Photosynthetic Pathways in Freshwater Aquatic Plants

Jon E. Keeley

Recent studies show that generalizations about photosynthetic pathways, derived from terrestrial plant studies, do not apply to aquatic plants. Crassulacean acid metabolism (CAM) photosynthesis is of selective value not only in arid environments, where it enhances water-use efficiency, but also in aquatic plants of oligotrophic waters, where it enhances competitive ability in carbon acquisition. C_4 photosynthesis is present in many aquatic species, but in these species it is not coupled with the specialized anatomy of terrestrial C_4 plants. The ratio of the stable carbon isotopes, $^{13}C/^{12}C$, in the biomass of terrestrial plants is a marker of their photosynthetic pathway. In aquatic environments, additional resistances to carbon-isotope fractionation make this technique of limited use in detecting photosynthetic pathways.

Much of our current understanding of photosynthetic pathways has been derived from studies of terrestrial plants, where we can distinguish three modes: C_3, C_4 and CAM (see Box 1 and Fig. 1). Recent studies on aquatic plant photosynthesis have revealed that many generalizations about photosynthetic pathways need to be reconsidered.

The aquatic milieu presents several factors not normally encountered by terrestrial species[1]. Due to the viscosity of water, gases diffuse 10^4 times slower than in air. Consequently, in the boundary layer around leaves, gases may undergo markedly greater diurnal changes than are observed in the atmosphere. During the day, photosynthetic consumption may deplete the boundary layer of carbon dioxide (CO_2) and generate elevated oxygen (O_2) concentrations. Under such conditions, some aquatic species are capable of utilizing other forms of inorganic carbon such as bicarbonate (HCO_3^-) (Ref. 2). This, however, requires expenditure of energy, and in some environments it seems that the benefits do not balance the costs; consequently, many species have not evolved the capacity for active uptake of HCO_3^-.

Jon Keeley is at the Dept of Biology, Occidental College, Los Angeles, CA 90041, USA.

Historically it was thought that because the total pool of inorganic carbon (CO_2, HCO_3^-, CO_3^{2-}) was orders of magnitude higher than in air, aquatic plants were seldom carbon-limited. Today, carbon limitation is considered to be a major driving force in the evolution of photosynthetic characteristics of aquatic plants.

Aquatic CAM plants

Crassulacean acid metabolism (CAM) is present in most terrestrial succulent plants of arid environments. Due to the reverse stomatal behavior (in which stomatal pores open at night and close during the day), CAM photosynthesis plays an important role in conserving water. In light of the adaptive significance of CAM in terrestrial species, the last place one might expect to find a CAM plant would be in water. However, we now know that a large number of aquatic plants have the CAM pathway. Included in this group is the rather large genus of primitive spore-bearing plants *Isoetes* (Isoetaceae) (Fig. 2)[3–6]. Other aquatic CAM species include higher plants, both monocots (Poaceae) and dicots (Crassulaceae and Plantaginaceae)[7–9].

It appears that ambient carbon limitation has been the driving force selecting for this pathway. Aquatic CAM plants are found worldwide, but only in certain aquatic habitats. Many are distributed in small temporary pools, which experience very marked diurnal fluctuations in CO_2 concentration (Fig. 3a). Due to the fact that such environments have high irradiances and are densely vegetated, the CO_2 is depleted very rapidly in the morning, at a rate in excess of the diffusion of CO_2 from the atmosphere into the water. Since aquatic CAM plants are unable to use HCO_3^- (Refs 10–12), they are carbon-limited throughout much of the day, and carbon uptake is greatly curtailed (Fig. 3b). However, nighttime respiration by the flora and invertebrate fauna replenishes the CO_2. Aquatic CAM plants take advantage of this plentiful supply of CO_2 available at night by fixing it and storing it overnight as malic acid (Fig. 3c). During the day, when the ambient CO_2 concentration is near zero, these plants break down the malic acid (as do terrestrial CAM plants) and thus generate their own intracellular supply of CO_2.

