

# Photosynthesis in Vernal Pool Macrophytes: Relation of Structure and Function

JON E. KEELEY

## Abstract

Structural and functional characteristics of the photosynthetic tissues in the 16 dominant macrophytes from selected southern California vernal pools are presented. The majority of species produced an isoetid growth form (a rosette of cylindrical leaves) as the initial submerged stage. In some taxa this form was later replaced by foliage specialized for an aerial environment, such as floating leaves, and in some species this stage was followed by a third set of foliage specialized for a terrestrial habitat. In nearly all species, leaves produced in the air were thinner and had substantially less intercellular airspace than did aquatic leaves. Aerial leaves had a similar order of magnitude increase in both chlorophyll and protein concentrations over the levels in submerged leaves. Overnight accumulation of malic acid, indicative of Crassulacean Acid Metabolism (CAM photosynthesis) was present in four taxa, one of which (*Orcuttia californica*), had not been reported previously. For all species CAM largely disappeared from aerial foliage. Short-term  $^{14}\text{C}$  fixation studies indicated that in the light some species fixed carbon into the  $\text{C}_3$  product phosphoglycerate, some fixed carbon initially into  $\text{C}_4$  organic acids, and other species had similar amounts of label fixed into both  $\text{C}_3$  and  $\text{C}_4$  products. Kranz anatomy, indicative of terrestrial  $\text{C}_4$  plants, was present in the floating leaves of *O. californica* and an anatomy with characteristics of Kranz was noted in the floating leaves of *Marsilea vestita*. In just about all cases the ratio of RuBP carboxylase: PEP carboxylase activity increased markedly in aerial foliage, suggesting that in many species PEP carboxylase-mediated  $\text{C}_4$  fixation is important in the aquatic environment but much less so in an aerial environment. The roles these structural and functional characteristics play in promoting coexistence of this relatively diverse vernal pool flora are discussed.

## Introduction

By their nature temporary bodies of water such as California vernal pools are seasonally variable environments. Perhaps less well known, but at least equally variable, are the diurnal changes in chemical and physical characteristics (Figure 1-A). These daily fluctuations produce a temporally heterogenous environment for photosynthetic organisms. As irradiance increases during the morning in these shallow, densely vegetated pools, photosynthetic activity depletes the free- $\text{CO}_2$  and supersaturates the water with  $\text{O}_2$  (Keeley, 1983). These rain-fed seasonal pools are poorly buffered and as a consequence, the depletion of carbon dioxide results in marked changes in pH; typically pre-dawn pH ranges between 6-7 but rises 2-4 pH units by early afternoon. Overnight, respiration by the pool flora and invertebrate fauna

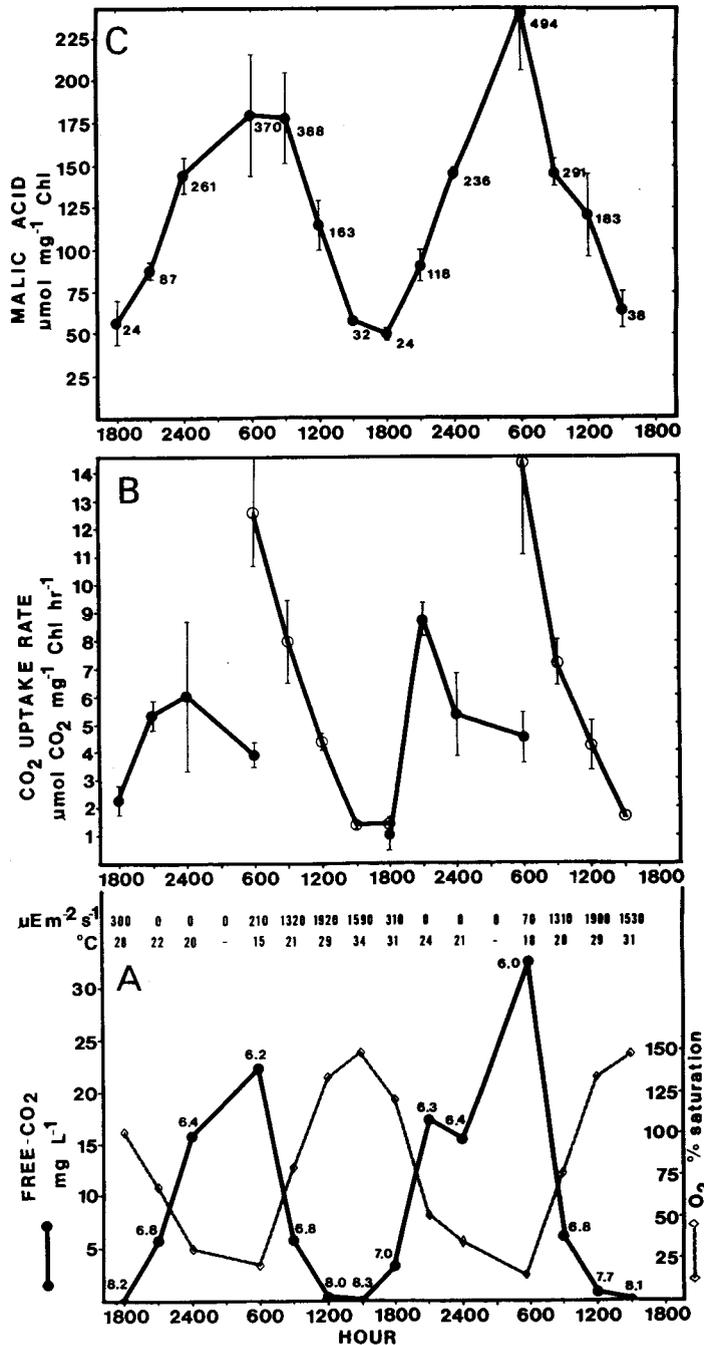


Figure 1. 24-hour cycle of (A) carbon dioxide levels (●) and oxygen levels (○) in the water of a vernal pool (pH is indicated adjacent to the carbon dioxide line), (B) carbon dioxide uptake and fixation rates in the dark (●) and in the light (○) by leaves of *Isoetes howellii* (vertical lines indicate the standard deviation,  $n = 3$ ), (C) malic acid concentration in *Isoetes howellii* leaves. Data taken in April from a pool of 20-25 cm depth in southern California (Mesa de Colorado, Riverside Co.).

# **Vernal Pool Plants Their Habitat and Biology**

---

Based on a symposium held at California State University, Chico  
Sponsored by  
Pacific Section, Botanical Society of America, and the  
Pacific Division, American Association for the Advancement of Science  
14 June 1989

## **Editors**

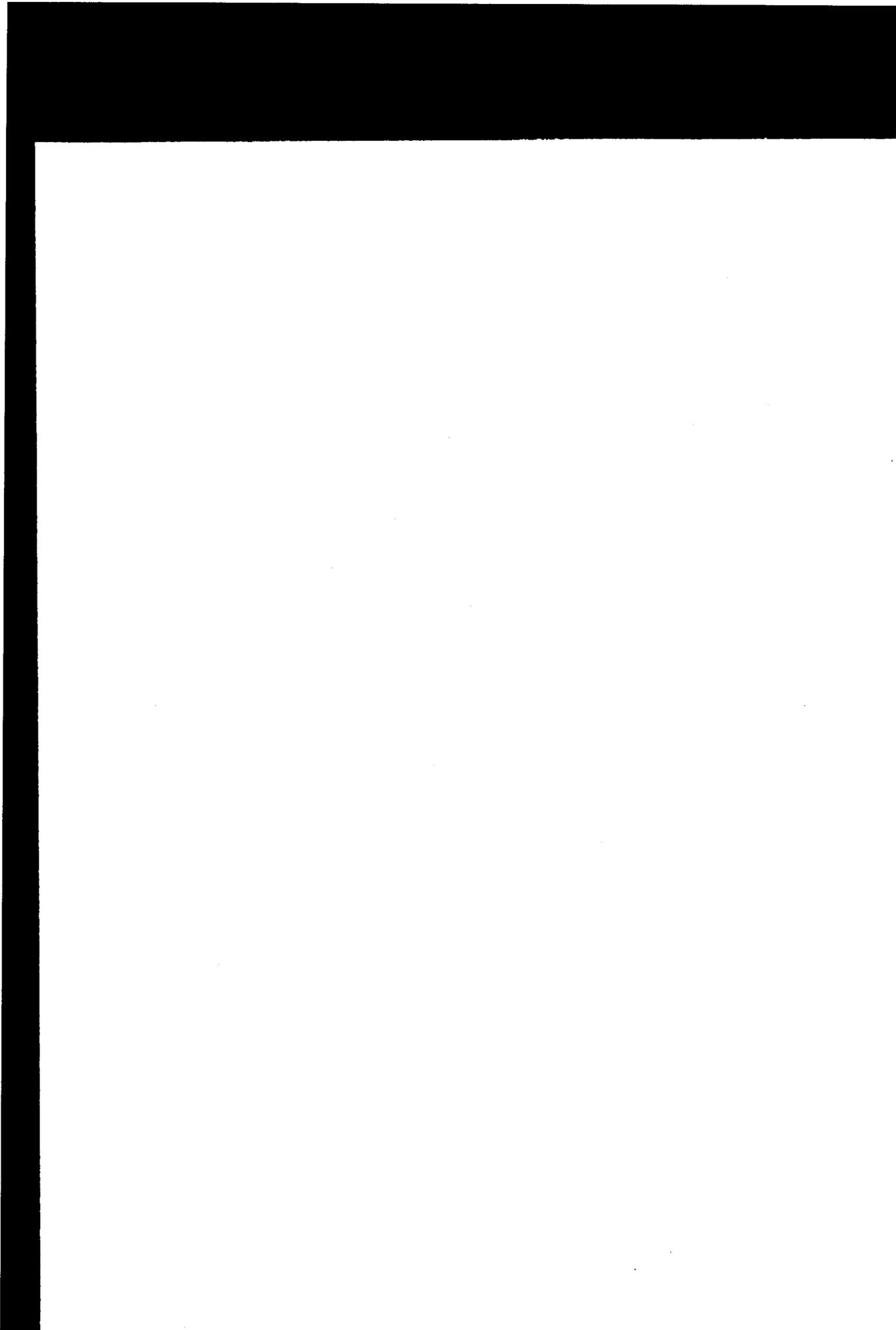
**Diane H. Ikeda  
Robert A. Schlising**

## **Assistant Editors**

**F. Jay Fuller  
Lawrence P. Janeway  
Paula Woods**

**Studies from the Herbarium  
California State University, Chico  
Number 8**

**June 1990**



replenish the free-CO<sub>2</sub> and reduce the O<sub>2</sub> levels. Such diurnal changes surpass seasonal changes observed over the course of a season in vernal pools (Kopecko and Lathrop, 1975) or between seasons in other aquatic environments (Wetzel, 1975), and result in a scale of temporal heterogeneity seldom observed in other ecosystems. Over a very short time scale, the vernal pool environment changes from a milieu conducive to photosynthesis to an environment less hospitable for photosynthetic organisms. As a consequence, adaptation to such an environment may require novel strategies not seen in terrestrial habitats.

