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Photosynthetic pathway diversity in a seasonal pool community

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Summary

1. Photosynthetic pathway diversity was evaluated for the dominant species in a seasonally aquatic community in the south-western USA using ^{14}C pulse-chase techniques.
2. Under submerged conditions, only about half of the species were clearly C_3, three of the 15 dominants were CAM, one species was C_4 and three were potentially assimilating carbon with both C_3 and C_4 fixation.
3. During the brief terrestrial stage in the life history of these amphibious plants, both the CAM and the $C_3 + C_4$ species switched to C_3, whereas the C_4 species did not switch.
4. Numerous variations were apparent; for example, the C_4 species, while exhibiting a biochemical pathway indistinguishable from terrestrial C_4 plants, lacked Kranz anatomy in the aquatic foliage. Also, despite well-developed CAM in several species, others exhibited low-level diel changes in acidity, apparently not indicative of CAM.
5. Species with C_4 or CAM CO_2 concentrating mechanisms lacked the capacity for bicarbonate uptake, an alternative CO_2 concentrating mechanism found in certain C_3 species in this community.
6. Rubisco/PEPC in aquatic foliage was higher in C_3 species than in C_4, CAM or putative $C_3 + C_4$ species. In the terrestrial phase, as expected, the switch from CAM or $C_3 + C_4$ to strictly C_3 assimilation was associated with a substantial increase in Rubisco/PEPC. Quite unexpected, however, was the substantial increase in this ratio in terrestrial C_3 foliage. It is hypothesized that submerged C_3 plants utilize PEPC for recycling of respiratory CO_2 and/or C_4 phototrophism under field conditions of limited CO_2 and O_2 saturation, and this is lost in the terrestrial foliage.

Key-words: C_3, C_4, CAM, community assembly, diversity, photosynthetic pathways

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Introduction

Aquatic plants have attracted attention in recent years because of the unexpected presence of CAM (e.g. Keeley 1981; Richardson *et al*. 1984) and C_4 (Bowes & Salvucci 1984; Spencer, Wetzel & Teeri 1996; Reiskind *et al*. 1997; Keeley 1998a) photosynthetic pathways. Indeed, it now appears that the aquatic environment is home to as great a diversity of photosynthetic pathways as terrestrial environments. While the environmental factors responsible for selection of these pathways in terrestrial species are reasonably well understood (Teeri & Stowe 1976; Kluge & Ting 1978; Ehleringer & Monson 1993; Paruelo & Lauenroth 1996), far less is known about the conditions determining the distribution of aquatic C_4 and CAM plants.

Despite numerous studies of terrestrial landscape changes in abundance and diversity of C_4 and CAM plants across various environmental gradients, relatively little attention has been given to the role of photosynthetic pathway diversity in controlling community composition. Factors determining the success of C_4 and CAM are sufficiently different so that sites where one dominates the other is often poorly represented. While terrestrial communities comprising a combination C_3, C_4 and CAM species are known, the role of photosynthetic diversity in community coexistence has not been considered. In aquatic systems even less is known about community patterns of photosynthetic diversity.

An aquatic community of particular interest is restricted to seasonal pools in California, USA, a habitat known as ‘vernal pools’. These are shallow rain-fed pools with large diel changes in physical and chemical characteristics (Fig. 1), which pose potential stresses for aquatic plant photosynthesis. On a typical spring day, water temperatures rise rapidly within the first few hours of the morning coupled with rapid photosynthetic production of O_2 and depletion of CO_2. Being rain-fed pools, they are poorly buffered and,

concomitant with the depletion of CO_2, there is a marked increase in pH. For many of the dominants, access to bicarbonate is limited (Keeley & Sandquist 1992), although water may increase to pH 9–10, suggesting the presence of bicarbonate-users in the community. In this mediterranean climate, standing water disappears as spring rains subside, leaving these amphibious plants exposed to an ephemeral terrestrial stage, which lasts until dormancy is imposed by the desiccating conditions of summer drought.

This study investigates, for a single vernal pool community, the photosynthetic pathways represented in the dominant species during the aquatic and terrestrial stages of their life history. These are evaluated relative to other structural and functional characteristics and the role of carbon assimilation in community coexistence is considered.

Vernal pool community and study materials

The Mesa de Colorado pool ('C1' of Lathrop & Thorne 1983) on the Santa Rosa Plateau in Riverside County, CA, (675 m) has been the focus of many floristic and ecological studies (e.g. Kopecko & Lathrop 1975; Collie & Lathrop 1976; Keeley 1983; Keeley & Busch 1984; Rosario & Lathrop 1984; Stagg & Lathrop 1984; Martin & Lathrop 1986). Substrate and seeds were returned to an outdoor growing area in Los Angeles and used to create artificial pools, maintained for over a decade on a normal seasonal cycle. Pools were filled (by rain or deionized water) in January and allowed to dry in mid-April, and were the source of foliage for biochemical studies. Diel cycles similar to those observed in the field (Fig. 1) were also evident in these artificial pools. Approximately two-thirds of the 30 species recorded for the site (Lathrop & Thorne 1983) established in the artificial pools, but only 15 were present in sufficient quantities to use in our studies. All studies presented here were on plants grown in these artificial pools.

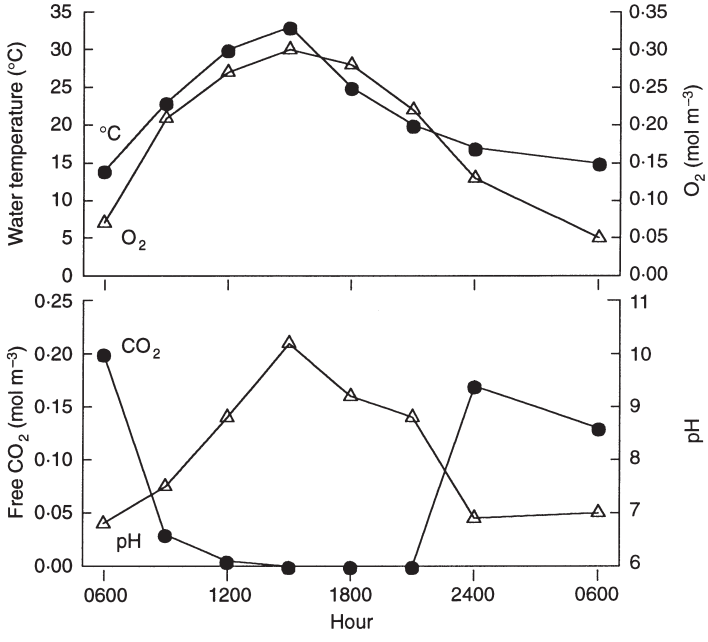

Fig. 1. Diel changes in temperature and O_2 (upper panel) and pH and CO_2 (lower panel) for a vernal pool in southern California in mid-spring (data from Keeley & Busch 1984).