In all aquatic plants, stomata are lacking or non-functional; therefore in aquatic CAM plants, the balance between nighttime and daytime carbon uptake is controlled by CO_2 availability rather than stomatal behavior, as in terrestrial CAM plants. Thus, the CAM pathway stands as an example of a biochemical pathway that has been selected in response to two very different sets of ecological conditions.

Temporary pools eventually dry down. When *Isoetes* and other aquatic CAM plants are exposed to the atmosphere they lose the CAM pathway and rely strictly on C_3 photosynthesis[11]. This loss of CAM activity occurs on a cell-by-cell basis; submerged leaf bases retain CAM, while aerial leaf tips largely lack CAM. The physiological mechanism behind this switch is unknown, but there are some clues as to the environmental stimuli responsible for the change from aquatic to aerial metabolism.

Two possible cues for CAM loss have been tested[13]: the aerial leaves could respond to the lower ratio of red–far-red light, or to the lower atmospheric water potential. *Isoetes howellii* plants were grown out of water in a high or low ratio of red–far-red light, combined with a high or low atmospheric humidity. Filtering light through water (which increases the ratio of red–far-red light) had no influence on overnight acid accumulation. However, when atmospheric humidity was maintained near saturation, CAM was not lost despite the fact that the plants were growing in an aerial environment. A similar finding has been reported for another CAM plant, *Littorella uniflora*[14].

Shallow temporary pools occur worldwide and often contain species of *Isoetes* and *Crassula*. Species in both genera from pools in North America, South America, Europe and Australia have been shown to possess CAM (Ref. 3 and Keeley, unpublished data). These two genera also occur in lacustrine environments. With rare exceptions, the only permanent bodies of water

regularly inhabited by aquatic CAM plants are oligotrophic lakes at high elevation or latitude. The low productivity of such environments is also a characteristic of terrestrial environments occupied by many CAM plants. Lacustrine environments are quite different from seasonal pools. Such habitats lack diurnal fluctuations in CO_2; however, carbon limitation is imposed by extremely low inorganic carbon levels in the water column[3,9,10,15]. Under these conditions, CAM allows plants to maintain above-saturating intercellular CO_2 concentrations throughout the day and night[16]. Depending upon the ambient carbon level, CAM may contribute from a third to over 90% of the total 24 h carbon uptake[3,8,17].

A number of freshwater species are capable of CO_2 fixation in the dark, but organic acids do not accumulate as they do in CAM plants, and it appears that these products may be metabolized in the dark[18,19]. Although it seems doubtful that dark CO_2 fixation plays a role in the photosynthesis of these species, it is unknown what contribution such dark carbon uptake makes to the overall carbon economy of these plants.

C_4 photosynthesis

Eleocharis vivipara is an example of an amphibious plant that uses C_3 photosynthesis in foliage produced under water, but C_4 photosynthesis in foliage produced on land[20]. Curiously, however, there are a number of aquatic macrophytes that have C_4 fixation of carbon while submerged. Commonly these aquatic plants have C_4 fixation of carbon simultaneously with C_3 fixation. Studies using ^{14}C labeling indicate that PEP-carboxylase-catalysed CO_2 incorporation in the light into C_4 acids may account for 20% (e.g. *Vallisneria spirilis*[18]), 50% (*Eleocharis acicularis*[19]) or more than 70% (*Orcuttia californica*[6] and *Hydrilla verticillata*[21]) of the initial products of carbon fixation.

The most thoroughly studied of these aquatic macrophytes with C_4 fixation is *Hydrilla*[21,22]. The photosynthetic role for the C_4 organic acids is indicated by the fact that they are decarboxylated and refixed through the C_3 pathway, as in terrestrial C_4 plants. *Hydrilla* lacks Kranz anatomy[23] (see Box 1), and consequently the spatial separation of carboxylase enzymes between mesophyll and bundle sheath cells, as seen in terrestrial C_4 plants, cannot occur. Enzyme localization studies using immunocytochemical gold labeling indicate that the C_3 enzyme RuBP carboxylase is located in the chloroplasts of all chlorenchyma cells in the leaf[24]. The C_4 enzyme PEP carboxylase is cytosolic and also present in the same cells.

