and endogenous CO_2 pools.

In aquatic plants, several factors inhibit CO_2 leakage during daytime decarboxylation of ıalate, thus creating a disequilibrium in CO_2 pools. The primary factor is the high diffusive :sistance of water (10^4 times greater than air). Also, water per se has an ameliorating effect n gas exchange, which, relative to leaves in air, inhibits outward diffusion of CO_2 (Steinberg, 996). The cuticle, a feature uncommon in aquatic plants (Sculthorpe, 1967), is quite apparent ı many aquatic CAM plants and may be an important resistance factor. Additionally, anatomiıl features play a role because chloroplasts are concentrated in mesophyll cells surrounding the lacunae, and consequently, sites of decarboxylation are several cell layers removed from the ambient environment, which constitutes a substantial diffusional resistance (Raven, 1977) and further contributes to disequilibrium. The standard to which these resistances are measured is the RUBISCO activity. For decarboxylation to be effective, CO_2 leakage must not be greater than the rate at which it can be fixed. Also, daytime PEPC activity may, through its substantially lower K_m, capture carbon and thus inhibit leakage (Osmond, 1984; Winter, 1985). Estimates of leakage rates for *Littorella* and *Isoëtes lacustris* indicate that only 1–2% inorganic carbon is lost, and leakage rate is not sensitive to CO_2 concentration (Søndergaard & Sand-Jensen, 1979a; Madsen, 1987b).

Habitats differ in the factors contributing to disequilibrium between ambient and endogenous CO_2 sources.

A. SEASONAL POOL CAM PLANTS

CAM plants in seasonal pools show diel patterns of carbon uptake in the light and dark that are correlated with changes in ambient CO_2. An example of one spring day for *Isoëtes howellii* shows that as available carbon declines during early morning (Fig. 7A), CO_2 uptake is suppressed (Fig. 7B). Tracking this decline is a rapid decarboxylation of vacuolar malic acid stores (Fig. 7B), as photosynthesis switches to increasing dependence upon this endogenous carbon source. Three of the four phases of CO_2 exchange recognized by Osmond (1978) for a "well-irrigated CAM plant" are evident in this aquatic (Fig. 7B).

Phase 1, the period of dark CO_2 uptake and assimilation, matches well with terrestrial CAM plants, including the suppressed uptake late in the dark phase (Fig. 7B). This depression is also observed under steady-state conditions in the lab (Keeley & Bowes, 1982) and may reflect feedback inhibition of malic acid on PEPC activity (Groenhof et al., 1988; Kluge & Brulfert, 1995).

Phase 2 shows an acceleration in uptake due to the light-induced switch to direct assimilation of carbon by the C_3 pathway, a pattern also seen in terrestrial CAM plants. It is not known how much of this initial burst in CO_2 uptake in the light results from a combination of both PEPC and RUBISCO activity. In Osmond's prototype CAM plant, Phase 2 is characterized by a rapid suppression of CO_2 uptake, resulting from stomatal closure, although there is much species-specific variation in rate of stomatal closure (Kluge & Ting, 1978; Borland & Griffiths, 1995; Winter & Smith, 1995a). Since functional stomata are lacking in aquatic plants, the drop in CO_2 uptake during Phase 2 is obviously not related to stomatal behavior; rather, it is due to the depletion of ambient CO_2 (Fig. 7A).

Phase 3 is a period of limited CO_2 uptake, controlled in terrestrial CAM plants by stomatal closure, which is a response to high internal $CO_2(p_i)$, generated by malate decarboxylation. Phase 3 in this aquatic CAM plant is controlled by the depletion of ambient CO_2.

Phase 4 in terrestrial plants is a period in which the Phase 3 suppression of CO_2 uptake is overcome because malate is depleted; as a consequence, $CO_2(p_i)$ decreases and this induces stomatal opening. Phase 4 is missing in this aquatic CAM plant because ambient CO_2 remains depleted, due to slow gas exchange with the atmosphere (Smith, 1985) and high pH resulting from bicarbonate uptake by other species in the community.

In *I. howellii* the pattern of acidification (Phase 1) and deacidification (Phases 2 & 3) track ambient CO_2 (Fig. 7). Deacidification is insignificant during the first three hours of Phase 2 and appears to be controlled by high ambient CO_2, as suggested by the fact that percentage deacidification is correlated with percentage CO_2 depletion of the water. Also, deacidification can be experimentally slowed by incubation under elevated CO_2 levels (Keeley, 1983a). A

ilar suppression of deacidification by elevated CO_2 is also observed in terrestrial CAM
its (Fischer & Kluge, 1985). In the aquatic habitat, *I. howellii* deacidification is correlated
ı irradiance, such that on cloudy days, decarboxylation of malate slows and ΔH^+ is sup-
;sed. This may be tied to the fact that lower PAR reduces photosynthetic demand for CO_2
he pool flora, causing CO_2 in the water to remain high through mid-day (Keeley & Busch,
4).

:ntegrating the area under the CO_2 uptake curve (Fig. 7B) shows that on this particular
:, CO_2 uptake contributed 49% of the total 24 hr gross carbon gain. Under shorter day
;ths and cooler temperatures earlier in the season, both total gross carbon uptake and the
‹ contribution are lower (Keeley & Busch, 1984).

A comparison of total CO_2 uptake in the dark and total CO_2 fixation in the dark (predicted
\H$^+$) indicates that carbon uptake never matches carbon assimilation. This is because dark
ıtion utilizes both ambient CO_2 and an endogenous source arising from respiration. Re-
ıtion of respiratory CO_2 is illustrated by the substantial overnight acid accumulation possi-
under CO_2-free conditions (Fig. 8). It is estimated that throughout the season this may
ount for 50–75% of the dark carbon fixation in *I. howellii* (Keeley & Busch, 1984) and in
ıssula helmsii (Newman & Raven, 1995).

In summary, dark fixation affects carbon balance both by extending the period of CO_2 up-
ɜ and by recycling CO_2. Terrestrial CAM plants are similar, in that a portion of overnight
l accumulation is due to refixation of respiratory carbon and this can be up to 100% in what
ɜferred to as "CAM-cycling" or "CAM-idling" (Griffiths, 1988; Martin, 1995).

Root uptake of CO_2 from interstitial water in the sediment may be substantial in many la-
trine isoetids (Section VIII.B.1) but is less significant in amphibious seasonal pool spe-
;. Although CO_2 concentration in these sediments is about one order of magnitude higher
n the peak water column levels (Keeley & Sandquist, 1991), soils are commonly fine clay
iments with small interstitial spaces. Also, seasonal pool *Isoëtes* have less intercellular air-
ce than do lacustrine species. Laboratory studies with leaves and roots in separate com-
tments show that for *I. howellii*, under CO_2 levels matching field conditions around leaves
l roots, uptake by leaves is about 5–10 times greater than by roots, and this is under condi-
ıs in which the solution surrounding the roots is stirred (Keeley, unpubl. data). When one
ısiders the diffusive resistances in these sediments, it is apparent they are not likely a major
bon source for these plants.

B. LACUSTRINE CAM PLANTS

The absence of diel changes in ambient CO_2 availability (Section VII.B) means that the
›lution of CAM in these environments has been driven by factors distinct from those effec-
ɜ in seasonal pools. There is evidence that both carbon and light may be limiting. In addi-
n, other nutrients are scarce in these infertile habitats, and the CAM pathway potentially
ıld enhance nitrogen-use efficiency (Griffiths, 1989; Robe & Griffiths, 1994). Evaluating
se factors is complicated by CO_2 uptake from both the water column and sediment.

