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Vernal Pools
and Intermittent Streams

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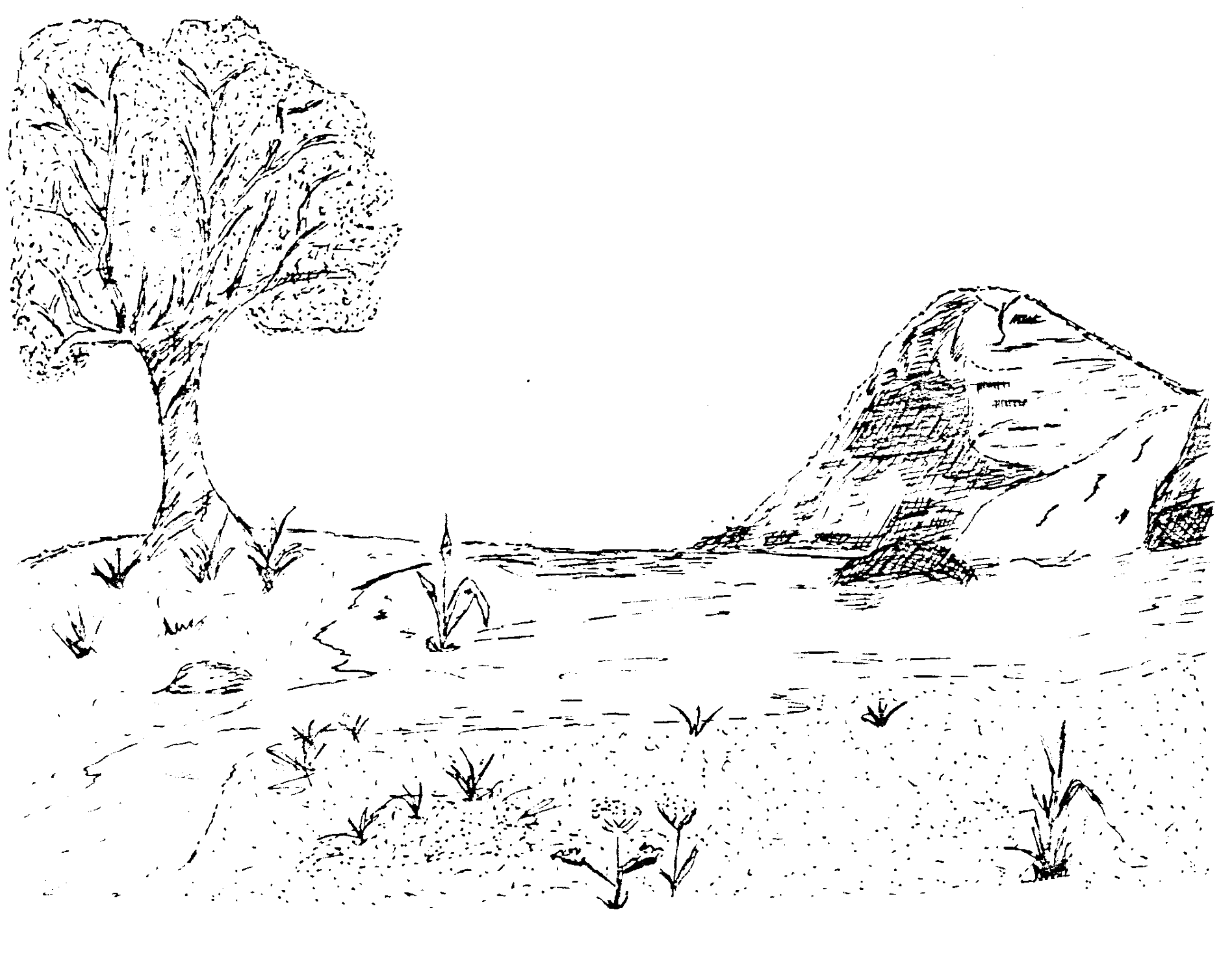

PHOTOSYNTHETIC CHARACTERISTICS OF CERTAIN VERNAL POOL SPECIES

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Abstract. Vernal communities are composed of CAM and presumably strictly C_3 species. Evidence from this study points to the role of diurnal changes in CO_2 availability in selection of CAM in vernal pools aquatics. Detailed studies of photosynthesis in both CAM and non-CAM vernal pool species are now underway in order to explore the adaptive significance of their physiological mechanisms.