Materials and methods

Each species was characterized morphologically and leaf cross-sections of all species were examined anatomically by light microscopy on thin-sections using anatomical techniques as described in Keeley (1998a).

Diel changes in acidity were determined by grinding *c.* 0·25 g of foliage in 10·0 ml distilled water and an aliquot deproteinized with 1 kmol m^{-3} HCl prior to malic acid determination (Gutmann & Wahlefeld 1974) and the remainder titrated with 10 mol m^{-3} NaOH to pH 6·4 (second pKa for malic acid).

^{14}C pulse-chase studies were carried out on leaves collected at midday (12.00 h ± 2 h) at three or more times throughout the season. Leaves were pulsed by injecting 5·5 Mbq of ^{14}C-sodium bicarbonate (2 Gbq mmol^{-1}) in a 25 ml serum vial with 25 mol m^{-3} MES pH 6·0 buffer (aquatic) or without buffer with leaves suspended above 1 ml of 100 mol m^{-3} HCl. Leaves were pulsed in the light (*c.* 500 μmol m^{-2} s^{-1}) for 5 s (aquatic) or 2 s (terrestrial) or in the dark for 3 h, followed either by immediate immersion in liquid nitrogen or by several rinses in distilled water and returned to ^{14}C-free buffer or ^{14}C-free air and 'chased' for various times. Leaves were extracted in ethanol-acetic acid, centrifuged at 116·4 km s^{-1} for 10 min, followed by two washes of the pellet in ethanol and water. After photo bleaching the supernatants and pellets were dried and re-suspended in 1·0 ml of distilled water then re-centrifuged. In one corner of a 20 cm × 20 cm thin layer cellulose plate 50 μl of supernatant was spotted for electrophoretic separation at 15 °C and 900 V (70–75 mA) in pyridine:glacial acetic acid:water (2:9:189) solvent, followed by chromatographic separation in the second dimension with sec-butanol:formic acid:water (6:1:2) solvent (Schürman 1969). ^{14}C-labelled compounds were located with autoradiographs (Kodak X-OMAT), scraped from the plate and radioactivity was counted (also for original pellets) in Bray's using an LKB 1214 Rackbeta scintillation counter. Compounds were identified by comparison of Rf values with standards run under identical conditions.

RuBP carboxylase (Rubisco) and PEP carboxylase (PEPC) were assayed from the same extract of leaves ground on ice in 50 mol m^{-3} Tris-HCl, 10 mol m^{-3} $MgCl_2$, 0·1 mol m^{-3} EDTA, 5 mol m^{-3} isoascorbate, 1% m/v PVP-400 at pH 8·0 and centrifuged for 5 min at 116·4 km s^{-1} (4 °C) and assayed immediately (at 25 °C) without further purification. Both enzymes were assayed using $^{14}CO_2$ as described by Lorimer, Badger & Andrews (1977) and Van, Haller & Bowes (1976)

followed by chlorophyll (Sesták, Katsky & Jarvis 1971) and protein (Lowry *et al*. 1951) determination on aliquots of the crude extract. Other important C_4 enzymes were assayed spectrophotometrically. NAD- and NADP-malic enzyme were assayed according to the procedure of Hatch & Kagawa (1974) as modified by Ueno, Takeda & Murata (1986). The ATP-dependent decarboxylating reaction of PEP carboxykinase was measured according to Hatch (1973) with modifications by Ueno *et al.* (1986). Pyruvate, Pi dikinase activity was determined after Ashton *et al*. (1990). Carbonic anhydrase was assayed as described by Wilbur & Anderson (1948).

Relative bicarbonate uptake capacity was evaluated by examining the final pH in pH-drift experiments across the bicarbonate range from 0·1 to 1·0 mol m^{-3} and *c.* 500 μmol m^{-2} s^{-1} PAR (Maberly & Spence 1983).

Results

From visual estimates in the field, the 15 species studied here (Table 1) represented the bulk (> 95%) of the pool biomass (not including *Eleocharis macrostachys*, a tall emergent perennial in deeper parts of the pool). All species germinated or sprouted underwater and maintained submerged aquatic foliage for several months.

STRUCTURAL VARIATION

There was a remarkable degree of convergence in the 'isoetid' growth form, with over half of the 15 dominant species producing a rosette of elongate (semi)terete leaves (Table 2). Aquatic foliage of all species had extensive aerenchymous air space and all species produced stomata, but were not observed to open underwater. Five species produced specialized laminate floating leaf blades on elongated petioles with stomata restricted to the aerial side of the leaf (Table 2). All 15 dominants (Table 1) were rhizophytes, but one free-floating colonial cyanobacterium, *Gloeotrichia* sp., was abundant in some years. None of the species produced aquatic foliage with Kranz anatomy but the floating leaves and the terrestrial leaves of *Orcuttia californica* had well developed Kranz anatomy. Floating leaves of *Marsilea vestita* had a dense chlorenchymous ring of bundle sheath cells, but chlorenchymous cells were throughout the mesophyll tissue as well.

As the water level dropped and foliage became aerial, five species underwent a marked metamorphosis, replacing isoetid aquatic foliage with caulescent laminate-leaf terrestrial foliage (Table 2). Typically the terrestrial stage lasted a few weeks to a month following the disappearance of standing water. Exceptions were *Chara contraria*, which desiccated within days and *O. californica*, which persisted for 2 months and delayed flowering until summer, by which time the clay substrate was dried and cracked. For the Anthophyta, with the exceptions of *Elatine californica* and *Lilaeae scilloides*, flowering was delayed until exposure to the aerial environment, whereas the Pterophyta, Lycophyta and Chlorophyta produced reproductive structures underwater. Eventually all plants survived summer and autumn only as dormant seeds or spores, and, in the species of Lycophyta, Pterophyta and one monocot (*Eleocharis acicularis*) and one dicot (*Eryngium aristulatum*), by perenniating vegetative structures.