1. Sediment CO_2 Uptake

In *Littorella*, the permeability for CO_2 transport across the root surface is 0.6–0.8 mm hr^{-1}
l across the leaf surface is 3.8–5.8 mm hr^{-1} (Madsen, 1987a). This, coupled with the sub-
ntially shorter source-to-sink path length in leaves, makes it no surprise that, under equal
$)_2$ concentrations, leaves exhibit greater CO_2 uptake (per unit surface area) than roots (Søn-

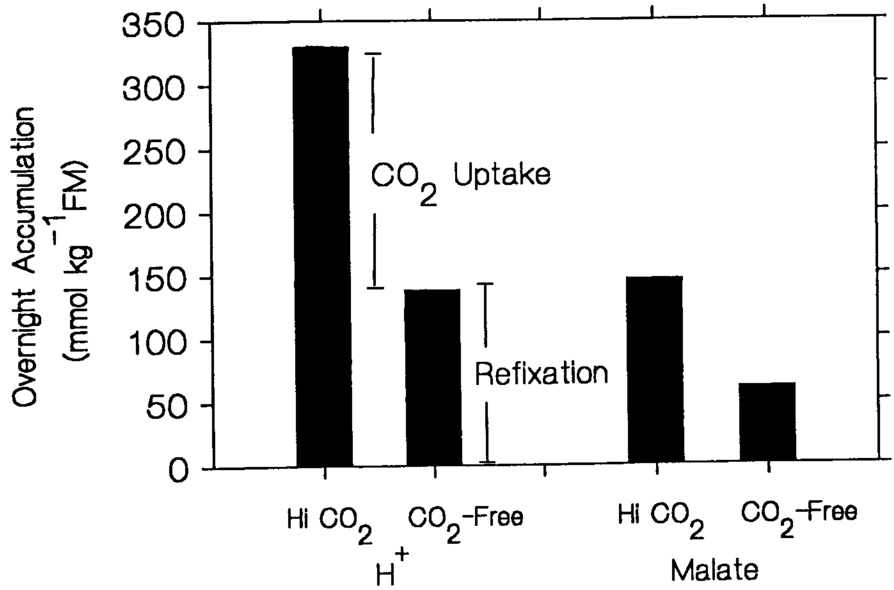

Fig. 8. Overnight H^+ and malate accumulation in the seasonal pool *Isoëtes howellii* under high-CO_2 (0.29 mol m^{-3}) and CO_2-free conditions. (Redrawn from Keeley & Busch, 1984.)

dergaard & Sand-Jensen, 1979a). However, oligotrophic lakes typically have carbon-rich sediments that may contain one to two orders of magnitude more free-CO_2 than the water column (Table IV). Macrophytes with the isoetid growth form, including both CAM and non-CAM species, capitalize on this rich carbon source and derive a substantial portion of their carbon from the sediment.

Under ambient CO_2 levels in the water column (0.015 mol m^{-3}) and sediment (>1 mol m^{-3}), for both *Littorella* and *Isoëtes* species, more than 95% of CO_2 uptake in the light is through the roots (Søndergaard & Sand-Jensen, 1979a; Boston et al., 1987). However, as the water column CO_2 level rises, root uptake may decline to <50% of the total uptake (Richardson et al., 1984; Sandquist & Keeley, 1990).

Dark CO_2 uptake shows a similar pattern where, under natural levels of CO_2 in the water column and sediment, all CO_2 uptake is through the roots (Fig. 9B). As root medium CO_2 level goes down, uptake from the water column increases (Fig. 9A), and when root medium levels are higher, there is net CO_2 evolution from the foliage (Fig. 9C). It is of some interest that the overnight acid accumulation in *Littorella*, which matches very closely the estimated total dark CO_2 fixation (= direct uptake from the water + root uptake from the sediment + refixation of respiratory carbon), does not differ significantly across the range from 0.7 to 3.1 mol m^{-3} sediment CO_2; rather, all that changes is the path of CO_2 uptake (Madsen, 1987a).

Root uptake results in a substantial increase in $CO_2(p_i)$ in the lacunae (Fig. 9 caption), and this endogenous CO_2 is an important source for carbon assimilation in both the light and the dark. In addition to being a rich carbon source, transport to chlorenchymous cells surrounding the lacunae is through the gas phase, and thus substantially faster than aqueous phase transport from the water column (Raven, 1984). This internal CO_2 supply can exceed demand at

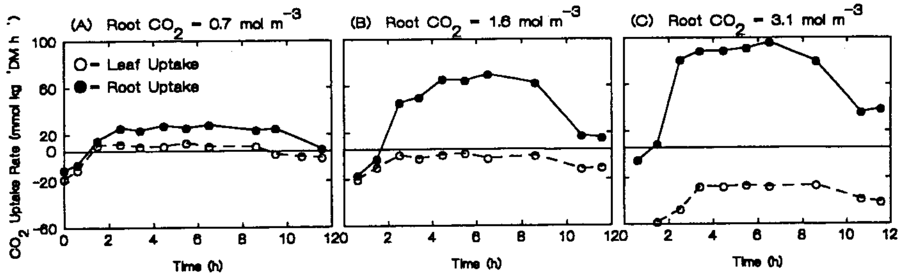

Fig. 9. Dark CO_2 uptake by roots vs. leaves of the lacustrine *Littorella uniflora* under varying condi-ions of root medium CO_2 concentration, with leaf medium held constant at 0.02 mol m^{-3} from natural ake water @ 15°C. Over the 12 h dark period average leaf lacunae CO_2 concentrations were **(A)** ~75, **(B)** ·150, and **(C)** ~425 mmol m^{-3}. (Redrawn from Madsen, 1987a.)

ıight, as evidenced by inorganic carbon leakage (Søndergaard, 1981), but may be limiting luring the day (Søndergaard & Sand-Jensen, 1979a; Madsen, 1987b). As this CO_2 source be-omes limiting, CAM—through decarboxylation of malate stores—enhances internal CO_2 oncentration (Robe & Griffiths, 1988). Under natural substrate levels of CO_2, it appears that AM is capable of maintaining endogenous CO_2 levels sufficient to suppress photorespiration ınd make PAR the limiting factor to photosynthesis (Robe & Griffiths, 1990).

Root uptake of CO_2 is by passive diffusion through airspaces in the roots, stems, and eaves (Raven et al., 1988; Keeley et al., 1994). There is also a net flow of O_2 into hypoxic ediments which has beneficial effects on nutrient uptake (Tessenow & Baynes, 1978; Sand-ensen et al., 1982; Smits et al., 1990; Pedersen et al., 1995).

Characteristics associated with the isoetid growth form which enhance carbon uptake rom the roots are 1) high root:shoot ratio, 2) short pathway from roots to leaves, 3) extensive ıir space, and 4) chloroplasts in cells surrounding the lacunae. Species with other growth orms, such as the non-CAM *Myriophyllum spicatum*, obtain very little carbon from the sedi-nent (Loczy et al., 1983; Raven et al., 1988). It appears that isoetids can alter their root per-neability in response to sediment characteristics—e.g., highest lacunal CO_2 concentrations vere observed in *Littorella* grown on the lowest CO_2 sediments (Robe & Griffiths, 1988)

Although *Crassula* species lack the isoetid growth form conducive to root uptake, they are generally prostrate and therefore may benefit from enhanced sediment CO_2; for instance, wa-er column CO_2 concentration a few centimeters above the sediment may be more than one or-ler of magnitude greater than the level in bulk water (Robe & Griffiths, 1992).

2. *Factors Affecting Acidification and Deacidification Patterns*

The decline in CO_2 uptake late in the dark period observed for *Littorella* (Fig. 9A–C) is imilar to that observed for the seasonal pool species *Isoëtes howellii* (Fig. 7B). Also in com-non with that seasonal pool species is the substantial role of nighttime refixation of respira-ory CO_2 in *Littorella* and *I. lacustris*: from ⅓ to ⅔ of the total acid accumulation (Madsen, 1987a; Robe & Griffiths, 1990; Richardson et al., 1984; Smith et al., 1985).

