Carbon Uptake Characteristics in Two High Elevation Populations of the Aquatic Cam Plant Isoetes bolanderi (Isoetacae)

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CARBON UPTAKE CHARACTERISTICS IN TWO HIGH ELEVATION POPULATIONS OF THE AQUATIC CAM PLANT ISOETES BOLANDERI (ISOETACAE)[1]

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ABSTRACT

In the aquatic CAM species *Isoetes bolanderi,* a resident of high elevation lakes in which ambient carbon levels are low and fairly constant throughout the day, CO_2 uptake by the leaves generally parallels changes in photosynthetic photon flux density as opposed to changes in ambient CO_2 levels. Overnight CO_2 uptake by the leaves of *I. bolanderi* contributed up to 33% of the total daily carbon assimilation from the water column which is typical of aquatic CAM species but low in comparison to the 30–50% seen in the seasonal pool congener *Isoetes howellii.* Two additional sources of carbon may supplement the 24-hour period for carbon assimilation conferred by CAM in *I. bolanderi*: the refixation of respiratory CO_2 and carbon acquisition from the interstitial sediment water via roots. CAM appears to play a significant role in enhancing carbon gain in *I. bolanderi* which dominates the littoral flora of Siesta and Ellery Lakes despite higher carbon uptake rates from the water column found in one associated macrophyte *Fontinalis antipyretica.*

CRASSULACEAN acid metabolism (CAM) is a photosynthetic pathway that was at one time known only as an adaptation to xeric environments (Kluge and Ting, 1978). CAM typically involves nighttime opening of stomata and fixation of CO_2 into organic acids, particularly malic acid, which are stored in the vacuole overnight. During the day, stomatal conductance to CO_2 is low, but photosynthesis continues utilizing the carbon provided by decarboxylation of these organic acids. In terrestrial plants of xeric environments selection for CAM may be attributed to its role in increasing water use efficiency. Thus, the discovery of CAM in a submerged aquatic plant, *Isoetes howellii* Engelmann (Keeley, 1981), was surprising and provided an additional perspective on the evolutionary, ecological, and functional significance of this photosynthetic pathway.

Isoetes howellii is found in shallow seasonal pools where photosynthetic demand by the pool's flora virtually eliminates the supply of free-CO_2 in the water column by midday (Keeley and Busch, 1984). Since all species of the genus *Isoetes* investigated thus far are unable to take up the other forms of inorganic carbon available in the pool (i.e., HCO_3 and CO_3), or do so very poorly, photosynthetic carbon uptake is limited throughout the majority of the light period (Keeley, 1983b; Boston and Adams, 1986). CAM, however, allows *I. howellii* to take up carbon dioxide at night when free-CO_2 is abundant in the water due to respiration by the pool's flora and invertebrate fauna. This carbon dioxide, stored overnight as malic acid and decarboxylated the following morning, provides a source for photosynthesis during the daytime when ambient free-CO_2 levels are again very low.

Previous field studies by Keeley and Busch (1984) have shown that peak CO_2 uptake by the leaves of *I. howellii* occurred in the early morning but declined rapidly as the ambient free-CO_2 level in the water dropped. Accordingly, carbon uptake in the dark increased as the ambient free-CO_2 levels rose. Thus, over the 24-hour cycle, carbon uptake from the water column by *I. howellii* closely tracked changes in the ambient free-CO_2 (Keeley and Busch, 1984).

