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REVIEW

Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms

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Abstract Minimum energy (as photon) costs are predicted for core reactions of photosynthesis, for photorespiratory metabolism in algae lacking CO_2 concentrating mechanisms (CCMs) and for various types of CCMs; in algae, with CCMs; allowance was made for leakage of CO_2 from the internal pool. These predicted values are just compatible with the minimum measured photon costs of photosynthesis in microalgae and macroalgae lacking or expressing CCMs. More energy-expensive photorespiration, for example for organisms using Rubiscos with lower CO_2–O_2 selectivity coefficients, would be less readily accommodated within the lowest measured photon costs of photosynthesis by algae lacking CCMs. The same applies to the cases of CCMs with higher energy costs of active transport of protons or inorganic carbon species, or greater allowance for significant leakage from the accumulated intracellular pool of CO_2. High energetic efficiency can involve a higher concentration of catalyst to achieve a given rate of reaction, adding to the resource costs of growth. There are no obvious mechanistic interpretations of the occurrence of CCMs algae adapted to low light and low temperatures using the rationales adopted for the occurrence of C_4 photosynthesis in terrestrial flowering plants. There is an exception for cyanobacteria with low-selectivity Form IA or IB Rubiscos, and those dinoflagellates with low-selectivity Form II Rubiscos, for which very few natural environments have high enough CO_2:O_2 ratios to allow photosynthesis in the absence of CCMs.

Keywords Carbon dioxide concentrating mechanisms · Environmental change · Nutrient use efficiency · Photosynthetically active radiation

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Introduction

This paper reviews the energetics of aquatic organisms with particular reference to differences between inorganic carbon acquisition by CO_2 diffusion and of CO_2 concentrating mechanisms (CCMs). Although energetic economy may not be the major selective factor favouring CCMs rather than diffusive CO_2 entry to ribulose bisphosphate carboxylase–oxygenase (Rubisco), it is highly relevant to the ecology of photosynthetic organisms, which may live in variable and often growth rate limiting fluxes of photosynthetically active radiation (PAR). Earlier analyses of the energy costs of CCMs and of diffusive CO_2 entry in cyanobacteria and algae include those of Raven (1984, 1991), Raven and Lucas (1985), Raven et al. (2000), Giordano et al. (2005), Hopkinson et al. (2011) and Raven et al. (2011, 2012). Analyses have also been made for CCMs in terrestrial C_4 plants (Sage and Zhu 2011; Sage et al. 2012; Sage 2013).

The paper starts with the background to assessing the costs of processes in organisms in general and of inorganic carbon supply in photosynthesis in particular, emphasising the importance of considering the resources, including energy, invested in the catalytic machinery as well as the energy costs of operating the machinery. A saving in operating costs by using one mechanism rather than another may be less obvious when the energy costs of producing the machinery are considered. This is particularly important for rapidly growing and (in nature) relatively short-lived microalgae. The next point considered is the minimum energy cost, based on known mechanisms of photosynthetic energy transduction and of either diffusive CO_2 entry, or CCMs based on active transport of one or more inorganic carbon species or protons, or on diffusive CO_2 entry to C_4 photosynthetic biochemistry. The energetic significance of leakage of carbon dioxide from the accumulated carbon dioxide pool for the overall energy requirements for CCMs is emphasised, since this energy cost has no parallel in diffusive carbon dioxide entry to Rubisco. These values are then compared with observed values for the cost in mol absorbed PAR photons per mol carbon assimilated in gross photosynthesis for algae expressing or not expressing CCMs grown, and experimented on, with air-equilibrium carbon dioxide and oxygen concentrations.

Efficiency and rate

The pursuit of energetic efficiency (energy useful to the organism as a fraction of energy input) in the growth of a photolithotrophic organism under PAR limiting conditions cannot be pursued in isolation, in natural selection. There is an inverse relationship, unfortunately poorly quantified, between efficiency and reaction rate. Higher efficiency generally means a lower rate and so, for an organism whose functioning requires a certain rate of product formation, a mechanism with a higher efficiency may well require larger quantities of catalysts, with corresponding requirements for not only energy but also carbon, nitrogen and, directly or indirectly via the RNA involved in the protein synthesis, phosphorus. (Odum and Pinkerton 1955; Raven 1984; Månsson and McGlade 1993; Patten 1993, 1995; Santillán et al. 1997; Raven 2012; Flamholz et al. 2013; see also Fabris et al. 2012). The saving in energy (and nitrogen and, possibly, phosphorus) in mechanisms of inorganic carbon acquisition that thereby needs less Rubisco per unit biomass to achieve a given rate of inorganic carbon fixation per unit biomass, assuming other catalysts do not compensate in resource use in synthesis, could be accounted for by the saving in synthesis of Rubisco (Raven 1991; Losh et al.2013; Raven 2013a, c).

