

## A preliminary survey for crassulacean acid metabolism (CAM) in submerged aquatic macrophytes in New Zealand

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**Abstract** This preliminary survey covered 13 aquatic macrophytes present at two sites in Lake Taupo and two sites in the Auckland region (northern half of the North Island, New Zealand). Three species previously unrecorded as CAM plants, *Isoetes kirkii* A. Braun, *Lilaeopsis lacustris* Hill, and *Vallisneria spiralis* Graeb., exhibited diurnal fluctuations in titratable acidity and malic acid content. The changes in titratable acidity varied seasonally from 97 (Dec. 1985) to 46 (June 1986) microequivalents per gram fresh weight for *I. kirkii*, and from 40 to 23 and 51 to 26 microequivalents per gram fresh weight for *L. lacustris* and *V. spiralis*, respectively. The absence of CAM both in other members of the low mixed community and in other hydrocharitacean species at the same sites suggests that CAM can not be unequivocally regarded as conferring an ecological advantage to a species.

**Keywords** crassulacean acid metabolism; CAM; *Isoetes kirkii*; *Lilaeopsis lacustris*; *Vallisneria spiralis*

### INTRODUCTION

Crassulacean Acid Metabolism (CAM) is the name given to a pathway of carbon fixation found in a number of terrestrial species but, most typically, in drought-resistant succulents (Kluge & Ting 1978). Dark fixation of CO<sub>2</sub> leads to the storage of malic acid in the vacuoles of green cells. During the next light period the acid is decarboxylated to produce CO<sub>2</sub> internally which is then fixed via the C<sub>3</sub> photosynthetic cycle. These changes result in diurnal rhythms of malic acid, a diagnostic feature of CAM (Szarek & Ting 1975), which may contribute significantly to total photosynthetic carbon acquisition in some species under certain environmental conditions (Neales 1975).

CAM was discovered only relatively recently in a freshwater plant, the fern ally *Isoetes howelli* Engell. (Keeley 1981a). In the photosynthetic tissues of the leaves, nocturnal increases in both titratable acidities and malic acid contents were demonstrated (Keeley 1981b, 1982). Fifteen other submerged members of the genus *Isoetes* from the Australian, European, and North American continents have now been recorded as exhibiting CAM (Aulio & Salin 1983; Keeley 1981a, 1981b, 1982, 1983a; Boston & Adams 1983; Richardson et al. 1984; Farmer & Spence 1985; Madsen 1985).

Thus far, only two species from other aquatic genera have been reported to show CAM, *Littorella uniflora* var. *americana* (Fern.) Gl. (Boston & Adams 1983) and *Crassula aquatica* (L.) Schoenl. (Keeley & Morton 1982). Other aquatics have been shown to have limited capacities (i.e., below the levels usually defined as CAM) for nocturnal accumulations of malate or increases in titratable acidity: *Hydrilla verticillata* L.f. Royle (Holaday & Bowes 1980) and *Scirpus subterminalis* Torr (Beer & Wetzel 1981). *Vallisneria spiralis* Graeb. has not been specifically tested for CAM but Sternberg et al. (1984) reported that the <sup>14</sup>C : <sup>13</sup>C ratio in tissues was lower than in the surrounding environment. Such preferential uptakes of <sup>13</sup>C are characteristic of CAM species.

Only a few general surveys of CAM in native aquatic populations have been published (Keeley & Morton 1982; Sternberg et al. 1984; Farmer & Spence 1985). This paper presents the first known survey of this kind in New Zealand.

## METHODS

Plants were collected at 0600 and 1800 h (NZST) from four sites in the northern half of the North Island, New Zealand, as indicated in Table 1. Samples (1–2 g) of green and non-green tissues were blotted dry, gently cleaned with tissue, homogenised in deionised water, and filtered (Whatman No. 1). Each filtrate was made up to 50 ml, and 40 ml of this titrated against 0.02 M NaOH to an end point of pH 8.3 (Orion 91–02 electrode and Digital Ionanalyser 501, Orion Research, Cambridge, Mass., United States).

For species exhibiting significant diurnal fluctuations in titratable acidities, the remaining 10 ml of the filtrate was analysed for malic acid by the enzymatic technique of Gutmann & Wahlefeld (1974). Malic dehydrogenase (bovine heart, 2–4000 units (mg protein)<sup>-1</sup>) and NAD were supplied by Sigma Chemical Co., St Louis, United States and the spectrophotometry was carried out with a Varian DMS-90 fitted with a flow-through sampler.