The genus *Isoetes* has several hundred species worldwide, most of which are aquatic (Tryon and Tryon, 1982), and many of these aquatic *Isoetes* species have been shown to possess CAM (Keeley, 1982, 1983a; Richardson et al., 1984; Farmer and Spence, 1985; Boston and Adams, 1986). While some *Isoetes* species are distributed in seasonal pools similar to the habitat of *I. howellii,* many species are true aquatics permanently submerged in lakes. These lat-

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ter species are usually restricted to oligotrophic conditions where habitat characteristics, with respect to carbon availability, are quite different from seasonal pools (Sand-Jensen and Søndergaard, 1979; Keeley, Walker, and Mathews, 1983; Madsen, 1985, 1987b; Boston and Adams, 1986, 1987); for example, the high elevation lakes in the Sierra Nevada Mountains of California are a common habitat of *I. bolanderi* Engelmann. In these lakes ambient levels of free-CO_2 in the water column do not vary diurnally, and total inorganic carbon levels are consistently much lower than those found in seasonal pools (Keeley et al., 1983).

The primary purpose of this study was to determine the diurnal course of carbon uptake by *I. bolanderi* which inhabits these high elevation oligotrophic lakes. Specifically we address the role of CAM and its contribution to carbon uptake by the shoots of *I. bolanderi.* As such, "carbon uptake" will refer to CO_2 acquisition by the shoots; this is not synonymous with total carbon assimilation which includes carbon gain from additional sources such as refixation of respiratory CO_2 and acquisition of CO_2 via roots. To address the potential role of these additional sources, however, subsequent investigations were undertaken and are reported in conjunction with the shoot data.

Since ambient carbon levels do not vary diurnally in these lakes (Keeley et al., 1983), we predicted that the patterns of carbon uptake by the leaves of *I. bolanderi* would be different from those shown for *I. howellii* but may be similar to that shown for other species of *Isoetes* of soft-water lakes (i.e., *I. macrospora* and *I. lacustris*). Such patterns may then put a premium on utilization of carbon from sediment water and/or refixation of respiratory CO_2.

For comparative purposes, CO_2 uptake from the water column was measured in the field on cut leaf samples as described by Keeley and and Busch (1984) for *I. howellii.* It is important to note, however, that these methods may underestimate the in situ rates of assimilation if intact plants are exposed to higher carbon levels resulting from interstitial diffusion of CO_2 from the carbon-rich sediment (Wium-Andersen, 1971; Richardson et al., 1984; Farmer and Spence, 1985; Boston, 1986; Boston and Adams, 1987; Boston, Adams, and Pienkowski, 1987a, b; Raven et al., 1988) or CO_2 evolved by internal respiration (Keeley and Busch, 1984; Farmer and Spence, 1985; Boston, 1986; Boston and Adams, 1986; Madsen, 1987a, b). Laboratory experiments were performed to determine the relative amount of CO_2 uptake from roots versus that of leaves. In the field, some leaf samples were incubated in water spiked with additional carbon in order to evaluate the potential response of *I. bolanderi* to higher carbon availability.

Finally, for comparative purposes, one sample of *Fontinalis antipyretica* Hedw., a non-CAM aquatic species (Keeley and Morton, 1982) found within the same macrophyte community as *I. bolanderi,* was also tested for carbon assimilation characteristics.

MATERIALS AND METHODS—Studies were conducted on *Isoetes bolanderi* from two lakes located along Tioga Road in the central Sierra Nevada mountain range of California; Siesta Lake (2,440 m, Tuolumne Co.) and Ellery Lake (2,905 m, Mono Co.) and are the same study sites described in Keeley et al. (1983). At each of three sampling dates, from early to late summer 1985, carbon uptake (leaf assimilation from the water column only) was determined every 3 hours (except at 0400 hr) for a 48-hour period at Siesta Lake and for a 24-hour period at Ellery Lake.

Carbon assimilation measures—Leaf samples of 0.15 g fresh weight (FW) were placed in 25-ml vials that had been filled with lake water collected at the time of sampling. Each vial was injected with 2.5 μmol $NaH^{14}CO_3$ (specific activity = 9 μCi μmol^{-1} $NaH^{14}CO_3$, 1 μCi = 37 kBq) and incubated for 30 minutes in a water bath under ambient temperature and light. This small addition does not significantly alter the absolute free-CO_2 concentration from that of the ambient water. Furthermore, it does not affect the pH of the water, and hence, the free-CO_2 to bicarbonate ratio in the sample vial does not differ from that of the lake waters. Three replicates were run at each sample period.

