

Crassulacean acid metabolism in the context of other carbon-concentrating mechanisms in freshwater plants: a review

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Abstract Inorganic carbon can be in short supply in freshwater relative to that needed by freshwater plants for photosynthesis because of a large external transport limitation coupled with frequent depleted concentrations of CO₂ and elevated concentrations of O₂. Freshwater plants have evolved a host of avoidance, exploitation and amelioration strategies to cope with the low and variable supply of inorganic carbon in water. Avoidance strategies rely on the spatial variation in CO₂ concentrations within and among lakes. Exploitation strategies involve anatomical and morphological features that take advantage of sources of CO₂ outside of the water column such as the atmosphere or sediment. Amelioration strategies involve carbon-concentrating mechanisms based on uptake of bicarbonate, which is widespread, C₄-fixation, which is infrequent, and crassulacean acid metabolism (CAM), which is of intermediate frequency. CAM enables aquatic plants to take up inorganic carbon in the night. Furthermore, daytime inorganic carbon uptake is generally not inhibited and therefore CAM is considered to be a carbon-conserving mechanism. CAM in aquatic plants is a plastic mechanism regulated by environmental variables and is generally downregulated when inorganic carbon does not limit photosynthesis. CAM is regulated in the long term (acclimation during growth),

but is also affected by environmental conditions in the short term (response on a daily basis). In aquatic plants, CAM appears to be an ecologically important mechanism for increasing inorganic carbon uptake, because the in situ contribution from CAM to the C-budget generally is high (18–55%).

Keywords Acclimation · CO₂ · Carbon-concentrating mechanism (CCM) · Elodeids · Inorganic carbon · Isoetids · Macrophytes · Regulation

Inorganic carbon availability in freshwater habitats

In terrestrial environments, autotrophic plants have evolved mechanisms and strategies that allow them to obtain the resources necessary for photosynthesis and growth such as water, light, nutrients and CO₂. Of these, atmospheric CO₂ is most constant and so, coupled with the relatively high rate of diffusion of CO₂ in the gas phase, it seldom limits productivity in natural systems, or directly affects ecological distribution. Nevertheless, some terrestrial plants have evolved carbon-concentrating mechanisms (CCMs), such as C₄ carbon fixation and CAM, that may maximise carbon uptake but also often solve problems caused by interaction with other environmental factors such as high temperature or shortage of water (e.g. Herrera 2009; Lüttge 2002; Keeley and Rundel 2003; Sage and Kubien 2003).

In contrast, in freshwaters, water is readily available but the concentration of CO₂ is highly variable and may range from close to 0 to more than 350 μmol l⁻¹ (Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991). Because of a high transport limitation caused by low-diffusion coefficients of CO₂ in water and substantial boundary layers, these concentrations are in the lower range of concentrations

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needed to saturate photosynthesis of freshwater macrophytes, where half-saturation concentrations often vary between 100 and 200 $\mu\text{mol l}^{-1}$ (Maberly and Spence 1983; Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Maberly and Madsen 1998). Furthermore, photosynthetic removal of CO_2 , which often generates very low- CO_2 concentrations (e.g. Maberly 1996), also generates high concentrations of oxygen, producing conditions that favour photorespiration via the oxygenase reaction of Rubisco. In situ measurements have demonstrated that photosynthesis and growth of freshwater plants can indeed be limited by inorganic carbon (Madsen and Maberly 1991; Vadstrup and Madsen 1995).

Responses to carbon limitation in freshwaters

Freshwater plants have evolved anatomical, morphological, biochemical, physiological and ecological strategies to counter this CO_2 -restriction (Bowes 1987; Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Raven 1995; Maberly and Madsen 2002; Raven et al. 2008). These strategies can be broadly classified as: ‘avoidance’, ‘exploitation’ and ‘amelioration’.

Avoidance strategies

This is perhaps the simplest strategy and relies on the ability of the plants to avoid low- CO_2 habitats or niches. In the aquatic habitat, avoidance of low CO_2 is possible due to the high within- and among-lake variation in concentration of CO_2 . For example, the freshwater moss *Fontinalis anti-pyretica*, which is restricted to the use of CO_2 (obligate CO_2 user), could survive in a lake with substantial summer CO_2 depletion by exploiting the niche just above the sediment surface with elevated CO_2 concentrations (Maberly 1985). Another example of plants avoiding low CO_2 is macrophytes from streams, which benefit from the continuous replacement of CO_2 -depleted water. Finally, macrophytes from unproductive lakes do not experience the same severe CO_2 depletion as plants from productive lakes and therefore macrophytes from these habitats are more likely to depend on CO_2 taken up from the water column than species from productive lakes (Maberly and Madsen 2002).

Exploitation strategies

Because some of the anatomical and morphological adaptations allow exploitation of alternative inorganic carbon sources besides CO_2 from the water, they are referred to as ‘exploitation strategies’. These include (1) floating or aerial leaves, which enable freshwater plants to make use of

atmospheric CO_2 ; (2) aerenchyma or lacunae within roots, stems and leaves, which allow gas transport by diffusion or mass flow and—linked to 2—(3) uptake of CO_2 from the interstitial water in the sediment (sediment CO_2). Carbon uptake by floating or aerial leaves can make a major contribution to the carbon balance of some freshwater plants (e.g. Prins and De Guia 1986, Nielsen and Borum 2008) and can also allow forced ventilation supplying oxygen and removing ethanol from the roots and hence promoting survival in anoxic sediments (Dacey 1980). The sediment CO_2 is transported through the roots to the leaves in the lacunae system (Bowes 1987; Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Madsen and Sand-Jensen 2006). Uptake of sediment CO_2 is only significant in the functional group of isoetids because of their large root-allocation, well-developed lacunae and short stature (Raven et al. 1988; Madsen et al. 2002). In addition to enabling the exploitation of sediment CO_2 , the lacunae facilitate transport of O_2 , produced in the leaves, to the roots.