Predicted photon yields for organisms with and without CCMs

Energetic stoichiometry of core photosynthetic processes

The efficiency of transfer of excitation energy from the chromophore that absorbed the photon to the reaction centre is very high (Collins et al. 2010; Hossein-Nejad et al. 2011; Hildner et al. 2011). The photon yield of primary photochemistry is one electron per absorbed photon in PSI and <0.8 electrons per absorbed photon in PSII (Falkowski and Raven 2007; Johnsen and Sakshaug 2007; Hogewoning et al. 2012); therefore, the reduction of two $NADP^+$ and production of one O_2 need at least $4 + (4/0.8) = 9$ photons (Table 1).

In non-cyclic electron transport, three protons are transported into the thylakoid lumen per electron moving from

Table 1 Stoichiometries of photons, electrons, protons and ATP in photosynthetic reactions at maximum efficiency, excluding photorespiration and CCMs considered in Table 1

Process	Value
Photon energy reaching reaction centre per photon absorbed by antenna pigments	1
Electron transferred through PSI per photon reaching PSI reaction centre	1
Electron transferred through PSII per photon reaching PSII reaction centre	0.8
Absorbed photons needed to transfer four electrons from H_2O to $2NADP^+$	9
Protons transferred from stroma/cytosol to thylakoid lumen per electron transferred from H_2O to CO_2	3
Absorbed photons needed to transfer 12 protons from stroma/cytosol to thylakoid lumen in electron transferred from H_2O to CO_2	9
Protons transferred from thylakoid lumen to stroma/cytosol per ADP phosphorylated (observed value; 4.67 needed from structural biology of CF_OCF_1 ATP synthase with 14 c subunits per three ADP binding sites)	4
ADP phosphorylated per 12 protons transferred from thylakoid lumen to stroma/cytosol per nine photons in non-cyclic electron flow	3
ATP needed per nine photons in assimilating one CO_2 by the Calvin-Benson cycle at CO_2 saturation	2
NADPH needed per nine photons in assimilating one CO_2 by the Calvin-Benson cycle at CO_2 saturation	2
Protons moved per electron cycled round cyclic electron flow in PSI using one photon	4
ADP phosphorylated per electron cycled round cyclic electron flow in PSI using one photon	1

water to $NADP^+$. One proton is deposited in the thylakoid lumen per electron removed from H_2O; one proton is removed from the stroma (cytosol in cyanobacteria) per electron transferred to CO_2. Between PSII and PSI, two protons are removed from the stroma (cytosol in cyanobacteria) and two protons are deposited in the thylakoid lumen per electron moving through the PQ-cytochrome b_6f-non-haem iron complex (Falkowski and Raven 2007). Since four electrons are moved per O_2 produced, three protons per electron means that 12 protons are moved per O_2 produced; so, with nine photons absorbed per 12 protons moved, 1.33 protons are moved per photon absorbed (Falkowski and Raven 2007; Petersen et al. 2012; Pogoryelov et al. 2012) (Table 1). For cyclic electron transport, maximally 4 (or perhaps only 3.5) protons are transported to the thylakoid lumen per electron cycling round PSI, assuming that both the NAD(P)H-PQ oxidoreductase and the PQ-cytochrome b_6f-non-haem iron complexes are involved (Falkowski and Raven 2007; Wikström and Hummer 2012). Two protons (or perhaps only 1.5) are transported to the lumen for each electron passing through the NAD(P)H-PQ oxidoreductase complex, and two protons are deposited in the thylakoid lumen per electron moving through the PQ-cytochrome b_6f-non-haem iron complex. With one electron moved for each photon absorbed by PSI, not more than four protons are moved per photon absorbed (Table 1).

There is still controversy over the number of protons moving out of the thylakoid lumen per ADP phosphorylated. On the basis of the stoichiometry of *c* subunits in the CF_oCF_1 ATP synthase, 14 protons move per three ADP phosphorylated in chloroplasts (4.67 protons per ADP), and 13–15 protons move per three ADP phosphorylated in cyanobacteria (4.33–5.0 protons per ADP) (Pogoryelov et al. 2012). However, only four protons move per ADP phosphorylated in experimental tests of the stoichiometry of proton movements per ADP phosphorylated (Petersen et al. 2012), suggesting that some of the *c* subunits do not participate in each catalytic cycle. Leaking of protons through a parallel pathway in the thylakoid membrane, or slipping through the ATP synthase, would mean more protons per ADP than predicted from the *c*-subunit stoichiometry, rather than fewer.