PHYSIOLOGICAL VARIATION

In nearly all species, floating and/or terrestrial foliage had higher chlorophyll and protein levels than aquatic foliage (Table 1).

Pulse-chase radioisotope experiments indicated operation of the C_3 pathway in the majority of species, as illustrated by *Plagiobothrys undulatus* and *Downingia bella*, in both aquatic and terrestrial foliage (Fig. 2). Preponderance of initial ^{14}C-label was in PGA and additional phosphorylated compounds, and these turned over into other soluble and insoluble compounds. Species not showing similar C_3 patterns of carbon assimilation are as follows.

CAM photosynthesis was evident in three species, *Isoëtes howellii*, *Isoëtes orcuttii* and *Crassula aquatica*. All had substantial diel changes in H^+ and malate (Table 1). In addition, these three species exhibited substantial ^{14}C uptake and fixation in the dark; gross carbon uptake in the dark was 20–50% of total combined light + dark uptake, whereas in all other species, dark uptake was only 1–5%. In the three CAM species a 3 h ^{14}C pulse, followed by a 9 h chase in the dark, demonstrated that 75–90% of the dark-fixed carbon remained in malate throughout the dark period, and the remainder ended up in citrate. Carbon assimilated in the dark was transferred out of malate and citrate in the light and accumulated in both soluble and insoluble fractions (Fig. 3a). In the light, under steady-state conditions of high CO_2 availability, these CAM plants were capable of direct assimilation through the C_3 pathway, both for aquatic foliage (Fig. 3b) and terrestrial foliage (Fig. 3c). The terrestrial foliage lacked diel acid changes (Table 1), indicating a switch to strictly C_3 fixation.

Two other species, *E. acicularis* and *O. californica*, exhibited low-level overnight acid accumulation (Table 1). In both these species low-level dark fixation resulted in initial labelling of malate but typically less than 25% remained in malate by the end of the dark period. Also, in the latter species there was substantial daytime malic acid accumulation in the foliage. *Downingia bella* exhibited the unusual pattern of overnight malate accumulation without any diel change in H^+ (Table 1).

One species, *O. californica*, had ^{14}C fixation patterns indicative of C_4 photosynthesis. In both

Table 1. Chlorophyll, protein and diel acid levels in submerged aquatic, floating and terrestrial foliage of species dominating the Santa Rosa Plateau vernal pool community. All are annuals except *Isoetes*, *Marsilea*, *Eryngium* and *Eleocharis*: n = 3–12

	Chlorophyll (g kg^{-1} FM) $\bar{X}$ + SD	Protein (g kg^{-1} FM) $\bar{X}$ + SD	Acidity (mol H^{+} m^{-3}) AM $\bar{X}$ + SD	Acidity (mol H^{+} m^{-3}) PM $\bar{X}$ + SD	Malic acid (mol m^{-3}) AM $\bar{X}$ + SD	Malic acid (mol m^{-3}) PM $\bar{X}$ + SD
Chlorophyta						
Characeae						
Chara contraria						
Aquatic	0·15 + 0·02	20·9 + 9·7	0 + 0	0 + 0	5 + 4	4 + 4
Lycophyta						
Isoetaceae						
Isoetes howellii						
Aquatic	0·36 + 0·03	2·8 + 0·5	161 + 44	14 + 11	97 + 26	34 + 10
Terrestrial	1·16 + 0·05	28·8 + 0·2	10 + 7	5 + 4	41 + 8	36 + 13
Isoetes orcuttii						
Aquatic	0·44 + 0·04	4·8 + 0·4	155 + 44	10 + 6	98 + 19	28 + 10
Terrestrial	1·21 + 0·05	31·2 + 0·1	15 + 3	8 + 5	21 + 5	16 + 3
Pterophyta						
Marsileaceae						
Marsilea vestita						
Aquatic	0·68 + 0·03	11·8 + 1·1	3 + 2	2 + 2	6 + 6	11 + 5
Floating	1·07 + 0·11	46·9 + 9·3	3 + 3	4 + 2	2 + 3	2 + 2
Anthophyta–Monocotyledonae						
Cyperaceae						
Eleocharis acicularis						
Aquatic	1·52 + 0·17	28·0 + 4·0	12 + 11	3 + 2	13 + 9	7 + 4
Terrestrial	2·26 + 0·27	50·8 + 1·6	2 + 3	1 + 1	19 + 10	11 + 7
Lilaeaceae						
Lilaeae scilloides						
Aquatic	0·32 + 0·78	3·1 + 1·1	4 + 2	1 + 1	18 + 10	21 + 18
Terrestrial	1·12 + 0·16	8·3 + 3·1	7 + 3	8 + 3	11 + 5	14 + 17
Poaceae						
Alopecuris howellii						
Aquatic	0·26 + 0·07	9·1 + 4·6	2 + 2	1 + 1	4 + 2	6 + 3
Floating	2·07 + 0·06	26·5 + 1·9	1 + 1	5 + 1	4 + 2	3 + 4
Terrestrial	2·17 + 0·41	45·2 + 3·2	2 + 2	4 + 1	7 + 2	3 + 1
Orcuttia californica						
Aquatic	0·63 + 0·05	9·30 + 1·2	12 + 2	1 + 1	16 + 2	4 + 1
Floating	1·96 + 0·11	17·41 + 1·9	11 + 2	5 + 1	24 + 5	16 + 3
Terrestrial	1·92 + 0·09	35·80 + 8·1	67 + 11	128 + 7	73 + 7	106 + 8
Anthophyta–Dicotyledoneae						
Apiaceae						
Eryngium aristulatum						
Aquatic	0·35 + 0·05	2·9 + 0·1	1 + 1	3 + 4	6 + 4	6 + 6
Terrestrial	1·14 + 0·15	4·5 + 1·1	3 + 3	5 + 2	7 + 4	11 + 2
Boraginaceae						
Plagiobothryus undulatus						
Aquatic	0·59 + 0·05	13·0 + 4·4	0 + 0	0 + 0	6 + 3	9 + 2
Terrestrial	0·45 + 0·02	37·7 + 4·5	0 + 0	0 + 0	14 + 5	7 + 4
Callitrichaceae						
Callitriche longipedunculata						
Aquatic	0·19 + 0·05	5·1 + 0·2	1 + 1	0 + 0	9 + 6	11 + 7
Floating	0·44 + 0·14	10·1 + 1·3	2 + 1	1 + 1	4 + 4	7 + 5
Campanulaceae						
Downingia bella						
Aquatic	0·28 + 0·19	1·4 + 0·7	5 + 8	1 + 1	17 + 6	11 + 3
Terrestrial	0·44 + 0·11	3·9 + 0·8	1 + 1	1 + 1	29 + 8	33 + 7
Crassulaceae						
Crassula aquatica						
Aquatic	0·45 + 0·03	2·2 + 0·5	129 + 29	7 + 3	67 + 21	14 + 3
Terrestrial	0·42 + 0·01	21·8 + 6·3	30 + 0	2 + 1	35 + 5	26 + 1