Additional replicate samples (N = 3) were used to evaluate the capacity of *I. bolanderi* to utilize carbon when present in quantities greater than ambient levels, a situation which may arise by either exposure to respiratory CO_2 or diffusion of CO_2 through the roots to photosynthetic tissue. The vials of each of these "spiked" samples were injected with enough $NaHCO_3$ (used in conjunction with an equivalent HCl concentration to maintain the ambient pH) to bring the CO_2 concentration to 0.8 mM above the lake CO_2 levels.

Samples in the light were run at 0700, 1000, 1300, 1600, and 1900 hr and in the dark (covered with aluminum foil) at 1900, 2200, 0100, and 0700 hr. Incubation periods were terminated by discarding the solution of lake water, rinsing the leaf material with 1.0 M HCl, and adding boiling 80% methanol. Retaining the

methanol, the vials were capped, photobleached in the sun, and returned to the lab where samples were ground and brought up to 25 ml with additional methanol. Subsamples of this solution were assayed by liquid scintillation for the incorporation of ^{14}C into stable compounds.

To further evaluate carbon acquisition via the roots, split-chamber experiments were performed in the lab under both light (1,000 μmol m^{-2} sec^{-1}) and dark conditions (as in Keeley, Osmond, and Raven, 1984). Intact plants were placed in split chambers such that the leaf and root environments could be controlled independently. Both root (bottom) and leaf (top) chambers were filled with 25 mM NaH_2PO_4 buffer at pH 6.5. Inorganic carbon, as $NaHCO_3$, was added to each chamber in approximate proportions as found in situ: 0.25 mM $NaHCO_3$ and 2.50 mM $NaHCO_3$ to the leaf and root chambers, respectively. Six replicates, three for each light condition, were run in which $NaH^{14}CO_3$ (4.7 mCi μmol^{-1}) was added to the leaf chamber and incubated for 120 minutes at 25 C with stirring, after which time the leaves, but not the roots, were removed for carbon assimilation analysis (see above). This same procedure was done under two other sets of conditions; in each case the samples were injected with $NaH^{14}CO_3$ into the root chamber and not the leaf chamber, but in one set the root chamber was stirred while in the other it was not. In these two latter experiments, the concentration of labeled carbon in the leaves can only be accounted for by acquisition through the roots.

Environmental measurements—The photosynthetic photon flux density (PPFD; 400–700 nm) was measured in air with a LiCor-188B (Lincoln, NE) integrating radiophotometer equipped with an Li-109SB sensor. Water temperature was measured at each sampling period with a standard mercury thermometer.

Lake water samples were collected in a glass bottle, free of air bubbles, and stored on ice in the dark until analyzed for O_2, pH, and alkalinity within a few hours of collection. Dissolved oxygen was measured with a YSI-57 (Yellow Springs, OH) meter and polarographic sensor. Determinations of pH were made with a Photovolt 126-A (New York, NY) pH meter, and alkalinity was determined by titrating the lake water to a pH of 5.1 with 0.02 N H_2SO_4. This endpoint is recommended by the American Public Health Association (1976) for waters of low alkalinity and was found to be close to the inflection point as indicated by preliminary titrations on water samples from both lakes. Free-CO_2 was determined from calculations based on the pH and alkalinity (Lind, 1979).

Interstitial sediment water was measured at the second and third sampling periods. Samples were taken from dialysis bags buried at the first sampling period, 20 and 56 days previously. These bags were initially filled with 200 ml of lake water, buried in 5–10 cm of sediment, and undisturbed until sampled, thus facilitating diffusion of the interstitial sediment water components into the dialysis bag. The chemistry of the interstitial sediment water was analyzed using the same techniques as those for the lake water (above).