Using the ratio of four protons per ADP phosphorylated, nine photons in non-cyclic electron flow could phosphorylate three ATP. With diffusive CO_2 entry at saturating CO_2, the photosynthetic carbon reduction cycle (PCRC) would not generate any phosphoglycolate from Rubisco oxygenase activity (but see Eisenhut et al. 2008); consequently, only three ATP and two NADPH are needed per CO_2 converted to carbohydrate (Table 1). This means that no additional ATP per NADPH needs to be synthesised by phosphorylation associated with the water–water cycle of non-cyclic electron transport, or with cyclic electron transport, to account for CO_2 fixation into carbohydrate. Assuming that four protons are moved for each electron in cyclic electron flow around PSI, a stoichiometry of four protons per ADP phosphorylated would mean one ATP produced per photon energising PSI (Table 1).

Rubisco oxygenase and the photorespiratory carbon oxidation cycle and related metabolism

Organisms lacking CCMs growing in the present atmosphere have carboxylase:oxygenase ratios that depend on the Rubisco selectivity factor for CO_2 relative to O_2. This selectivity increases with decreasing temperature in the few cases examined (Long 1999). The in vivo carboxylase:oxygenase ratio also depends on the extent of CO_2 drawdown and O_2 build-up at the Rubisco active site. The steady-state concentration of CO_2 and O_2 at the site of Rubisco activity is determined by the relative magnitudes of the diffusive conductance to CO_2 and O_2 and the biochemical conductance (Raven and Glidewell 1981). The conductances are the quotient of the steady fluxes of the solutes (mol per square metre of mesophyll area exposed to intercellular gas space per second) and the concentration difference for the solute (mol per cubic metre). For diffusion, the difference is that between the concentrations at the ends of the diffusion pathway; for the biochemistry of photosynthesis, it is the difference in the CO_2 concentration between the active site of Rubisco and the CO_2 compensation concentration. In the steady state, the diffusive flux of CO_2 is equal to the biochemical flux.

For the highest known CO_2:O_2 selectivity factor of Rubisco of 238 (Raven et al. 2000), with air-equilibrium concentrations of CO_2 and O_2 at the site of such a Rubisco, the net synthesis from CO_2 of one carbon in carbohydrate by the PCRC–PCOC (photorespiratory carbon oxidation cycle (PCOC), using glycolate dehydrogenase and glycine decarboxylase) requires 2.25 NADPH and 3.21 ATP (Raven et al. 2000, their Table A2). It is important to note that such calculations rely not only, entirely, on the selectivity factor, but also, crucially, on the values of the determinants of the selectivity factor, i.e., the half-saturation concentrations of carbon dioxide and of oxygen for the carboxylase and oxygenase functions of the Rubisco, and the substrate-saturated rates of the carboxylase and oxygenase functions of the Rubisco. In the absence of determinations of these kinetic values, they must be estimated. Accordingly, the values of energy costs given below must be regarded as illustrative rather than definitive.

With nine photons used to produce two NADPH, this net carbon fixation pathway needs 10.125 absorbed photons and generates a surplus of 0.165 ATP. If this ATP had to be produced using cyclic electron flow at a cost of 0.165

photons, the net photon cost would be 10.125−0.165 or 9.96 photons (Table 2). For the tartronate semialdehyde pathway (=glycerate pathway), the requirement is 2.25 NADH and 3.17 ATP (Raven et al. 2000; their Table A2), so the photon requirement is still 10.125 absorbed photons but with an excess of 0.205 ATP. Again, assuming that the excess ATP would be made by cyclic electron flow, the net cost of the metabolism is 10.125−0.205 or 9.92 photons (Table 2). Any other combination of assumptions about the Rubisco selectivity and the pathway of phosphoglycolate metabolism (and, especially, the excretion of glycolate) increases the photon requirement of the net conversion of 1 CO_2 to carbohydrate.

The high selectivity for CO_2 relative to O_2 (and a high affinity for CO_2) of some Rubiscos is mechanistically related to a low maximum specific reaction rate with units of mol CO_2 assimilated per unit time per unit mass of Rubisco (Tcherkez et al. 2006). This relationship means that the low running cost of the PCOC for the high-selectivity Rubisco is traded off against a high energy cost, as well as cost of synthesis of the machinery in terms of carbon and nitrogen and, through the involvement of RNA in protein synthesis, of phosphorus (Raven 2012).