Table 1. *Continued.*

	Chlorophyll (g kg^{-1} FM) $\bar{X}$ + SD	Protein (g kg^{-1} FM) $\bar{X}$ + SD	Acidity (mol H^+ m^{-3}) AM $\bar{X}$ + SD	Acidity (mol H^+ m^{-3}) PM $\bar{X}$ + SD	Malic acid (mol m^{-3}) AM $\bar{X}$ + SD	Malic acid (mol m^{-3}) PM $\bar{X}$ + SD
Elatinaceae						
Elatine californica						
Aquatic	0·75 + 0·07	11·4 + 3·6	1 + 1	0 + 0	10 + 3	5 + 1
Terrestrial	0·70 + 0·03	9·2 + 0·5	2 + 1	1 + 1	12 + 2	9 + 2
Ranunculaceae						
Ranunculus aquatilis						
Aquatic	1·14 + 0·07	19·6 + 3·8	2 + 2	0 + 0	7 + 4	6 + 2
Floating	1·06 + 0·07	24·4 + 8·0	1 + 1	1 + 1	7 + 4	5 + 1

aquatic and terrestrial leaves, 95–100% of the initial carbon fixation products were C_4 products, primarily, malic acid (Fig. 4). The rapid turnover of organic acids, coupled with an increase in labelled phosphorylated compounds, indicated transfer of carbon from the C_4 to the C_3 cycle, pointing to the presence of a functional C_4 pathway in both aquatic and terrestrial leaves, despite the fact that only the terrestrial foliage had Kranz anatomy.

A number of species had substantial initial carbon assimilation into both C_3 and C_4 products, suggesting carbon assimilation through both pathways, although the percentage of ^{14}C label in organic acids often varied between experiments, e.g. *C. contraria* 42–95%, *M. vestita* 37–57%, *E. acicularis* 20–55%. Pulse-chase studies were inconclusive; labelled phosphorylated compounds decreased with time but changes in labelled organic acids were variable between species and experiments. Two other less common pool species could be added to the list of potential C_3 + C_4 intermediate species; based on initial fixation studies in the light, substantial organic acid fixation was observed in *Pilularia americana* (Pterophyta), and in the blue–green colonial alga *Gloeotrichia* sp. However, these two species were sufficiently uncommon in our pools to preclude their inclusion in further studies. The apparent C_3 + C_4 intermediate pattern was restricted to aquatic foliage (floating leaves in the case of *M. vestita*) and switched to strictly C_3 in the terrestrial stage.

Based on the ^{14}C labelling studies, a summary of the distribution of photosynthetic pathways in this community is shown in Table 2.

Carboxylating enzyme activities in aquatic foliage showed the Rubisco/PEPC ratio varied between species by more than an order of magnitude (Table 3). Species with substantial C_4 acid fixation, including CAM, C_4

Table 2. Distribution of growth forms and photosynthetic pathways in the Santa Rosa Plateau vernal pool community

Assimilation pathway	Species	Bicarbonate uptake	Floating leaves	Submerged isoetid	Terrestrial metamorphosis	Origin*
C_3	*Alopecuris howellii*	No	+	+/–	+/–	T
	Lilaeae scilloides	No	–	+	–	A
	Callitriche longipedunculata	No	+	–	–	A
	Downingia bella	No	–	+	+	T
	Elatine californica	No	–	–	–	A
	Eryngium aristulatum	No	–	+	+	T?
	Plagiobothryus undulatus	No	–	+	+	T
	Ranunculus aquatilis	Yes	+	–	–	A
CAM	*Isoetes howellii*	No	–	+	–	A
	Isoetes orcuttii	No	–	+	–	A
	Crassula aquatica	No	–	–	–	A
C_4	*Orcuttia californica*	No	+	+	+	T
C_3 + C_4	*Chara contraria*	Yes	–	–	–	A
	Marsilea vestita	No	+	–	–	A
	Eleocharis acicularis	No	–	+	–	A
	Gloeotrichia sp.	Yes	–	–	–	A
	Pilularia americana	No	–	+	–	A

*Presumed origin: T, localized endemics with near relatives terrestrial; A, cosmopolitan aquatic species and/or genera. See text.