In *Littorella*, incubation for several weeks under a 12 hr photoperiod of low photosyntheti-ally active radiation (PAR = 40–50 μmol m^{-2} s^{-1}) greatly reduces overnight acid accumulation Madsen, 1987c; Robe & Griffiths, 1990). This damping effect of low light also has been reported for *Isoëtes kirkii* (Rattray et al., 1992). Perhaps this is due to low stores of starch for glycolytic PEP production or the extra ATP required to drive the tonoplast transfer of malate (Smith et al., 1995; Lüttge, 1987) and is consistent with the high photon costs of net CO_2 fixation by CAM plants (Raven & Spicer, 1995). A similar effect of low daytime PAR inhibiting ΔH^+ is observed in terrestrial CAM plants (Osmond, 1978). Seasonal changes in light and temperature also contribute to lower levels of CAM in autumn and winter for the aquatic *I. macrospora* (Boston & Adams, 1985) and *I. lacustris* (Gacia & Ballestros, 1993).

When light is less limiting (450–500 μmol m^{-2} s^{-1}), CAM activity is maintained at CO_2 levels between 0.01 and 1.5 mol m^{-3}) but reduced or eliminated at 5.5 mM free-CO_2 (Madsen, 1987b, 1987c; Robe & Griffiths, 1990). In *Littorella*, a CO_2 level sufficient to suppress CAM is 3.0 mol m^{-3} around the leaves, but 5.4 mol m^{-3} is required around the roots, reflecting the substantially greater resistances, less surface area, and longer path length from roots to the site of carboxylation (Madsen, 1987b). Inhibition of CAM by elevated CO_2 operates by suppressing daytime decarboxylation, as indicated by the fact that high (>1 mol m^{-3}) CO_2 in the dark phase produces high ΔH^+ but the same CO_2 level in the light phase causes an immediate suppression of CAM (Madsen, 1987b; Hostrup & Wiegleb, 1991a).

3. *Contribution of CAM*

Calculation of a carbon budget is complicated by the necessity to include carbon uptake from both leaves and roots, and carbon fixation in the light and dark, as well as refixation of respiratory carbon. Light is potentially limiting, and its effect is likely to differ between species. *Littorella*, which occupies shallow water, typically experiences mid-day photosynthetically active radiation (PAR) levels of 100–200 μmol m^{-2} s^{-1}) at the leaf tips and receives an annual photon flux density (PFD) estimated at 1760 mol m^{-2} yr^{-1} (Sand-Jensen & Madsen, 1991). *Isoëtes lacustris* is distributed more deeply (PFD = 455 mol m^{-2} yr^{-1}) and, in response to these zonation differences, has higher chlorophyll levels, lower light-saturated net photosynthesis, and higher photosynthetic rates under low irradiance than *Littorella* (Sand-Jensen, 1978). The extent to which these factors affect differences in expression of CAM (e.g., stoichiometry of uptake: fixation in both the dark and light) has not been explored.

Field studies of *I. bolanderi* showed that daytime carbon uptake tracked irradiance and that substantial uptake was restricted to about a 6 hr period around mid-day (Sandquist & Keeley, 1990). In this study dark CO_2 uptake contributed about 30% of the gross carbon uptake, which approximates the 28% calculated for the contribution of dark CO_2 uptake by *I. lacustris* (Richardson et al., 1984).

A reasonably complete carbon budget for *Littorella* has been provided by Robe and Griffiths (1990), under natural carbon conditions and little or no light limitation (Fig. 10):

1. 55% of the total carbon gain is derived from dark CO_2 uptake
2. CO_2 uptake accounts for only 30% of the dark fixation (i.e., there is substantial refixation of respiratory CO_2)
3. 81% of the CO_2 supply for daytime photosynthesis is derived from decarboxylation of malate.

The importance of CAM is further demonstrated by the lack of congruence in O_2 evolution and CO_2 uptake (Fig. 11); during the day, *Littorella* exhibits substantial O_2 evolution but minimal CO_2 uptake. This seeming disconnection of the light reactions and carbon reduction reactions is because carbon assimilation is utilizing endogenous CO_2 sources, such as that derived from decarboxylation of malate. A consequence of using this endogenous CO_2 source is a re-

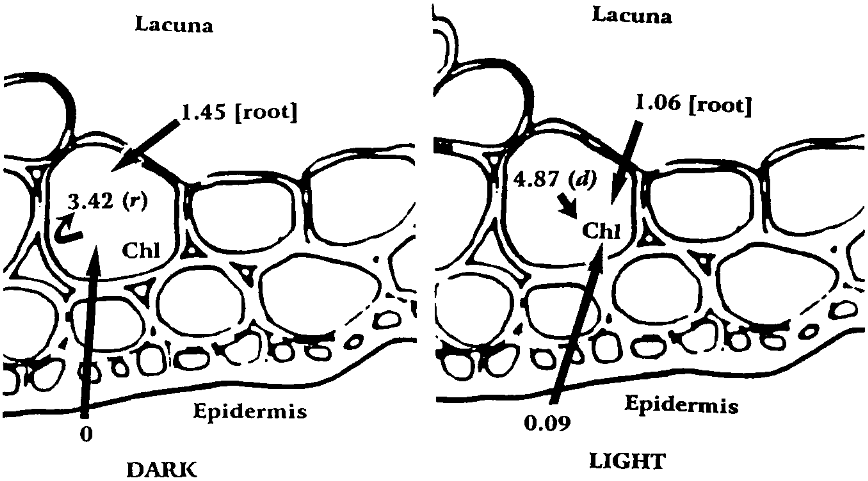

Fig. 10. Carbon sources (mmol C kg^{-1} FM h^{-1}) in dark and light for *Littorella uniflora* photosynthe- under 12 h photoperiod of 300 μmol m^{-2} s^{-1} PAR @ 19–20°C under CO_2 concentrations typical for wa- and sediment from Esthwaite (see Table IV). Chl, chloroplast; [root], uptake from sediment; *r*, ixation of respiratory CO_2; *d*, CO_2 from decarboxylation of malate pool. Both *r* and *d* represent net ex- ınge and could involve exchanges with lacunal gas space (data from Robe & Griffiths, 1990), drawn modified leaf illustration from Hostrup & Wiegleb, 1991b.

ction in the CO_2 compensation point and increase in carboxylation efficiency (Madsen, 87b, 1987c).

Limitations of nutrients other than carbon appear to play a relatively minor role in control- g CAM activity (Madsen, 1987c; Robe & Griffiths, 1994). However, evolution of carbon- ncentrating mechanisms such as CAM, in plants on infertile sites, potentially makes nutri- ts other than carbon the limiting resource in primary productivity (Raven, 1995). Even ›ugh nutrient limitations may have minimal proximal effect, ultimately the infertility of gotrophic lakes has likely been a strong selective influence on growth rates (Boston, 1986; ›ston & Adams, 1987). Reflective of these CAM plants' adaptation to nutrient-poor habitats the observation that *Littorella* plants grown on the lowest sediment CO_2 concentrations ıintained the highest levels of lacunal CO_2, ΔH^+, and photosynthesis (Robe & Griffiths, 88).