The model that best explains C_4 fixation in aquatic plants is one in which the two carboxylase enzymes (PEP carboxylase and RuBP carboxylase) are segregated intracellularly rather than intercellularly as in terrestrial C_4 plants. As CO_2 enters the cell, some is fixed in the cytosol by PEP carboxylase, while some may diffuse into the chloroplast and be fixed directly by RuBP carboxylase. The cytosolic C_4 fixation products (organic acids such as malate) are transported into the chloroplast for decarboxylation by NADP malic enzyme, and the CO_2 is refixed by RuBP carboxylase and enters the carbon reduction pathway to glucose.

This double CO_2 fixation process would concentrate CO_2 at the site of RuBP carboxylase and act to reduce photorespiration. Indeed, the presence of C_4 fixation in *Hydrilla* during the summer results in a reduction in photorespiration; at other times of the year the rate of photorespiration is higher[21]. Species with substantial C_4 fixation are largely unknown from oligotrophic habitats, and its role in reducing photorespiration under eutrophic conditions may be the key to its presence in aquatic plants.

The absence of Kranz anatomy in *Hydrilla* and other aquatic species[25,26] with C_4 photosynthesis requires some explanation. In species such as *Hydrilla*, the leaf lamina is only two cell layers thick and this simple leaf design precludes Kranz anatomy[23]. However, such an explanation cannot account for the lack of Kranz anatomy in species such as *Orcuttia californica*[6] (an annual grass that is endemic to seasonal pools in California, USA). In these species, the seeds germinate under water and the first leaves are several cell layers thick, similar to many terrestrial grass leaves, except for the extensive intercellular airspace and the semi-cylindrical shape due to the folding of the leaf lamina. These submerged leaves lack Kranz anatomy; however, over 70% of the initial carbon fixation is via β-carboxylation into organic

Box 1. C_3, C_4 and CAM photosynthetic pathways

From terrestrial plant studies, three photosynthetic modes are recognized: C_3, C_4 and CAM (crassulacean acid metabolism).

In the majority of plants, atmospheric CO_2 is taken up by photosynthetic cells and fixed into phosphoglycerate (PGA), which enters the photosynthetic carbon reduction (PCR) pathway leading to glucose. The initial carbon fixation step occurs in the chloroplast and is catalysed by ribulose bisphosphate carboxylase (RuBP carboxylase). Since the first product of photosynthesis is a three-carbon molecule, species with this pathway are known as C_3 plants.

C_4 species, which are known from many plant families, have coupled this C_3 pathway with a prior carboxylation step catalysed by phosphoenolpyruvate carboxylase (PEP carboxylase). This enzyme occurs in the cytosol of mesophyll cells near the stomatal pores, and the first stable products of fixation are four-carbon organic acids such as malate and aspartate. These products are transported to specialized 'bundle sheath cells' (Fig. 1), which surround the vascular system in the interior of the leaf. Here the organic acids are decarboxylated and the CO_2 that is generated is fixed a second time with the C_3 enzyme RuBP carboxylase and enters the PCR pathway to glucose. Most plants have bundle sheath cells but only in C_4 plants do they contain chloroplasts, producing a distinct ring-like pattern known as Kranz anatomy (Fig. 1). Thus, C_4 plants have an intracellular separation of PEP carboxylase in the cytosol of mesophyll cells and RuBP carboxylase in the chloroplasts of bundle sheath cells.

CAM plants are capable of CO_2 fixation at night, catalysed by PEP carboxylase, and organic acids are stored overnight in the cell vacuole. During the day these acids are decarboxylated, in the same cells, and this carbon is assimilated through the C_3 cycle. In CAM plants the two carboxylation events are separated temporally, rather than spatially as in C_4 plants. The typical or prototype CAM plant shows high CO_2 conductance at night and low CO_2 conductance, and low water loss, during the day.