RESULTS—Throughout the season, the higher elevation Ellery Lake had a higher pH than Siesta Lake and less than half the free-CO_2 in the water column (Fig. 1, 2). Oxygen levels, however, were relatively similar at both lakes during the season; Ellery Lake ranged from 0.19 to 0.24 mM O_2, and Siesta Lake from 0.15 to 0.22 mM O_2. At both sites the levels of free-CO_2 in the interstitial sediment water differed dramatically from that of the lake water. The means for free-CO_2 from the interstitial sediment water samples were an order of magnitude higher than those for the lake water at Ellery Lake (0.99 mM free-CO_2 in the sediment vs. 0.05 mM free-CO_2 in the water column) and at Siesta Lake (1.59 mM free-CO_2 in the sediment vs. 0.12 mM free-CO_2 in the water column).

The free-CO_2 concentration in both lakes showed no consistent diurnal pattern at any sampling period during the season (Fig. 1, 2), yet carbon uptake from the water column by *I. bolanderi* did show marked diurnal fluctuations. Typically this uptake peaked around midday, between 1000 and 1300 hr, and dropped off rapidly thereafter. In general, carbon uptake in the light tracked changes in PPFD with moderate, but significant, correlations (log-log plots; for Siesta Lake plants $r = 0.53$, $P < 0.005$, $N = 30$; and for Ellery Lake plants $r = 0.52$, $P < 0.05$, $N = 15$). Leaf uptake in the dark exhibited far less variation; only a tendency towards slightly enhanced rates of assimilation near the end of the dark period (Fig. 1, 2).

By integrating the area under the carbon uptake curves of Fig. 1, 2, the contribution of dark carbon uptake to the total 24-hour gross carbon uptake by the leaves was estimated (Table 1). Throughout the season, each lake showed a fairly consistent proportion (20–30%) of total carbon uptake from the water column being contributed by dark uptake (Table 1).

In the samples from Ellery Lake that were spiked with additional carbon, the free-CO_2