C. PRODUCTIVITY

Most studies of aquatic CAM production concern lacustrine species from infertile rbon-poor habitats. Standing above-ground biomass of macrophytes in oligotrophic ces is commonly one to three orders of magnitude lower than in eutrophic lakes lacking .M species (Sculthorpe, 1967; Wetzel, 1975). Within the littoral zone dominated by mac- ›hytes, standing crops often are 0.1–2.0 mg oven-dry mass ha^{-2} (Sand-Jensen & Sønder- ard, 1979; Toivonen & Lappalainen, 1980; Keeley et al., 1983a; Boston & Adams, 1987; ıcia & Ballestros, 1994). Growth rates are generally low and, even when placed under en- hed carbon conditions, species (both CAM and non-CAM) from such oligotrophic lakes ve rates lower, by an order of one magnitude or more, than species from more meso- or

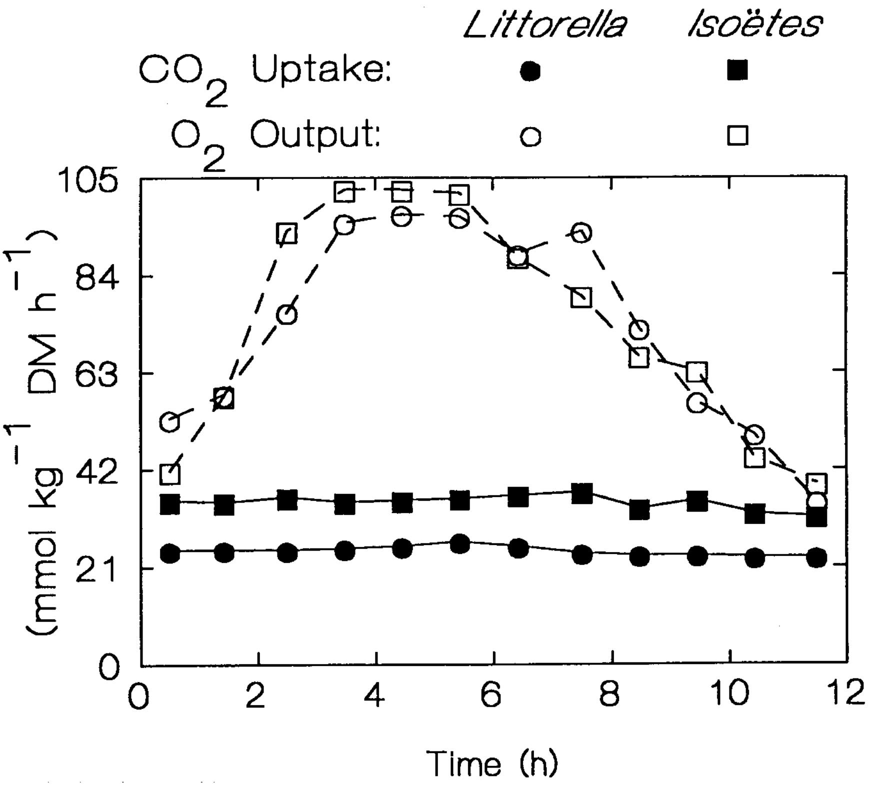

Fig. 11. Comparison of two measures of photosynthesis in *Littorella uniflora* (●○) and *Isoëtes lacustris* (■□) using carbon uptake (●■) and oxygen evolution (○□) with an external CO_2 concentration of 0.125 mol m^{-3} @ 15°C and 300 μmol m^{-2} s^{-1} PAR. (Redrawn from Madsen, 1987b.)

eutrophic habitats (Boston et al., 1989). In the lacustrine habitat, the CAM pathway contributes about 50% of the total annual carbon gain, largely through the extension of the carbon assimilation period (Boston & Adams, 1986). This nocturnal carbon contribution was equivalent to the total 24 hr dark respiration and a critical component to success in these lakes.

Seasonal pools are densely vegetated with as much as 10 mg dry mass ha^{-2} yr^{-1} production each growing season (Keeley & Sandquist, 1991). While not a record for CAM plant productivity (Nobel, 1995), it is significantly higher than the productivity of many arid CAM habitats. In one study, gross CO_2 uptake was about 10% higher for *Isoëtes* than associated non-CAM species (Keeley & Sandquist, 1991). Gross measures of productivity (i.e., biomass changes during the growing season) showed *I. howellii* production at 9.9 ± 0.1 g dry mass m^{-2} day^{-1}; this species represented 37% of the biomass early in the season and 53% late in the season. These seasonal pools are mesotrophic habitats, and under the right conditions certain CAM plants are capable of considerable productivity, potentially outcompeting other species, as evidenced by the aggressive invasive ability of the aquatic CAM *Crassula helmsii* (Dawson & Warman, 1987; Newman & Raven, 1995).

IX. Aquatic CAM Plants in an Aerial Environment

Seedlings of terrestrial CAM species commonly are C_3 and switch to CAM later in development (Raven & Spicer, 1995), whereas amphibious CAM species exhibit an opposite pattern. During early stages of development underwater they exhibit CAM, but upon exposure to an aerial environment amphibious species switch off CAM and rely strictly on the C_3 pathway. This has been demonstrated both by diminished ΔH^+ (Table V) and ^{14}C-labeling studies (Keeley, 1998b). This switch occurs on a cell-by-cell basis as the emergent tips of leaves will reduce overnight acid accumulation, whereas submerged bases retain CAM (Keeley, 1988). As the dry season approaches, and these aerial plants are exposed to increasing aridity, they do not regain the CAM pathway. Many eulittoral lacustrine species also will switch off CAM upon exposure (Table V).

Aquatic CAM plants exhibit further plasticity in their adaptation to a terrestrial existence; stomata become functional or are initiated *de novo*, and there are increases in protein, total chlorophyll, percentage chlorophyll a, $^{RUBISCO}/_{PEPC}$ ratio, and photosynthetic rate (Table III; Groenhof et al., 1988; Keeley, 1990, 1998b). Coupled with these physiological changes are subtle changes in leaf anatomy, such as increased stomatal density, thicker cuticle, and smaller lacunae (Keeley, 1990; Hostrup & Wiegleb, 1991b). It is apparent that water potential changes at the leaf surface are involved in switching off CAM, as *I. howellii* maintained at >90% relative humidity will retain CAM in the aerial environment (Keeley, 1988), as does *I. setacea* (Gacia & Ballestros, 1993) and *Littorella* (Aulio, 1986b). These structural and functional changes are likely mediated by hormonal changes induced by lower water potentials (e.g., Schmitt et al., 1995).

Switching off CAM in the aerial environment is ultimately a response to enhanced availability of CO_2. Despite the fact that atmospheric partial pressure of CO_2 is lower than in most aquatic habitats, substantially lower diffusional resistances in air dramatically reduce carbon limitation in the leaf boundary layer. As with terrestrial species exhibiting similar photosynthetic flexibility (e.g., Bloom & Troughton, 1979), the shift from CAM to C_3 is potentially tied to enhanced productivity in these amphibious species as well.

Some aquatic characteristics are retained in the terrestrial environment—e.g., sediment-based CO_2 uptake continues in terrestrial populations of *Littorella* (Nielsen et al., 1991) as well as in the non-CAM *Lobelia dortmanna* (Pedersen & Sand-Jensen, 1992). High cuticular resistance of the terrestrial leaves was noted by these authors as reason for hypothesizing a terrestrial origin for this mode of nutrition. However, all aquatic plants possess cuticles (Raven, 1984), and it is particularly prominent in many lacustrine *Isoëtes*, although thickness is not a reliable indicator of permeability (Kerstiens, 1996). With respect to both sediment-based nutrition and CAM, there are clear selective advantages to cuticular development in aquatic plants.

Not all lacustrine *Isoëtes* switch off CAM upon emergence. Some tropical alpine species, for instance, retain CAM for at least six months in an aerial environment with low humidity (Table V), and leaves initiated under terrestrial conditions fail to produce stomata.