Many submerged plants have evolved thin or dissected leaves—resulting in a large surface:volume ratio—and have chloroplasts positioned in the outermost cell layers of the leaf (Madsen and Sand-Jensen 1991) that may help to minimise transport limitation. Thin leaves may also match low areal-amounts of photosynthetic machinery to low areal-rates of inward carbon flux (Black et al. 1981).

Although these anatomical and morphological adaptations may have evolved to reduce inorganic carbon limitation, their evolution could have been triggered by other environmental factors such as removal of water shortage, response to shear-stress from water flow and availability of nutrients or light.

Amelioration strategies

Physiological or biochemical adaptations, as opposed to the anatomical and morphological adaptations, most likely evolved to ameliorate inorganic carbon limitation. They are generally referred to as CCMs because they increase the concentration of inorganic carbon around the active site of Rubisco (Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Maberly and Madsen 2002; Raven et al. 2008).

CCMs are not ubiquitous in freshwater plants because their operation has both costs and benefits. The benefits may include increased carbon uptake, reduced photorespiration, reduced photoinhibition and increased nutrient use efficiency (Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Raven et al. 2008). The photorespiration cycle is an energy- and carbon-expensive mechanism, because one CO_2 equivalent is lost when two O_2 equivalents are fixed by Rubisco. Photorespiration is enhanced by

a high $[O_2]:[CO_2]$ ratio near the active site of Rubisco and thus by CO_2 depletion, high- O_2 concentrations and high temperature (Bowes 1991). By increasing the internal CO_2 supply and thereby increasing the $[CO_2]:[O_2]$ ratio internally, the operation of the CCM can reduce photorespiration. Due to the higher internal CO_2 supply, the CCM may also alleviate photoinhibition, because surplus energy may be dissipated via photosynthetic carbon assimilation (Osmond et al. 1993; White et al. 1996). Theoretically, the CCM, which increases the concentration of CO_2 around Rubisco, may increase the nutrient use efficiency because of higher efficiency of the carboxylase activity of Rubisco (Ehleringer and Monson 1993). Higher carboxylase efficiency could reduce the Rubisco needed for a given amount of carbon fixation and thereby result in higher nitrogen-use efficiency (NUE). However, bicarbonate use is not increased under nutrient-deficient conditions, but rather depends on a sufficient nutrient supply (Baatrup-Pedersen 1996). Similarly, for *Littorella uniflora*, the relation between CAM and photosynthetic NUE could not be verified experimentally, although CAM was still present at low nitrogen concentrations (Baatrup-Pedersen and Madsen 1999).

On the flip side of the CCM-coin are the extra costs in terms of energy and nutrient demand needed to produce, maintain and run the CCM apparatus in addition to the basic costs of the C_3 -pathway into which it is an accessory (Madsen and Sand-Jensen 1991; Lüttge 2002; Madsen et al. 2002). Investment of nitrogen in various CCM enzymes or transport proteins may have a negative impact in a low-nutrient habitat. In low-light habitats or locations, the energetic cost of the CCM may be significant (Raven and Spicer 1996), because ATP and NADPH production limit photosynthesis at low light. However, in high-light habitats, the energetic costs of the CCM are most likely irrelevant—or potentially affect plant performance positively by reducing photoinhibition.

The amelioration mechanisms include (1) bicarbonate (HCO_3^-) uptake (2) C_4 -fixation and (3) crassulacean acid metabolism (CAM).

HCO_3^- uptake

Uptake of bicarbonate from the bulk medium into the cell (HCO_3^- use) appears favourable in most freshwaters because its concentration exceeds that of CO_2 at pH values higher than ca. 6.4 (Maberly and Spence 1983; Vestergaard and Sand-Jensen 2000; Madsen and Sand-Jensen 2006). However, the affinity for bicarbonate is lower than the CO_2 affinity and thus CO_2 is the preferred inorganic carbon source when concentrations of HCO_3^- and CO_2 are similar (Bowes and Salvucci 1989; Maberly and Spence 1989; Prins and Elzenga 1989). Bicarbonate use is by far the most

frequently observed physiological mechanism for increasing inorganic carbon uptake and has been reported in about 50% of the investigated submerged angiosperms (Maberly and Madsen 2002). Transport of bicarbonate into the cell can occur directly via a HCO_3^-/H^+ symporter or indirectly via acidification of the boundary layer, thereby shifting the chemical equilibrium towards CO_2 , which thereafter can diffuse into the cell (Prins and Elzenga 1989). Bicarbonate users have a competitive advantage and are generally most abundant in alkaline habitats, where pH and the absolute concentration of bicarbonate often are high (Maberly and Spence 1983; Vestergaard and Sand-Jensen 2000). In addition to energy costs, species that are able to use bicarbonate have a lower affinity for CO_2 than species restricted to CO_2 alone (obligate CO_2 users; Maberly and Madsen 1998; Madsen and Maberly 2003), which may impose an ecological cost at some sites.

