Crassulacean acid metabolism in *Isoetes bolanderi* in high elevation oligotrophic lakes

Jon E. Keeley, Cindy M. Walker, and R. Patrick Mathews
Department of Biology, Occidental College, Los Angeles, CA 90041, USA

Summary. *Isoetes bolanderi* dominates the littoral flora of Siesta (elevation 2,440 m) and Ellery (2,905 m) lakes in the Sierra Nevada Range of California, USA. Both lakes are sparsely vegetated and *I. bolanderi* maintained aboveground oven dry weight of 30–50 m^{-2} through most of the 1981 summer growing season. Plants at the higher elevation Ellery Lake were half as large as plants at Siesta Lake and had substantially more biomass in corms. Titratable acidity levels in *Isoetes* leaves showed a diurnal fluctuation < 50 μeq g^{-1} fresh weight early in the season at the highest elevation site but this increased to ~ 300 μeq g^{-1} FW by mid-summer; starch and chlorophyll levels likewise increased in the leaves over this time. Throughout the season the magnitude of the diurnal acid change was comparable in *Isoetes* from both lakes but the dynamics of daytime deacidification were not. Averaged over the season, total daytime deacidification at Ellery Lake was 65% complete by noon whereas at Siesta Lake it was only 22% complete by noon. It is suggested that this may be related to the fact that Siesta Lake was more acidic and thus more carbon was in the form of free CO_2. In both lakes water chemistry showed no consistent diurnal fluctuation in pH or free CO_2 though total inorganic carbon levels were at the extreme low end for aquatic habitats. The studies reported here suggest that under extremely low inorganic carbon levels there may be selection for nighttime CO_2 assimilation. Consistent with this hypothesis is the observation that emergent *I. bolanderi* plants, resulting from fluctuating water levels, initiated leaves with stomata (unlike adjacent submerged plants) and, although these leaves had substantially higher chlorophyll levels, they showed an order of magnitude less acid fluctuation than submerged leaves.

Introduction

Crassulacean acid metabolism (CAM) is a photosynthetic pathway that is widespread in xeric adapted terrestrial plants. CAM plants fix carbon at night, store it overnight in the form of organic acids and during the day release the carbon and refix it through the C_3 pathway (Osmond 1978). The prototype CAM plant ('Super-CAM' of Kluge and Ting 1978) has this pathway coupled with a diurnal pattern of low stomatal conductance during the day when water deficits are greatest and high stomatal conductance

at night. In most cases it appears that CAM was selected as a means of increasing water use efficiency in relatively xeric environments.

Recently crassulacean acid metabolism has been discovered in the submerged aquatic *Isoetes howellii* Engelmann (Isoetaceae) where it undoubtedly was selected for a function other than increasing water use efficiency (Keeley 1981a, b; Keeley and Bowes 1982). In this species there is substantial net CO_2 uptake in the dark which is fixed largely into malic acid. Maximum malic acid production rates can be accounted for by rates of net CO_2 uptake in the dark which in turn can be accounted for by PEP carboxylase activity. Daytime deacidification results in a diurnal fluctuation of 100–300 μequivalents titratable acidity g^{-1} fresh weight. Tracer studies show that dark fixed carbon moves from organic acids to PGA and phosphorylated sugars in the light. CO_2 uptake may occur in the light through C_3 type reactions. *Isoetes howellii* have stomata though they are apparently non-functional while submerged (Sculthorpe 1967; Keeley 1981b).

It has been hypothesized that the primary selective advantage of CAM in *I. howellii* is that it provides an internal CO_2 source during the day when CO_2 becomes limiting to C_3 photosynthesis. The pools which *I. howellii* typically inhabits are heavily vegetated, shallow, clear and relatively stagnant. By noon on a sunny day the pH of the pool may increase 2 units and free CO_2 may be completely depleted (Keeley 1981b, 1983a). Under these conditions CO_2 uptake rates in the day are substantially reduced.