Photosynthesis in an aquatic setting is affected by factors not encountered in a terrestrial environment. Due to the viscosity of water, gases diffuse four orders of magnitude slower than in air; consequently, changes in CO<sub>2</sub> and O<sub>2</sub> observed in the pools (Figure 1-A) are greatly exacerbated in the boundary layer around leaves. Also, in water, forms of inorganic carbon other than CO<sub>2</sub>, such as HCO<sub>3</sub><sup>-</sup>, are potential carbon sources for photosynthesis. Thus, even though carbon dioxide may be depleted from the water during part of the day, substantial bicarbonate remains. Typically total inorganic carbon levels fluctuate from about 0.6 mols m<sup>-3</sup> at sunrise to about 0.3 mols m<sup>-3</sup> at sunset (Keeley, unpublished). However, since bicarbonate is ionized it does not diffuse freely across membranes as does carbon dioxide and thus it is energetically costly for plants to use this carbon source (Lucas and Berry, 1985). As a result, many aquatic plants have not evolved the capacity for utilizing bicarbonate.

The vernal pool quillwort, *Isoetes howellii* (nomenclature according to Lathrop and Thorne, 1985) is an example of a species that has little, if any, capacity for utilizing bicarbonate. Thus, as free-CO<sub>2</sub> is depleted from the pools during the early morning, carbon uptake is markedly inhibited and remains low throughout the afternoon (Figure 1-B). This aquatic macrophyte, however, has the capacity for substantial carbon uptake in the dark. Carbon is fixed in the dark by phosphoenolpyruvate carboxylase (PEPcase) and stored overnight as malic acid (Figure 1-C). During the day, the malic acid is decarboxylated and the carbon is utilized as an internal source of CO<sub>2</sub> for photosynthesis, just as in terrestrial plants with the Crassulacean Acid Metabolism (CAM) photosynthetic pathway (Keeley, 1987). It has been hypothesized that this pathway has been selected for as a means of competing for carbon in the carbon-limited vernal pool environment.

Few other species in these pools possess CAM (Keeley and Morton, 1982) and therefore, we might presume, other mechanisms exist for competing for carbon. However, a field study comparing carbon uptake for three non-CAM species, *Downingia bella*, *Eleocharis acicularis*, and *Plagiobothrys undulatus* (Keeley and Sandquist, in press) showed that daytime carbon uptake was similar to that observed in Figure 1-B for *I. howellii*. This raises the question, to what extent does community coexistence of vernal pool macrophytes

depend upon differing mechanisms of carbon competition? The answer will require a thorough knowledge of the photosynthetic characteristics of the pool flora. Here I will summarize what is known about the photosynthetic tissues, both in terms of structure and function, for vernal pool macrophytes.

## Materials and Methods

All plants were grown in artificial pools maintained for several years at Occidental College, Los Angeles County, California. These pools were begun with seeds or corms and substrate collected from pools on the Santa Rosa Plateau in Riverside County. Each year, beginning in December, the pools were kept filled with deionized water until May, after which they were allowed to dry.

### Anatomical Preparations

Leaves and stems from several representative individuals of each species were fixed in FAA and then dehydrated through an alcohol series followed by xylene. Portions were embedded in paraffin, sectioned on a microtome, and stained with safranin and fast green. These were examined under the light microscope, and photomicrographs were printed. The cross-sectional area occupied by airspace was estimated by cutting out the airspace from enlarged prints and comparing the mass of the photographic paper before and after.

### Physiological Studies

Diurnal changes in acidity were determined by collecting photosynthetic tissues (0.2-0.5 g fresh weight) at 0600 hr and 1700 hr. These were washed, blotted dry, and weighed. After grinding with 15 ml cold CO<sub>2</sub>-free deionized H<sub>2</sub>O, a 10 ml sample was immediately titrated with CO<sub>2</sub>-free 0.01 NaOH to pH 6.4 and/or pH 7.0. A 1 ml sample was deproteinized with an equal volume of 0.6 N HClO<sub>4</sub> and then enzymatically assayed for malic acid (Gutmann and Wahlefeld, 1974).

Carboxylase enzymes were assayed by grinding tissues on ice in buffer (50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 5 mM isoascorbate, 1% w/v PVP-40), one sample at pH 8.0 and one at pH 8.5. Aliquots were taken for chlorophyll and protein assays; these were maintained on ice in the dark until the enzyme assay was completed. The enzyme extract was centrifuged at 11,870 xg for 5 min at 4 C. The supernatant was assayed immediately. Both enzymes were assayed at 25 C, using <sup>14</sup>CO<sub>2</sub> fixation techniques and liquid scintillation counting of acid stable products. RuBP carboxylase was assayed in the active form as described by Lorimer et al. (1977). Experiments were initiated by addition of RuBP substrate and terminated after 1 min by the addition of 6M HCl. PEP carboxylase was assayed according to the procedure of Van et al. (1976) with a 2 min incubation. Experiments were initiated by

addition of PEP substrate and terminated by addition of 6M HCl saturated with 2,4-dinitrophenylhydrazine to stabilize OAA. Use of this compound required a separate quench curve. Chlorophyll was determined with the method of Arnon (1949) after correcting for the small absorption at 710 nm as suggested by Sestak et al. (1971). Soluble protein was determined with the Lowry Method as modified by Bergmeyer (1974, vol. I, p. 172-174).

Short-term steady-state  $^{14}\text{C}$  labeling experiments were conducted in order to evaluate the initial products of carbon fixation. Foliage samples of 0.10 to 0.70 g were tied with a small thread into loose bundles. These were immersed in 25-ml stoppered serum vials filled with 60 mM sodium potassium phosphate pH 6.0 buffer prepared fresh daily. Prior to injection of the isotope, samples were pre-incubated in the light for 15 min. Experiments were initiated by injection of 75  $\mu\text{Ci}$   $\text{NaH}^{14}\text{CO}_3$  (9  $\mu\text{Ci}$   $\mu\text{mole}^{-1}$ ). Vials were not stoppered, and after 5 seconds, experiments were terminated by immersing leaf bundles in boiling 80% methanol on a heating plate, and boiling was continued for several minutes. The sample was homogenized in a glass grinder and centrifuged at 11,870  $\times g$  for 20 min. The pellet was washed once in deionized  $\text{H}_2\text{O}$  and both supernatants combined. These were evaporated dry at 80 C and then resuspended in 2 ml deionized  $\text{H}_2\text{O}$ . This was evaporated down to approximately 500  $\mu\text{l}$  and then centrifuged in capillary blood serum separator microcentrifuge tubes. An aliquot of this sample was taken for determination of total activity by liquid scintillation counting. Another aliquot was utilized for determination of labeled photosynthetic products.

Thin-layer chromatography (TLC) and electrophoresis were used to separate labeled products. Samples of 100  $\mu\text{l}$  were spotted with capillary tubes on TLC cellulose (250  $\mu\text{m}$ ) covered glass plates (20 x 20 cm). Separation in the first dimension was with electrophoresis in pyridine: glacial acetic acid:  $\text{H}_2\text{O}$  (2:9:200) at pH 4.0 with solvent made fresh daily. The unit was an LKB Multiphor II with water circulating cooling plate (21 x 27 cm) maintained at 15 C. Separation was run for 50 min at 900 V and 70-75 ma. Separation in the second dimension was done chromatographically in sec-butanol: acetic acid:  $\text{H}_2\text{O}$  (6:1:2) solvent made fresh daily. The solvent front was allowed to rise twice, the first time to 11 cm above the origin, after which the plates were blown dry under a cool airstream, and again to 15 cm above the origin. Autoradiographs were made by placing film sheets on the plates, wrapping tightly and exposing for several days. Spots were identified by using the same separation techniques on authentic compounds and visualizing them by combinations of various stains. Spots detected by the autoradiographs were scraped from the plates, eluted in  $\text{H}_2\text{O}$  and counted with liquid scintillation counting with an automatic quench curve for the cellulose.