C_4 -metabolism

In addition to bicarbonate use, two inorganic carbon uptake mechanisms exist in freshwater plants that are based on C_4 -metabolism. They depend on carbon fixation via the enzyme phosphoenol pyruvate carboxylase (PEPcase) either during the day (C_4) or during the night (CAM), involving either a spatial (C_4) or temporal (CAM) separation of inorganic carbon fixation through PEPcase and Rubisco (Bowes and Salvucci 1989; Ehleringer and Monson 1993; Keeley and Rundel 2003). The light-dependent PEPcase fixation of inorganic carbon in freshwater plants is analogous to the terrestrial C_4 photosynthetic pathway, but in contrast to terrestrial C_4 —which is normally expressed constitutively—freshwater C_4 is a plastic mechanism, induced under inorganic carbon limitation (Van et al. 1976; Salvucci and Bowes 1981; Reiskind et al. 1997). Furthermore, freshwater C_4 plants do not have Kranz-anatomy like most terrestrial C_4 plants. However, single-cell C_4 -metabolism has recently been observed in terrestrial plants and may be an overseen phenomenon in freshwater plants (Edwards et al. 2004). C_4 -metabolism appears to be relatively rare in freshwater plants, it has been observed in *Hydrilla verticillata*, *Egeria densa* (Bowes and Salvucci 1989; Madsen and Sand-Jensen 1991; Casati et al. 2000) and a number of freshwater grasses (Keeley 1998a, Ueno et al. 1988).

CAM

CAM is primarily known from desert plants as an adaptation to enhance water conservation (Kluge and Ting 1978; Osmond 1978; Winter and Smith 1996; Cushman 2001; Dodd et al. 2002; Silvera et al. 2010). It enables CO_2 to be taken up and fixed via nighttime-PEPcase activity and

the C_4 product stored in the cell vacuole as malate, causing a decline in cell-sap acidity. During the day, malate is decarboxylated, resulting in de-acidification and the released CO_2 is fixed by Rubisco and enters the Calvin cycle (Fig. 1; Groenhof et al. 1988; Winter and Smith 1996; Nimmo 2000).

However, CAM is also present in some freshwater plants where it serves a different function. Unlike terrestrial CAM plants, where stomata are closed during the day, freshwater CAM plants have no stomata, and CO_2 can potentially be taken up 24 h a day (Osmond 1978; Keeley 1998b). In freshwater plants, the inorganic carbon source for PEPcase fixation (HCO_3^-) is derived from endogenous (respiratory CO_2) or exogenous sources (CO_2 from the bulk water or sediment CO_2). Use of HCO_3^- as the inorganic carbon specimen being transported into the cell has not been observed in aquatic CAM plants (Maberly and Madsen 2002). In addition to minimising or preventing respiratory carbon loss (potentially a positive carbon gain) in the night, freshwater CAM plants are able to concentrate CO_2 internally during the decarboxylation phase and thus CAM functions as both a carbon-conserving mechanisms and a CCM (Keeley 1998b; Madsen et al. 2002). Freshwater CAM has been observed in five freshwater genera, *Isoetes*, *Littorella*, *Crassula*, *Sagittaria* and *Vallisneria* (Keeley 1998b) and is thus present in isoetids and elodeids.

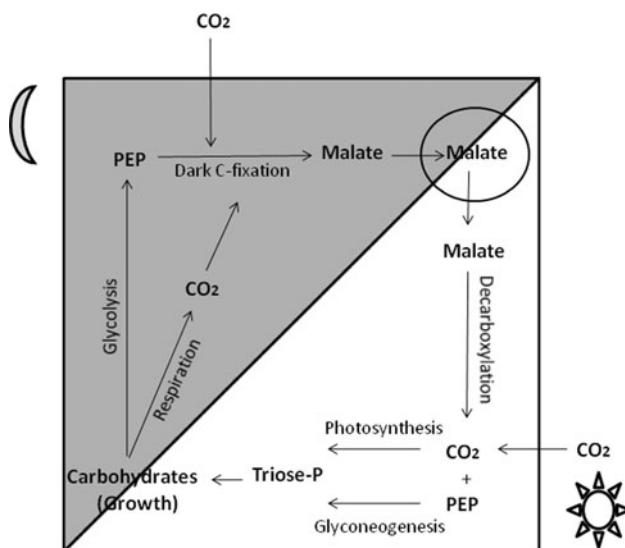


Fig. 1 The crassulacean acid metabolism cycle. Dark- CO_2 fixation occurs through the enzyme, PEPcase, and the sources of inorganic carbon are either of endogenous origin (respiration) or of exogenous origin (water or sediment CO_2). The grey area represents reactions occurring in the dark, whereas the white area contains daytime reactions. The round circle symbolises the cell vacuole. Modified from Winter and Smith (1996)

Habitats with CAM plants

For CAM (and other CCMs) to be of ecological benefit, the plants with CAM must be growing in a habitat with limited inorganic carbon. One such low-carbon habitat is soft-water lakes, which are characterised by relatively low pH, very low total inorganic carbon concentration and bicarbonate concentrations that are often too low to support bicarbonate use. Here, plants with CAM are likely to have an ecological advantage, because inorganic carbon can be taken up throughout the day increasing carbon gain and thus enhancing the chance of survival. In agreement with this, several CAM species—including the isoetids *Isoetes* spp. and *Littorella uniflora*—belong to the plant community typical of oligotrophic soft-water lakes (Sand-Jensen and Søndergaard 1997; Keeley 1996; Madsen et al. 2002).