X. Diel Acid Changes in Other Aquatic Species

Not all 69 species demonstrating significant ΔH^+ (Table I) have been included in this discussion of aquatic CAM. In addition to the five genera already discussed, others may deserve this designation. For example, *Lilaeopsis lacustris* (Apiaceae) was reported to have substantial overnight accumulation of acidity and malate (Table I), but was not included due to the

Table V

Diel changes in titratable acidity (ΔH^+) under submerged and aerial conditons for aquatic and terrestrial species

								(ΔH^+)[d] (mmol kg^{-1} F.M. 24-h^{-1})	
Taxa	Habitat[a]	Habit[b]	Latitudinal zone	Data source[c]	Country	Latitude	Elev. (m)	Submerged ($\bar{x}$ + SD)	Aerial ($\bar{x}$ + SD)
Isoëtes howellii	Seas. pool	Sum. decid	Temperate	3	U.S.A.	34°N	610	294 + 22	14 + 4
Crassula aquatica	Seas. pool	Sum. decid.	Temperate	4	U.S.A.	34°N	610	103 + 9	28 + 1
C. natans	Seas. pool	Sum. decid.	Temperate	7	S. Africa	33°N	200	100	4
Isoëtes bolanderi	Lacustrine	Win. decid.	Temperate	5	U.S.A.	38°N	2900	187 + 9	32 + 3
I. macrospora	Lacustrine	Evergreen	Temperate	7	U.S.A.	47°N	100	182 + 10	4 + 2
Littorella uniflora	Lacustrine	Evergreen	Temperate	1	Finland	61°N	—	141 + 12	1 + 6
Isoëtes palmeri	Lacustrine	Evergreen	Tropical	7	Colombia	4°N	3650	68 + 13	80 + 21
I. karstenii	Lacustrine	Evergreen	Tropical	7	Colombia	4°N	3650	98 + 5	85 + 6
I. nuttallii	Terrestrial	Sum. decid.	Temperate	2	U.S.A.	38°N	500	2 + 1	1 + 1
I. butleri	Terrestrial	Sum. decid.	Temperate	2	U.S.A.	35°N	500	1 + 1	1 + 1
I. stellenbosensis	Terrestrial	Sum. decid.	Temperate	7	S. Africa	33°S	1200	1 + 1	2 + 1
Crassula erecta	Terrestrial	Sum. decid.	Temperate	4	U.S.A.	34°N	610	3 + 1	2 + 1
C. oblanceolata	Terrestrial	Sum. decid.	Temperate	7	S. Africa	33°S	1200	3 + 1	2 + 1
Isoëtes andicola	Terrestrial	Evergreen	Tropical	6	Peru	11°S	4135	—	90 + 15
I. andina	Terrestrial	Evergreen	Tropical	6	Colombia	4°N	3650	—	182 + 22
I. novo-granadensis	Terrestrial	Evergreen	Tropical	6	Ecuador	0°	4050	—	142 + 25

[a] Seas. pool, seasonal pool.

[b] Sum. decid., summer deciduous; Win. decid., winter deciduous.

[c] 1, Aulio, 1985; 2, Keeley, 1983b; 3, Keeley & Busch, 1984; 4, Keeley & Morton, 1982; 5, Keeley et al., 1983a; 6, Keeley et al., 1994; 7, Keeley, unpubl. data.

[d] N ≥3

c of other supporting data and absence of ΔH^+ in other aquatic species of *Lilaeopsis*. *Scir- subterminalis* likewise has not been included for lack of further data and the low ampli- e of ΔH^+ (Table I), which, of course, does not preclude presence of the CAM pathway.

Prudence is justified, as some species with significant ΔH^+ clearly are not CAM. For exam- , *Orcuttia* spp. (Poaceae) have a low but consistent ΔH^+ (Table I; Keeley, 1998a), and la- ing studies indicate that malate is the first stable product of dark fixation. However, dark se–dark chase studies show nearly all label fixed in the dark is transferred out of the malate l in the dark, and a substantial proportion ends up in insoluble compounds (Fig. 12A). By end of the dark period, over 50% of the label is in citrate (not shown), suggesting that dark- ed carbon has been transported to the mitochondria (Kalt et al., 1990; Olivares et al., 1993). *ocharis acicularis* (Cyperaceae) exhibits a similar pattern of malate turnover in the dark eley, unpubl. data). *Hydrilla verticillata* was early documented as exhibiting dark fixation l slight acid accumulation (Holaday & Bowes, 1980). It, too, metabolizes a substantial por- 1 of the dark-fixed carbon in the dark in apparently non-autotrophic metabolism (Fig. 3). These observations do not conclusively demonstrate absence of the CAM pathway, as n well-recognized terrestrial CAM plants utilize some portion of dark-fixed carbon for 1-autotrophic metabolism (Lüttge, 1988). However, when coupled with data on rates of up- e, it appears that dark CO_2 fixation in these species may not contribute significantly to au- ophism. Typological designations such as CAM are always problematic when dealing with nomena that vary quantitatively.

Downingia bella has CO_2 fixation in the dark, and the fact that malate accumulates (Fig. C) suggests it may contribute to autotrophism, but this species lacks certain CAM criteria: It libits a highly significant Δmalate, but, despite repeated sampling, there is no indication of [+ (Table I). It is comparable to *Isoëtes* in the $^{RUBISCO}/_{PEPC}$ ratio, and activity of NADP Malic zyme and pyruvate, P_i-dikinase (Keeley, 1998b). This plant deserves further study, as it is a me candidate for the scheme proposed by Raven et al. (1988) for a CAM mechanism that uld couple H^+ disposal with K^+ uptake. They envisioned an autotrophic pathway that uld simulate CAM in most details, except malate^{2-} + $2K^+$ would be stored in the vacuole, ulting in significant Δmalate but no ΔH^+, as is observed in *D. bella* (Table I).

Some marine algae in all three of the major phyla have long been noted for their dark CO_2 ation (e.g., Joshi et al., 1962; Akagawa et al., 1972b; Willenbrink et al., 1979; Church et al., 33), and certain of the brown algae (Phaeophyta) have significant ΔH^+ (Table I). This, cou- d with evidence of photosynthetic use of endogenous CO_2 (Ryberg et al., 1990), has oked labels of CAM and CAM-like for several brown algae (Johnston & Raven, 1986; Raven Samuelsson, 1988; Axelsson et al., 1989; Raven et al., 1989; Raven & Osmond, 1992). One h species is the well-studied *Ascophyllum nodosum*, which has been reported to accumu- e 10–20 mmol H^+ kg FM (Surif & Raven, 1983; Johnston & Raven, 1986). Deviations from M are evident in the type of carboxylating enzyme (PEP carboxykinase: Kremer, 1979; rby & Evans, 1983) and lack of carbon storage in malate; only 5% of dark-fixed carbon re- ins in malate at the end of the 12 hr dark period (Fig. 13). Products labeled in the dark in- de glutamate, aspartate, succinate, and various amino acids, but during the dark period ase, most label accumulates in fumarate and citrate (Keeley, unpubl. data), which are or- nic acids not likely to act as carbon storage compounds for autotrophism (Lüttge, 1988). ese labeling patterns are not markedly different from those observed for other brown algae kagawa et al. 1972a; Kremer, 1979; Coudret et al., 1992).