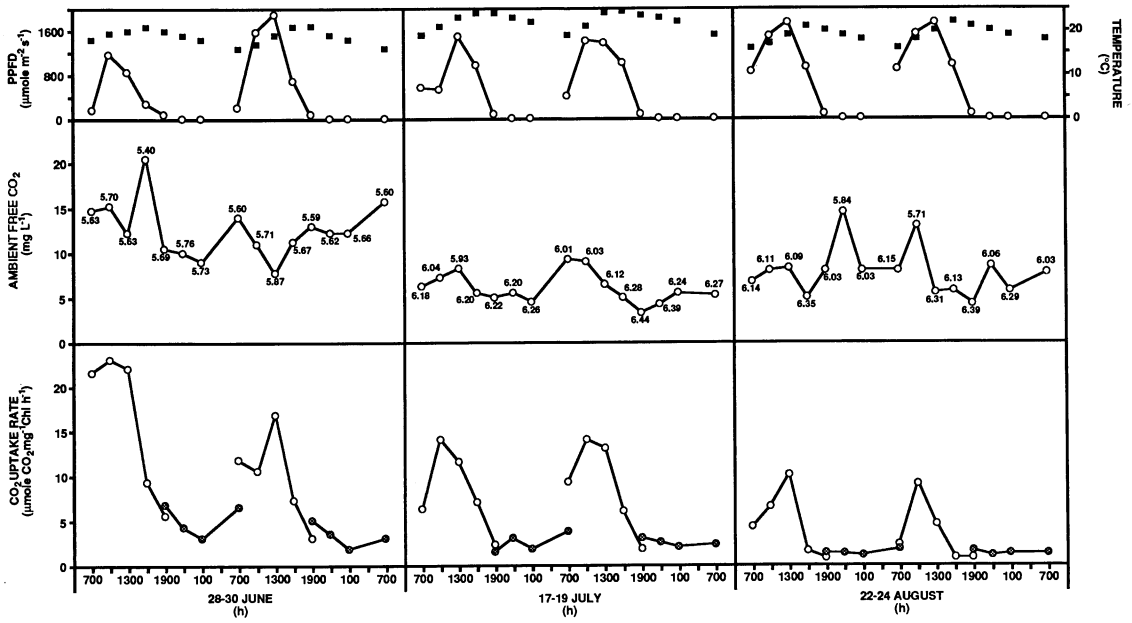

Fig. 1. Environmental parameters and carbon uptake by *Isoetes bolanderi* at Siesta Lake for the three sampling dates, summer 1985. Top panel: daily course of PPFD (open circles) and temperature (closed squares). Middle panel: daily course of ambient free-CO_2 (pH given next to line). Bottom panel: daily course of carbon uptake rates by *Isoetes bolanderi* leaves incubated in lake water (open circles = daytime; closed circles = nighttime; at 1900 hr and 0700 hr two samples are shown, one in daylight and one covered with foil for nighttime).

levels in each vial (0.92 mM free-CO_2 on the average) closely approximated that of the sediment water (0.99 mM free-CO_2). In these samples, peak uptake rates were 79–94 μmol CO_2 mg^{-1} chlorophyll hr^{-1} in the light and 32–33 μmol CO_2 mg^{-1} Chl hr^{-1} in the dark (709 μg Chl g^{-1} FW for Ellery Lake plants on average). The mean total 24-hour carbon gain under these conditions was approximately 1,160 μmol CO_2 mg^{-1} Chl with dark uptake contributing an average of 29%. By comparison, leaf samples under ambient water column conditions showed peak daytime and nighttime uptake rates of 26–35 and 11–20 μmol CO_2 mg^{-1} Chl hr^{-1}, respectively, with total carbon uptake estimated at about 360 μmol CO_2 mg^{-1} Chl and 29% contribution by nighttime uptake. Interestingly, the contribution by nighttime uptake is identical at both low (water column) and high (sediment) carbon concentrations (29%). However, if sediment carbon is contributing to the daily carbon gain of *I. bolanderi,* and these spiked samples accurately represent the contribution of carbon by this interstitial supply, the values reported in Fig. 1, 2 (water column carbon uptake) may represent less than 50% of the total daily assimilation.

The laboratory split-chamber experiments, which assessed the degree to which roots may contribute to carbon gain in *I. bolanderi,* are summarized in Table 2. For both the light and dark samples, root uptake appears to contribute a substantial amount of free-CO_2 (up to two-thirds) to the total carbon gain of the plant. However, since the roots were incubated in the absence of sediment, and diffusion rates may be lower in the presence of sediment, these results may be overestimates of the in situ root contribution (but see Madsen, 1987a).

Fontinalis antipyretica, another submerged aquatic species commonly associated with *Isoetes bolanderi,* showed higher carbon uptake rates from the water column throughout the day (31 July–1 August, at Siesta Lake) than did *I. bolanderi.* For *F. antipyretica,* only a small percentage (2.7%) of its 24-hour carbon gain was derived from nighttime fixation, as is typical of non-CAM plants. However, over that 24-hour period, total uptake from the water column was nearly an order of magnitude greater than that observed for *I. bolanderi* (1,325 vs. 146 μmol CO_2 mg^{-1} Chl; 2,497 μg Chl g^{-1} FW for *F. antipyretica,* 574.3 μg Chl g^{-1} FW for *I. bolanderi* at Siesta Lake). Its peak uptake rate (153.5 μmol CO_2 mg^{-1} Chl hr^{-1}) was at 1300 hr, and uptake was much more strongly correlated with PPFD ($r = 0.97$, $P < 0.005$, log-log plot) than was seen for *I. bolanderi.*

DISCUSSION—The diurnal course of carbon uptake by the leaves of the high elevation lake species *Isoetes bolanderi* is distinctly different from that observed for the congener *Isoetes*