INTRODUCTION

My current interest in photosynthesis of vernal pool plants began with the observation that the leaves, but not the corms, of submerged Isoetes howellii (Isoetaceae) undergo a marked diurnal fluctuation in malic acid (Keeley 1981). This was a surprising discovery since the only place in the plant kingdom from which such a phenomenon was known was in xerophytic succulent CAM plants.

CAM is an acronym for Crassulacean Acid Metabolism which is characterized by the unique combination of all of the following characteristics: Dark-fixation of CO_2 takes place in photosynthetic tissues; malic acid is the major product of dark CO_2-fixation, and accumulates in the vacuole overnight. Malic acid is decarboxylated during the day and CO_2 is refixed in the C_3 pathway, such that diurnal cycle of nighttime acidification and daytime deacidification involves a diurnal fluctuation of 75-200 equivalents gm^{-1} fresh weight. The evidence presently available (Keeley, 1981; unpublished data) indicates that submerged plants of Isoetes howellii possess all these characteristics.

The role of CAM in the net carbon gain of terrestrial xerophytic succulents is variable. In the prototype CAM plant the bulk of CO_2-uptake occurs at night and is controlled by a diurnal change in stomatal behaviour. Isoetes howellii have stomata but as in other aquatics these are apparently nonfunctional while submerged, thus CO_2-uptake is probably controlled largely by CO_2 availability. Isoetes howellii have the capability for substantial CO_2 uptake in the light (Keeley 1981) but, as discussed below, diurnal changes in CO_2 availability may produce conditions under which dark CO_2-fixation is the major contributor to the total carbon gain.

The focus of this paper will be to address two questions: 1) Is Crassulacean Acid Metabolism common in and among aquatic plants unique to vernal pools? 2) What is the adaptive significance of CAM to an aquatic plant?

Distribution of CAM

Diurnal changes in titratable acidity is often taken as an indication of CAM. Bryce Morton and I have surveyed some of the more common submerged vernal pool species for titratable acidity fluctuations. Table 1 indicates that of one algal species and 8 angiosperms surveyed, only one (Crassula aquatica) showed any evidence of CAM. It is perhaps not surprising to some that Crassula aquatica is CAM since most of the family Crassulaceae is CAM. It is noteworthy, however, that we have been unable to detect diurnal changes in acidity in the closely related terrestrial Crassula erecta.

Table 1. Morning and evening fluctuations in titratable acidity (to pH 6.4) for common vernal pool species.

Species	Family	Tritratable Acidity (equivalents gm^{-1} fresh weight)	
Chara sp.	(Characeae)	0	0
Ranunculus aquatilis	(Ranunculaceae)	3	0
Elatine chilensis	(Elatinaceae)	2	1
Plagiobothrys undulatus	(Boraginaceae)	0	0
Crassula aquatica	(Crassulaceae)	111	9
Downingea cuspidata	(Campanulaceae)	0	0
Callitriche longipedunculata	(Callitrichaceae)	1	0
Lilaea scilloides	(Lilaeaceae)	1	1
Eleocharis acicularis	(Cyperaceaea)	11	3

Thus it is apparent that vernal pool communities consist of a mixture of CAM and non-CAM species. Table 2 indicates that aquatic CAM plants are not restricted to vernal pools. Across the largely aquatic genus Isoetes, CAM appears to be common. It is found not only in the vernal pool members I. howellii and I. orcuttii but also in species from high elevation oligotrophic lakes as well as species from tidal flats. Thus it is found in species which are seasonally submerged such as in vernal pools or permanently submerged in lakes or diurnally submerged in tidal flats.

Adaptive Significance

The possession of CAM in the vernal pool aquatics, Isoetes howellii, I. orcuttii, and Crassula aquatica raises an important question as to its functional significance. The key to understanding CAM is the fact that daytime decarboxylation of malic acid generates an internal CO_2 source. In terrestrial CAM plants this is undoubtedly selected for because it allows the plant to carry on photosynthesis with stomata closed, thereby conserving water. I hypothesize that in certain vernal pool species CAM was selected for as an adaptation to limited daytime CO_2 availability.

Table 2. Morning and afternoon fluctuations in titratable acidity (to pH 6.4) for greenhouse maintained *Isoetes* species (sample size N=2).