## Results

Morphological and anatomical characteristics of the species used in this study are given in Table 1. These 16 species are the common dominants found in the Santa Rosa Plateau pools. The growth forms in this community illustrate degrees of diversity as well as convergence. For more than half of the species, the first stage of development was a rosette of cylindrical leaves, often described as the "isoetid" growth form (Sculthorpe, 1967). In both species of *Isoetes*, and *Eryngium aristulatum*, leaves arose from a corm, and in other perennials such as *Pilularia americana* and *Eleocharis acicularis* rosettes of leaves were produced at intervals along a horizontal rhizome. In several annuals, the first leaves formed a rosette, which persisted in *Lilaea scilloides*, but in other species was replaced with a caulescent growth form, (e.g. *Plagiobothrys undulatus*), or floating leaves, (e.g. *Orcuttia californica*; Figure 2). The cylindrical leaf shape in the grasses *Alopecurus howellii* and *Orcuttia californica* was generated by the folding around of an otherwise laminate leaf design (e.g. Figure 2). In *Downingia bella* the leaves were extremely reduced but the stem formed an enlarged cylinder not unlike an inverted carrot.

*Isoetes howellii*, *Eryngium aristulatum*, and *Lilaea scilloides* produced a rather robust isoetid growth form, with leaves 15-25 cm tall and 1-2 mm thick, whereas other species with this growth form were quite diminutive.

Some degree of leaf heterophylly was characteristic of all vernal pool species. Distinctly different leaf shapes were produced between aquatic and aerial environments by *Marsilea vestita*, *Callitriche longipedunculata*, *Downingia bella*, *Eryngium aristulatum*, *Plagiobothrys undulatus*, *Ranunculus aquatilis*, *Alopecurus howellii*, and *Orcuttia californica*. In *Isoetes* species, *Pilularia americana*, *Crassula aquatica*, *Elatine californica*, *Eleocharis acicularis*, and *Lilaea scilloides* the leaf shape remained unchanged between the aquatic and aerial environments; however, there were quantitative changes in several characteristics. Regardless of whether or not there was a change in leaf shape, leaves produced in the aquatic milieu were generally thicker than similar leaf types produced in air and the volume of intercellular air space was much greater (Table 1).

Distribution of stomata was variable. All species had stomata on organs initiated in an aerial environment although the location on upper and lower leaf surfaces was variable (Table 1). Not surprisingly, floating leaves had stomata restricted to the upper epidermis. All other aerial leaves had stomata distributed around the circumference of cylindrical leaves or on both upper and lower surfaces of laminate leaves. On submerged leaves, stomata were present in most species although they were typically much less frequent than on aerial organs and were non-functional due to occlusion by wax. For *Downingia* and *Orcuttia*, stomata were lacking on submerged leaves.

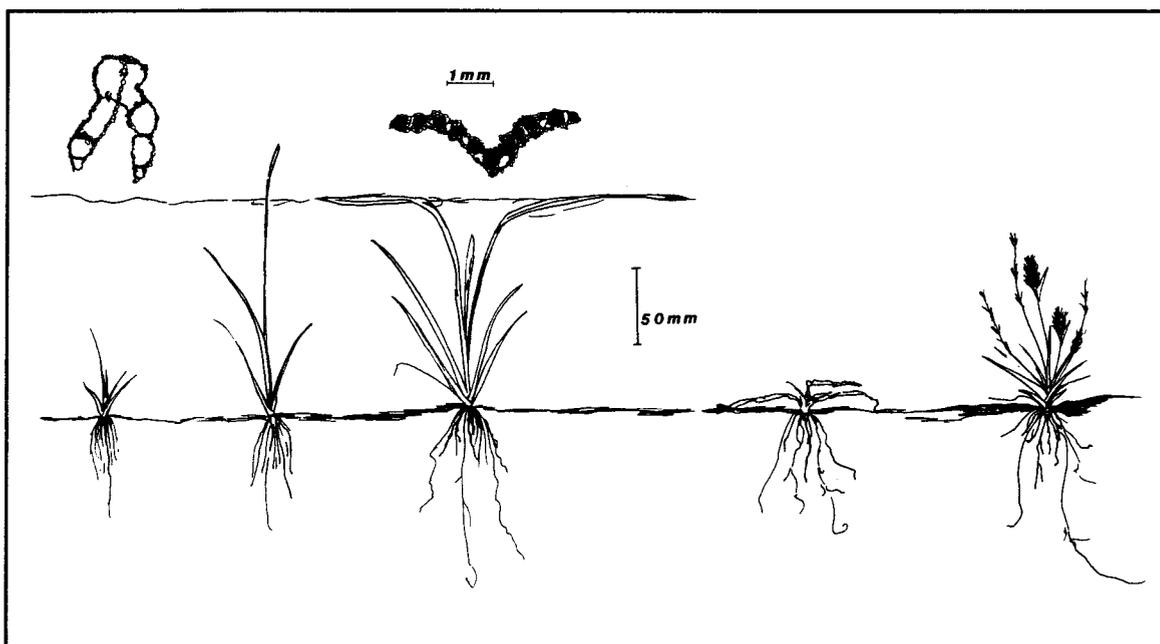


Figure 2. Schematic drawing of the submerged leaves, floating leaves, and terrestrial foliage of *Orcuttia californica*. Leaf cross sections are drawn above the first two stages.

Epidermal cells were variable in size; in *Eleocharis* they were less than one-tenth the size of the mesophyll cells whereas in *Elatine* they were double the size of the adjacent mesophyll cells. Cuticle thickness also varied. Submerged leaves of most species had some cuticle but it was much thicker on *Isoetes* species and *Crassula*. Submerged leaves of *Alopecurus* and *Orcuttia* had no obvious cuticle.

Distribution of chloroplasts was variable. In aerial leaves, chloroplasts were lacking from the epidermis. Additionally, they were lacking in the epidermis of submerged leaves in all species except *Marsilea* and *Ranunculus*; in both *Isoetes* species occasional chloroplasts were evident in some epidermal cells. In submerged leaves of *Isoetes* species, *Pilularia*, and *Crassula* chloroplasts were distributed throughout the mesophyll but were most concentrated in the cells bordering the lacunal air chambers. This was also true for the submerged stems of *Downingia*. In other species, chloroplasts in submerged leaves were concentrated directly beneath the epidermis; this was particularly striking in *Marsilea*, *Callitriche*, *Elatine*, *Ranunculus*, and *Lilaea*.

The  $C_4$  Kranz anatomy, with chloroplasts concentrated in the bundle sheath cells, was present in the floating leaves (Figure 2) and terrestrial leaves (Figure 3) of *Orcuttia*, but not in the submerged leaves. Floating leaves of

Table 1. Growth form and structural characteristics of plant species typical of the Santa Rosa Plateau vernal pools

	Growth form	Leaf shape	Leaf thickness (mm)	Airspace (% cross-sectional area)	Stomata	Kranz Anatomy
<b>CHLOROPHYTA</b>						
<i>Chara contraria</i> (Characeae) - A <sup>a</sup>						
Submerged	caulescent	cylindric <sup>b</sup>	0.10 (dia)	83	n.a. <sup>c</sup>	no
<b>LYCOPHYTA</b>						
<i>Isoetes howellii</i> (Isoetaceae) - P						
Submerged	rosette	cylindric	1.80 (dia)	82	present	no
Emergent	rosette	cylindric	1.10 (dia)	42	present	no
<i>Isoetes orcuttii</i> (Isoetaceae) - P						
Submerged	rosette	cylindric	0.60 (dia)	70	present	no
Emergent	rosette	cylindric	0.35 (dia)	20	present	no
<b>PTEROPHYTA</b>						
<i>Marsilea vestita</i> (Marsileaceae) - P						
Submerged	petiole	cylindric	0.40 (dia)	23	few	no
Emergent	floating	laminar	0.13 (thick)	14	upper	intermediate
<i>Pilularia americana</i> (Marsileaceae) - P						
Submerged	rosette	cylindric	0.35 (dia)	59	present	no
Emergent	rosette	cylindric	- d	-	-	no
<b>ANTHOPHYTA -- DICOTYLEDONEAE</b>						
<i>Callitriche longipedunculata</i> (Callitrichaceae) - A						
Submerged	caulescent	laminar	0.15 (thick)	62	few	no
Emergent	floating	laminar	0.20 (thick)	26	upper	no
<i>Crassula aquatica</i> (Crassulaceae) - A						
Submerged	caulescent	semi-cylindric	0.45 (thick)	52	present	no
Emergent	caulescent	semi-cylindric	0.45 (thick)	35	present	no
<i>Downingia bella</i> (Campanulaceae) - A						
Submerged	columnar	cylindric <sup>e</sup>	1.90 (dia)	75	not evident	no
Emergent	caulescent	laminar	0.30 (thick)	39	bottom	no

Table 1. Continued

	Growth form	Leaf shape	Leaf thickness (mm)	Airspace (% cross-sectional area)	Stomata	Kranz Anatomy
<i>Elatine californica</i> (Elatinaceae) - A						
Submerged	prostrate	lamine	0.14 (thick)	68	upper/lower	no
Emergent	prostrate	lamine	-	45	-	-
<i>Eryngium aristulatum</i> (Apiaceae) - P						
Submerged	rosette	cylindric	1.00 (dia)	59	present	no
Emergent	caulescent	lamine	0.25 (thick)	23	upper/lower	no
<i>Plagiobothrys undulatus</i> (Boraginaceae) - A						
Submerged	rosette	cylindric	0.65 (thick)	64	present	no
Emergent	caulescent	lamine	0.25 (thick)	26	upper/lower	no
<i>Ranunculus aquatilis</i> (Ranunculaceae) - A						
Submerged	caulescent	cylindric	0.15 (dia)	16	few	no
Emergent	floating	lamine	0.20 (thick)	17	upper	no
ANTHOPHYTA -- MONOCOTYLEDONEAE						
<i>Alopecurus howellii</i> (Poaceae) - A						
Submerged	"rosette"	cylindric	0.12 (thick)	48	few	no
Emergent	floating	lamine	0.10 (thick)	20	upper	no(?)
Terrestrial	caulescent	lamine	-	-	-	-
<i>Eleocharis acicularis</i> (Cyperaceae) - P						
Submerged	"rosette"	cylindric	0.40 (dia)	68	present	no
Emergent	"rosette"	cylindric	0.30 (dia)	12	present	no
<i>Lilaea scilloides</i> (Lilaeaceae) - A						
Submerged	rosette	cylindric	1.40 (dia)	56	present	no
Emergent	rosette	cylindric	-	-	-	-
<i>Orcuttia californica</i> (Poaceae) - A						
Submerged	rosette	cylindric	0.14 (thick)	57	not evident	no
Emergent	floating	lamine	0.10 (thick)	21	upper	Kranz
Terrestrial	caulescent	lamine	0.15 (thick)	1	upper/ lower	Kranz

