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# Short note
# Report of diurnal acid metabolism in two aquatic Australian species of *Isoetes*

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## Abstract

*Previous studies have demonstrated the presence of crassulacean acid metabolism in the North American submerged aquatic* Isoetes howellii *(Isoetaceae). Diurnal changes in titratable acidity and malic acid levels indicate the presence of this pathway in two Australian species of* Isoetes.

## Introduction

The North American submerged aquatic *Isoetes howellii* Engelmann (Isoetaceae) possesses crassulacean acid metabolism (CAM) comparable to that found in terrestrial CAM plants (Keeley 1981; Keeley & Bowes 1982). Substantial net $CO_2$ uptake occurs in the dark and is stored as malic acid. Overnight acidification is followed by daytime deacidification of the same magnitude as observed in terrestrial CAM plants. Maximum malic production rates can be accounted for by net dark $CO_2$ uptake rates which in turn can be accommodated by PEP carboxylase activities. This pathway is shut off in the light though $C_3$ type $CO_2$ assimilation is possible during the day. Although day and night $CO_2$ uptake is known from terrestrial plants exhibiting CAM, submerged *I. howellii* differ from the prototype CAM plant in that they lack a diurnal pattern of changes in stomatal conductance which (in terrestrial CAM plants) result in the bulk of the carbon gain occurring at night. Although *I. howellii* possess stomata they apparently are non-functional while submerged and gas exchange involves diffusion across the epidermis. There is some evidence that diurnal changes in $CO_2$ availability in the shallow pools *I. howellii* inhabits may result in the bulk of carbon assimilation occurring at night (Keeley 1983).

The North American *I. howellii* is a member of a worldwide genus, several of which occur in Australia. The purpose of this study was to determine if diurnal acid metabolism was present in the indigenous Australian *I. australis* Williams and *I. drummondii* A. Br. These species provide an interesting contrast in that they both occupy seasonal pools (similar to *I. howellii* habitats) and stomata are present in *I. drummondii* but lacking in *I. australis*.

## Methods

*Species collection and maintenance* *Isoetes australis* was collected from granite outcrop pools along the Albany Hwy, near Glen Eagle, in Western Australia (see Bayly 1982 for description of pools in this region). *I. drummondii* was collected from small pools around the Pine Oval in Belair National Park, South Australia. Plants were maintained submerged in aquaria with sand substrate in growth chambers with 12 h photoperiod (0600–1800 h), photon flux density of 500 $\mu E\ m^{-2}$ per s, and 25°C day/15°C night.

*Assay*

Leaf samples were collected at 0600 h and 1800 h, washed with distilled water, blotted dry, weighed, then ground in cold $CO_2$ free distilled water in a Ten Broech. After centrifuging, a sample of supernatent was deproteinized and saved for malic acid determination. Another aliquot was immediately titrated with $CO_2$ free 0.01N NaOH to pH 6.4 and pH 8.3; these represent the range of endpoints used in the CAM literature. Malic acid was determined with the enzymatic endproduct assay of Gutmann & Wahlefeld (1974).

## Results and discussion

Table 1 shows a marked diurnal fluctuation in both

TABLE 1. Overnight changes in titratable acidity (to pH 6.4) and malic acid in leaves of two Australian *Isoetes* species (with s.d., $n = 2$)

| | Titratable acidity (μEq/g FW) | | | | Malic acid (μmol/g FW) | | | |
|---|---|---|---|---|---|---|---|---|
| | p.m | | a.m. | | p.m. | | a.m. | |
| | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. |
| *I. australis* | 16 | 0 | 74 | 0 | 18 | 2 | 46 | 3 |
| *I. drummondii* | 24 | 1 | 106 | 11 | 10 | 3 | 49 | 3 |

titratable acidity and malic acid levels in leaves of both *I. australis* and *I. drummondii*. Titratable acidity is presented for the pH 6.4 endpoint which accounted for 94–100% of the diurnal flux in acidity at the pH 8.3 endpoint. The diurnal titratable acidity flux varied from 58 to 82 μEq/g FW in *I. australis* and *I. drummondii*, respectively. The diurnal malic acid flux (expressed as equivalents) varied from 56 to 78 μEq/g FW in these two species. *I. australis* apparently has a large malate pool in the leaves which is not involved in the acid cycling; afternoon 'malic acid' levels are twice as high in *I. australis* than in *I. drummondii* but the diurnal flux is *c.* 30% less.

These data suggest that crassulacean acid metabolism is present in aquatic Australian *Isoetes*. This is not surprising in light of the habitat similarity between *I. howellii* and the *Isoetes* spp. studies here. A point worthy of further study (though not possible here due to limited plant material) is the effect of emergence upon acid metabolism. Upon emergence, *I. howellii* loses CAM, stomata become functional and carbon uptake is apparently restricted to daytime $C_3$-type assimilation (Keeley 1983). *I. australis* should provide an interesting ecophysiological comparison since it likewise becomes emergent as the pools dry but it lacks stomata.

## References

Bayly I. A. E. (1982) Invertebrate fauna and ecology of temporary pools on granite outcrops in southern Western Australia. *Aust. J. Mar. Freshw. Res.* **33**, 599–606.

Gutmann I. & Wahlefeld A. W. (1974) l-Malate: determination with malate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis* (ed. H. U. Bermeyer), pp. 1585–9. Academic Press, New York.

Keeley J. E. (1981) *Isoetes howellii*: a submerged aquatic CAM plant? *Am. J. Bot.* **68**, 420–4.

Keeley J. E. (1982) Crassulacean acid metabolism in the seasonally submerged aquatic *Isoetes howellii*. Oecologia, in press.

Keeley J. E. & Bowes G. (1982) Gas exchange characteristics of the submerged aquatic crassulacean acid metabolism plant, *Isoetes howellii*. *Plant Physiology* **70**, in press.

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