Perennially submerged *Isoetes* species usually occur in sparsely vegetated oligotrophic lakes, a habitat which contrasts sharply with the seasonal pool environment of *I. howellii*. The purpose of this study was to examine the dynamics of the diurnal acidification/deacidification cycle of *I. bolanderi* Engelmann from two high elevation oligotrophic lakes and the associated water chemistry changes.

Methods

Species and study sites

Isoetes bolanderi is widespread throughout western North America in oligotrophic lakes at high elevation or high latitude. This species is morphologically similar to others in the genus; it produces a rosette of quillike leaves arising from an underground corm and each leaf has four longitudinal air canals separated by septa. Unlike other *Isoetes*

species from oligotrophic lakes, *I. bolanderi* leaves do not overwinter. This is surprising in light of the observation some *Isoetes* species may be photosynthetically active under winter ice cover (Boylen and Sheldon 1976). Possibly more severe winters at the high elevation *I. bolanderi* habitats precludes this.

Two lakes in the Sierra Nevada of California, USA were selected which represent the elevational range for the species in this region: Siesta Lake (2,440 m), Tuolumne Co. and Ellery Lake (2,905 m), Mono Co.

Water sampling

Quantum solar radiation (QSR) was measured with a Li Co LI-188SB integrating meter with the LI-190SB quantum sensor at, and perpendicular to, the water surface and with the LI-192SB underwater quantum sensor at the underwater level of the plants. Specific conductance of the water was measured with a YSI-33 conductivity meter at 25° C. A dissolved oxygen meter (YSI-57) with polarographic sensor (YSI-5700) was used for oxygen determinations. A Photovolt 126-A pH meter was used for pH and titrations. Carbon dioxide was determined titrimetrically on water samples maintained on ice till assayed (usually within an hour) by titrating to pH 8.3 with CO_2-free 0.0227 N NaOH (APHA 1976). Alkalinity was determined by titrating with 0.02 N H_2SO_4; a pH 5.1 endpoint was used because of the very low alkalinity concentrations as recommended by APHA (1976). Sediment organic matter content was determined on samples oven dried to constant weight and combusted at 700 C in a muffle furnace.

Plant sampling

Samples were kept on ice till extraction, usually within an hour. Leaf samples (0.2–0.5 g) were washed, blotted dry and weighed on an Ohaus 300 electronic balance. These were ground in a Ten Broeck with 15.0 ml cold CO_2-free deionized water and spun down with a desk top centrifuge. A 1.0 ml sample of supernatant was deproteinized with an equal volume of 0.6 N perchloric acid and returned to the lab for malic acid determination with the enzymatic assay of Gutmann and Wahlefeld (1974). A 10.0 ml sample of supernatant was titrated with CO_2-free 0.01 N NaOH to pH 6.4 and pH 8.3. The diurnal fluctuation at the pH 6.4 endpoint accounted for 92% (S.D. = 9) of the diurnal fluctuation at pH 8.3 and will be the only one reported here. Intact plants were kept on ice (1–3 days) and returned to

the lab for chlorophyll determination using the procedure of Arnon (1949); small absorbances at 710 nm were substracted prior to calculations as suggested by Sestak et al. (1971). Samples were prepared for starch determination as follows. Plants were kept on ice 1–2 h and then dried 1 h at 120° C in a gravity oven. These samples were kept on ice and returned to the lab and dried for 24 h at 60° C. Dried samples were pulverized to pass a 40 mesh screen and assayed colorometrically using the assay of Hudson et al. (1976) as modified by Clark and Burk (1980) and further modified using a sodium acetate buffer of pH 4.5 and an incubation temperature of 55° C. Biomass estimates were made by collecting above and belowground parts of all plants inside a 15 cm dia hoop. Within the *Isoetes* zone five random samples were taken at each lake on each date and returned to the lab and divided by species into leaves and stems, oven dried to constant weight and weighed.