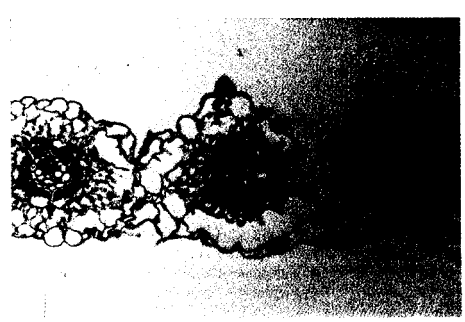

Fig. 1. Cross-section of a floating leaf of *Orcuttia californica*, illustrating the rings of chloroplast-laden bundle sheath cells surrounding the vascular system – a pattern known as Kranz anatomy.

Fig. 2. A rosette of sporophylls of *Isoetes howellii*, an aquatic quillwort, which grows in temporary pools of water in the western United States.

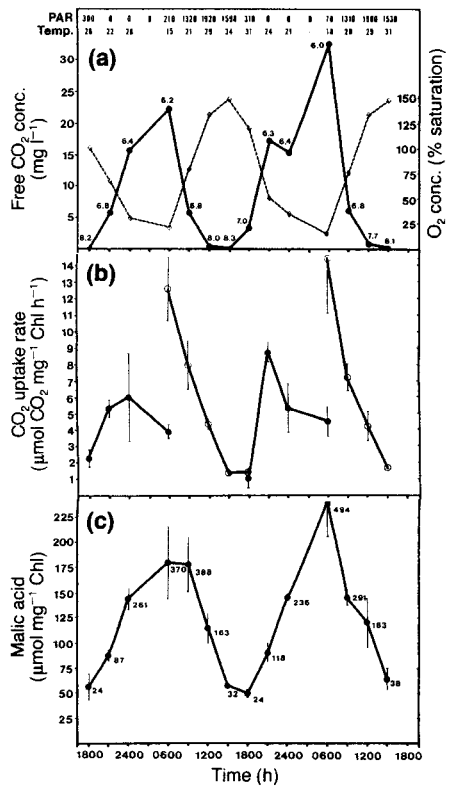

Fig. 3. Photosynthesis in *Isoetes howellii* in a seasonal pool. (a) Concentrations of CO_2 (solid line; adjacent numbers are pH values) and O_2 (dashed line) in water in a pool over 48 h; photosynthetically active radiation (PAR, μE m^{-2} s^{-1}) and water temperature (°C) are given above the curves. (b) Carbon assimilation rates in the light (open circles) and dark (filled circles), and (c) malic acid levels (numbers indicate titratable acidity, μmol H^+ mg^{-1} chlorophyll), in leaves of *Isoetes howellii*. *Adapted from Ref. 11, with permission.*

acids, largely aspartate, and pulse-chase studies reveal a turnover of these products similar to that observed in terrestrial C_4 plants (Keeley, unpublished). Later in development the submerged leaves are replaced with leaves that float on the water surface[6]. Floating leaves also have C_4 fixation but, unlike the submerged leaves, they have well-developed Kranz anatomy (Fig. 1); this is also true of the third set of leaves, produced once the pools have dried down.

The tribe Orcuttieae comprises a dozen species, all of which are endemic to Californian seasonal pools and all of which are C_4 (Keeley, unpublished data). The affinities of these grasses indicate that they are derived from terrestrial ancestors. The most primitive member of the tribe, *Neostapfia colusana*, produces submerged leaves with Kranz anatomy as well as stomata. The more advanced *Orcuttia californica* represents an evolutionary loss of Kranz anatomy, as well as loss of other structures such as stomata. Thus, we can surmise that in *Orcuttia*, and possibly other aquatic plants, there may be an advantage to combining C_4 photosynthesis with intracellular compartmentation of carboxylating enzymes, rather than intercellular compartmentation as in terrestrial C_4 plants.