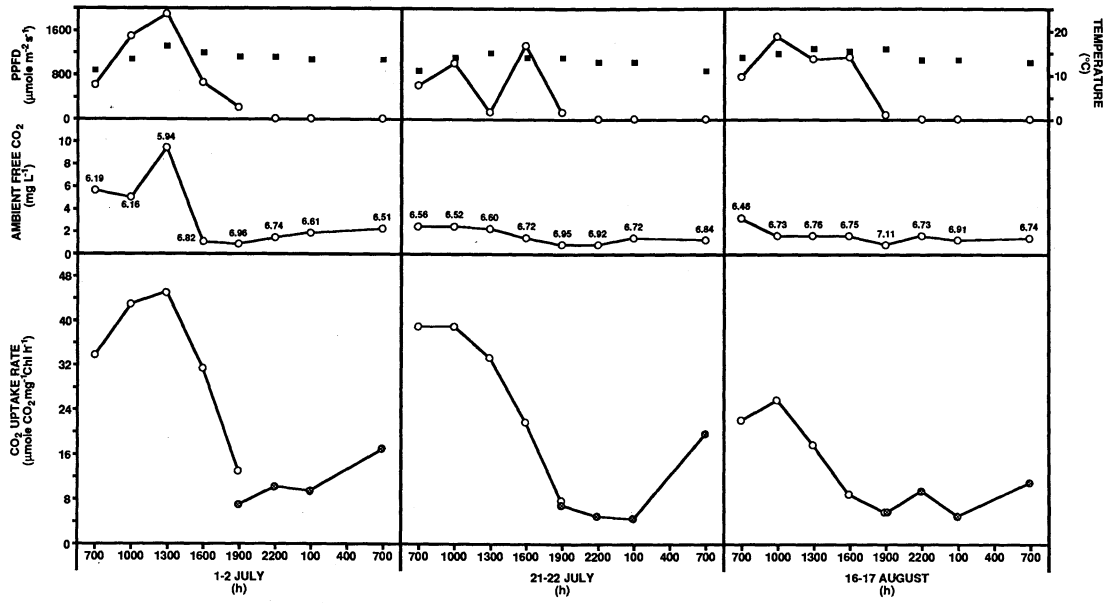

Fig. 2. Environmental parameters and carbon uptake by *Isoetes bolanderi* at Ellery Lake for the three sampling dates, summer 1985. Top panel: daily course of PPFD (open circles) and temperature (closed squares). Middle panel: daily course of ambient free-CO_2 (pH given next to line). Bottom panel: daily course of carbon uptake rates by *Isoetes bolanderi* leaves incubated in lake water (open circles = daytime; closed circles = nighttime; at 1900 hr two samples are shown, one in daylight and one covered with foil for nighttime).

howellii, an inhabitant of seasonal pools. In the latter species, peak CO_2 uptake typically occurs early in the morning when ambient free-CO_2 levels in the water are very high and PPFD levels are low (Keeley and Busch, 1984). In the pools occupied by *I. howellii,* free-CO_2 in the water is usually depleted by noon; consequently carbon uptake by this species is greatly reduced from midmorning throughout the rest of the day (Keeley and Busch, 1984). In the oligotrophic lakes where *I. bolanderi* is found, however, ambient free-CO_2 levels have no apparent diurnal pattern and are consistently low (Fig. 1, 2). Rather than tracking ambient CO_2 levels, it appears that carbon uptake by the leaves of *I. bolanderi* more closely tracks variation in light intensity throughout the day. Madsen (1987c) has suggested that this behavior may be of adaptive significance under conditions where isoetids grow in deep waters and light may be the limiting factor to photosynthesis. This is a possibility for the deeper Ellery Lake population as well as in the smaller and shallower Siesta Lake where a dense pine stand surrounding the lake may produce the same effect (Keeley et al., 1983). However, all samples used in this study were growing in shallow, well-lighted waters, in which case carbon may then be the most limiting factor (Madsen, 1987c). Clearly, photosynthesis is restricted to some degree by both carbon and light limitations; however, nighttime carbon acquisition by *I. bolanderi* may circumvent this stress in a limited, yet significant way.