Results

Both Ellery and Siesta Lakes are relatively shallow, ice covered in the winter and fed by runoff from melting snow. The sediment consists of a thick silt overlaying granitic rock material. Sediment organic matter was approximately 25% greater at the lower elevation lake: organic matter (by weight) was 15.0% (S.D. = 0.9, $N = 2$) at Ellery and 20.6% (± 0.1) at Siesta. On 1 June 1981 both lakes were free of ice but no *Isoetes bolanderi* leaves were apparent above the sediment. By mid-June substantial leaf biomass had been produced at Siesta Lake but much less at the higher elevation Ellery Lake (Table 1). The aboveground biomass was significantly greater at Siesta Lake on this date ($P < 0.01$, with the Student's t-test) but by early July and through most of the season there was no significant difference ($P > 0.05$). The lower biomass level observed at Ellery Lake in late August is most likely attributable to sampling error arising from the very patchy distribution of *Isoetes* in that lake. At the earliest sampling date leaves accounted for 16–21% of the total biomass whereas through much of the season they accounted for $^1/_3$ (Ellery) to $^1/_2$ (Siesta) of the total biomass. Throughout the season, *Isoetes* were significantly ($P < 0.01$) smaller and had more of their biomass tied up in corms at the higher elevation Ellery Lake (Table 2). In the biomass samples *Eleocharis acicularis* L. was occasionally encountered, though through the season it amounted to only a fraction of the total

Table 1. *Isoetes bolanderi* biomass at Ellery and Siesta Lakes during the 1981 growing season, sampled between 50 to 75 cm depth at both sites, $\bar{X} \pm$ S.D., $N = 5$

Ellery lake	14 June	4 July	27 July	18 August	4 September
Leaves (g ODW m^{-2})[a]	4.8 ± 1.2	37.0 ± 7.0	37.1 ± 3.2	16.8 ± 7.2	55.2 ± 20.2
% of total above and below ground biomass as leaves	16%	39%	31%	36%	32%

Siesta Lake	17 June	2 July	28 July	19 August	6 September
Leaves (g ODW m^{-2})[a]	14.6 ± 6.5	32.4 ± 10.2	34.9 ± 5.0	36.1 ± 13.7	38.7 ± 10.1
% of total above and below ground biomass as leaves	21%	47%	46%	51%	47%

[a] Oven dry weight

Table 2. Biomass comparison of individual *Isoetes bolanderi* at Ellery and Siesta Lakes averaged over the 1981 growing season

	N	Total biomass (g ODW)		% as leaves	
		$\bar{X}$	SD	$\bar{X}$	SD
Ellery lake	1,035	17.7	13.8	33.7	12.5
Siesta lake	697	35.8	22.4	42.8	15.2

biomass at Ellery Lake and was not encountered at Siesta Lake.

The distribution of *Isoetes* at this time, and through the season, differed in these two lakes. At Siesta Lake *Isoetes* occurred below 50 cm depth and down to the maximum which was only ~1 m. At Ellery Lake *Isoetes* occurred from the shoreline down to several meters and water level fluctuations resulted in some plants being left exposed on the shore at times during the season. Leaves on plants exposed for several weeks possessed stomata unlike more or less adjacent submerged plants. *Isoetes* at Siesta Lake remained submerged through the season and these plants lacked stomata.

The seasonal changes in surface quantum solar radiation, water chemistry and acidity levels in leaves are shown in Tables 3 and 4. Most sampling dates were clear sunny days and averaged over the summer the photon flux density at the underwater level of the plants (50–75 cm) was 71% (S.D. = 14) at 0600 h, 84% (S.D. = 10) at 1200 h and 74% (S.D. = 13) at 1800 h.