Carbon isotopes

The ^{12}C isotope accounts for 99% of carbon in nature[27]. Most of the remaining carbon consists of atoms of the heavier isotope, ^{13}C. Stable isotopes such as these are measured on a mass spectrometer and expressed as:

$$\frac{^{13}C/^{12}C \text{ ratio of sample}}{^{13}C/^{12}C \text{ ratio of standard}} - 1$$

This number is multiplied by 1000, expressed as 'per mil' (‰) and abbreviated as $\delta^{13}C$ (the carbon isotope ratio). In plant matter, it is always negative; the more negative the ratio, the less ^{13}C present.

In terrestrial plants, $\delta^{13}C$ values of photosynthetic tissues vary from −8‰ to −15‰ in C_4 plants, and from −20‰ to −35‰ in C_3 plants[27]. CAM plants may range from C_4-like to C_3-like in their carbon isotope ratio, depending upon the balance between nighttime and daytime stomatal conductance. Atmospheric CO_2 is −8‰, and during photosynthesis plants discriminate against ^{13}C; this is most pronounced in C_3 plants. Discrimination comes about largely because the C_3 enzyme RuBP carboxylase shows a marked preference for the ^{12}C isotope. Since ^{13}C is discriminated against, it will accumulate, but readily diffuses away as it is mixed with atmospheric air passing through the stomatal pores. C_4 plants fix atmospheric carbon with PEP carboxylase, which has much less discrimination against ^{13}C. Even though the products of C_4 fixation are broken down and the CO_2 is refixed with RuBP carboxylase, discrimination at this stage is largely prevented because RuBP carboxylase is restricted to the interior of the leaf in the Kranz-type bundle sheath cells. Although the ^{13}C isotope is discriminated against, it diffuses out of the bundle sheath cells very slowly; thus, it accumulates within these cells and eventually the enzyme is forced to fix this isotope.

In aquatic plants, the $\delta^{13}C$ value is not indicative of the photosynthetic pathway[28,29]. There are several reasons for this. As mentioned earlier, some aquatics can take up HCO_3^- in addition to CO_2. When inorganic carbon species are at equilibrium in solution, HCO_3^- will have a less negative $\delta^{13}C$ than CO_2; depending upon pH and temperature, this may result in an 8–10‰ difference. Thus, theoretically, two aquatic plants with identical photosynthetic pathways may differ substantially if one utilizes HCO_3^- and the other is restricted solely to CO_2 uptake[29,30].

Another profound influence is created by the boundary-layer effects resulting from the aquatic milieu. In aquatic C_3 plants, RuBP carboxylase discrimination against the ^{13}C isotope will lead to the accumulation of this isotope in the boundary layer. As it accumulates, the higher concentration will overcome discrimination and, in effect, the enzyme will be forced to fix the ^{13}C and thus discrimination will be minimal. In many aquatic environments, diffusional resistance to CO_2 will be the dominant factor influencing $\delta^{13}C$ values[28–31].

Another factor in aquatic environ-

ments is that the $\delta^{13}C$ value of the inorganic carbon varies depending upon the source; for example, in one study values from +1‰ to −21‰ were reported for carbonate from different aquatic habitats[31]. In environments such as the temporary pools discussed earlier, much of the CO_2 is autogenic, being generated by respiration from the pool flora and fauna. Such carbon already reflects discrimination during the previous fixation events. Thus the carbon source for nighttime CAM fixation in *Isoetes* is respiratory carbon released by the pool flora, many of which are C_3 plants. This would explain why the CAM species have $\delta^{13}C$ values similar to the non-CAM species with which they coexist[29].

It has been suggested that $\delta^{13}C$ measurements may have some potential for evaluating diffusional resistances to carbon uptake in aquatic plants[28,31]. For aquatic plants that use CO_2 and rely exclusively on C_3-type RuBP carboxylase fixation, the $\delta^{13}C$ value of the biomass approaches that of the source carbon as diffusional resistances to carbon uptake increase. Plants from stagnant pools of water have less negative $\delta^{13}C$ values than plants from fast-moving streams, because the boundary-layer effect results in the accumulation of ^{13}C and swamps out the discrimination by RuBP carboxylase.