The contribution of nighttime carbon uptake by the shoots of *I. bolanderi* was remarkably constant (Table 1) despite variation in total carbon uptake from the water column throughout the season (Fig. 1, 2). Although the 20–30% nighttime contribution is lower than that shown for the seasonal pool *I. howellii* (30–50%; Keeley and Busch, 1984), it is remarkably close to the 22% and 36% reported for two other oligotrophic CAM macrophytes, *Isoetes lacustris* L. and *Littorella uniflora* (L.) Aschers, respectively (Madsen, 1985).

Interspecific comparisons of carbon uptake for oligotrophic species of *Isoetes* are difficult due to the variety of conditions under which these studies have been performed (Sand-Jensen, 1978; Richardson et al., 1984; Madsen, 1985, 1987b; Boston and Adams, 1986; Boston et al., 1987a; Raven et al., 1988). However, in those studies with conditions similar to the ones reported here, we find a general agreement in daily CO_2 uptake characteristics. Daytime uptake rates from the water column for *Isoetes macrospora* Duriev. (Boston and Adams, 1986) and for *Isoetes lacustris* (Sand-Jensen, 1978; Madsen, 1987b) fall within the range we demonstrate for *I. bolanderi* at Siesta Lake. Studies of nighttime uptake rates for *I. lacustris* (Madsen, 1985) are also in agreement. Interestingly, these same studies, and results from the Siesta

TABLE 1. *Total 24-hour CO_2 uptake from ambient lake conditions and the percentage contributed by nighttime assimilation in* Isoetes bolanderi *of Siesta and Ellery Lakes*

Sampling dates and days	Total 24-hour CO_2 uptake (μmol CO_2 mg^{-1} Chl)	Contribution by dark uptake (%)
Siesta Lake:		
28–30 June		
Day 1	262	21
Day 2	164	23
17–19 July		
Day 1	145	33
Day 2	148	30
22–24 August		
Day 1	79	20
Day 2	63	22
Ellery Lake:		
1–2 July	565	24
21–22 July	419	26
16–17 August	298	32

TABLE 2. *Free-CO_2 uptake rates by the leaves of* Isoetes bolanderi *from Siesta Lake in the light and dark, where labeled carbon was added to either the leaf chamber or the root chamber in approximate proportions as found in situ (N = 3 for all experiments)*

	$^{14}CO_2$ fed to leaves	$^{14}CO_2$ fed to roots	
	With stirring (μmol CO_2 mg^{-1} Chl h^{-1}; mean ± S.E.)	With stirring (μmol CO_2 mg^{-1} Chl h^{-1}; mean ± S.E.)	Without sitrring (μmol CO_2 mg^{-1} Chl h^{-1}; mean ± S.E.)
Light	8.3 ± 1.4	17.5 ± 6.9	8.7 ± 5.9
Dark	0.3 ± 0.1	0.6 ± 0.5	0.5 ± 0.4

Lake population, showed little correspondence in uptake rates to those of *I. bolanderi* in Ellery Lake. With few exceptions, leaf uptake rates in Ellery Lake plants were three- to six-fold higher than that of Siesta Lake plants and *I. lacustris* and *I. macrospora* in both light and dark periods (Sand-Jensen, 1978; Richardson et al., 1984; Madsen, 1985, 1987b; Boston and Adams, 1986). The relative contribution of nighttime carbon uptake to total carbon uptake by the shoots for all these taxa, however, was similar. The higher uptake rates in the Ellery Lake population are even harder to explain when the differences in the diffusion gradient between the sediment and water column are considered. The ratio of sediment CO_2 to water column CO_2 is higher in Ellery Lake than it is in Siesta Lake (20:1 in Ellery, 13:1 in Siesta), and it has been suggested that a higher gradient may decrease the proportion of CO_2 taken up from the water column (Boston et al., 1987a; Madsen, 1987b; Raven et al., 1988).