At Ellery Lake (Tables 3 a–e) there was a marked change from mid-June to early July; water temperatures doubled and diurnal acidity fluctuations quadrupled. Maximum acidity changes approached 300 µequivalents g^{-1} fresh weight later in the season. Acidity levels at 1800 h were always less than 20 µeq g^{-1} FW with one exception; 17 August was the only overcast day encountered (noontime photon flux density was less than a quarter full sunlight) and at 1800 h submerged leaves still had 99 µeq acidity g^{-1} FW. Emergent plants were sampled on two separate occasions: 3–4 July and 17–18 August. Water table records from the Ellery Lake Reservoir indicate the plants on the first date had been exposed 21 days prior to sampling and the plants sampled on 17–18 August had been exposed for only 5 days (S.C.E Hydro Division, unpublished reservoir report). On both dates the emergent leaves had greatly reduced diurnal fluctuations in acidity and the magnitude of reduction was closely linked to the length of time emergent.

Table 3. Quantum solar radiation at the water surface, physical and chemical characteristics of the water and levels of titratable acidity (to pH 6.4) and malic acid in *Isoetes bolanderi* from Ellery Lake (2,905 m) during the 1981 growing season. Water conductivity varied from 18–27 µmhos cm^{-1} through the season. Water level fluctuations allowed for sampling of emergent plants on two dates

a) 12–14 June 1981

	Hour								
	0600	1200	1800	2400	0600	1200	1800	2400	0600
Water									
QSR (µE m^{-2} sec^{-1})	95	1960	615	0	50	1960	800	0	35
Temperature (°C)	7	7	9	7	2	10	9	8	4
Oxygen (% saturation)	94	99	103	92	87	101	103	103	90
pH	6.6	6.7	6.6	6.7	6.8	6.5	6.8	6.7	6.6
Free-CO_2 (mg L^{-1})	2.4	2.0	2.6	3.1	3.7	2.6	1.8	2.8	3.1
HCO_3^- and CO_3^{-2} (mg L^{-1})[a]	5.0	4.9	4.9	5.9	6.0	4.9	4.8	4.1	4.9
Submerged leaves									
Acidity (µeq g^{-1} FW)[b]	56±11	17±1	9±1	33±1	43±1	30±1	8±1	23±8	39±3
Malic acid (µmol g^{-1} FW)[b]	56±6	18±1	16±6	18±1	35±2	32±6	25±4	33±1	32±1

b) 2–4 July 1981

	Hour								
	0600	1200	1800	2400	0600	1200	1800	2400	0600
Water									
QSR (µE m^{-2} sec^{-1})	40	1925	730	0	30	1870	630	0	25
Temperature (°C)	13	16	18	14	14	18	19	17	15
Oxygen (% saturation)	102	120	123	117	109	127	121	115	106
pH	6.6	6.7	6.9	6.6	6.5	6.7	6.8	6.7	6.5
Free-CO_2 (mg L^{-1})	3.9	2.1	1.8	3.6	2.7	2.8	2.7	2.6	3.7
HCO_3^- and CO_3^{-2} (mg L^{-1})	5.3	5.2	4.8	4.3	3.9	4.9	4.2	5.9	6.4
Submerged leaves									
Acidity (µeq g^{-1} FW)	210±27	66±18	16±1	131±8	194±26	80±15	13±2	151±9	200±15
Malic acid (µmol g^{-1} FW)	132±24	61±13	35±3	94±7	116±15	61±6	*	63±8	134±41
Emergent leaves									
Acidity (µeq g^{-1} FW)	–	–	–	–	36±5	21±4	3±2	32±0	35±4
Malic acid (µmol g^{-1} FW)	–	–	–	–	48±2	42±5	*	51±2	55±2

Table 3 (continued)

c) 25–27 July 1981	Hour								
	0600	1200	1800	2400	0600	1200	1800	2400	0600
Water									
QSR ($\mu E\ m^{-2}\ sec^{-1}$)	20	1870	385	0	20	1865	725	0	90
Temperature (°C)	15	18	17	16	14	18	19	17	16
Oxygen (% saturation)	75	79	88	89	79	87	89	74	69
pH	6.8	6.7	7.0	6.7	7.1	6.7	6.8	6.8	7.0
Free-CO_2 (mg L^{-1})	1.8	2.4	1.8	1.6	3.3	2.8	2.2	2.0	1.7
HCO_3^- and CO_3^{-2} (mg L^{-1})	5.1	4.8	4.3	4.9	4.7	4.6	4.1	4.6	5.5
Submerged leaves									
Acidity ($\mu eq\ g^{-1}$ FW)	237±73	142±8	18±1	169±4	211±10	89±4	15±7	139±16	191±3
Malic acid ($\mu mol\ g^{-1}$ FW)	118±4	93±2	29±1	88±9	101±24	64±4	30±1	69±7	121±12