Results are expressed as microequivalents per gram fresh weight (Moradshahi et al. 1977), abbreviated here as  $\mu\text{eq (g FW)}^{-1}$ .

## RESULTS

There was no evidence of diurnal fluctuations in titratable acidities in the non-green tissues of any of the plants listed in Table 1. In the plants sampled from Three Mile Bay, Lake Taupo, only the leaves of *Isoetes kirkii* and of *Lilaeopsis lacustris* showed titratable acidity changes of sufficient magnitude to be considered as significant in terms of the standard definition of CAM (Kluge & Ting 1978). Seasonally, in the *Isoetes* species, the diurnal changes in acid ranged from 97 (Dec 1985) to 46 (June 1986)  $\mu\text{eq (g FW)}^{-1}$  (Fig. 1; Table 1). *Lilaeopsis lacustris* exhibited smaller, but still significant changes, ranging from 40 to 23  $\mu\text{eq (g FW)}^{-1}$ .

**Table 1** The diurnal changes in titratable acidities and malic acid contents of green parts of aquatic macrophytes from four sites in the North Island of New Zealand. Total inorganic carbon and acidities of the lake waters were: Lake Taupo TIC = 8.5 mg C l<sup>-1</sup>, pH = 7.4; Lake Pupuke TIC = 81.5 mg C l<sup>-1</sup>, pH = 8.7; Meola Creek TIC = 12.0 mg C l<sup>-1</sup>, pH = 6.8. Plants were sampled at 0600 and 1800 h (NZST). Values in  $\mu\text{eq (g FW)}^{-1}$  are the mean of five replicates; TA, change in titratable acids; MA, change in malic acid; % M, malate as a percentage of titratable acids; n.d., not determined.

Species	Location	Month	TA	MA	%M
<i>Isoetes kirkii</i> A. Braun†	L. Taupo	Aug 1985	51 ± 9	54	105
		Dec 1985	97 ± 7	n.d.	n.d.
		Apr 1986	79 ± 8	87	110
		Jun 1986	46 ± 4 (n=4)	47	104
		Sep 1986	48 ± 12	47	98
		Dec 1986	81 ± 8	60	74
<i>Lilaeopsis lacustris</i> Hill†	L. Taupo	May 1986	23 ± 4	28	123
		Dec 1986	41 ± 7	48	116
<i>Vallisneria spiralis</i> Graeb.	L. Pupuke	Sep 1986	26 ± 9	27	103
		Dec 1986	51 ± 1	54	105
<i>Lagarosiphon major</i> (Ridley) Moss	L. Taupo	Dec 1985	6 ± 6		
		Sep 1986	8 ± 5		
<i>Myriophyllum triphyllum</i> Orchard†	L. Taupo	Dec 1985	2 ± 3		
	L. Pupuke	Sep 1986	3 ± 4		
<i>M. propinquum</i> A. Cunn†	L. Taupo	May 1986	5 ± 2		
<i>Ruppia polycarpa</i> R. Mason†	L. Taupo	Sep 1986	0 ± 1		
<i>Ranunculus fluitans</i> Auct. NZ†	L. Taupo	May 1986	1 ± 1		
<i>Scirpus</i> sp.†	L. Taupo	May 1986	2 ± 2		
<i>Elodea canadensis</i> Michx.	L. Taupo†	May 1986	6 ± 4		
<i>Egeria densa</i> Planch.	Meola Creek	Sep 1986	1 ± 1 (n=3)		
	L. Pupuke	Dec 1986	2 ± 1		
<i>Ottelia ovalifolia</i> (R.Br.) Rich	Meola Creek	Sep 1986	7 ± 5 (n=3)		
<i>Potamogeton crispus</i> L.	L. Pupuke	Dec 1986	1 ± 0		

†Member of the low mixed community (Brown 1975).

‡Two Mile Bay; all other Lake Taupo samples were from Three Mile Bay.

N.B. *Glossostigma elatinoides* Benth. was not investigated in this study, as it had been shown previously not to exhibit a diurnal acid cycle (Hermans unpubl. data).