Thus, whereas $\delta^{13}C$ measurements are of value in distinguishing biochemical aspects of photosynthesis in terrestrial plants, they may be of more value in studies of physical aspects of photosynthesis in aquatic plants.

Acknowledgements

This work was supported by grant BSR-8705250 from the US National Science Foundation and a fellowship from the John Simon Guggenheim Foundation.

References

1 Raven, J.A. (1984) *Energetics and Transport in Aquatic Plants*, Alan R. Liss
2 Lucas, W.J. and Berry, J.A. (1985) *Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms*, American Society of Plant Physiologists
3 Keeley, J.E. (1987) in *Plant Life in Aquatic and Amphibious Habitats* (Crawford, R.M.M., ed.), pp. 113–128, Blackwell Scientific Publications
4 Boston, H.L. and Adams, M.S. (1983) *Aquat. Bot.* 15, 381–386
5 Farmer, A.M. and Spence, D.H. (1985) *Ann. Bot.* 56, 347–350
6 Richardson, K., Griffiths, H., Reed, M.L., Raven, J.A. and Griffiths, N.A. (1984) *Oecologia* 61, 115–121
7 Keeley, J.E. *Am. J. Bot.* (in press)
8 Keeley, J.E. and Morton, B.A. (1982) *Photosynthetica* 16, 546–553
9 Madsen, T.V. (1987) *New Phytol.* 106, 35–50
10 Boston, H.L. and Adams, M.S. (1985) *Oecologia* 65, 573–579
11 Keeley, J.E. and Busch, G. (1984) *Plant Physiol.* 76, 525–530
12 Madsen, T.V. (1987) *J. Exp. Bot.* 238, 367–377
13 Keeley, J.E. (1988) *Plants Today* 1, 127–132
14 Aulio, K. (1986) *Ann. Bot.* 58, 273–275
15 Madsen, T.V. (1987) *Physiol. Plant.* 70, 183–188
16 Robe, W.E. and Griffiths, H. (1987) *J. Exp. Bot.* 39, 1397–1410
17 Boston, H.L. and Adams, M.S. (1986) *Oecologia* 68, 615–622
18 Helder, R.J. and van Harmelen, M. (1982) *Acta Bot. Neerl.* 31, 281–295
19 Keeley, J.E. and Sandquist, D.R. *Ecology* (in press)
20 Ueno, O., Samejima, M., Muto, S. and Miyachi, S. (1988) *Proc. Natl Acad. Sci. USA* 85, 6733–6737
21 Holaday, A.S., Salvucci, M.E. and Bowes, G. (1983) *Can. J. Bot.* 61, 229–236
22 Salvucci, M.E. and Bowes, G. (1983) *Plant Physiol.* 73, 488–496
23 Bowes, G. and Salvucci, M.E. (1984) in *Advances in Photosynthesis Research* (Vol. III) (Sybesma, C., ed.), pp. 829–832, Junk Publishers
24 Reiskind, J.B., Berg, R.H., Salvucci, M.E. and Bowes, G. (1989) *Plant Sci. Lett.* 61, 43–52
25 Hough, R.A. and Wetzel, R.G. (1977) *Aquat. Bot.* 3, 297–313
26 Beer, S. and Wetzel, R.G. (1982) *Plant Physiol.* 70, 488–492
27 Rundel, P.W., Ehleringer, J.R. and Nagy, K.P., eds (1989) *Stable Isotope Ratios in Ecological Research*, Springer-Verlag
28 Raven, J.A., Griffiths, H. and Macfarlane, J.J. (1987) in *Plant Life in Aquatic and Amphibious Habitats* (Crawford, R.M.M., ed.), pp. 129–149, Blackwell Scientific Publications
29 Keeley, J.E., Sternberg, L.O. and DeNiro, M.J. (1986) *Aquat. Bot.* 26, 213–223
30 Raven, J.A., Beardall, J. and Griffiths, H. (1982) *Oecologia* 53, 68–78
31 Osmond, C.B., Valaane, N., Haslan, S.M., Uotila, P. and Roksandic, Z. (1981) *Oecologia* 50, 117–124