d) 16–18 August 1981	Hour								
	0600	1200	1800	2400	0600	1200	1800	2400	0600
Water									
QSR ($\mu E\ m^{-2}\ sec^{-1}$)	25	1830	410	0	10	390	560	0	0
Temperature (°C)	16	20	19	19	15	17	16	15	15
Oxygen (% saturation)	76	84	80	82	76	76	79	73	72
pH	6.9	7.0	7.6	6.8	6.9	7.2	7.3	6.8	6.8
Free-CO_2 (mg L^{-1})	2.9	2.7	3.2	2.5	3.8	2.0	2.0	2.5	2.4
HCO_3^- and CO_3^{-2} (mg L^{-1})	8.2	7.0	6.0	6.2	6.8	6.4	5.9	5.9	5.6
Submerged leaves									
Acidity ($\mu eq\ g^{-1}$ FW)	274±29	81±3	8±0	133±4	217±47	142±4	99±5	259±42	*
Malic acid ($\mu mol\ g^{-1}$ FW)	135±4	60±13	28±6	81±8	116±17	75±3	44±10	118±16	*
Emergent leaves									
Acidity ($\mu eq\ g^{-1}$ FW)	–	–	–	–	101±1	72±2	25±5	45±8	95±10
Malic acid ($\mu mol\ g^{-1}$ FW)	–	–	–	–	86±47	63±1	32±5	62±22	73±8

e) 2–4 September 1981	Hour								
	0600	1200	1800	2400	0600	1200	1800	2400	0600
Water									
QSR ($\mu E\ m^{-2}\ sec^{-1}$)	10	1755	310	0	1	1745	400	0	1
Temperature (°C)	13	16	17	14	14	17	17	17	13
Oxygen (% saturation)	67	77	92	63	57	79	78	87	76
pH	7.0	7.1	6.8	7.2	6.6	6.9	7.4	7.2	7.2
Free-CO_2 (mg L^{-1})	3.1	1.9	4.1	1.5	2.7	2.4	1.2	1.4	1.1
HCO_3^- and CO_3^{-1} (mg L^{-1})	9.0	7.9	5.0	5.0	5.1	5.0	5.1	5.9	5.8
Submerged leaves									
Acidity ($\mu eq\ g^{-1}$ FW)	302±66	95±11	11±1	133±1	222±33	68±11	16±4	105±5	166±8
Malic acid ($\mu mol\ g^{-1}$ FW)	134±23	33±14	22±19	72±17	96±6	40±8	16±2	49±1	73±22

* Sample lost

[a] As $CaCO_3$ [b] $\bar{X}$±S.D., $N=2$, FW = Fresh weight

At Siesta Lake (Table 4a) the early season diurnal acidity changes in *Isoetes* leaves were more than double those observed at the higher elevation Ellery Lake. These levels increased and eventually exceeded 250 $\mu eq\ g^{-1}$ FW late in the season (Table 4b–e).

A major difference between the *Isoetes* in these two lakes was the dynamics of the acidification/deacidification cycle. At Ellery Lake the total daytime deacidification was 65% complete by noon (averaged over the season the range was 37–75%). In contrast the total daytime deacidification was only 22% complete by noon at Siesta Lake (range 0–35%). This is striking in light of the very similar magnitude of acid fluctuations involved. Overnight acidification rates were more comparable; by midnight they were 68% complete at Ellery Lake and 57% complete at Siesta Lake (averaged over the season).

Water chemistry was quite similar in these two lakes (Tables 3 and 4). Oxygen seldom reached saturation level.