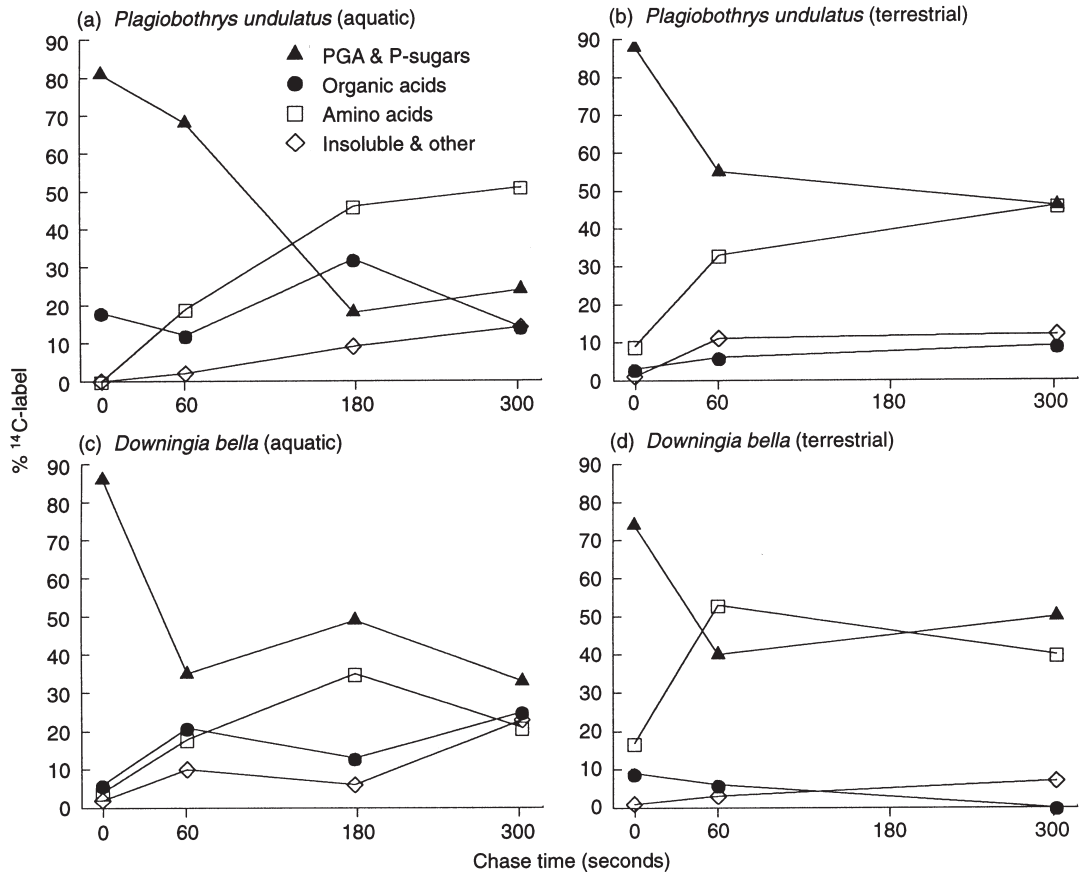

Fig. 2. Distribution of ^{14}C label in pulse-chase experiments in the light for *Plagiobothrys undulatus* (a) aquatic and (b) terrestrial foliage, and *Dowingia bella* (c) aquatic and (d) terrestrial foliage. Each point represents the mean of three or more samples.

and putative $C_3 + C_4$ intermediates, had Rubisco/PEPC ratios from 2 to 7 and C_3 plants ranged from 7 to 64. Aquatic CAM and aquatic C_4 species had ratios substantially lower than a typical C_3 plant, such as Spinach, *Spinacia oleracea*, but not as low as a terrestrial C_4 (Corn, *Zea mays)* or CAM (Pineapple, *Annanas comosus*) plant (Table 3).

When the pools dried and these amphibious species entered their terrestrial stage, the Rubisco/PEPC ratios, with one exception, increased to within the range of a typical terrestrial C_3 plant (Table 3). This parallels similar changes observed above with respect to photosynthetic pathway; CAM and $C_3 + C_4$ intermediates switched to strictly C_3 assimilation in the terrestrial stage. Terrestrial *O. californica*, on the other hand, retained the C_4 pathway and the Rubisco/PEPC ratio decreased.

C_4 and CAM species had substantial NADP malic enzyme activity, which was likely the prime decarboxylating enzyme because PEP carboxykinase was not detected (Table 3). Pyruvate, Pi-dikinase was detectable in the aquatic foliage of species with CAM,

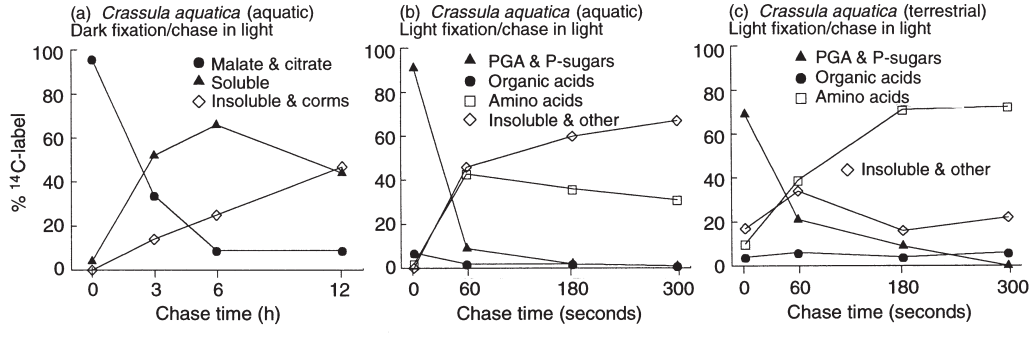

Fig. 3. Distribution of ^{14}C label in pulse-chase experiments for *Crassula aquatica* (a) 3 h dark pulse/12 h chase in the light for aquatic foliage, (b) pulse-chase in the light for aquatic and (c) terrestrial foliage. Each point represents the mean of three or more samples.

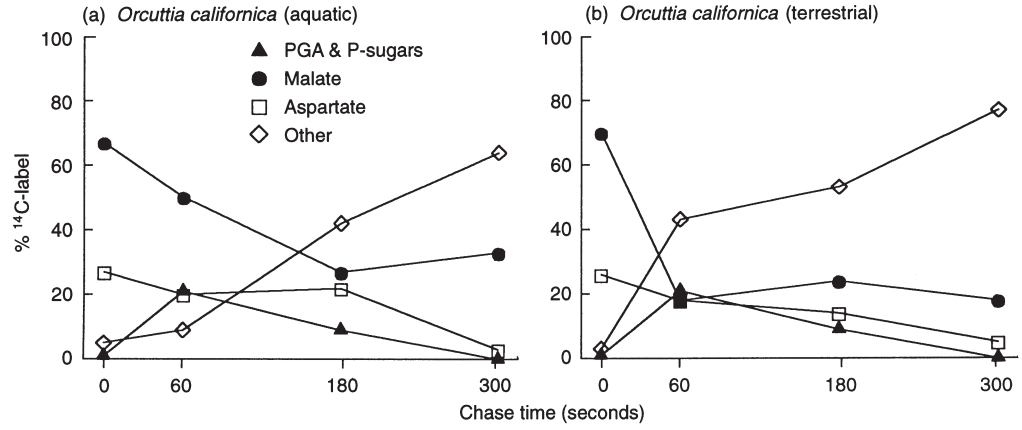

Fig. 4. Distribution of ^{14}C label in pulse-chase experiments in the light for *Orcuttia californica* (a) aquatic and (b) terrestrial foliage. Each point represents the mean of three or more samples.