CAM may not only raise the competitive ability of the plants in soft-water lakes but also in habitats with large fluctuations in the CO_2 concentration. Large daily CO_2 variations occur in low- and high-alkaline lakes with a high productivity, thereby giving rise to low daytime and high nighttime- CO_2 concentrations in the open water (Maberly 1996) and especially in weed beds (Van et al. 1976). In these lakes with large CO_2 fluctuations, plants with CAM are (1) able to take up inorganic carbon in the night, where the CO_2 concentration is higher and where competition for inorganic carbon with non-CAM species is eliminated and (2) less dependent on external CO_2 in the daytime—and thus CAM confers a competitive advantage on these species relative to non-CAM species in these habitats. In accordance with this, isoetid-CAM species are often found in ‘seasonal-pools’, whereas CAM species such as the invasive *Crassula helmsii* can be found in high-alkaline more eutrophic lakes (Keeley 1996, 1999; Dawson and Warman 1987). Thus, even in high-alkaline habitats with a relatively high inorganic carbon concentration during the daytime, CO_2 may be limiting and thus make the possession of CAM favourable. However, the reason why high-alkaline lakes are not a typical CAM-plant habitat is likely to be caused by the direct competition with bicarbonate users, which can take advantage of the high bicarbonate concentration and tend to be larger, faster-growing species.

CAM plasticity

CAM is a plastic mechanism in freshwater plants which is consistent with its function as a carbon-conserving and carbon-concentrating mechanism: The regulation ensures that resource allocation to energy- and nutrient-demanding uptake mechanisms is avoided when inorganic carbon does not limit photosynthesis (Bowes and Salvucci 1989; Maberly and Madsen 2002; Madsen et al. 2002). The

regulation can involve long-term acclimation over weeks or months or short-term responses (during the 24-h cycle) to external conditions and has been documented in isoetids and elodeids.

Long-term regulation of CAM

Regulation of CAM is dependent on various environmental variables e.g. light, CO₂, temperature, nutrients and water level (Aulio 1985; Madsen 1987a; Robe and Griffiths 1990; Hostrup and Wiegleb 1991; Klavsen and Maberly 2009; 2010, Klavsen unpubl. data). However, the outcome of regulation of CAM is dependent on the interaction between these variables (Table 1).

Light and CO₂ interact in the regulation of CAM, and for the invasive elodeid, *C. helmsii*, low light causes downregulation, independent of the CO₂ concentration (Klavsen and Maberly 2010; Table 1). For the isoetid *L. uniflora*, downregulation of the CAM apparatus has also been observed at low light, although in this species downregulation depends on the CO₂ availability during growth, with low-CO₂ grown plants not reducing CAM activity (Madsen 1987a; Klavsen Unpublished; Table 1). In a low-light regime, and particularly at moderate or high CO₂, CO₂ becomes saturating for photosynthesis which most likely triggers downregulation of CAM. Downregulation of CAM at low light is ecophysiologicaly favourable because it removes the energy cost associated with maintaining and running the CAM cycle (Raven and Spicer 1996). Maintenance of the CAM apparatus in a low-light regime may also be too costly in terms of nutrients. When CAM is not needed to enhance inorganic carbon uptake, nutrients associated with CAM can be allocated to acquisition of more limiting resources such as investments in light harvesting.

At light intensities saturating for photosynthesis and low-CO₂ availability, CAM is generally upregulated (Madsen 1987a; Robe and Griffiths 1990; Klavsen and Maberly 2010). At saturating light, CAM is generally decreased with raised CO₂ (Table 1). However, the CO₂ concentration, at which downregulation is triggered, is very different in *C. helmsii* and *L. uniflora*. The reason for the differences in the absolute CO₂ concentrations causing downregulation is likely to be related to the CO₂ concentration needed to saturate photosynthesis, which for isoetids is relatively high (half-saturation around 500–600 μmol l⁻¹ CO₂; Madsen et al. 2002). For *C. helmsii*, the half-saturation concentration of CO₂ is lower and was estimated to be ca. 100 μmol l⁻¹ from the data from Klavsen and Maberly (2010). Regarding *L. uniflora*, contrary results on CAM regulation at high light have been found, because CAM downregulation is not triggered by high CO₂ per se (Table 1; Madsen 1987a;

Baatrup-Pedersen and Madsen 1999), thereby emphasizing the interactive effect of environmental variables on CAM.

In agreement with light affecting the regulation of CAM, CAM varies with season and thus light intensity (Boston and Adams 1985; Klavsen and Maberly 2009). Indirectly, seasonal regulation indicates regulation of CAM by temperature in *L. uniflora* and *C. helmsii*. For *L. uniflora*, regulation of CAM by temperature has been observed, because *L. uniflora* appears to optimize CAM at or close to ambient temperature (Klavsen Unpublished). This implies that *L. uniflora* performed better at low than high temperature in the winter months (Q_{10} of 0.6–0.7). In the summer, CAM was stimulated by raised temperature and Q_{10} was 1.4–1.8 (Klavsen Unpublished). In contrast to terrestrial CAM plants, the seasonal variation in CAM cannot easily be determined by differences in $\delta^{13}\text{C}$, because the $\delta^{13}\text{C}$ values in aquatic plants vary depending on factors such as inorganic carbon source and diffusion resistance (Keeley and Sandquist 1992).