The pH was neutral to slightly acidic and total inorganic carbon was very low. Neither lake showed any consistent diurnal change in pH or free CO_2 level. The lower elevation Siesta Lake tended to be substantially warmer throughout the season. Siesta Lake also was more acidic than Ellery Lake and, although total inorganic carbon levels were relatively similar, Siesta had higher free CO_2 levels; averaged over the season, total CO_2 was 9.3 mg L^{-1} (S.D. = 1.3, N = 24) at Siesta and 7.3 ± 1.3 (N = 45) at Ellery whereas free CO_2 was 5.0 ± 1.7 at Siesta and 2.5 ± 0.7 at Ellery.

Chlorophyll levels in submerged leaves peaked in late August and were very similar in plants from both lakes (Table 5). Plants at Ellery Lake which had been emergent for nearly 3 weeks (2 July) had substantially higher chlorophyll levels than submerged plants. Recently emergent plants (viz., those emergent on 18 August) had chlorophyll levels similar to submerged plants. In both lakes, chlorophyll levels in *Isoetes* leaves declined late in the season.

Starch levels increased in the leaves during the day and decreased markedly overnight (Table 6). Afternoon starch

Table 4. Quantum solar radiation at the water surface, physical and chemical characteristics of the water and levels of titratable acidity (to pH 6.4) and malic acid in *Isoetes bolanderi* from Siesta Lake (2,440 m) during the 1981 growing season. Water conductivity varied from 9–27 μmhos cm^{-1} through the season

a) 16–17 June 1981

		Hour				
		0600	1200	1800	2400	0600
Water	QSR (μE m^{-2} sec^{-1})	25	1430	825	0	25
	Temperature (°C)	16	19	23	18	16
	Oxygen (% saturation)	93	96	113	99	91
	pH	6.2	5.7	5.7	5.6	5.5
	Free-CO_2 (mg L^{-1})	5.1	6.6	7.1	8.5	9.3
	HCO_3^- and CO_3^{-2} (mg L^{-1})[a]	3.9	2.0	2.1	2.2	2.5
Submerged leaves	Acidity (μeq g^{-1} FW)[b]	153±16	139±11	14±1	52±12	211±50
	Malic acid (μmol g^{-1} FW)	112±1	80±13	26±2	35±4	118±26

b) 1–2 July 1981

		Hour				
		0600	1200	1800	2400	0600
Water	QSR (μE m^{-2} sec^{-1})	40	1825	910	0	40
	Temperature (°C)	17	22	26	22	19
	Oxygen (% saturation)	89	125	134	110	95
	pH	6.8	6.6	6.7	6.5	6.4
	Free-CO_2 (mg L^{-1})	5.0	3.8	5.2	6.3	3.5
	HCO_3^- and CO_3^{-2} (mg L^{-1})	7.8	4.9	4.5	4.1	4.2
Submerged leaves	Acidity (μeq g^{-1} FW)	217±30	146±7	0±0	155±5	272±49
	Malic acid (μmol g^{-1} FW)	115±14	91±2	29±16	97±1	145±48

c) 27–28 July 1981

		Hour				
		0600	1200	1800	2400	0600
Water	QSR (μE m^{-2} sec^{-1})	20	1730	765	0	20
	Temperature (°C)	19	22	27	22	19
	Oxygen (% saturation)	65	74	90	*	65
	pH	6.6	6.2	6.7	*	6.4
	Free-CO_2 (mg L^{-1})	4.4	2.3	3.6	*	4.8
	HCO_3^- and CO_3^{-2} (mg L^{-1})	6.1	6.8	5.2	*	5.2
Submerged leaves	Acidity (μeq g^{-1} FW)	193±30	197±57	12±1	184±6	229±19
	Malic acid (μmol g^{-1} FW)	106±28	108±12	21±9	100±10	123±5