C_4 and C_3 + C_4 (Table 3). The failure to detect this enzyme in *C. contraria* (a putative C_3 + C_4 intermediate) and presence of this enzyme in *D. bella* (an apparent C_3 plant) was surprising.

Carbonic anhydrase exhibited marked variation that was more species-specific than tied to the aquatic *vs* terrestrial conditions. Most species exhibited very limited activity relative to a species such as spinach, however, *L. scilloides*, *Callitriche longipedunculata* (floating leaf) and *D. bella* were relatively high.

pH-drift experiments showed that in this community, relatively few species were potential bicarbonate users (Table 2). The majority of species could not drive final pH much above 8·0, suggesting little capacity for bicarbonate uptake. In contrast, three species (*Ranunculus aquatilis*, *C. contraria* and *Gloeotrichia* sp.) were capable of driving the solution pH above pH 9·5.

Discussion

This aquatic community comprises a rich diversity of photosynthetic characteristics that may reflect selection for competitive ability for limiting resources; primarily carbon limitation in the aquatic stage and water limitation in the terrestrial stage. Under equilibrium conditions, competitive exclusion may replace species that overlap too greatly in their resource requirements, thus, producing a plant assemblage with greater diversity of resource acquisition strategies (e.g. Tilman 1982; Grace 1990). Evolution is expected to respond to stress by selecting for both structural and physiological characteristics that can be categorized as either **avoidance** or **tolerance** responses. In these amphibious species such responses are recognizable in both the aquatic and terrestrial stages.

AQUATIC STAGE

Avoidance strategy

A third of the dominant species in this community avoid carbon limitations of the aquatic environment by production of floating leaves with functional stomata (Table 2). Under well-stirred conditions, maximum photosynthetic rates of submerged leaves of amphibious species may not exceed those observed for aerial leaves (Nielsen 1993). However, in densely vegetated stagnant pools, boundary-layer effects are likely to be a critical limitation to carbon assimilation. Atmospheric mixing around floating leaves reduces the likelihood of boundary layer CO_2 depletion, in contrast to the situation with submerged leaves (Longstreth 1989). This, coupled with an inexhaustible supply of water (albeit ephemeral), reduces selection for CO_2 concentrating mechanisms. Consistent with this is the lack of CAM species with floating leaves. However, C_4 floating leaves in *O. californica* would seem to contradict this conclusion, but phylogeny may play a role by limiting available developmental pathways; the laminate leaf blade plus Kranz anatomy in floating leaves are pleisomorphic characteristics, seemingly homologous to similar structures in the terrestrial stage (Keeley 1998a). The apparent C_3 + C_4 assimilation pattern in *M. vestita*, coupled with the 'intermediate' Kranz condition of bundle sheath chloroplasts surrounded by a densely chlorenchymous mesophyll (Gaudet 1964; Keeley 1990), is described for some terrestrial species where it appears to play a role in reducing photorespiration under high light conditions (Monson *et al*. 1986).

Another means of avoiding daytime carbon stress is to gain access to sediment CO_2, which, in this pool, is an order of magnitude higher than the peak water concentration (Keeley & Sandquist 1991). The isoetid growth form is uniquely adapted to utilizing sediment CO_2 (Sculthorpe 1967; Keeley *et al*. 1994; Raven 1995) and is common in this aquatic flora (Table 2). All photosynthetic types were represented by one or more species with the isoetid-rosette growth form. Root uptake of CO_2 has been shown in *I. howellii*, although it is significantly less important than for congeneric species in lacustrine habitats (Keeley 1998c). Those species lacking the isoetid form were diminu-

Table 3. Activity (mol kg^{-1} Chl h^{-1}) for carboxylating enzymes, Rubisco and PEP carboxylase, and other photosynthetic enzymes in submerged aquatic foliage, floating leaves and terrestrial leaves of species dominating the Santa Rosa Plateau vernal pool community: n = 2–3

	Rubisco	PEPcase	Rubisco/ PEPcase	Malic enzyme		PEPckinase	Pyr, Pi dikinase	Carbonic anhydrase
				NAD^+	NADP			
Chlorophyta								
Characeae								
Chara contraria								
Aquatic	46	10	4·6	129	16	ND	ND	6
Lycophyta								
Isoetaceae								
Isoetes howellii								
Aquatic	256	36	7·1	2	37	ND	110	26
Terrestrial	553	18	30·7	ND	42	ND	186	55
Isoetes orcuttii								
Aquatic	225	46	4·9	–	–	–	–	–
Terrestrial	480	15	32·0	–	–	–	–	–
Pterophyta								
Marsileaceae								
Marsilea vestita								
Aquatic	184	30	6·0	–	–	ND	–	36
Floating	316	26	12·2	ND	6	ND	–	99
Anthophyta–Monocotyledonae								
Cyperaceae								
Eleocharis acicularis								
Aquatic	224	80	2·8	ND	24	ND	71	1
Terrestrial	675	36	18·8	ND	15	ND	ND	12
Lilaeaceae								
Lilaeae scilloides								
Aquatic	846	72	11·2	–	–	–	–	1877
Terrestrial	1501	67	22·4	ND	56	ND	–	1058
Poaceae								
Alopecuris howellii								
Aquatic	316	36	8·8	–	–	–	–	40
Floating	1335	61	21·9	–	–	–	–	7
Terrestrial	1635	83	20·0	ND	100	ND	–	
Orcuttia californica								
Aquatic	183	34	5·4	4	47	ND	67	21
Floating	330	66	5·0	–	–	–	–	–
Terrestrial	299	157	1·9	8	41	ND	160	5
Anthophyta–Dicotyledoneae								
Apiaceae								
Eryngium aristulatum								
Aquatic	698	104	6·7	ND	26	ND	–	–
Terrestrial	996	22	45·3	ND	ND	ND	–	–
Boraginaceae								
Plagiobothryus undulatus								
Aquatic	335	16	20·9	–	–	–	–	–
Terrestrial	455	11	41·4	–	–	–	–	–
Callitrichaceae								
Callitriche longipedunculata								
Aquatic	127	2	63·5	–	–	–	–	121
Floating	811	11	73·7	–	–	–	–	3929
Campanulaceae								
Downingia bella								
Aquatic	198	37	5·6	ND	55	ND	216	918
Terrestrial	992	99	10·0	ND	21	ND	161	741
Crassulaceae								
Crassula aquatica								
Aquatic	392	178	2·2	2	78	ND	208	15
Terrestrial	854	45	19·0	4	156	ND	ND	–