<sup>a</sup> P = perennial, A = annual. <sup>b</sup> refers to branches (no true stems or leaves). <sup>c</sup> n.a. = not applicable. <sup>d</sup> indicates no observations. <sup>e</sup> refers to stems (leaves are greatly reduced)

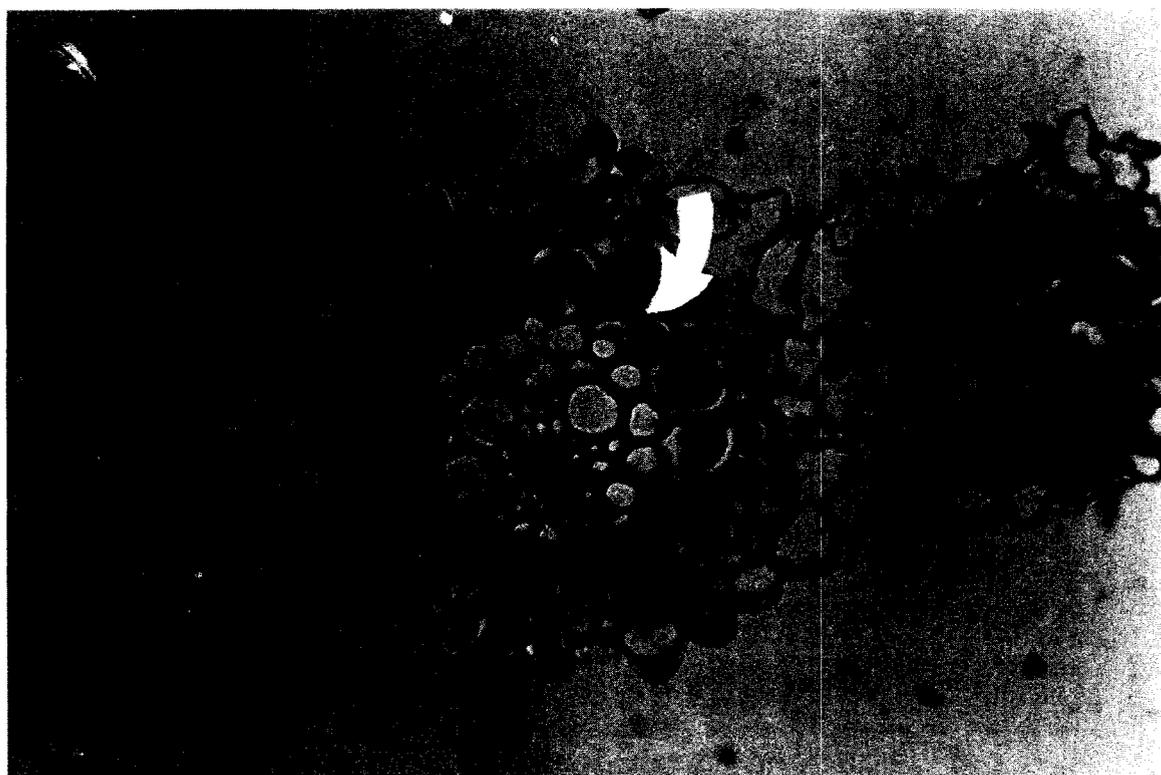


Figure 3. Cross-section of terrestrial leaf of *Orcuttia californica*. Arrow indicates bundle sheath cells.

*Marsilea* were intermediate between Kranz and  $C_3$  leaf anatomy; chloroplasts occurred in the bundle sheath cells as well as mesophyll cells (Figure 4). In the floating leaves of *Alopecurus*, chloroplasts were present in most bundle sheath cells but were far more concentrated in the mesophyll cells.

Chloroplast density was, in nearly all cases, higher in emergent leaves compared to submerged leaves. This is perhaps best quantified in terms of chlorophyll (Table 2). In addition, there were qualitative changes; the proportion of chlorophyll *a* increased in emergent leaves. Protein concentration, per unit fresh weight, also increased in the emergent foliage (Table 2). Presumably this was due to an increase in photosynthetic enzymes since there was little difference between submerged and emergent foliage in protein concentration per unit chlorophyll; e.g. for *Isoetes howellii* it was 24.4 and 24.7 mg protein  $mg^{-1}$  chl, and for *Crassula*, 44.7 and 48.0, respectively, for submerged and emergent leaves. Expressed on this basis, protein typically ranged from 20-40  $mg\ mg^{-1}$  chl.

Overnight acid changes in photosynthetic tissues are indicative of Crassulacean Acid Metabolism (CAM) and several vernal pool species have been previously reported to be CAM. These data, as well as data for most



Figure 4. Cross-section of floating leaf of *Marsilea vestita*. Arrow indicates bundle sheath cells.

other vernal pool macrophytes are summarized in the Appendix. In addition to species previously reported to be CAM (i.e. *Isoetes* and *Crassula*), the submerged leaves of *Orcuttia* also had a significant ( $P < 0.01$ , with two-tailed t-test) overnight increase in malic acid. In all cases, this nighttime increase in acid nearly disappeared in the emergent leaves. The terrestrial leaves of *Orcuttia* had substantial levels of malic acid and showed the surprising characteristic of daytime accumulation of this acid. It is doubtful that this characteristic is indicative of some novel photosynthetic pathway since the malic acid is secreted onto the surface of the leaf; leaves wiped gently with a

Table 2. Chlorophyll and protein levels in submerged and emergent foliage of Santa Rosa Plateau vernal pool species

	Chlorophyll ( $\text{mg g}^{-1}$ fresh weight)		Soluble protein ( $\text{mg g}^{-1}$ fresh weight)
	Total Chl $\bar{X} \pm \text{SD}$	% chl a $\bar{X} \pm \text{SD}$	$\bar{X} \pm \text{SD}$ (n)
<b>CHLOROPHYTA</b>			
<i>Chara contraria</i>			
Submerged	$0.15 \pm 0.04$	$72.7 \pm 1.0$	$20.9 \pm 23.8(6)$
<b>LYCOPHYTA</b>			
<i>Isoetes howellii</i>			
Submerged	$0.34 \pm 0.04$	$74.3 \pm 0.6$	$8.3 \pm 3.1 (6)$
Emergent	$1.16 \pm 0.10$	$76.3 \pm 0.8$	$28.7 \pm 3.4 (6)$
<b>PTEROPHYTA</b>			
<i>Marsilea vestita</i>			
Submerged	$0.68 \pm 0.04$	$76.0 \pm 5.0$	- (2)
Floating	$1.03 \pm 0.12$	$78.3 \pm 0.2$	- (2)
<b>ANTHOPHYTA -- DICOTYLEDONEAE</b>			
<i>Callitriche longipedunculata</i>			
Submerged	$0.44 \pm 0.06$	$72.6 \pm 0.7$	$12.4 \pm 1.4 (6)$
Floating	$0.92 \pm 0.28$	$76.3 \pm 1.2$	$18.4 \pm 3.8 (6)$
<i>Crassula aquatica</i>			
Submerged	$0.38 \pm 0.08$	$73.8 \pm 0.8$	$17.0 \pm 2.6 (8)$
Emergent	$0.51 \pm 0.06$	$75.2 \pm 0.5$	$24.5 \pm 4.8 (4)$
<i>Downingia bella</i>			
Submerged	$0.30 \pm 0.01$	$73.1 \pm 0.7$	$6.4 \pm 3.7 (4)$
Emergent	$0.47 \pm 0.08$	$76.0 \pm 0.8$	$15.0 \pm 3.3 (6)$
<i>Elatine californica</i>			
Submerged	$0.72 \pm 0.24$	$72.3 \pm 0.9$	$19.1 \pm 3.1 (6)$
Emergent	$0.39 \pm 0.08$	$76.2 \pm 0.1$	$34.8 \pm 6.3 (2)$
<i>Eryngium aristulatum</i>			
Submerged	$0.45 \pm 0.08$	$73.8 \pm 0.6$	$14.6 \pm 2.6 (6)$
Emergent	$1.05 \pm 0.09$	$76.9 \pm 0.3$	$36.6 \pm 9.4 (4)$