d) 18–19 August 1981

		Hour				
		0600	1200	1800	2400	0600
Water	QSR (μE m^{-2} sec^{-1})	45	1770	750	0	35
	Temperature (°C)	18	24	24	18	17
	Oxygen (% saturation)	65	79	87	65	68
	pH	6.2	6.6	6.9	6.2	6.4
	Free-CO_2 (mg L^{-1})	4.5	4.8	4.2	4.8	5.1
	HCO_3^- and CO_3^{-2} (mg L^{-1})	6.5	6.8	5.5	5.2	5.8
Submerged leaves	Acidity (μeq g^{-1} FW)	251±10	167±11	8±1	163±22	265±36
	Malic acid (μmol g^{-1} FW)	113±4	100±4	26±5	88±9	122±19

Table 4 (continued)

e) 5-6 September 1981		Hour				
		0600	1200	1800	2400	0600
Water	QSR (μE m^{-2} sec^{-1})	1	1690	540	0	1
	Temperature (°C)	13	26	24	18	16
	Oxygen (% saturation)	59	90	96	74	63
	pH	6.6	6.6	7.0	6.2	6.4
	Free-CO_2 (mg L^{-1})	4.9	3.2	2.3	5.3	4.6
	HCO_3^- and CO_3^{-2} (mg L^{-1})	5.0	5.0	5.0	5.2	5.1
Submerged leaves	Acidity (μeq g^{-1} FW)	207±47	151±16	21±8	169±16	239±12
	Malic acid (μmol g^{-1} FW)	104±20	62±15	23±5	93±5	121±3

* Sample lost

[a] As $CaCO_3$ [b] $\bar{X}$±S.D., N=2, FW=fresh weight

Table 5. Chlorophyll (μg g^{-1} fresh weight) levels in submerged leaves (unless otherwise indicated) of *Isoetes bolanderi* from Ellery and Siesta Lakes during the 1981 growing season ($\bar{X}$±S.D., N=2). Dates approximate (±1 day) date of collection*

-	Chlorophyll (μg g^{-1} FW)	
	Ellery lake	Siesta lake
15 June	527±5	521±10
2 July	682±57	589±15
(Emergent)	1,294±125	–
18 August	850±8	872±64
(Emergent)	810±109	–
5 September	696±94	495±29

* Samples from sampling period number 3 were lost

Table 6. Early and mid-season starch levels expressed as glucose equivalents (μmol g^{-1} oven dry weight) in leaves and corms of *Isoetes bolanderi* from Ellery and Siesta Lakes

	Starch (μmol g^{-1} ODW)					
	Ellery lake			Siesta lake		
	Leaves		Corms	Leaves		Corms
	1800	0600		1800	0600	
Mid-June						
$\bar{X}$	28	0	306	8	0	117
SD	28	0	137	9	0	51
N	4	4	8	2	2	4
Early July						
$\bar{X}$	536	95	204	1,159	48	80
SD	390	85	207	650	19	57
N	6	4	10	2	2	4

levels in the leaves increased markedly from mid-June to early July. Starch levels in the corms tended to drop from mid-June to early July. Using a leaf fresh/dry weight ratio of 16.5 (±1.3, N=10) these data were converted to a fresh weight basis for comparison with the overnight changes in malic acid. Early in the season, the overnight drops in glucose equivalents of starch was 2 and 1 μmol g^{-1} fresh weight for plants at Ellery and Siesta respectively which

is insufficient to account for the malic acid accumulation. In July starch levels dropped 27 and 67 μmol g^{-1} FW overnight at Ellery and Siesta which is more nearly sufficient to account for the acid accumulation.

Discussion

Despite the marked difference in environments, crassulacean acid metabolism is remarkably similar between *Isoetes bolanderi* in high elevation oligotrophic lakes and *I. howellii* in low elevation seasonal pools (Keeley 1981a, 1981b, 1983a). The magnitude of diurnal acid fluctuation is commonly 200 to 300 μeq g^{-1} FW in both species suggesting it is of considerable physiological importance.