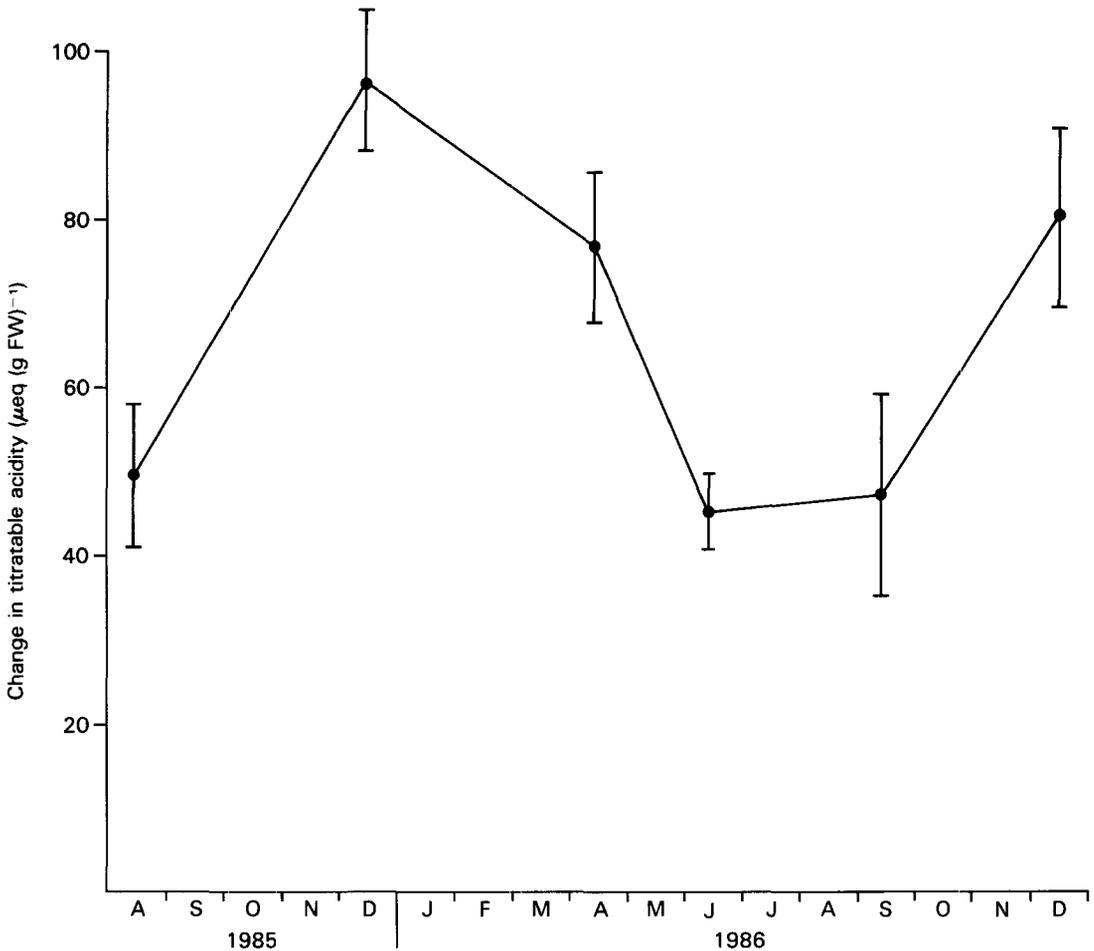


Fig. 1 Seasonal changes in titratable acidity differences between 0600 and 1800 h (NZST) in the green portions of leaves of *Isoetes kirkii* A. Braun from Three Mile Bay, Lake Taupo, New Zealand.

Of the plants sampled at the other three sites, *Vallisneria spiralis* was the only one to exhibit marked diurnal fluctuations in titratable acids (51 to 26  $\mu\text{eq (g FW)}^{-1}$ ). The results reported for this plant relate only to the apical 10 cm portions of leaves which were greater than 1 m in total length. Titratable acidity fluctuations decreased with distance from the apex.

Malic acid assays for *I. kirkii*, *L. lacustris*, and *V. spiralis* gave complementary results to those for titratable acidities. The total acid which could be accounted for by malic acid concentrations was over 74% in all three species (Table 1). Values over 100% of the titratable acidity can be explained by internal salt formation of some of the malic acid accumulated.

## DISCUSSION

In the total of 13 species tested, only three exhibited marked diurnal titratable acidity fluctuations. That malic acid could account for most of these changes and that they occurred only in chlorophyll-containing tissues lends confidence to the view that CAM metabolism was occurring in these plants. Thus, *Isoetes kirkii*, *Lilaeopsis lacustris* (both endemic to New Zealand), and *Vallisneria spiralis* can be added to the list of aquatic CAM species. *Lilaeopsis* is the first known aquatic member of the family Umbelliferae shown to possess CAM characteristics.