The seasonal regulation of CAM by light and temperature is in agreement with CAM acting as a CCM to enhance inorganic carbon uptake under environmental conditions with inorganic carbon depletion. In the summer—where CAM is highest (Fig. 2; Boston and Adams 1985; Klavsen and Maberly 2009)—high temperature and irradiance as well as long daylength enhance the photosynthetic rate and the overall daily photosynthesis and thus increase the inorganic carbon demand and the need for CAM. The need for an upregulated CCM is further accentuated by a potentially higher photorespiration because of higher temperature in summer.

Regulation by nutrients appears to be of minor importance, although nutrient depletion lowers CAM in *L. uniflora* grown at high light (Madsen 1987a; Robe and Griffiths 1994; Baatrup-Pedersen and Madsen 1999). This is consistent with the higher nutrient demand in the production and maintenance of the CAM apparatus, including CAM-related enzymes and tonoplast transporters. Theoretically, but not experimentally verified (Baatrup-Pedersen and Madsen 1999), a higher NUE due to the operation of CAM may have balanced the extra nitrogen cost.

Freshwater CAM plants growing in the near-shore area of the littoral zone or in seasonal pools can be exposed to air. In the water–land transition, CAM is often fully or partially downregulated (Keeley et al. 1983; Keeley and Busch 1984; Aulio 1985; Keeley 1999; Robe and Griffiths 2000). This is explained by higher inorganic carbon availability caused by the 10⁴ times higher diffusion rate in air compared to water. Contemporary with CAM being downregulated, *L. uniflora* also acclimates to the aerial life by traits such as low lacunal volume, high Rubisco activity and production of stomata, which enables the terrestrial

Table 1 Regulation of CAM in aquatic CAM plants

Species	Free CO ₂ ($\mu\text{mol l}^{-1}$)	Light ($\mu\text{mol m}^{-2} \text{s}^{-2}$)	Temp. (°C)	Actual CAM ($\mu\text{eq g}^{-1} \text{FW}$)	Potential CAM ($\mu\text{eq g}^{-1} \text{FW}$)	References
<i>L. uniflora</i>	60	40	15	35	65	Klavsen (Unpublished)
	60	200	15	50	125	Klavsen (Unpublished)
	100	450	15	66	87	Madsen (1987a)
	100	200	15	60	–	Boston et al. (1987)
	100	1,000	15	52	–	Boston et al. (1987)
	130	300 ^a	18–28	50	55	Baatrup-Pedersen and Madsen (1999)
	300	200	15	50	–	Boston et al. (1987)
	300	1,000	15	46	–	Boston et al. (1987)
	500	40	15	15	60	Klavsen (Unpublished)
	500	200	15	90	130	Klavsen (Unpublished)
	900	300 ^a	18–28	140	180	Baatrup-Pedersen and Madsen (1999)
	1,000	1,000	15	46	–	Boston et al. (1987)
	1,500	43	15	–4	30	Madsen (1987a)
	1,500	450	15	70	79	Madsen (1987a)
	5,500	450	15	–2	36	Madsen (1987a)
	50/1,000 ^b	50	19–20	35	–	Robe and Griffiths (1990)
	50/1,000 ^b	300	19–20	112	–	Robe and Griffiths (1990)
80/600 ^c	350	18–19	110	–	Robe and Griffiths (1994)	
20/1,000 ^c	50	18–19	60	–	Robe and Griffiths (1994)	
<i>C. helmsii</i>	3	200	20	14	70	Klavsen (Unpublished)
	20	40	15	–	83	Klavsen (Unpublished)
	20	150	15	–	109	Klavsen (Unpublished)
	22	30	20	15	23	Klavsen and Maberly (2009)
	22	150	20	30	44	Klavsen and Maberly (2009)
	22	23	20	–2	12	Klavsen and Maberly (2010)
	22	230	20	35	60	Klavsen and Maberly (2010)
	230	23	20	2	8	Klavsen and Maberly (2010)
	230	230	20	18	30	Klavsen and Maberly (2010)
	290	40	15	–	19	Klavsen (Unpublished)
	290	150	15	–	12	Klavsen (Unpublished)

Means of available data are presented. ‘–’ indicates ‘not determined’. Plants have been growing and acclimated to conditions of CO₂ and light according to the ones given in the table. Actual CAM was measured as the diurnal change in acidity under growth conditions. Potential CAM was determined as the maximum diurnal acidity change: In the daytime, plants were placed in low CO₂ (ca. atmospheric equilibrium) and high light (thereby increasing decarboxylation) and in the night, plants were incubated in a medium with high CO₂ (>500 mmol m⁻³; thereby increasing nighttime-CO₂ uptake via CAM)