Species	Titratable Acidity (equivalents gm^{-1} fresh weight) 7 AM	3 PM	Habitat	Habitat
I. bolanderi	138	19	Shallow oligotrophic lake	perennially submerged
I. echinospora ssp. *maritima*	136	21	Fresh water tidal creek	diurnally submerged
I. engelmanni	52	8	Pond	seasonally submerged
I. howellii	67	14	Vernal Pool	seasonally submerged
I. lacustris	108	24	Oligotrophic lake	perennially submerged
I. lithophila	163	40	Granite outcrop pool	seasonally submerged
I. mexicana	88	15	Shallow pool	seasonally submerged
I. occidentalis	93	38	Oligotrophic Lake	perennially submerged
I. orcuttii	85	20	Vernal Pool	seasonally submerged
I. raparia	117	33	Freshwater tidal bay	diurnally submerged
I. storkii	99	11	Oligotrophic lake	± perennially submerged
I. tegetiformans	95	51	Granite outcrop pool	seasonally submerged

The availability of carbon to submerged aquatic plants is often limiting to photosynthesis because the diffusive resistance to CO_2 transfer in water is quite high and not all of the inorganic carbon is in available form. Free-CO_2 is the "preferred" substrate for photosynthesis by both terrestrial and aquatic plants (Raven 1970). During the day, as free CO_2 is depleted, HCO_3^- becomes the dominant form of inorganic carbon. Some aquatic plants are capable of assimilating HCO_3^- but many are not. Preliminary work indicates that *I. howellii* uses HCO_3^- poorly if at all. $^{14}CO_2$-uptake studies in the light show over an 80% reduction in uptake between pH 5.4 (where nearly all the carbon is free-CO_2) and pH 8.0 (where most of the carbon is in bicarbonate).

Field studies in which we have tracked the diurnal changes in acidity along with changes in the availability of free-CO_2 support the above hypothesis . Table 3 shows data for *I. howellii* and *I. orcuttii* from vernal pools in both San Diego and Riverside counties. In both cases acid metabolism is associated with a diurnal change in the availability of free-CO_2. In the early morning malic acid levels are highest at which time there are appreciable levels of free-CO_2 in the water. At the end of the day malic acid levels in the leaves are greatly reduced and there is no detectable free-CO_2 in the water.

Table 3. Diurnal changes in physical and chemical parameters of two vernal pools and malic acid concentration of Isoetes leaves submerged in those pools.

	Pool Parameters				Malic Acid (mg/gm FW) in Leaves			
PST hr	C°	ppmO_2	pH	Free CO_2 (mg/l)	Isoetes howelii		Isoetes orcuttii	
	Miramar - San Diego 8 April 1979							
6 PM	22.9	12.0	9.0	0	1.8±0.4	(6)	- -	-
6 AM	16.0	2.1	6.8	7.5	7.1±1.5	(6)	- -	-
	Mesa de Colorado-Riverside 26 April 1980							
6 PM	21.0	11.0	8.5	0	3.3±0.2	(2)	3.7±1.4	(2)
6 AM	12.5	7.4	6.3	9.5	12.5±2.8	(2)	13.1±2.6	(2)

Further evidence of the influence of diurnal changes in free-CO_2 availability in the water on diurnal acid metabolism in Iosetes howellii was provided by data, collected in spring 1981 from vernal pools on the Santa Rosa Plateau in Riverside county. The dynamics of daytime deacidification are variable and perhaps dependent upon immediate weather conditions. April 5 was a cloudless day with noontime quantum radiation levels at full sunlight, also water temperature was 26°C, oxygen exceeded 150% saturation and free-CO_2 was depleted. In contrast April 18 was foggy and overcast with noontime quantum radiation approximately a third full sunlight, water temperature 16°C, oxygen at saturation and substantial free-CO_2 still present. Associated with these different conditions was a very marked difference in the pattern of daytime deacidification.

In light of the facultative nature of CAM in many terrestrial succulents, a further prediction from this hypothesis is that diurnal acid metabolism should be lost as I. howellii becomes emergent. Table 4 shows acid levels in submerged and nearby emergent leaves of I. howellii. Emergent plants do show a weak overnight acid accumulation, however, morning levels are an order of magnitude less than in submerged plants.

Table 4. Titratable acidity (to pH 6.4) in submerged and emergent leaves of Isoetes howellii from the Santa Rosa Plateau, 17-18th April 1981 (sample size N=2).

	Titratable Acidity (equivalents gm^{-1} fresh weight) Hour		
	1800	2400	0600
Submerged	26	188	271
Emergent	11	19	28

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