Table 2. Continued

	Chlorophyll ( $\text{mg g}^{-1}$ fresh weight)		Soluble protein ( $\text{mg g}^{-1}$ fresh weight)
	Total Chl X $\pm$ SD	% chl a X $\pm$ SD	X $\pm$ SD (n)
<i>Plagiobothrys undulatus</i>			
Submerged	0.27 $\pm$ 0.05	74.6 $\pm$ 0.5	13.0 $\pm$ 4.4 (5)
Emergent	0.79 $\pm$ 0.08	76.3 $\pm$ 0.1	37.7 $\pm$ 4.5 (4)
<i>Ranunculus aquatilis</i>			
Submerged	1.14 $\pm$ 0.07	72.0 $\pm$ 1.5	19.6 $\pm$ 3.8 (5)
Floating	1.06 $\pm$ 0.07	75.3 $\pm$ 0.4	24.4 $\pm$ 8.0 (4)
ANTHOPHYTA -- MONOCOTYLEDONEAE			
<i>Alopecurus howellii</i>			
Submerged	0.22 $\pm$ 0.01	73.6 $\pm$ 0.5	9.1 $\pm$ 1.5 (5)
Floating	2.07 $\pm$ 0.14	75.7 $\pm$ 0.3	26.5 $\pm$ 4.3 (5)
Terrestrial	1.00	85.9	45.0 $\pm$ 3.2 (2)
<i>Eleocharis acicularis</i>			
Submerged	1.36 $\pm$ 0.18	74.1 $\pm$ 0.4	28.0 $\pm$ 9.9 (6)
Emergent	2.13 $\pm$ 0.23	76.5 $\pm$ 0.6	50.8 $\pm$ 3.2 (4)
<i>Lilaea scilloides</i>			
Submerged	0.32 $\pm$ 0.16	72.5 $\pm$ 4.1	- (4)
Emergent	0.87 $\pm$ 0.02	75.6 $\pm$ 1.8	- (2)
<i>Orcuttia californica</i>			
Submerged	0.63 $\pm$ 0.13	74.7 $\pm$ 0.6	9.3 $\pm$ 3.3 (8)
Floating	1.96 $\pm$ 0.30	79.5 $\pm$ 0.4	17.4 $\pm$ 5.0 (7)
Terrestrial	1.92 $\pm$ 0.21	79.7 $\pm$ 0.7	35.8 $\pm$ 19.8(6)

damp cloth prior to assaying showed no daytime increase in malic acid. In other words, there was approximately a 30  $\mu\text{mol (g}^{-1}$  fresh weight) accumulation of malic acid on the leaf surface during the day, which disappeared overnight.

Activities of RuBP carboxylase and PEP carboxylase are shown in Table 3. For both enzymes, activity was generally greater at pH 8.0 than at pH 8.5. For most species the submerged leaves had RuBPCase activities between 100 and 200  $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ chl h}^{-1}$ , although *Lilaea* was significantly higher than this

Table 3. Maximum activities for RuBP carboxylase and PEP carboxylase in submerged and emergent foliage of Santa Rosa Plateau vernal pool species assayed at pH 8.0 and pH 8.5 (n = 3)

	Carboxylase activity ( $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ chl h}^{-1}$ )				
	RuBPcase		PEPcase		RuBPcase PEPcase
	pH 8.0 X $\pm$ SD	pH 8.5 X $\pm$ SD	pH 8.0 X $\pm$ SD	pH 8.5 X $\pm$ SD	
<b>CHLOROPHYTA</b>					
<i>Chara contraria</i>					
Submerged	46 $\pm$ 46	34 $\pm$ 41	10 $\pm$ 15	8 $\pm$ 14	4.6
<b>LYCOPHYTA</b>					
<i>Isoetes howellii</i>					
Submerged	65 $\pm$ 28	70 $\pm$ 5	6 $\pm$ 3	8 $\pm$ 2	8.8
Emergent	226 $\pm$ 85	181 $\pm$ 59	11 $\pm$ 6	8 $\pm$ 5	20.5
<b>ANTHOPHYTA -- DICOTYLEDONEAE</b>					
<i>Callitriche longipedunculata</i>					
Submerged	17 $\pm$ 3	11 $\pm$ 5	<1 $\pm$ 1	<1 $\pm$ 1	28.3
Floating	102 $\pm$ 49	88 $\pm$ 55	6 $\pm$ 5	8 $\pm$ 7	12.8
<i>Crassula aquatica</i>					
Submerged	134 $\pm$ 46	84 $\pm$ 28	56 $\pm$ 24	42 $\pm$ 11	2.4
Emergent	337 $\pm$ 45	223 $\pm$ 66	14 $\pm$ 2	15 $\pm$ 4	22.5
<i>Downingia bella</i>					
Submerged	214 $\pm$ 58	175 $\pm$ 46	19 $\pm$ 9	22 $\pm$ 9	9.8
Emergent	377 $\pm$ 50	257 $\pm$ 43	11 $\pm$ 7	14 $\pm$ 8	26.9
<i>Elatine californica</i>					
Submerged	95 $\pm$ 23	55 $\pm$ 27	5 $\pm$ 3	3 $\pm$ 4	19.0
Emergent	117 a	63	1	1	117.0
<i>Eryngium aristulatum</i>					
Submerged	247 $\pm$ 7	172 $\pm$ 30	16 $\pm$ 1	12 $\pm$ 3	15.1
Emergent	205 $\pm$ 134	197 $\pm$ 7	4 $\pm$ 2	5 $\pm$ 1	41.0
<i>Plagiobothrys undulatus</i>					
Submerged	147 $\pm$ 84	115 $\pm$ 82	16 $\pm$ 5	15 $\pm$ 6	9.2
Emergent	129 $\pm$ 5	83 $\pm$ 13	9 $\pm$ 1	7 $\pm$ 3	14.3
<i>Ranunculus aquatilis</i>					
Submerged	149 $\pm$ 83	63 $\pm$ 12	2 $\pm$ 1	2 $\pm$ 1	74.5
Floating	486 $\pm$ 75	294 $\pm$ 60	13 $\pm$ 9	10 $\pm$ 7	37.4

Table 3. Continued

	Carboxylase activity ( $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ chl h}^{-1}$ )				
	RuBPcase		PEPcase		RuBPcase
	pH 8.0 X $\pm$ SD	pH 8.5 X $\pm$ SD	pH 8.0 X $\pm$ SD	pH 8.5 X $\pm$ SD	PEPcase
<b>ANTHOPHYTA -- MONOCOTYLEDONEAE</b>					
<i>Alopecurus howellii</i>					
Submerged	118 $\pm$ 6	77 $\pm$ 22	36 $\pm$ 14	29 $\pm$ 18	3.3
Floating	329 $\pm$ 178	247 $\pm$ 71	12 $\pm$ 8	10 $\pm$ 5	27.4
Terrestrial	381 a	357	36	30	10.6
<i>Eleocharis acicularis</i>					
Submerged	173 $\pm$ 39	136 $\pm$ 24	15 $\pm$ 2	12 $\pm$ 2	11.5
Emergent	296 $\pm$ 86	170 $\pm$ 14	15 $\pm$ 2	13 $\pm$ 2	19.7
<i>Lilaea scilloides</i>					
Submerged	554 $\pm$ 209	449 $\pm$ 114	15 $\pm$ 2	13 $\pm$ 2	36.9
Emergent	1283 $\pm$ 250	970 $\pm$ 214	11 $\pm$ 2	8 $\pm$ 2	116.6
<i>Orcuttia californica</i>					
Submerged	117 $\pm$ 31	57 $\pm$ 14	19 $\pm$ 9	22 $\pm$ 12	5.3
Floating	167 $\pm$ 31	113 $\pm$ 16	13 $\pm$ 5	12 $\pm$ 5	12.8
Terrestrial	153 $\pm$ 19	121 $\pm$ 9	17 $\pm$ 9	18 $\pm$ 6	8.5

and *Callitriche* was significantly lower. PEPcase activity was highest in submerged leaves of *Crassula* followed by *Alopecurus*, *Orcuttia*, and *Downingia*. In nearly all instances there was a rather striking change in the ratio of RuBPcase:PEPcase when submerged leaves were replaced with emergent leaves. In general, this was due to a marked jump in the level of RuBPcase upon emergence.

The initial products of photosynthesis in these species are indicated by the distribution of radioactive C after a brief steady-state exposure to  $^{14}\text{C}$  (Table 4). Some species, e.g. submerged leaves of *Marsilea*, *Plagiobothrys*, *Ranunculus*, and *Lilaea*, showed a pattern characteristic of  $\text{C}_3$  species, where most of the  $^{14}\text{C}$  was detected in phosphoglycerate (PGA). Submerged as well as floating leaves of *Orcuttia californica* were clearly  $\text{C}_4$ -like in their labeling patterns. Many species exhibited significant levels of activity in both  $\text{C}_3$  and  $\text{C}_4$  products.

Table 4. Distribution of  $^{14}\text{C}$  label into the  $\text{C}_3$  product phosphoglycerate (PGA) and into the  $\text{C}_4$  organic acids, after 5 seconds steady state labeling in foliage of Santa Rosa Plateau vernal pool species

	Percentage in		(n)
	<u>Phosphoglycerate</u> X $\pm$ SD	<u>Organic acids</u> X $\pm$ SD	
<b>CHLOROPHYTA</b>			
<i>Chara contraria</i> Submerged	14 $\pm$ 1	42 $\pm$ 14	(3)
<b>LYCOPHYTA</b>			
<i>Isoetes howellii</i> Submerged	35 $\pm$ 10	46 $\pm$ 15	(8)
<b>PTEROPHYTA</b>			
<i>Marsilea vestita</i> Submerged	59 $\pm$ 6	34 $\pm$ 12	(2)
Floating	46 $\pm$ 2	37 $\pm$ 3	(2)
<b>ANTHOPHYTA -- DICOTYLEDONEAE</b>			
<i>Callitriche longipedunculata</i> Submerged	46 $\pm$ 5	22 $\pm$ 21	(5)
Floating	32 $\pm$ 10	39 $\pm$ 15	(3)
<i>Crassula aquatica</i> Submerged	20 $\pm$ 9	48 $\pm$ 9	(2)
<i>Downingia bella</i> Submerged	48 $\pm$ 12	40 $\pm$ 6	(5)
<i>Elatine californica</i> Submerged	48 $\pm$ 13	42 $\pm$ 9	(5)
<i>Eryngium aristulatum</i> Submerged	48 $\pm$ 13	43 $\pm$ 9	(3)
<i>Plagiobothrys undulatus</i> Submerged	56 $\pm$ 14	33 $\pm$ 9	(10)
<i>Ranunculus aquatilis</i> Submerged	54 $\pm$ 9	28 $\pm$ 7	(6)
Floating	49 $\pm$ 1	39 $\pm$ 4	(2)

Table 4. Continued

	Percentage in		(n)
	<u>Phosphoglycerate</u> X ± SD	<u>Organic acids</u> X ± SD	
ANTHOPHYTA -- MONOCOTYLEDONEAE			
<i>Alopecurus howellii</i>			
Submerged	36 ± 12	36 ± 12	(2)
Floating	23 ± 14	51 ± 7	(4)
<i>Eleocharis acicularis</i>			
Submerged	35 ± 8	55 ± 9	(4)
<i>Lilaea scilloides</i>			
Submerged	55 ± 5	31 ± 3	(2)
<i>Orcuttia californica</i>			
Submerged	9 ± 7	79 ± 12	(4)
Floating	22 ± 1	71 ± 2	(2)

### Discussion

Data presented here cover all of the 16 common species in the Santa Rosa Plateau vernal pools, although there are approximately twice this number of species recorded from these pools (Lathrop and Thorne, 1985). Here I will summarize the degree of structural and functional convergence in the flora and then examine how divergence in structure and function may contribute to coexistence.