Documenting the potential non-autotrophic uses of dark-fixed carbon is beyond the scope this review. However, it is worth noting that dark CO_2 fixation may contribute carbon to veral pathways, though not necessarily tied to acid accumulation. Non-autotrophic uses of

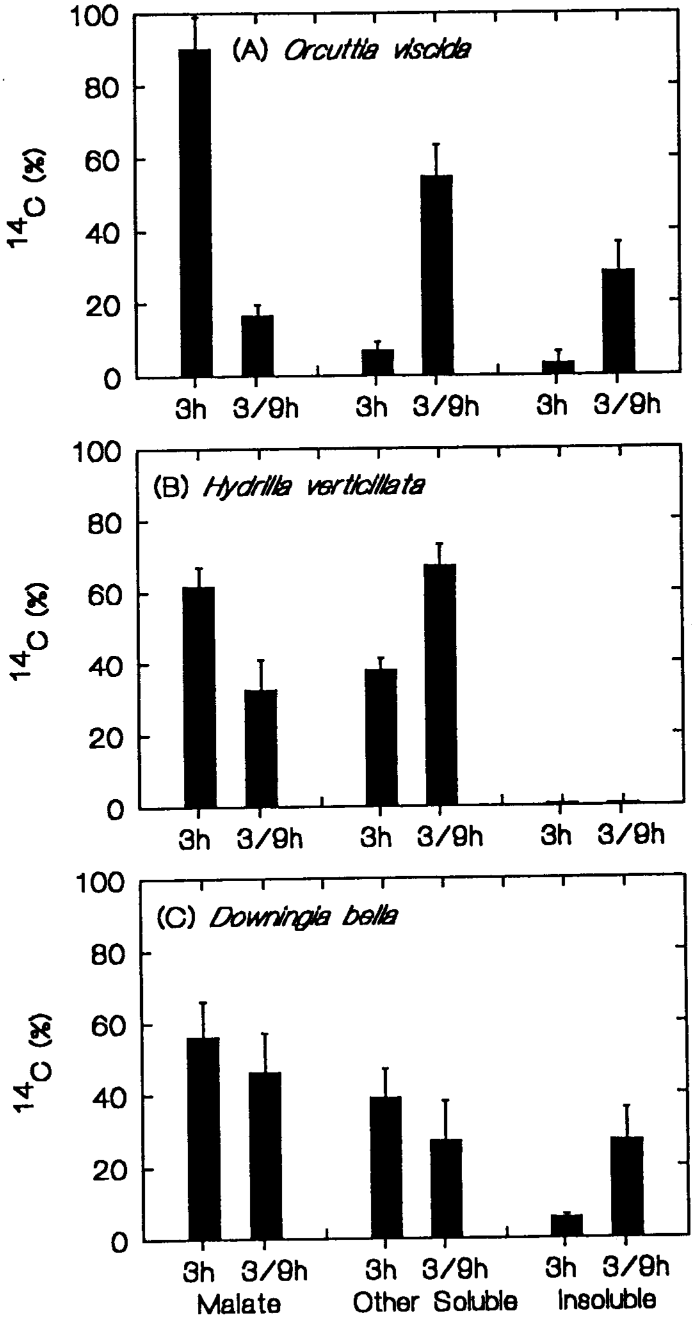

Fig. 12. Distribution of 3 h dark ^{14}C fixation and 3 h dark ^{14}C-pulse followed by 9 h ^{14}C-free-chase in the dark for species with low levels of ΔH^+ or Δmalate (see Table I) @ ~20°C and 10 mol m^{-3} MES buffer pH 6.0 (Keeley, unpubl. data).

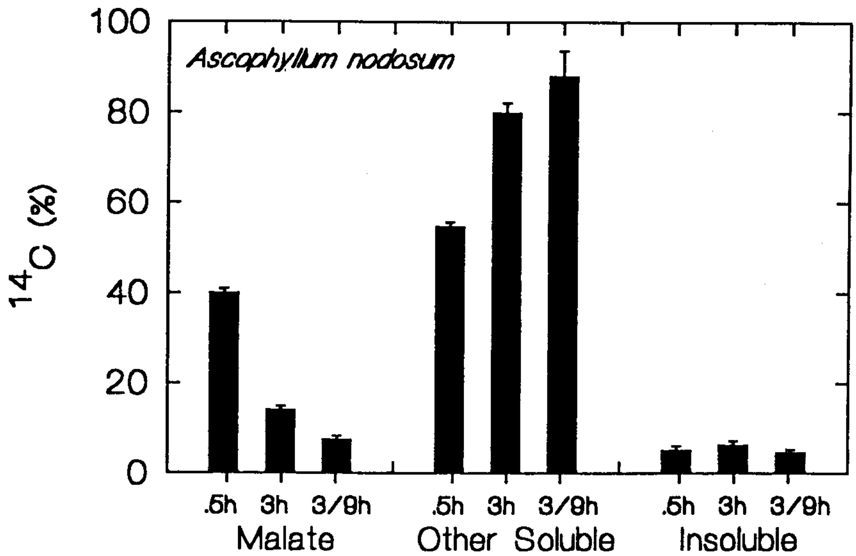

Fig. 13. Distribution of dark-labeled products after 0.5 and 3 h pulse and after a 3 h dark pulse plus a 9 :hase in the dark for the marine alga *Ascophyllum nodosum* (Keeley, unpubl. Data).

rk-fixed carbon include involvement as a pH stat mechanism for reducing cytoplasmic ıic disequilibrium (Raven, 1986) or in anaplerotic reactions related to nitrogen assimilation urpin et al., 1991). Relevant to the latter mechanism, dark CO_2 fixation in the macrophytic own algae *Ascophyllum nodosum* can be stimulated under enhanced nitrogen conditions .eeley, unpubl. data).

XI. Systematic Distribution

Significant ΔH^+ has not been detected in either the Chlorophyta or Rhodophyta, and the idification cycle in the brown algae (Phaeophyta) may not represent CAM (Section X). Ap- rent restriction of CAM to the Tracheophyta may be explained in part by the greater carbon ocation to cell wall material in these macrophytes, resulting in C acquisition being a more :e-limiting step than N, P, or Fe acquisition (Raven & Spicer, 1995).

Within the vascular plant flora, aquatic CAM plants are from widely unrelated taxa, such as :opods, monocots, and dicots. Of the 134 vascular plant species reported here, 37% had .M but more could be added with additional information. Estimating the proportion of the ›rld's aquatic flora with CAM is problematic due to incomplete information on the total mber of amphibious species. If we restrict our attention to just those 33 characteristic uatic families listed by Sculthorpe (1967), thus removing species of *Crassula* and *Littorella* ›m our analysis, and assuming all aquatic *Isoëtes* are CAM, it is calculated that 6% of the uatic flora is CAM, which compares exactly with the 6% reported for terrestrial floras (Win- · & Smith, 1995a).

Of course, such comparisons are phylogenetically biased because of the potential linkage CAM and the aquatic habitat in certain lineages. While lacking a precise phylogenetically corrected comparison (Eggleton & Vane-Wright, 1993), we can obtain a less biased view of aquatic and terrestrial CAM distribution by focusing at the family level. Of the 33 aquatic families (representatives in about one-half have been tested), three (Isoetaceae, Alismataceae, and Hydrocharitaceae) have evolved CAM, or 9% of the aquatic plant families. For comparison with the distribution of terrestrial CAM, most attention has been focused on flowering plants, where there are 26 families with CAM (Smith & Winter, 1995). Based on an estimated 321 "terrestrial" families [349 flowering plant families reported by Stebbins (1974), minus the aquatic families considered above], gives an estimate that 8% of the terrestrial plant families have CAM, quite comparable to the aquatic flora. This suggests that CAM has had an equal likelihood of evolving in water as on land.

XII. Evolution of Aquatic CAM Plants

Being restricted to the Tracheophyta means that CAM is found only in secondarily aquatic plants. Did CAM originate in an aquatic milieu or was it present in terrestrial ancestors?

