Crassulacean acid metabolism in *Isoetes bolan-deri* 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 CO₂. In both lakes water chemistry showed no consistent diurnal fluctuation in pH or free CO₂ 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 CO₂ 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 C₃ 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 CO₂ uptake in the dark which is fixed largely into malic acid. Maximum malic acid production rates can be accounted for by rates of net CO₂ 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 pequivalents 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. CO₂ uptake may occur in the light through C₃ type reactions. *Isoetes howellii* have stomata though they are apparently non-functional while submerged (Seulthorpe 1967; Keeley 1981 b).

It has been hypothesized that the primary selective advantage of CAM in *I. howellii* is that it provides an internal CO₂ source during the day when CO₂ becomes limiting to C₃ 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 CO₂ may be completely depleted (Keeley 1981 b, 1983 a). Under these conditions CO₂ 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. bolan-deri* 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 endpoint accounted for 92% (S.D. = 9) of the diurnal fluctuation at pH 6.4 and pH 8.3. The diurnal fluctuation at the pH 6.4 lab for malic acid determination with the enzymatic assay equal volume of 0.6 N perchloric acid and returned to the lab for chlorophyll determination using the procedure of Arnon (1949); small absorbances at 710 nm were subtracted 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 biomass.

**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, ± S.D., *N* = 5

<table>
<thead>
<tr>
<th>Ellery lake</th>
<th>14 June</th>
<th>4 July</th>
<th>27 July</th>
<th>18 August</th>
<th>4 September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves (g ODW m⁻²)⁶</td>
<td>4.8 ± 1.2</td>
<td>37.0 ± 7.0</td>
<td>37.1 ± 3.2</td>
<td>16.8 ± 7.2</td>
<td>55.2 ± 20.2</td>
</tr>
<tr>
<td>% of total above and below ground biomass as leaves</td>
<td>16%</td>
<td>39%</td>
<td>31%</td>
<td>36%</td>
<td>32%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Siesta Lake</th>
<th>17 June</th>
<th>2 July</th>
<th>28 July</th>
<th>19 August</th>
<th>6 September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves (g ODW m⁻²)⁶</td>
<td>14.6 ± 6.5</td>
<td>32.4 ± 10.2</td>
<td>34.9 ± 5.0</td>
<td>36.1 ± 13.7</td>
<td>38.7 ± 10.1</td>
</tr>
<tr>
<td>% of total above and below ground biomass as leaves</td>
<td>21%</td>
<td>47%</td>
<td>46%</td>
<td>51%</td>
<td>47%</td>
</tr>
</tbody>
</table>

---

⁶ Oven dry weight