Zedler (1987) has pointed out that the vernal pool flora is comprised of a combination of widespread taxa and localized endemics. The widespread taxa are in genera such as *Isoetes*, *Marsilea*, *Pilularia*, *Crassula*, *Callitriche*, *Elatine*, *Ranunculus*, *Eleocharis*, and *Lilaea* which, except for *Crassula*, are largely aquatic genera and thus the vernal pool species were likely derived from aquatic ancestors by evolving an ability to persist through the dry season. Both perennial and annual life histories were able to adapt to this amphibious habitat. Vernal pool endemics are mostly annual species in genera such as *Downingia*, *Plagiobothrys*, *Alopecurus*, and *Orcuttia* and are aligned with terrestrial taxa; and thus adaptation to the amphibious environment required the acquisition of aquatic adaptations. The presence of

certain structural and functional characteristics in both widespread and endemic species of diverse origins illustrates the strong selective pressure which has led to convergence in this flora.

The isoetid growth form (a rosette of cylindrical leaves) is widespread in the vernal pool flora, being found in species from aquatic lineages as well as in endemic vernal pool taxa (Table 1). This isoetid growth form may have been selected for, indirectly, because of the carbon limitations of the pool environment. Under stagnant flow conditions, the thickness of the leaf boundary layer would be a considerable limitation to photosynthesis. The size of the boundary layer is affected by leaf shape; Silvester and Sleigh (1985) reported that it would be thinner around an isoetid-type cylinder than a laminate plate. Additionally, any factor which increases the surface-area:cell-volume ratio should increase carbon gain. Increasing the volume of intercellular airspace will increase this ratio and as the amount of airspace increases the cylindrical isoetid leaf shape may confer added structural support for the leaf. One caveat, however, is that an increased volume of airspace may increase the mesophyll resistance to carbon dioxide diffusion (Nobel, 1983), which would decrease the rate of carbon assimilation. The net effect will depend upon the species. In *Lilaea*, for example, chloroplasts are concentrated around the periphery and thus the mesophyll resistance would be low, whereas in *Isoetes* the chloroplasts are distributed throughout the leaf, a factor which would increase the mesophyll resistance. This, however, assumes all carbon flow is across the epidermis. In *Isoetes*, a portion of the photosynthetic carbon is derived internally from decarboxylation of malic acid and possibly by diffusion from the sediment (e.g. Raven et al., 1988).

The presence of stomata in submerged foliage of most species requires some discussion since presumably they are non-functional (Sculthorpe, 1967). In taxa such as *Isoetes*, *Pilularia*, *Crassula*, *Elatine*, *Eryngium*, *Eleocharis*, and *Lilaea* the submerged leaves also function as aerial leaves at which time the stomata become functional. In the other taxa the submerged foliage is replaced with morphologically distinct leaves; however, in most cases stomata are present on both submerged and aerial foliage. In *Marsilea*, *Callitriche*, *Ranunculus*, *Alopecurus*, and *Orcuttia* the submerged foliage is replaced with floating leaves specialized for the aerial environment with stomata restricted to the upper leaf surface. The presence of stomata on submerged foliage is perhaps best viewed as a relictual trait that has been conserved but serves no function. Consistent with this interpretation is the fact that stomata are relatively infrequent on these tissues. *Orcuttia* is of interest because stomata are not present on submerged leaves. I suggest this is a derived condition since the submerged leaves of the more primitive member of the tribe Orcuttiaea, *Neostapfia colusana* (Davy) Davy, have stomata (Keeley, unpublished). The absence of stomata on submerged foliage of *Downingia* may also be a derived condition.

The presence of CAM photosynthesis in aquatic taxa as diverse as *Isoetes*, *Crassula* and *Orcuttia* is a reflection of its polyphyletic origin (Table 3). The loss of CAM from these species when growing in an aerial environment attests to its role in the aquatic milieu. By fixing carbon at night, these species have access to a plentiful carbon source and this may provide a competitive advantage over other species that must rely on the acquisition of carbon during the day when it is in short supply (Figure 1-A). Aquatic CAM photosynthesis differs from terrestrial CAM in that gas exchange is not controlled by stomatal conductance. In these aquatics, stomata, when present, are non-functional and gases diffuse across the epidermis. Carbon uptake is dictated by the gradient of CO<sub>2</sub> concentration between the inside and outside of the leaf. Consequently, early in the day carbon uptake continues as long as ambient carbon is available; this is also true of terrestrial CAM plants if stomatal resistance is overcome by removing the epidermis. However, uptake of ambient carbon in the light is largely restricted to a narrow window of time in the early morning (Figure 1-B). When ambient carbon becomes limiting, the overnight stores of malic acid are decarboxylated (Figure 1-C) and serve as an internal source of CO<sub>2</sub> for photosynthesis. The cuticle on submerged *Isoetes* and *Crassula* may serve to inhibit the outward diffusion of CO<sub>2</sub> released by decarboxylation, and chloroplasts distributed around the lacunae would facilitate assimilation of carbon dioxide that accumulated in the intercellular airspaces.

Previous estimates for *I. howellii* indicate that 30-50% of the carbon gain comes from dark fixation (Keeley and Busch, 1984). In light of this it is curious that the activity of PEPcase is not more similar to that of RuBPCase (Table 3). The explanation for this may be that high RuBPCase activity is necessary to take advantage of the narrow window of time in the early morning when ambient carbon is plentiful. Also, the fact that the water is supersaturated with oxygen during much of the day suggests that the carboxylation efficiency of this enzyme may be low much of the time. Low PEPcase activity may be tied to the much longer period of high carbon availability at night and the fact that carbon assimilation in the dark is limited by the volume of the vacuole for storing malic acid (Kluge and Ting, 1978).

Despite the lack of overnight acid accumulation in *Downingia*, *Plagiobothrys*, and *Eleocharis*, submerged leaves of these species are capable of significant levels of carbon fixation in the dark (Keeley and Sandquist, in press). The products of dark fixation are malate, citrate, and aspartate, and these apparently are further metabolized in the dark. At this point it is uncertain as to the role, if any, this pathway plays in the carbon gain of these species.

The biochemical pathway of carbon acquisition during the day by submerged foliage appears to vary with the species. Some such as *Marsilea*, *Plagiobothrys*, *Ranunculus*, and *Lilaea* clearly have active C<sub>3</sub> fixation (Table 4). Others such as *Isoetes*, *Crassula*, and *Eleocharis* have substantial fixation

in the light into  $C_4$  products. In aquatic and aerial leaves of *Orcuttia* the  $C_4$  pathway predominates (Table 4). However, in the submerged leaves this is not coupled with Kranz anatomy, whereas it is in the floating leaves (Figure 2) and terrestrial leaves (Figure 3). The lack of Kranz anatomy in the submerged leaves is best interpreted as an evolutionary reduction in this taxon similar to the hypothesized loss of stomata mentioned earlier. It is of particular note that the submerged leaves of the more primitive vernal pool member of the Orcuttieae, *Neostapfia colusana*, has Kranz anatomy in both submerged and emergent leaves (Keeley, unpublished).

Bowes and Salvucci (1984) found high  $C_4$  activity in the submerged aquatic *Hydrilla verticillata* Royal which also lacks Kranz anatomy. They provided evidence that both RuBPCase and PEPcase were active in the same cells. They hypothesized that PEPcase-mediated fixation in the cytosol was able to concentrate  $CO_2$  around the RuBPCase enzyme in the chloroplast, analogous to the carbon concentrating gradient between mesophyll and bundle sheath cells seen in terrestrial  $C_4$  plants. The subcellular distribution of carboxylases in vernal pool plants is as yet unexplored, but Bowes and Salvucci's model may apply. The apparent loss of Kranz by submerged *Orcuttia* leaves, and lack of Kranz in the aquatic  $C_4$  *Hydrilla verticillata*, suggests that the  $C_4$  pathway is more effective in aquatic foliage if the spatial separation of carboxylation events is intracellular as opposed to intercellular. The advantage to utilizing this pathway would lie in the fact that the PEPcase enzyme is not oxygen inhibited as is RuBPCase. In light of the relatively high oxygen levels observed in vernal pools during the day (Figure 1-A) this potentially could be of great advantage.