^a Estimate based on an irradiance of 10–16 mol photons m⁻² day⁻¹

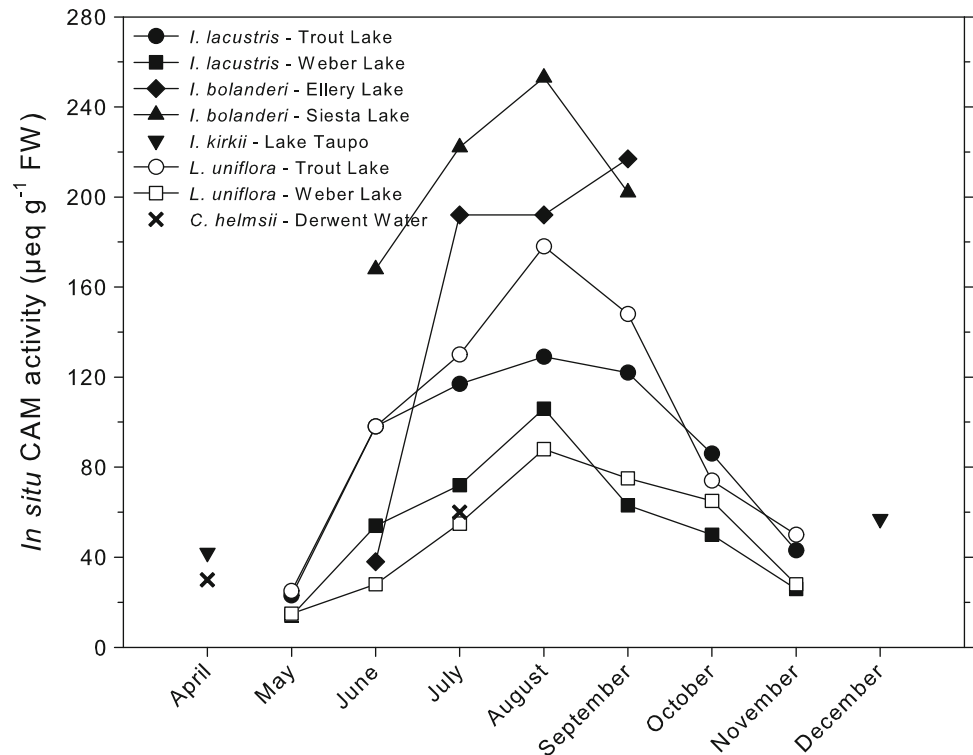
^b Plants were grown in natural sediments. The free-CO₂ concentrations of the water and interstitial water were 50 and 1,000 $\mu\text{mol l}^{-1}$, respectively

^c Plants were grown in natural sediments. The free-CO₂ concentrations of the water and interstitial water were either 20 or 80 and 600 and 1,000 $\mu\text{mol l}^{-1}$, respectively

life-form to make use of CO₂ from the air and makes the plant less dependent on CO₂ from the sediment and from CAM. However, contrary results on CAM regulation in the shift from water to land occur, because CAM is not always downregulated in the land-form (Farmer and Spence 1985; Aulio 1986) and exposure to atmospheric CO₂ per se therefore does not trigger downregulation. The factor triggering CAM regulation in the land-form may be water-

vapour concentration, thereby downregulating CAM when the water-vapour concentration is low (Aulio 1986). However, because the land-form of *L. uniflora* can still rely on sediment CO₂ and dark CO₂ uptake via CAM (Nielsen et al. 1991), the CO₂ concentration experienced by the plant may not differ from the CO₂ experienced under water—and this may be the reason for the lack of CAM downregulation.

Fig. 2 In situ CAM activity measured in the isoetids *Isoetes lacustris*, *I. bolanderi*, *I. kirkii* and *Littorella uniflora* and in the elodeid *Crassula helmsii*. Data are modified from Keeley et al. (1983), Boston and Adams (1985), Rattray et al. (1992) and Klavsen and Maberly (2009)



Short-term regulation of CAM

Light and CO₂ do not only affect the diel CAM cycle in the long term (after an acclimation period) but also in the short term and thus on a daily basis and this effect has been observed in both laboratory and field (Keeley et al. 1983; Keeley and Busch 1984; Boston and Adams 1985; Madsen 1987a; Hostrup and Wiegler 1991; Robe and Griffiths 1990; Rattray et al. 1992; Klavsen and Maberly 2010; Klavsen Unpublished). Generally, malate decarboxylation appears to be dependent on the demand for inorganic carbon relative to its supply rate during the day. Thus, it has been found that high-CO₂ availability and/or reduced light intensity, e.g. caused by an overcast sky, affect the amount of malate being decarboxylated, thereby resulting in lower decarboxylation rates—or complete inhibition of decarboxylation—and/or higher minimum acidity level at the end of the light period. Contrary, a high photosynthetic carbon demand increases the decarboxylation rate and lowers the minimum acidity level obtained in the evening (Boston and Adams 1985; Madsen 1987b; Robe and Griffiths 1990; Rattray et al. 1992; Klavsen and Maberly 2010). However, in *C. helmsii* grown under low and high CO₂, decarboxylation rates did not vary between CO₂ treatments, but the decarboxylation period was longer and the minimum acidity level lower for low-CO₂ grown plants (Klavsen and Maberly 2010). In *L. uniflora*, the rate of decarboxylation was generally high under low external-CO₂ concentration, but could be fully inhibited by high CO₂ (Madsen 1987c). This indicates that CAM in *L. uniflora*

operates under most natural CO₂ conditions, although the long-term regulation of CAM, e.g. due to seasonal changes, will affect the actual CAM activity (Boston and Adams 1985; Klavsen and Maberly 2009).

Light not only affects decarboxylation but also affects photosynthesis and eventually the pool of starch being synthesised during the day. In the night, starch is broken down in glycolysis and serves as the precursor for phosphoenol pyruvate (PEP)—the acceptor molecule for nighttime fixation of inorganic carbon via PEPcase (see Fig. 1; Kluge and Ting 1978; Osmond 1978; Winter and Smith 1996). Thus, the light intensity the previous day can potentially have implications for malate (and thereby acidity) accumulation in the night. This indirect effect of light on CAM has been observed in *C. helmsii*, where high concentration of CO₂ only had significant effect on the acidity buildup in the night after exposure to high daytime light intensity (Klavsen and Maberly 2010). It should be noted that in *I. bolanderi*, the starch pool is not always sufficient to account for the malate buildup in the night (Keeley et al. 1983), indicating a role for another carbohydrate precursor molecule or alternatively that starch production occurs from other carbohydrates simultaneously with starch breakdown.