We hypothesize that crassulacean acid metabolism has been selected for in aquatic *Isoetes* to enhance carbon gain under carbon limiting conditions. Studies of *Isoetes howellii* in seasonal pools show that diurnally fluctuating pH and inorganic carbon conditions limit CO_2 uptake during much of the day and favor uptake at night (Keeley 1983a). In contrast, Siesta and Ellery Lakes showed no consistent diurnal change in pH or free CO_2. Total inorganic carbon levels however were at the extreme low end for aquatic habitats and are 5 to 10 times lower than levels observed in low elevation seasonal pools. Considering the diffusive resistance of water to CO_2, the stillness of these lakes, coupled with the depth of the plants, it is not unlikely that active CO_2 uptake could generate a boundary layer with carbon levels below the CO_2 or HCO_3^- compensation point, as has been demonstrated for other aquatic macrophytes under much greater carbon levels (Prins and Helder 1981; Prins et al. 1980). Such conditions could put a premium on day and night carbon assimilation.

Several observations are consistent with the hypothesis of CAM having been selected to enhance carbon gain in carbon limiting aquatic environments. The loss of CAM upon emergence from the water would be predicted based on the several orders of magnitude greater CO_2 diffusivity in the atmosphere. *Isoetes bolanderi* as seen in this study and *I. howellii* (Keeley 1983a) both show an order of magnitude drop in acid fluctuation upon emergence. Consistent with this is the observation that while all aquatic *Isoetes* species so far tested are CAM (Keeley 1982) the terrestrial *I. nuttallii* A. Br. ex. Engelmann and *I. butleri* Engelmann are not (Keeley 1983b). Another observation which suggests a link between daytime carbon demand and acid me-

tabolism is the reduced deacidification on overcast days. *Isoetes bolanderi* in this study and *I. howellii* (Keeley 1983a) both show 50% less malic acid consumption on heavily overcast days, acid levels in the leaves remain ≥ 100 μeq g^{-1} FW at the end of the day in both species under such conditions.

An observation from this study which is particularly intriguing is the marked difference in rate of daytime deacidification observed between *I. bolanderi* in Ellery Lake and plants in Siesta Lake. In summary, *Isoetes* in Ellery Lake utilize the bulk of their malic acid pool in the morning whereas *Isoetes* in Siesta Lake consume the bulk of their malic acid in the afternoon. Subtle differences in carbon conditions may be involved. Siesta Lake has slightly higher carbon levels and because of the slightly more acidic water more of it is readily available; free CO_2 levels are twice as high in Siesta Lake throughout the season. If boundary layers, which are below the CO_2 (HCO_3^-) compensation point, developed during the day as suggested above, they might develop more rapidly under the carbon conditions observed at Ellery Lake.

Carbon limiting conditions may be an important characteristic of *Isoetes* habitats. There are 60 species distributed worldwide largely in "temperate" climates and freshwater aquatic habitats (Pfeiffer 1922). In general, *Isoetes* segregate into "seasonal pool" species or soft water oligotrophic lake species. The latter are known to grow well under eutrophic and hard water conditions (Seddon 1965; Lee and Belknap 1970) but their normal absence from such habitats appears to be due to poor competitive ability under high carbon conditions. Their ability to dominate oligotrophic waters may stem from a competitive advantage provided by nighttime CO_2 uptake.

Carbon limitation to photosynthesis is perhaps widespread in submerged aquatic macrophytes (Westlake 1967; Adams et al. 1978). One means of enhancing carbon conditions, particularly under oligotrophic conditions, would be carbon uptake by the roots. This has been shown for two species with the "isoetid" growth form. *Lobelia dortmanna* L. and *Littorella uniflora* (L.) Aschers dominant the littoral flora of European oligotrophic lakes and both take up CO_2 from the interstitual water in the sediment and transport it from the roots to the leaves (Wium-Anderson 1971; Søndergaard and Sand-Jensen 1979) and the latter species appears to be CAM (Keeley and Morton 1982; Boston and Adams in press). It is unknown if this is an important source of carbon for *Isoetes bolanderi*.

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