Once the pools dry and the plants exist as terrestrials, there are changes in carbon fixation pathways. As already noted, aquatic CAM species lose CAM in aerial foliage. This happens on a cell by cell basis as the tips of leaves will lose CAM even though the submerged bases retain CAM (Keeley and Busch, 1984). It appears that the loss of CAM is cued by changes in water potential since aerial leaves maintained at high humidity do not lose CAM (Keeley, 1988).

For many of the species the change in carboxylase activity (Table 3) suggests a change to relatively little reliance upon  $C_4$  fixation in the aerial or terrestrial foliage. Perhaps this reflects less carbon limitation to photosynthesis due to lower diffusive resistance in air. This would be supported by the fact that emergent leaves of *Isoetes* (Keeley and Busch, 1984) and *Downingia*, *Plagiobothrys*, and *Eleocharis* (Keeley and Sandquist, in press) do not show a mid-day depression in carbon uptake typical of submerged leaves (e.g. Figure 1-B). Consequently, in the aerial environment total carbon gain is greater than under water and this would account for the higher chlorophyll and protein levels upon emergence (Table 2). Particularly striking is the observation that in *Marsilea*, and to a lesser extent *Alopecuris*, the floating

leaf type is associated with an anatomy that could be interpreted as intermediate to Kranz (Figure 4) such as has been described for terrestrial C<sub>3</sub>-C<sub>4</sub> plants (Ku et al., 1983). This leaf type has been described previously for the aquatic macrophyte *Potamogeton praelongus* Wulf., but was not associated with C<sub>4</sub>-type carbon fixation (Hough and Wetzel, 1977). In *Alopecurus* the proportion of <sup>14</sup>C label that is fixed into organic acids is greater in the floating leaf type than in the submerged leaf. At this point the significance of these characteristics is unknown. The presence of the C<sub>4</sub> pathway in the terrestrial foliage of *Orcuttia californica* (and all members of the Orcuttieae tribe) accounts for the persistence of this grass long after the pools have dried. Commonly this taxon will reach anthesis in mid-summer, months after the water and all other species have disappeared.

In light of the degree of convergence in structure and function of vernal pool species, one may be tempted to question the degree to which structural and functional divergence contributes to coexistence within these aquatic communities. However, there are numerous ways these species differ in their mode of competing for carbon, including size, leaf morphology, photosynthetic pathway, bicarbonate uptake, and spatial segregation.

For example, *Isoetes howellii*, and *I. orcuttii* are indistinguishable photosynthetically, yet there is a marked difference in size that could affect competition for carbon. *Isoetes howellii* is a large robust species which may benefit from the fact that portions of its leaves become aerial relatively early in the spring. The more diminutive *I. orcuttii* on the other hand may gain an advantage by capturing carbon leaking from the sediment; the free-CO<sub>2</sub> concentration in the sediment is an order of magnitude greater than the highest level observed in the water column and it is not depleted during the day (Keeley and Sandquist, in press). The prostrate *Elatine* may be even more efficient at capturing sediment carbon. These tradeoffs in size may help account for the persistence of other robust (*Eryngium* and *Lilaea*) and diminutive (*Pilularia*, *Plagiobothrys*, *Alopecurus*, *Eleocharis*, and *Orcuttia*) isoetid species. Floating leaves are another means of escaping the limitations of the aquatic milieu and thus gaining an advantage in carbon assimilation (Figure 2). This mode is quite prevalent (Table 1) and appears to have evolved in vernal pool endemics as well as in the more widespread taxa.

The presence of CAM and daytime C<sub>4</sub> type fixation in some species and C<sub>3</sub> fixation in others may promote coexistence of this diverse flora. These different pathways of fixing carbon could reduce direct competition since conditions in the morning (high carbon dioxide and low oxygen) would favor C<sub>3</sub> photosynthesis. Conditions in the afternoon (low carbon dioxide and high oxygen) would favor C<sub>4</sub> photosynthesis. Nighttime conditions would obviously favor CAM fixation.

Ability to utilize bicarbonate is another means by which the carbon resource could be differentially divided up in a way which would promote coexistence. Free-CO<sub>2</sub> is readily available in the morning and absent in the afternoon, at which time bicarbonate is the main carbon source. A precise answer as to which vernal pool species are capable of utilizing bicarbonate is not available. The pattern of carbon fixation for *I. howellii*, shown in Figure 1-B, is consistent with the conclusion that this species utilizes bicarbonate poorly if at all. Studies with three other vernal pool species (*Downingia*, *Plagiobothrys*, and *Eleocharis*) showed patterns of carbon uptake nearly identical to *Isoetes*, suggesting that these species also lack bicarbonate uptake (Keeley and Sandquist, in press). Thus, for some of the vernal pool species, the expenditure of energy required for bicarbonate uptake may not be worthwhile since free-CO<sub>2</sub> is abundant at some time during the day. However, as more species compete for this early morning carbon we might expect the selection pressure for bicarbonate uptake to increase. Obviously, some species have this capacity since the pH of the pools has been observed to rise above pH 10 during the afternoon in the late spring (Keeley and Sandquist, in press).

Obtaining carbon dioxide from the sediment, via internal diffusion through the roots, is an important mode of carbon gain in isoetids from oligotrophic lakes (Raven et al., 1988). Annual vernal pool species have very small root systems and thus it is probably not important for them. The relative root contribution to carbon gain has been evaluated for *Isoetes howellii* (Keeley, unpublished). In these experiments leaves and roots of intact plants were exposed to different carbon levels (leaves 2 mM, roots 10 mM, pH 6.5) and the rate of leaf carbon assimilation determined by exposing either leaves or roots to <sup>14</sup>C. The rate generated by uptake from the roots was only half that from direct assimilation by leaves; *in situ* root contribution is likely to be even less since the root solution in these experiments was stirred. When the same experiment was done with the oligotrophic lake species *Isoetes bolanderi*, fixation of carbon diffusing from the roots was twice that measured for fixation directly from the solution around the leaves. Thus, the contribution of sediment carbon to photosynthesis by the vernal pool *I. howellii* is minor compared to the contribution by the water column and far less than for oligotrophic species of *Isoetes*. Part of the reasons for this must lie in the fact that the densely packed clay soils typical of vernal pools would generate a very high diffusive resistance to carbon dioxide movement.

Spatial segregation of vernal pool taxa could promote coexistence. Zedler (1987) for example has shown that species can be characterized by their position along a gradient of duration of inundation. In the Santa Rosa Plateau pools there is spatial separation of CAM species; *Crassula* dominates the outer edges of the pools and *Isoetes* species dominate the center (Keeley, 1987). Similar patterns are evident in the San Diego vernal pools studied by Zedler

(1987). Much remains to be discovered about the spatial division of resources in vernal pool communities.

Lastly, coexistence within this diverse community may be enhanced by the stochastic pattern of annual rainfall which produces very different levels of inundation. This would create a disequilibrium such that each year a different collection of species would be favored. For example, the aerial foliage of *Downingia* and *Plagiobothrys* have substantially higher photosynthetic rates than aerial foliage of *Isoetes howellii*, whereas the opposite is true of submerged foliage (Keeley and Sandquist, in press). In very wet years the latter species may proliferate, whereas in drier years *Downingia* and *Plagiobothrys* may gain. The observation that the population size of *Pilularia* (Thorne and Lathrop, 1970) and species of *Orcuttia* (Griggs and Jain, 1983) is extremely variable from year to year supports the idea that different years favor different species.

## Conclusions

There are numerous physiological solutions to surviving the heterogeneity of the vernal pool habitat. In addition, this environmental variability provides many ecological opportunities for the coexistence of a diverse flora.

## Acknowledgements

I acknowledge the assistance of Teresa Montygierd-Loyba and Daryl Peterson. This research was supported by NSF grant BSR-8705250 and a fellowship from the John Simon Guggenheim Foundation.

## Literature Cited

- Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. *Plant Physiol.* 24: 1-15.
- Bergmeyer, H. U. [ed.]. 1974. *Methods of enzymatic analysis*. Volume 1. Academic Press, New York.
- Bowes, G., and M. E. Salvucci. 1984. *Hydrilla*: Inducible C<sub>4</sub>-type photosynthesis without Kranz anatomy. In C. Sybesma [ed.], *Advances in photosynthesis research*, Volume III, 829-832. Dr. W. Junk, The Hague.
- Griggs, F. T., and S. K. Jain. 1983. Conservation of vernal pool plants in California, II. Population biology of a rare and unique grass genus *Orcuttia*. *Biol. Conserv.* 27: 171-193.

Gutmann, I., and A. W. Wahlefeld. 1974. L-malate: determination with malate dehydrogenase and NAD. In H. U. Bergmeyer [ed.], *Methods of enzymatic analysis*, Volume 4, 1585-1589. Academic Press, New York.

Hough, R. A., and R. G. Wetzel. 1977. Photosynthetic pathways of some aquatic plants. *Aq. Bot.* 3: 297-313.

Keeley, J. E. 1983. Crassulacean acid metabolism in the seasonally submerged aquatic *Isoetes howellii*. *Oecologia* 58: 57-62.

\_\_\_\_\_. 1987. The adaptive radiation of photosynthetic modes in the genus *Isoetes* (Isoetaceae). In R. M. M. Crawford [ed.], *Plant life in aquatic and amphibious habitats*, 113-128. Blackwell Scientific Publications, Oxford.

\_\_\_\_\_. 1988. Photosynthesis in quillworts, or why are some aquatic plants similar to cactus? *Plants Today* 1: 127-132.

\_\_\_\_\_, and G. Busch. 1984. Carbon assimilation characteristics of the aquatic CAM plant, *Isoetes howellii*. *Plant Physiol.* 76: 525-530.

\_\_\_\_\_, and B. A. Morton. 1982. Distribution of diurnal acid metabolism in submerged aquatic plants outside the genus *Isoetes*. *Photosynthetica* 16: 546-553.