Decarboxylation and O₂:CO₂ ratios

The regulatory pattern of CAM indicates that CAM functions as a CCM in freshwater macrophytes. However, for

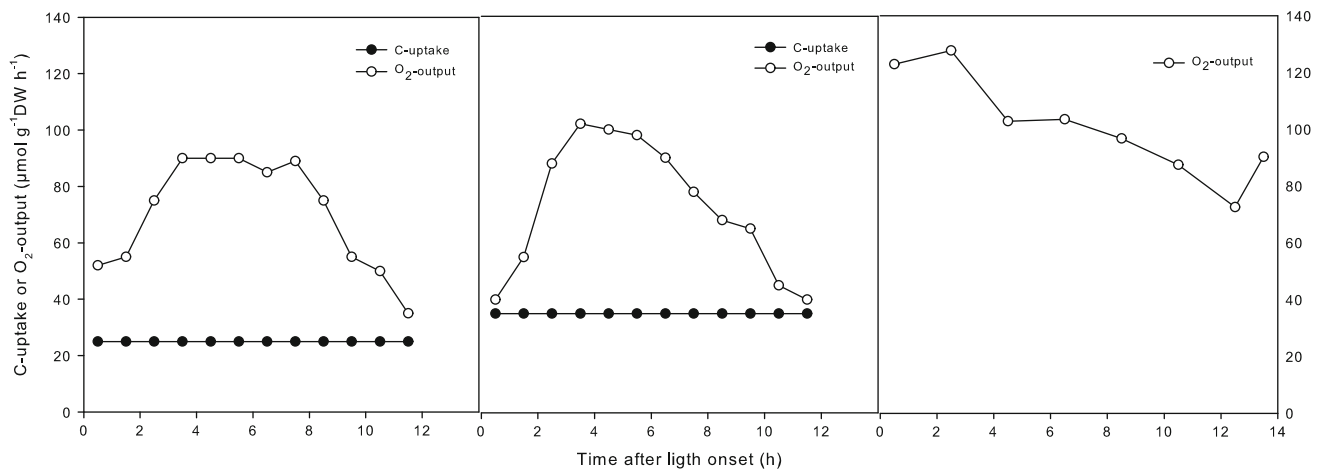


Fig. 3 Rates of inorganic carbon uptake and oxygen evolution in the isoetids *Littorella uniflora* (left panel) and *Isoetes lacustris* (middle panel) and oxygen evolution in the elodeid *Crassula helmsii* (right panel). *Crassula helmsii* was grown and photosynthesis measured at low CO_2 (22 mmol m^{-3}), but decarboxylation did not start until after

2 h after light onset. High CAM activity results in high $\text{O}_2:\text{CO}_2$ ratios (*L. uniflora* and *I. lacustris*), if external inorganic carbon uptake is not inhibited by decarboxylation. Data modified from Madsen (1987b) and Klavsen and Maberly (2010)

CAM to act as an effective CCM, the photosynthetic rate should at least balance the rate of decarboxylation, because CO_2 derived from CAM could otherwise be lost. In *L. uniflora*, this was verified experimentally because less than 2% of the CO_2 resulting from daytime decarboxylation was lost (Smith et al. 1985; Madsen 1987b) and because the photosynthetic rate exceeds the decarboxylation rate in both *L. uniflora* and *C. helmsii* (Klavsen and Madsen 2008; Klavsen and Maberly 2009). In agreement with this, photosynthesis and CAM have been shown to be positively coupled in *L. uniflora* (Klavsen and Madsen 2008).

For CAM to operate efficiently as a CCM, and thus for decarboxylation to influence the rate of photosynthesis positively, it would be anticipated that the O_2 evolution relative to the external- CO_2 uptake (and thus the $\text{O}_2:\text{CO}_2$ ratio) will be well above 1 during the decarboxylation phase. This was found for *L. uniflora* and *I. lacustris* (Madsen 1987b), where the $\text{O}_2:\text{CO}_2$ ratio was up to 3.5 during decarboxylation (Fig. 3). If the oxygen evolution does not increase considerably and thus give rise to $\text{O}_2:\text{CO}_2$ ratio above 1 during decarboxylation this may be because either (1) external CO_2 is so high that decarboxylation is inhibited or (2) the high internal CO_2 obtained during decarboxylation inhibits external- CO_2 uptake. This implies that the CCM is working less efficiently and external CO_2 will not be taken up 24 h a day, thereby minimising C-gain. For *C. helmsii*, no considerable change in oxygen evolution was observed during decarboxylation (Fig. 3). This may question the concept of CAM as a CCM in this species. However, because decarboxylation appears to be delayed in *C. helmsii*, maybe due to a circadian rhythm or daytime C_4 activity, the plant may benefit from CAM, because decarboxylation occurs around midday, where the

inorganic carbon demand is likely to be greatest (Klavsen and Maberly 2010). Furthermore, CAM may help conserve carbon, because respiratory CO_2 can be re-captured in the night.