The seasonal patterns of diurnal changes clearly demonstrated for *Isoetes kirkii* (Fig. 1) and indi-

cated by the results for both *Lilaeopsis* and *Vallisneria* (Table 1) are also consistent with CAM metabolism. Both light intensity and daylength influence diurnal changes in a number of terrestrial CAM plants (e.g., Klug & Ting 1978).

This survey was not exhaustive since some of the species occurring with *Isoetes* and *Lilaeopsis* in the low mixed community (Brown 1975) were present in too small a quantity to permit adequate sampling. *Crassula sinclairii* (Hook. f.) Druce & Given (formerly *Tillea sinclairii* Hook. f.) deserves further investigation. Its aquatic congener in the United States, *C. aquatica* (L.) Schaerl., exhibits diurnal changes in acidity up to  $100 \mu\text{eq (g FW)}^{-1}$  (Keeley & Morton 1982) as do many terrestrial members of the family after which the CAM phenomenon is named. An increased frequency of seasonal sampling might reveal other New Zealand CAM species since diurnal cycling of malic acid is "facultatively" dependent on environmental  $\text{CO}_2$  stress in some plants including, possibly, the aquatic *Hydrilla verticillata* Royle (Holaday & Bowes 1980).

Keeley & Morton (1982) suggested that CAM in aquatic plants could be linked to the small rosette growth form ("isoetid" by the definition of den Hartog & Segal 1964), especially in waters low in photosynthetically available carbon. These suggestions are not supported by our results. Neither of the lakes (nor, for that matter, any New Zealand lake) can be regarded as low in total inorganic carbon (TIC). Further, *Lilaeopsis*, whilst it has quill-like "leaves", does not possess a rosette growth form and *Vallisneria*, technically an isoetid, is certainly not small. Moreover, the absence of CAM in other small rosette species such as *Ruppia polycarpa* and the *Scirpus* sp. (Table 1) adds to the existing reports of similar absences elsewhere (Richardson et al. 1984; Farmer & Spence 1985; Madsen 1985). It appears unlikely that aquatic CAM can be firmly linked to either a specific growth habit or available carbon for photosynthesis.

Madsen (1985) used cover area and abundance as a measure of the success of CAM species over non-CAM plants whereas Keeley (1983b) found little correlation in this respect for *Isoetes howellii*. In the low mixed community *Isoetes kirkii* often formed up to 100% of the ground cover at 1.2 to 4 m water depth in Three Mile Bay, Lake Taupo (Webb 1987), whereas *Vallisneria spiralis* is the almost exclusive dominant throughout its depth range in Lake Pupuke. However, in Lake Taupo, both *Isoetes* and *Lilaeopsis* are much less dominant at shallower depths and it appears more probable that successional stages due to physical factors (Chapman et al. 1971) are determinants of distributions rather than CAM status.

In Lake Pupuke, *Vallisneria* occupies the depth zones dominated elsewhere in New Zealand by *Elodea canadensis*, *Lagarosiphon major*, or *Egeria densa* (Brown 1975) despite the fact that these three species are also present in this lake. As shown in Table 1, the Total Inorganic Carbon of Pupuke water is relatively high ( $81.5 \text{ mg C l}^{-1}$ ) but, because of the pH of 8.7, free  $\text{CO}_2$  is less than  $1 \mu\text{M}$  during daylight hours (Webb 1987). Thus, it is tempting to define the success of *Vallisneria* in Lake Pupuke as due to the presence of CAM in a very low photosynthetically available-carbon situation. This would accord with Keeley (1981a) that aquatic CAM is associated with low available-carbon waters, albeit not in the context of the oligotrophic, low TIC waters which led to his suggestion. However, *Vallisneria* has been reported to use bicarbonate for photosynthesis and this reason for its dominance cannot be excluded despite the facts that such photosynthesis occurs only at relatively low rates and is also present in *Egeria* (Browse et al. 1979). Detailed balance sheet comparisons of the contributions of bicarbonate photosynthesis and of free  $\text{CO}_2$  photosynthesis in all these tall macrophyte species, as well as of CAM contributions to total carbon fixation in *Vallisneria*, are required before conclusions can be drawn.

Moreover, in Lake Taupo ( $< 1.2 \mu\text{M}$  free  $\text{CO}_2$  in the water), the absence of CAM in other members of the low mixed community (Table 1) and the dominances of *Elodea* and *Lagarosiphon* at other depths (Howard-Williams & Vincent 1983) suggest that the environmental successes of aquatic species may be far more complex than the "induction" of a single carbon gaining pathway. Further investigations of other accessory photosynthetic mechanisms and facets of growth are necessary before CAM in aquatics can be regarded as always conferring a significant ecological advantage to a species.

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