Table 3. *Continued.*

	Rubisco	PEPcase	Rubisco/ PEPcase	Malic enzyme NAD$^+$	Malic enzyme NADP	PEPckinase	Pyr, Pi dikinase	Carbonic anhydrase
Elatinaceae								
Elatine californica								
Aquatic	300	10	30·0	ND	32	ND	–	–
Terrestrial	478	8	59·8	ND	3	ND	–	–
Ranunculaceae								
Ranunculus aquatilis								
Aquatic	245	23	10·7	ND	69	ND	–	12
Floating	486	13	37·4	ND	89	ND	–	49
Zea mayes	462	842	0·5	–	–	–	289	–
Spinacea oleracea	865	54	16·0	–	–	–	ND	1698
Ananas comosus	–	–	–	–	83	908	–	–
Hoya carinosa	–	–	–	ND	106	–	–	–
Kalanchoe daigremontiana	–	–	–	96	73	–	–	–

tive or prostrate annuals (e.g. *E. californica*) with close sediment contact, or were larger, with highly dissected leaves and access to bicarbonate (e.g. *R. aquatilis*, Table 2).

The aquatic CAM strategy

While the majority of species appear to be C_3 (Table 2), a significant component of the flora is CAM (over one-third by biomass, Keeley & Sandquist 1991). These species are widely distributed within the pool with the diminutive annual, *C. aquatica*, being restricted to the periphery, whereas the deepest parts of the pools are dominated by the more robust perennial *I. howellii*.

Evidence for CAM in *Isoëtes* has been well established (Keeley 1995) and evidence of this pathway in *C. aquatica* is presented here (Table 1, Fig. 3). Fixation patterns in the light indicate exclusively C_3 uptake, a pattern also observed in terrestrial CAM plants with incomplete stomatal closure (Kluge & Ting 1978). Under field conditions terrestrial CAM plants typically have limited C_3 uptake owing to stomatal closure. In vernal pools, C_3 uptake is limited by the depletion of ambient CO_2 during much of the day (Fig. 1), coupled with an inability to utilize bicarbonate (Keeley & Busch 1984; Keeley 1995).

In these aquatic CAM plants PEPC activity, while sufficient to account for levels of overnight acid accumulation, is very low relative to terrestrial CAM species (Dittrich, Campbell & Black 1973). I hypothesize that the difference is not the result of lower CAM activity, as aquatic species accumulate as much acid overnight as terrestrial species. I hypothesize this is owing to the substantially higher ambient CO_2 levels at night in these pools, relative to air. Under elevated carbon conditions the typically high carbon affinity of PEPC would result in maximum vacuolar storage capacity being reached earlier in the dark period, thus, favouring reduced PEPC concentration as an energy- or nutrient-conserving response. This is supported by the observed inverse relationship between levels of ambient CO_2 and PEPC activity in both aquatic (Groenhoef, Smirnoff & Bryant 1988) and terrestrial (Nobel, Israel & Wang 1996) CAM plants, despite the maintenance of acid accumulation under both high and low CO_2 levels. Daytime deacidification is catalysed largely by NADP malic enzyme and this pathway, coupled with the lack of PEP carboxykinase activity, is consistent with the high activities for pyruvate, Pi-dikinase (Black *et al*. 1995).

It is hypothesized that decarboxylation of the endogenous malic-acid pool contributes to a CO_2-pumping mechanism, despite the absence of daytime stomatal closure, and this is owing, at least in part, to the high diffusive resistance of water inhibiting CO_2 leakage. In summary, CAM plants compete for carbon by taking advantage of elevated night-time levels in the water and generating endogenous CO_2 when light energy is sufficient to drive carbon reduction.

Weak overnight acid accumulation in aquatic foliage of *O. californica* (Table 1) has also been observed in the related *Orcuttia viscida* from northern California, but does not appear to be indicative of CAM photosynthesis and possibly involves a non-phototrophic pathway (Keeley 1998b).

Downingia bella has a diel change in malate but not H^+ (Table 1). As a matter of speculation, this species may be utilizing a CAM-like pathway with tonoplast transport of malate + K^+ rather than malate + H^+, a model proposed by Raven *et al*. (1988). If so, this may explain the activity of the C_4 enzyme pyruvate, Pi-dikinase (Table 3), an expected characteristic for an apparent C_3 plant (Fig. 2).

The aquatic C_4 strategy

C_4 photosynthesis in aquatic *O. californica*, in the absence of Kranz anatomy, is similar to the pattern documented for the related *O. viscida* (Keeley 1998a).

Although apparently lacking intercellular separation of Rubisco and PEPC, there is substantial reason to suspect intracellular separation of these carboxylases (e.g. Bowes & Salvucci 1984; Reiskind *et al*. 1989, 1997; Ueno 1996). Despite the lack of suberized bundle sheath cells, it has been hypothesized that the high diffusive resistance of water, may contribute to this pathway acting as a CO_2-pumping mechanism in aquatic *Orcuttia* spp. (Keeley 1998a).

Combined C_3 + C_4 assimilation

As in other aquatic habitats (e.g. Salvucci & Bowes 1983), some vernal pool species have both C_3 and C_4 fixation in the light (Table 2). Conclusive evidence that ^{14}C-labelling of C_4 acids derives from β-carboxylation, and is indicative of phototrophic carbon assimilation, requires more detailed study. Prudence is advised as there is evidence that such C_4-labelling patterns in some blue–green algae are tied to other biosynthetic pathways (Coleman 1989). This is a likely explanation for C_4-labelling in *C. contraria* (Table 2), because it apparently lacks the key C_4 pathway enzyme, pyruvate, Pi-dikinase (Table 3). However, other apparent C_3 + C_4 species, such as aquatic *E. acicularis*, have significant activity of key C_4 enzymes and a very low C_4-like Rubisco/PEPC ratio (Table 3).