In *Isoëtes*, the earliest aquatic CAM plants, the view that they represent recent herbaceous descendants of a long linear reduction sequence from the arborescent Lepidodendrales (Stewart, 1983), could be interpreted as suggesting a terrestrial or at least emergent-aquatic origin for the group. However, recent evidence disputes this view and suggests that *Isoëtes*'s origins are tied to similar aquatic corm-bearing plants well developed in the Carboniferous, which coexisted with arborescent Lycophyta (Taylor, 1981; Skog & Hill, 1992; Kovach & Batten, 1993; DiMichele & Bateman, 1996). Several recent studies have shown complete *Isoëtes* specimens in early Triassic (>230 Ma) sediments, apparently forming dense monocultures in ephemeral pools (Wang, 1996; Retallack, 1997). Throughout the Triassic, these *Isoëtes* coexisted with other herbaceous Lycophyta, such as the extinct *Tomiostrobus* (Retallack, 1997) and *Isoëtites* Münster (Ash & Pigg, 1991; Pigg, 1992), both of which were amphibious, and remarkably similar to extant *Isoëtes*. Indeed, Hickey (1986, 1990) suggests that the three neotropical *Isoëtes* that form subgenus *Euphyllum* are basal to the genus and "represent relictual morphotypes" of the extinct *Isoetites*. These species (and perhaps *I. wormwaldii* from South Africa) have in common a laminate leaf, which clearly separates them from the rest of *Isoëtes*. Although *Isoetites* were cosmopolitan, these primitive *Isoëtes* have populations that are highly restricted (and mostly extirpated), but like *Isoëtites* they are aquatic.

Based on a cost–benefit evaluation of atmospheric conditions, Raven and Spicer (1995) speculated that terrestrial environments conducive to CAM were unlikely during geological periods relevant to the early evolution of the Isoetaceae. Their arguments, however, apply less to aquatic habitats, where biogenic processes buffer the system from the impact of atmospheric changes in CO_2. Carbon-limiting factors conducive to aquatic CAM evolution, such as diel changes in CO_2 availability in shallow seasonal pools, could have been present since the early Triassic history of the Isoetaceae. In addition, the rising temperature of the early Triassic (Spicer, 1993) would have exacerbated the tendency for sharp diel changes in CO_2 availability in shallow pools.

This scenario is supported by other observations. Based on the widespread distribution of derived traits, it is apparent that the initial morphological divergence from ancestral aquatic *Isoetites*, giving rise to modern *Isoëtes*, was in traits conducive to surviving dry dormant periods, indicative of an amphibious origin for the group (Hickey, 1986; Taylor & Hickey, 1992). Such an amphibious lifecycle is also supported by the presence of stomata in the earliest known *Isoëtes* and in other paleoecological characteristics (Retallack, 1997). Possibly the origin of *Isoëtes* was in amphibious habitats at the edges of Triassic swamps. Such habitats would have ıd diel changes in carbon limitation, which would have favored the evolution of CAM. High :ganic matter in these swamp sediments may also have favored CO_2 uptake from the sediment, ; suggested by the similarity in lacunal volume between *Isoëtes* roots and fossil roots of the exnct *Stigmaria* (Karrfalt, 1980) and *Pleuromeia* (Munster) Corda (Grauvogel-Stamm, 1993), ıd this in turn would have favored the evolution of CAM (Osmond, 1984).

The Cretaceous radiation of modern *Isoëtes* (Pigg, 1992), into less fertile lacustrine habi-.ts (Hickey, 1986), may reflect increasing competition from faster-growing aquatic flowerıg plants (Section VII). If so, CAM would have been an important pre-adaptation to ɔlonizing these oligotrophic lacustrine habitats.

An amphibious origin for CAM keeps alive Cockburn's (1981) "stomatal-hypothesis," but ther biochemical origins are equally reasonable (Osmond, 1984; Winter, 1985). Griffiths's .989) suggestion that CAM evolution proceeded from dark refixation of respiratory carbon to ark uptake, would not apply to aquatic CAM plants, since CO_2 uptake in these plants is not de-endent on evolution of unique stomatal behavior. The near-ubiquitous presence of CAM photo-nthesis in *Isoëtes* suggests that CAM has had a long and monophyletic relationship with the roup and therefore *Isoëtes* represents the oldest clade of CAM plants (Winter & Smith, 1995b). hus, the evolution of CAM photosynthesis dates back to the Paleozoic or shortly thereafter.

Despite this apparently very early origin for CAM, its widespread and highly disjunct phy-ɪgenetic distribution leads to the inescapable conclusion that, within the Tracheophyta, it is ot a homologous trait (Lüttge, 1987; Monson, 1989; Ehleringer & Monson, 1993). Further ısights into the evolution of aquatic CAM photosynthesis are possible through comparative :udies of certain taxa. Particularly promising are *Isoëtes* and *Crassula*, which are large genra (100–200 species), dominated by CAM species but also having non-CAM species. Comarison of these genera is of interest because *Isoëtes* comprises mostly aquatics with a few :rrestrial species, whereas *Crassula* is mostly terrestrials, with very few aquatic species.

A. PATTERNS OF RADIATION IN *ISÖETES*

Cladistic analysis indicates that radiation of modern *Isoëtes* has been from seasonal pools ıto both terrestrial habitats and infertile lacustrine habitats (Hickey, 1986; Taylor & Hickey, 992).

1. Putative Amphibious-to-Terrestrial Transitions

Evolutionary changes in photosynthetic biology occurred in the transition from water to ınd. Strictly terrestrial[1] species *I. nuttallii* and *I. butleri*, of western and eastern North Amer-:a, respectively, and *I. stellenbossiensis*, from the Cape Province of South Africa, lack CAM ven when artificially submerged (Table V); possibly the terrestrial *I. durieui* of Europe is

[1] The designation "terrestrial" has not been used consistently in *Isoëtes* literature. Bold et al. (1980) re-:rved the term for very few species such as *I. butleri*. To my knowledge, in North America *I. nuttallii* is ıe only other truly terrestrial *Isoëtes*, although there may be terrestrial ecotypes in *I. engelmanni* ʾarker, 1943). Because of constitutive physiological differences in their capacity for CAM (Table V), I elieve it is important to make the distinction between true terrestrial *Isoëtes*—here defined as ones ɔever" experiencing inundation—from amphibious species that initiate growth underwater, followed y a brief terrestrial stage prior to dormancy; others also make this distinction (e.g., Hickey, 1986). Tay-ɪr and Hickey (1992), on the other hand, used the term "terrestrial" more broadly to include all species ʾith a terrestrial stage and thus did not make a distinction between terrestrial and amphibious species. pecies in the latter category seldom establish on sites that are not inundated during early growth.

similar (Richardson et al., 1984). Lack of CAM, high $\Delta^{13}C$ values, and the absence of Kranz anatomy indicates that these terrestrials are C_3, which is consistent with their summer-deciduous nature, as there are few, if any, examples of C_4 or CAM terrestrial geophytes. These Temperate Zone terrestrial species are summer-deciduous plants restricted to vernally moist sites with relatively short growing seasons. They have functional stomata and develop rapidly until dormancy is imposed by drought, even in summer-rain climates (Baskin & Baskin, 1979). Normal growing conditions are similar to those experienced by amphibious species following dry-down of the seasonal pool habitat. An aquatic ancestry is supported by the presence of four lacunal chambers, structures that are atypical for terrestrial plants and missing from terrestrial outgroups in the Lycophyta (Hickey, 1986). Consistent with this model is the placement of terrestrial *I. butleri* as an offshoot of a clade that has radiated into various amphibious habitats (Hickey et al., 1989). On the other side of the continent, a similar origin applies to the terrestrial *I. nuttallii,* which would appear to be a recent derivative of the amphibious *I. orcuttii*; these species are so close that they have been synonymized in some taxonomic treatments.

In summary, *I. nuttallii, I. butleri, I. stellenbossiensis*, and *I. durieui*—plus an unnamed species from Chile (Keeley & Hickey, unpubl. data) and probably species from Australia (Keeley, unpubl. data)—are secondarily terrestrial and secondarily C_3. Systematic (Pfeiffer, 1922) and cladistic (Hickey, 1986; Hickey et al., 1989; Taylor & Hickey, 1992) analyses suggest a polyphyletic origin for this terrestrial syndrome.