CAM in relation to C-gain

For CAM to be of ecological significance as a carbon-conserving mechanism, CAM must first of all be present in the field. Although the in situ CAM activity is dependent on long-term (e.g. season) and short-term regulation (e.g. day-to-day changes in, for example, irradiance), significant in situ CAM activities have been found in several aquatic CAM species (Fig. 2; (Keeley et al. 1983; Boston and Adams 1985; Rattray et al. 1992; Klavsen and Maberly 2009). In addition to CAM being present under natural conditions, CAM must contribute considerably to the carbon gain to act as a carbon-conserving mechanism. For *L. uniflora*, CAM undoubtedly contributes in a net positive carbon gain, because decarboxylation does not inhibit the external inorganic carbon uptake (resulting in large $\text{O}_2:\text{CO}_2$ ratios [Fig. 3]). Due to the plasticity of CAM, the influence of nighttime- CO_2 uptake on daily CO_2 uptake in photosynthesis can vary significantly depending on the environmental conditions. Thus, the contribution from CO_2 derived from CAM to daily photosynthesis varies from 0 to 95%. The latter estimate of the contribution from CAM was found for *L. uniflora* and *I. lacustris* at an external- CO_2 concentration of $30 \mu\text{mol l}^{-1}$. At higher external- CO_2 concentrations, the nighttime- CO_2 uptake via CAM in relation to daily photosynthetic carbon uptake was reduced to 34–38% (Madsen 1987b) due to higher uptake of

external CO₂ and potentially partial inhibition of decarboxylation. In *L. uniflora* grown at low light, the contribution from CAM-derived CO₂ to photosynthesis was high (62%), but lower than in plants grown at high light (81%) (Robe and Griffiths 1990). Also in *I. howellii*, ca. 30–50% of daily CO₂ uptake in photosynthesis was estimated to derive from nighttime uptake through CAM (Keeley and Busch 1984). Another estimate of the contribution from CAM to the carbon budget was made on *L. uniflora* in which 40–55% of the annual carbon gain derived from CAM (Boston and Adams 1985, 1986).

For the elodeid *C. helmsii*, however, no oxygen peak is observed during decarboxylation (Fig. 3) and thus the benefit from CAM is in principle lost. However, CAM may still be favourable to the C-gain of the plant, if the external-CO₂ concentration is low. In *C. helmsii*, the in situ contribution from CAM to daily photosynthesis varied from 18 to 42%, depending on depth of growth and time of year (Klavnsen and Maberly 2009). Most likely these estimates are valid as contributions from CAM to the daily carbon balance, because almost all respiratory CO₂ in the night was refixed via CAM and because roots make up a very small part of the total plant biomass in this species.

Thus, in natural populations of freshwater CAM species, CAM appears to be of high ecophysiological significance for the carbon balance. These estimates are in agreement with estimates for terrestrial facultative CAM plants in which 10 to nearly 100% of the carbon fixation in daily photosynthesis derive from CAM (Winter and Holtum 2002; Lüttge 2004).

Night time CO₂ uptake

CAM potentially enables the plants to take up inorganic carbon 24 h a day, although this is probably not realised in all species (Keeley 1998b; Madsen et al. 2002; Klavnsen and Maberly 2010). Even though external CO₂ is not taken up at night, CAM can still be considered a carbon-conserving mechanism, because re-capture of respiratory endogenous produced CO₂ through the operation of CAM can reduce or eliminate C-loss in the night and thereby influence C-gain positively (Keeley and Busch 1984; Madsen 1987c; Robe and Griffiths 1990; Madsen et al. 2002). The contribution of re-captured respiratory CO₂, otherwise lost to the surroundings, to the total-CO₂ uptake via CAM is dependent on the external-CO₂ concentration, but often makes up a substantial part of the nighttime inorganic carbon fixation. For *L. uniflora*, between 30 and 99% of nighttime-CO₂ uptake via CAM derives from CO₂ produced in respiration (Richardson et al. 1984; Smith et al. 1985; Madsen 1987b, c; Boston et al. 1987; Robe and Griffiths 1990) and for *I. howellii*, values of 50–66% have been found (Keeley and

Busch 1984). Because respiratory CO₂ under natural conditions rarely makes up the total nighttime-CO₂ uptake, this implies that CO₂ uptake through CAM is at least partly dependent on the external-CO₂ availability, which potentially can lead to inorganic carbon limitation at night (Klavnsen and Maberly 2010). However, the length of the night period—although not realised under field conditions—can compensate for low external-CO₂ availability (Keeley and Bowes 1982; Madsen et al. 2002). Thus, plants relying on CO₂ primarily derived from endogenous sources can reach the same maximum CAM activity as plants incubated in a high-CO₂ medium. Respiratory CO₂ can potentially make up the entire nighttime-carbon uptake through CAM under low external CO₂ in both *C. helmsii* and *L. uniflora*, because the rate of respiration can exceed the rate of CO₂ uptake through CAM (assuming a constant CO₂ uptake in CAM, a constant respiratory rate and a respiratory quotient of 1; Boston et al. 1987; Klavnsen and Maberly 2010).

Conclusions

CAM is found in aquatic plants belonging to both the functional group of isoetids and elodeids. In both types of CAM plants, CAM is regulated in relation to environmental cues—in agreement with CAM functioning as a CCM in aquatic plants. For both isoetid CAM-species (*Isoetes spp.* and *L. uniflora*) and the elodeid *C. helmsii*, CAM appears to be of high ecological importance, because inorganic carbon uptake via CAM contributes significantly to the carbon budget. For *C. helmsii*—but not the isoetid CAM plants—external inorganic carbon uptake seems to be inhibited by decarboxylation, which will lower the significance of CAM. However, CAM may still help conserve carbon, because respiratory CO₂ loss can be eliminated by re-fixation through PEPcase in the night. Furthermore, for *C. helmsii*, CAM may be beneficial when the external concentration of CO₂ in the water is low.

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