Despite fixation patterns indicative of C_3 + C_4 intermediates, carbon assimilation pathways and mechanisms for avoiding 'futile' cycling of carbon are unknown. There are, however, models of how this might be accomplished (Winter 1985; Bowes & Salvucci 1989; Reiskind *et al*. 1997).

Assuming the C_3 + C_4 labelling patterns reflect phototrophism through both pathways, it may not be possible to ascertain their real role through steady-state laboratory studies run under high CO_2 conditions. In the pools, during much of the day, CO_2 is limiting and O_2 is above saturation (Fig. 1), conditions that would favour C_4 carbon assimilation. I hypothesize that as daytime CO_2 and O_2 levels change, the contribution of these two pathways varies and the roughly equal and seemingly simultaneous contribution of C_3 and C_4 assimilation observed in laboratory studies may not be representative of what happens in the field. This proposal is supported by the observation that similar changes in assimilation occur over relatively short distances in mats of *Hydrilla verticillata*; midday depletion of CO_2 in the centre of the mat apparently results in elevated C_4 fixation, relative to plants on the periphery (Spencer, Teeri & Wetzel 1994). Also, it has been shown for *H. verticillata* that the contribution of C_3 *vs* C_4 assimilation changes in response to seasonal changes in carbon availability (Bowes & Salvucci 1984). In vernal pools the diel changes in carbon availability exceed, by orders of magnitude, the seasonal changes (Keeley & Busch 1984; Keeley & Sandquist 1991), supporting the hypothesis that the contribution of C_3 *vs* C_4 carbon assimilation may change through the day in response to changes in carbon availability.

Additionally, perhaps many seemingly C_3 species in these pools (Table 2) possess such an ability. This is suggested by the substantially lower Rubisco/PEPC ratios in aquatic *vs* terrestrial foliage for nearly all species (Table 3). Of course other roles for elevated PEPC activity, such as refixation of respiratory CO_2, have been proposed for both aquatic (Spencer, Wetzel & Teeri 1996) and terrestrial (Monson 1989) species.

Other factors

While certain carbon assimilation pathways are effective means of competing for carbon, there are other CO_2 concentrating mechanisms at work in these pools. Three species (Table 2) are efficient bicarbonate users, indicating potential temporal separation of carbon competition (e.g. Fig. 1). Active uptake of inorganic carbon coupled with carbonic anhydrase can act as a CO_2 concentrating mechanism (Raven 1995). The very high activity of this enzyme in some vernal pool species suggests a further avenue of research (Table 3). In order to evaluate this potential, however, much more information is required as it may be involved in non-phototrophic processes (Raven & Newman 1994) and it may occur as an extra-cellular, cytoplasmic or chloroplastic enzyme (Smith & Bidwell 1989). Lastly, there are other physiological characteristics that enhance carbon assimilation in aquatic plants (e.g. Salvucci & Bowes 1983; Badger 1987; Raven 1991; Beer 1994), not addressed in this study.

TERRESTRIAL STAGE

These amphibious species have a relatively brief terrestrial stage but even so this is likely to be a critical period; for example, during this stage there is substantial starch accumulation in corms of *I. howellii* (Keeley 1983) and reproduction of most flowering plants is restricted to this time period. Increased chlorophyll and protein in all species (Table 1) is indicative of enhanced photosynthetic capacity, as documented for several species (Keeley & Sandquist 1991).

Greater CO_2 availability in the aerial environment likely accounts for the switch from CAM to strictly C_3 under terrestrial conditions (Fig. 3). This may also explain the switch from C_3 + C_4 assimilation to C_3 in *E. acicularis*.

In contrast, Ueno *et al*. (1988) have shown *Eleocharis vivipara* switches from an aquatic C_3 to a terrestrial C_4 pathway. In vernal pools, species of *Orcuttia* are C_4 underwater (but lack Kranz anatomy) and, in the terrestrial stage, retain this pathway (with Kranz), and this undoubtedly contributes to their ability to persist well into the summer drought (Keeley 1998a).

Terrestrial foliage of *Orcuttia* spp. also exhibit substantial daytime malic-acid accumulation, which is

apparently unrelated to photosynthetic pathway and speculated to play a role as an herbivore defence mechanism (Keeley 1998b).

Although all species show at least some quantifiable level of morphological and anatomical change between the aquatic and terrestrial foliage (Keeley 1990), four species studied here exhibited radical metamorphosis between the submerged aquatic stage and the terrestrial stage. Phylogeny may play a role. These species are in the genera *Orcuttia, Eryngium, Plagiobothrys* and *Downingia*, which have many vernal pool species distributed throughout California. Cladistic analysis indicates that the vernal pool species are relatively recently derived from terrestrial ancestors (Keeley & Zedler 1998; Spencer & Rieseberg 1998; Keeley 1998a); thus, they apparently have retained the capacity for producing specialized terrestrial foliage. In contrast, the other 11 genera, which undergo little morphological change in the terrestrial environment, represent widespread cosmopolitan aquatic groups and the vernal pool representatives are probably derived from aquatic ancestors (Keeley & Zedler 1998). Under terrestrial conditions it is noted that species in the former group have substantially greater gross carbon gain than species representative of the latter group (Keeley & Sandquist 1991).

ROLE OF DISEQUILIBRIUM IN COMMUNITY COEXISTENCE

The observation that community composition in vernal pools often changes markedly from year to year (Thorne & Lathrop 1970; Holland & Jain 1984; Zedler 1987; J. E. Keeley, personal observations), suggests that disequilibrium conditions may play a role in promoting coexistence. The annual cycle in these seasonal environments produce potential disequilibrium conditions, induced by yearly differences in rates of filling and in rates of drying. Species-specific differences in inundation tolerance (Zedler 1987), sets the stage for annual variation in competitive success. On top of this there are marked differences between species in the period of maximum carbon gain, some peak early in the aquatic stage, others later and still others under terrestrial conditions (Keeley & Sandquist 1991). Annual weather variation is also likely to produce annual variation in reproductive potential, as non-flowering vascular plants in these pools produce reproductive structures while submerged, whereas most Anthophyta initiate flowering on aerial foliage. The extent to which annual variation in pool conditions drives demographic changes is unknown but it is apparent that photosynthetic pathways represent only one factor determining community coexistence. It is hoped that the studies reported here will provide a framework sufficient to test more thoroughly the importance of photosynthetic diversity in determining community composition.

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