2. *Putative Amphibious-to-Lacustrine-to-Terrestrial Transitions*

Given the absence of many plesiomorphic traits, it appears that lacustrine species of *Isoëtes* are more recently derived from amphibious ancestors (Hickey, 1986; Taylor & Hickey, 1992). CAM would have assisted in the invasion of these infertile lakes, and these sites would have enhanced further development of sediment-based CO_2 uptake. Many of these aquatic species have retained the facultative responses to emergence so that, under terrestrial conditions, they develop stomata and switch off CAM (Section IX).

However, in some neotropical alpine lacustrine species, adaptations to the aquatic environment appear to be genetically fixed; when grown in air, they retain CAM and fail to produce stomata (Section IX). This constitutive response could reflect a much earlier origin, an idea consistent with the neotropical distribution of the most primitive *Isoëtes* (Hickey, 1990). These neotropical alpine species often grow in relatively flat lake basins subject to siltation, and as a consequence many have very long leaves, with the lower ⅔ buried in the sediment.

Adjacent to many lakes, from Peru to Colombia, are terrestrial *Isoëtes* that are likewise "buried" in the sediment. They are evergreen with astomatous leaves and are the only extant terrestrial species of Tracheophyta lacking stomata. One of these terrestrial species is *I. [Stylites] andicola*, which has roots extending >2 m in depth and a below-ground:above-ground biomass ratio >15. These plants obtain most of their carbon from the sediment by diffusion through hollow roots and are CAM. These patterns have been verified experimentally (Keeley et al., 1984, 1994) and with isotopes; depletion in ^{14}C, relative to contemporary atmospheric levels, supports the conclusion of sediment-based nutrition, and high deuterium verifies the importance of CAM (Sternberg et al., 1985). Retention of CAM (Table V) in these neotropical terrestrial *Isoëtes* would be favored by the accumulation of lacunal CO_2 at night and by the highly cutinized astomatous leaves, which provide diffusive resistance to CO_2 leakage during daytime deacidification.

The fact that these terrestrial species have retained the conservative lacunal leaf architec-ıre suggests an aquatic ancestry for these species. These terrestrials are all high polyploids 2n = 44–132: J. Hickey, pers. comm.), and a polyphyletic origin for this syndrome is sup-orted both by flavonoid patterns between terrestrial and nearby lacustrine species and by the resence of this terrestrial syndrome in widely disjunct *Isoëtes* in South America and Papua Jew Guinea (Keeley et al., 1994).

B. PATTERNS OF RADIATION IN *CRASSULA*

All terrestrial perennial species of *Crassula* have the CAM pathway, although stomatal be-avior and gas exchange patterns are plastic (Pilon-Smits et al., 1995), and nearly all are re-tricted to Southern Africa (Tölken, 1977). Annual species, on the other hand, occur ıroughout the world and include both aquatic and terrestrial plants. Aquatic annuals from our continents, occurring in both seasonal pools and lakes, have been tested: All are CAM Table I) and all are closely related in the subgenus *Disporocarpa* (Tölken, 1977, 1981; By-rater & Wickens, 1984). Two terrestrial annual species in *Disporocarpa* lack CAM and CAM an not be induced (Table V), and these are perhaps the only members of the family com-letely lacking the CAM pathway (cf. Pilon-Smits et al., 1995). Arguments similar to those roposed above for the loss of CAM in Temperate Zone terrestrial *Isoëtes* would apply to these errestrial *Crassula*, which occupy similar seasonal environments.

The present distribution of *Crassula* suggests a South African origin for the group and ong-distance dispersal of the annual species or their progenitors, likely accounts for their lobal distribution. Such dispersal is most probable for aquatic species, which are distributed ı habitats more likely to be frequented by migrating birds, and the seeds (dispersed into the ıud) have a high probability of sticking to long-distance dispersers (Raven, 1963). Thus, the errestrial annuals are probably secondarily terrestrial and secondarily C_3. Since the rest of ıe Crassulaceae family is both terrestrial and CAM, it would perhaps be prudent to suggest ıat CAM was present in terrestrial ancestors giving rise to aquatic CAM species. However, pecies in *Disporocarpa* are apparently basal to the genus (Tölken, 1977), which makes it at east plausible that terrestrial CAM plants in *Crassula* may be derived from aquatic CAM spe-ies.

XIII. Conclusions and Areas for Future Research

CAM is a CO_2-concentrating mechanism. The immediate or proximal selective advantage ; the provision of an endogenous CO_2 source for photosynthesis. This has arisen in two envi-onments with different selective forces. On land the ultimate selective factor has been to en-ance water use efficiency, and in aquatic habitats the ultimate selective factor has been to iminish the threat of carbon starvation—the "desiccation vs. starvation" dilemma of Lüttge 1987). As a concentrating mechanism, a primary function of CAM is to enhance the $CO_2(p_i)$ ufficiently to overcome photorespiratory effects. This requires daytime decarboxylation of vernight malate stores in a system with sufficient diffusional resistances to allow accumula-on of CO_2 and prevent leakage. In terrestrial plants this requires increasing stomatal resis-ınce, whereas in aquatic plants this is largely effected by the 10^4 greater diffusional esistance of the water. An additional factor may be the relatively thick cuticle characteristic f most *Isoëtes*, although little is known about their permeability characteristics. A valuable ontribution would be comparative studies of resistances contributing to CO_2 disequilibria in quatic plants.

In both terrestrial and aquatic CAM plants, dark CO_2 fixation may result in net carbon uptake plus the conservation of carbon by refixation of respiratory CO_2. In aquatic plants, CAM's contribution to the total carbon budget is variable. Exemplary studies of the contribution of CAM to the carbon budget, such as those by Boston and Adams, Madsen, and Robe and Griffiths for lacustrine species, are needed in a greater range of habitats. Quantitative estimates of the CAM contribution to the carbon budget are likely to provide more insights than attempts to typologically categorize variation with terms such as "idling," "cycling," AAM, SCAM, TAAM, and so forth.

Although we have a reasonably good understanding of the selective factors favoring CAM in seasonal pools and oligotrophic lakes, there are other habitats (Section VII.C) where the role of CAM is not apparent. These species need to be examined in greater detail.

Future research should focus on species with predictable diel acid fluctuations, but with characteristics that do not fit recognized criteria for CAM. Of particular interest is the seasonal pool species *Downingia bella* (Campanulaceae), which may reflect an innovative CAM mechanism. Other roles for dark CO_2 fixation should be examined. Dark CO_2 fixation may be important as a source of carbon skeletons for both carbon and nitrogen assimilation, particularly in nutrient-poor habitats.

Of practical concern is the manner in which lake acidification and eutrophication alter carbon budgets (e.g., Robe & Griffiths, 1994). Also, in many parts of the globe aquatic CAM species are threatened: *I. andicola* of Peru, for instance, is clearly threatened by habitat loss (Leon & Young, 1996), and two of the three primitive *Isoëtes*, morphologically similar to the extinct *Isoetites*, are apparently extinct (Hickey, 1986). At the other extreme, the aquatic CAM *Crassula helmsii* is an aggressive alien (Dawson & Warman, 1987), in need of further studies such as those of Newman and Raven (1995) in a greater range of habitats.

Isoëtes, being the oldest lineage of CAM plants, potentially holds further interesting discoveries with respect to photosynthetic patterns. The most primitive species in the group are distinct in their lack of the typical terete "isoetid" leaf. These species are restricted to isolated sites in South America and have seldom been collected. They are apparently basal to the group, sharing the laminate leaf characteristic with the extinct and possibly ancestral *Isoetites* (Hickey, 1986). The hypothesized amphibious origin for CAM suggests the possibility that these primitive species may lack CAM. Further study of the photosynthetic metabolism and habitat characteristics of these would be a stimulating contribution to the story of aquatic CAM photosynthesis. Here, and in other aspects of aquatic CAM photosynthesis, a multitude of possibilities are presented with new molecular genetic techniques, now being applied to terrestrial CAM plants (Cushman & Bohnert, 1997).

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