In other studies of *Isoetes* species, nighttime uptake of carbon by the leaves accounted for only a small fraction of the overnight malic acid accumulation (Keeley and Busch, 1984; Madsen, 1985, 1987b; Boston and Adams, 1986). It appears that this same conclusion is applicable for *I. bolanderi.* For example, overnight malic acid accumulation for this species in Siesta Lake, observed during mid- and late-summer, was 123 and 122 μmol g^{-1} FW, respectively (Keeley et al., 1983). In the present study, carbon uptake from the water column over the 12-hour dark period in mid- and late-summer was estimated at 15.7 μmol g^{-1} FW and 11.6 μmol g^{-1} FW; about 10% of the overnight malic acid accumulation reported for these plants by Keeley et al. (1983). Assuming an equal molar relationship between CO_2 fixation and malic acid production, it is apparent that rates of nighttime carbon uptake by the leaves are insufficient to account for acid accumulation. Other investigators have documented similar patterns in related isoetids and suggest that the discrepancy between nighttime malic acid accumulation and nighttime carbon uptake from the water column is due to refixation of respiratory carbon and/or acquisition of carbon from the interstitial sediment water via the roots (Keeley and Busch, 1984; Richardson et al., 1984; Farmer and Spence, 1985; Madsen, 1985, 1987a, b; Smith, Boston, and Adams, 1985; Boston, 1986; Boston and Adams, 1986, 1987; Boston et al., 1987a, b; Raven et al., 1988). Accession of carbon through the roots of *I. bolanderi* is supported by our results from the split-chamber experiment. Furthermore, dark carbon fixation rates measured in lake water spiked with additional carbon, comparable to that of the sediment water, could more than account for the accumulation of malic acid overnight.

From the results of our split-chamber experiment, and evidence shown for other *Isoetes* species, it is reasonable to conclude that the leaves of *I. bolanderi* are fixing some CO_2 transported from the sediment water via the roots (Table 2). In addition, assimilation rates by the leaves were markedly higher when supplementary carbon was present in sample vials indicating that these leaves are capable of assimilation at carbon levels greater than water column concentrations, whether by refixation of respiratory CO_2 or by root acquisition.

These results suggest that total carbon gain by *I. bolanderi* is not restricted to uptake from the lake water column. Thus, the observed rates of CO_2 uptake from the water column are clear-

ly underestimates of in situ assimilation. Although total daytime and nighttime carbon uptake from the water column is much lower for *I. bolanderi* than for the associated non-CAM, non-rooted plant *F. antipyretica,* the acquisition of carbon through the roots of *I. bolanderi* and the possibility of refixation of respiratory CO_2 appear to provide substantial amounts of carbon for this isoetid that may account, in part, for its dominance of the macrophyte flora in Siesta and Ellery Lakes.

CONCLUSION—Contrary to observations in seasonal pool species of *Isoetes,* diurnal patterns of carbon uptake from the water column for *Isoetes bolanderi* appear to be relatively independent of ambient CO_2 levels in situ. Rather, daytime carbon uptake is more closely linked to changes in PPFD. Assimilation of carbon from interstitial sediment water may also play an important role in the carbon economy of this species.

The presence of CAM contributes up to 33% of the total carbon gain, by the leaves, from ambient lake water due to nighttime fixation of free-CO_2 and may contribute more through overnight fixation of CO_2 from the sediment and respiration. Thus, the presence of CAM in *I. bolanderi* appears to extend the period of carbon gain to 24 hours. This may confer a selective advantage for *I. bolanderi* under conditions of very low carbon concentrations which occur in Siesta and Ellery Lakes, as well as other oligotrophic habitats in which this genus resides.

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