\_\_\_\_\_, and D. R. Sandquist. In press. Diurnal photosynthesis cycle in CAM and non-CAM seasonal pool aquatic macrophytes. *Ecology*.

Kluge, M., and I. P. Ting. 1978. *Crassulacean acid metabolism*. Springer-Verlag, Berlin.

Kopecko, K. J. P., and E. W. Lathrop. 1975. Vegetation zonation in a vernal marsh on the Santa Rosa Plateau of Riverside County, California. *Aliso* 8: 281-288.

Ku, M. S. B., R. K. Monson, R. O. Littlejohn, H. Nakamoto, D. B. Fisher, and G. E. Edwards. 1983. Photosynthetic characteristics of C<sub>3</sub>-C<sub>4</sub> intermediate *Flaveria* species. I. Leaf anatomy, photosynthetic responses to O<sub>2</sub> and CO<sub>2</sub>, and activities of key enzymes in the C<sub>3</sub> and C<sub>3</sub> pathways. *Plant Physiol.* 71: 944-948.

Lathrop, E. W., and R. F. Thorne. 1985. A flora of the Santa Rosa Plateau. *Southern California Botanists, Special Publ.*, No. 1.

Lorimer, G. H., M. R. Badger, and T. J. Andrews. 1977. d-Ribulose-1,5 biphosphatase carboxylase-oxygenase. Improved methods for the activation and assay of catalytic activities. *Analyt. Biochem.* 78: 66-75.

Lucas, W. J., and J. A. Berry [eds.]. 1985. Inorganic carbon uptake by aquatic photosynthetic organisms. *Symposium proceedings published by the American Society of Plant Physiologists, Baltimore, MD.*

Nobel, P. S. 1983. *Biophysical plant physiology and ecology*. W.H. Freeman and Company, San Francisco.

Raven, J. A., L. L. Handley, J. J. Macfarlane, S. McInroy, L. McKenzie, J. H. Richards, and G. Samuelsson. 1988. The role of CO<sub>2</sub> uptake by roots and

CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. *New Phytol.* 108: 125-148.

**Sculthorpe, C. D.** 1967. The biology of aquatic vascular plants. E. Arnold Publishers, London.

**Sestak, Z., J. Katsky, and P. G. Jarvis [eds.].** 1971. Plant photosynthesis production. Manual of methods. Dr. W. Junk, The Hague.

**Silvester, N. R. and M. A. Sleight.** 1985. The forces on microorganisms at surfaces in flowing water. *Freshw. Biol.* 15: 433-448.

**Thorne, R. F., and E. W. Lathrop.** 1970. *Pilularia americana* on the Santa Rosa Plateau, Riverside County, California. *Aliso* 7: 149-155.

**Van, T. K., W. T. Haller, and G. Bowes.** 1976. Comparison of the photosynthetic characteristics of three submersed aquatic plants. *Plant Physiol.* 58: 761-768.

**Wetzel, R. G.** 1975. *Limnology.* W.B. Saunders Co., Philadelphia, PA.

**Zedler, P. H.** 1987. The ecology of southern California vernal pools: a community profile. U.S. Fish Wildl. Serv. Biol. Rep. 85(7.11).

---

Jon E. Keeley  
Department of Biology  
Occidental College  
Los Angeles, CA 90041

---

Keeley, J. E. 1990. Photosynthesis in vernal pool macrophytes: relation of structure and function. *In* D. H. Ikeda and R. A. Schlising [eds.], *Vernal pool plants—their habitat and biology*, 61-87. Studies from the Herbarium No. 8, California State University, Chico.

Appendix. Titratable acidity and malic acid concentration in submerged and emergent foliage of Santa Rosa Plateau vernal pool species

	(n)	Titratable acidity ( $\mu\text{mol g}^{-1}$ fresh weight)				Malic acid ( $\mu\text{mol g}^{-1}$ fresh weight)	
		to pH 6.4		to pH 7.0		AM	PM
		X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD
<b>CHLOROPHYTA</b>							
<i>Chara contraria</i>							
Submerged	(4)	0 $\pm$ 0	0 $\pm$ 0	-	-	5 $\pm$ 4	4 $\pm$ 4
<b>LYCOPHYTA</b>							
<i>Isoetes howellii</i>							
Submerged	(20)	161 $\pm$ 44	14 $\pm$ 11	-	-	97 $\pm$ 26	34 $\pm$ 10
Emergent	(10)	10 $\pm$ 7	5 $\pm$ 4	-	-	41 $\pm$ 8	36 $\pm$ 13
<i>Isoetes orcuttii</i>							
Submerged	(3)	155 $\pm$ 44	10 $\pm$ 6	-	-	98 $\pm$ 19	28 $\pm$ 10
Emergent	(3)	3 $\pm$ 7	5 $\pm$ 4	-	-	23 $\pm$ 11	26 $\pm$ 13
<b>PTEROPHYTA</b>							
<i>Marsilea vestita</i>							
Submerged	(6)	3 $\pm$ 2	2 $\pm$ 2	13 $\pm$ 3	9 $\pm$ 2	6 $\pm$ 6	11 $\pm$ 5
Floating	(3)	3 $\pm$ 3	4 $\pm$ 2	17 $\pm$ 3	15 $\pm$ 2	2 $\pm$ 3	2 $\pm$ 2
<i>Pilularia americana</i>							
Submerged	(6)	0 $\pm$ 0	0 $\pm$ 0	-	-	9 $\pm$ 4	4 $\pm$ 4
<b>ANTHOPHYTA – DICOTYLEDONEAE</b>							
<i>Callitriche longipedunculata</i>							
Submerged	(6)	1 $\pm$ 1	0 $\pm$ 0	14 $\pm$ 2	9 $\pm$ 1	9 $\pm$ 6	11 $\pm$ 7
Floating	(3)	2 $\pm$ 1	1 $\pm$ 1	7 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 4	7 $\pm$ 5
<i>Crassula aquatica</i>							
Submerged	(3)	129 $\pm$ 29	7 $\pm$ 3	-	-	67 $\pm$ 21	14 $\pm$ 3
Emergent	(2)	30 $\pm$ 0	2 $\pm$ 1	-	-	35 $\pm$ 5	26 $\pm$ 1
<i>Downingia bella</i>							
Submerged	(12)	5 $\pm$ 8	1 $\pm$ 1	9 $\pm$ 5	11 $\pm$ 1	17 $\pm$ 6	11 $\pm$ 3
Emergent	(6)	1 $\pm$ 1	1 $\pm$ 1	46 $\pm$ 6	41 $\pm$ 4	29 $\pm$ 8	33 $\pm$ 7
<i>Elatine californica</i>							
Submerged	(6)	1 $\pm$ 1	0 $\pm$ 0	-	-	10 $\pm$ 3	5 $\pm$ 1

## Appendix. Continued

		Titratable acidity ( $\mu\text{mol g}^{-1}$ fresh weight)				Malic acid ( $\mu\text{mol g}^{-1}$ fresh weight)	
		to pH 6.4		to pH 7.0		AM	PM
(n)		X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD	X $\pm$ SD
<i>Eryngium aristulatum</i>							
Submerged	(6)	1 $\pm$ 1	3 $\pm$ 4	15 $\pm$ 5	43 $\pm$ 31	6 $\pm$ 4	6 $\pm$ 6
<i>Plagiobothrys undulatus</i>							
Submerged	(12)	0 $\pm$ 0	0 $\pm$ 0	21 $\pm$ 7	19 $\pm$ 6	6 $\pm$ 3	9 $\pm$ 2
Emergent	(6)	0 $\pm$ 0	0 $\pm$ 0	24 $\pm$ 2	25 $\pm$ 2	14 $\pm$ 5	7 $\pm$ 4
<i>Ranunculus aquatilis</i>							
Submerged	(6)	2 $\pm$ 2	0 $\pm$ 0	13 $\pm$ 5	8 $\pm$ 1	7 $\pm$ 4	6 $\pm$ 2
Floating	(6)	1 $\pm$ 1	1 $\pm$ 1	-	-	7 $\pm$ 4	5 $\pm$ 1
ANTHOPHYTA – MONOCOTYLEDONEAE							
<i>Alopecurus howellii</i>							
Submerged	(6)	2 $\pm$ 2	1 $\pm$ 1	12 $\pm$ 2	11 $\pm$ 1	4 $\pm$ 2	6 $\pm$ 3
Floating	(3)	4 $\pm$ 1	5 $\pm$ 1	18 $\pm$ 24	24 $\pm$ 2	4 $\pm$ 4	3 $\pm$ 4
<i>Eleocharis acicularis</i>							
Submerged	(16)	12 $\pm$ 11	3 $\pm$ 2	37 $\pm$ 24	24 $\pm$ 19	13 $\pm$ 9	7 $\pm$ 4
Emergent	(6)	2 $\pm$ 3	1 $\pm$ 1	43 $\pm$ 16	28 $\pm$ 6	19 $\pm$ 10	11 $\pm$ 7
<i>Lilaea scilloides</i>							
Submerged	(8)	4 $\pm$ 2	1 $\pm$ 1	-	-	18 $\pm$ 10	21 $\pm$ 18
<i>Orcuttia californica</i>							
Submerged	(6)	7 $\pm$ 3	0 $\pm$ 0	12 $\pm$ 1	1 $\pm$ 1	16 $\pm$ 3	4 $\pm$ 5
Floating	(3)	0 $\pm$ 0	0 $\pm$ 0	11 $\pm$ 4	5 $\pm$ 3	24 $\pm$ 3	16 $\pm$ 5
Terrestrial	(6)	50 $\pm$ 12	104 $\pm$ 19	67 $\pm$ 12	28 $\pm$ 15	73 $\pm$ 8	106 $\pm$ 17