Although CCMs decrease the Rubisco oxygenase activity of Rubisco relative to the carboxylase activity by increasing the carbon dioxide to oxygen ratio at the active site, there always seems to be a residual Rubisco oxygenase activity and flux through one or other of the pathways which metabolise 2-phosphoglycolate to 3-phosphoglycerate. This was elegantly shown for cyanobacteria by knocking out one or other, or both, of the pathways of 2-phosphoglycolate metabolism (Eisenhut et al. 2008). This means that there is a small carbon and energy flux through 2-phosphoglycolate and the PCOC or its equivalent (Roberts et al. 2007a, b; Raven et al. 2012), noting that gaps in the enzymology of the PCOC are slowly being filled for fully sequenced diatoms (Fabris et al. 2012).

Before turning to CCMs, with their much decreased costs related to the PCOC but with their own running and synthetic costs, an important point in Raven et al. (2000) is worth reiterating. This point is that the carbon and energy flux though the Rubisco oxygenase and the PCOC as a

Table 2 Estimates of minimum net photon yield of processes converting one mole of extracellular (bulk medium) inorganic carbon into one mol of carbon in carbohydrate, assuming no loss processes in the CCMs. Also indicated is the requirement for carboxysomes or pyrenoids

Process	Requirement of mol photons absorbed in converting one mol bulk phase inorganic carbon into one mol carbon in carbohydrate assuming no leakage of carbon dioxide in CCMs (unbracketted values) and with leakage equal to the rate of photosynthesis (CCM values in square brackets)	Involvement of carboxysomes or pyrenoids
No CCM (diffusive CO_2 flux from bulk medium to Rubisco, aided by parallel HCO_3^- flux in compartments containing carbonic anhydrase; PCOC)	9.96	Neither
No CCM (diffusive CO_2 flux from bulk medium to Rubisco, aided by parallel HCO_3^- flux in compartments containing carbonic anhydrase; Tartronate semialdehyde pathway	9.92	Neither
CCM with only energised step being proton pumping in thylakoid lumen for carbonic anhydrase-catalysed conversion of HCO_3^- to CO_2	9.25 [9.50]	Pyrenoids
CCM with only energised step being the extracellular conversion of HCO_3^- to CO_2 involving active proton efflux to produce acid zones, with or without extracellular carbonic anhydrase	9.5 (ATP from cyclic photo-phosphorylation) [10.0] 9.9 (ATP from respiration of photosynthate) [10.8]	Neither
CCM with only energised step being the conversion of CO_2 to HCO_3^- by NAD(P)H-PQ oxidoreductase	9.5 [10]	Carboxysomes
CCM with the only energised step being the influx of HCO_3^- at one membrane between the medium and Rubisco	9.5 (2 HCO_3 moved per ATP) [10] 10 (1 HCO_3^- moved per ATP) [11]	Carboxysomes (cyanobacteria); + or − pyrenoids in eukaryotes
CCM with only energised step being the ATP-requiring decarboxylation of oxaloacetate by PEPCK in C_4 photosynthesis	10 [11]	None

fraction of the carbon and energy transformed in photosynthesis is independent of the PAR level at which the organism is growing (Raven et al. 2002). Indeed, the fraction may even decrease at low irradiances for growth, if acclimation to low irradiances means a higher capacity for gas exchange relative to the achieved rate of carboxylation and downstream processes (Raven et al. 2002). The outcome is that a constant, or decreasing, fraction of the resources used in the synthesis, maintenance and running of photosynthesis that is allocated to the PCOC is independent of PAR, or decreases at low PAR. By contrast, leakage and slippage processes in CCMs decrease as a fraction of net carbon flux through the CCM and photosynthesis pathways as PAR for growth is decreased (Raven et al. 2000).

CCM: CO_2 production from HCO_3^- in the thylakoid lumen

For CCMs, the minimum photon cost is found for a mechanism based on observations on *Chlamydomonas* suggesting that the CCM involves HCO_3^- entry to the thylakoid lumen through an (as yet uncharacterised) anion channel. This HCO_3^- entry is driven by the light-powered proton pump, which generates both an electrical potential difference (inside positive) and a pH difference (inside acid) across the thylakoid membrane (Raven 1997, 2010). Equilibration of HCO_3^- and CO_2 ensues in the thylakoid lumen, in those parts of the thylakoid which penetrate into the pyrenoid, and is accelerated by catalysis via the luminal carbonic anhydrase *Cah3* (Raven 1997; Moroney and Ynalvez 2007; Raven 2010). At the low pH in the thylakoid lumen, the equilibrium HCO_3^-:CO_2 ratio is as little as 0.01 of that in the stroma. It is a diffusive flux, perhaps facilitated by selective proteinaceous pores, of CO_2 from the thylakoid lumen to Rubisco in the pyrenoid that provides the higher CO_2 at the active site of Rubisco than is found in the medium as the outcome of the CCM (Raven 1997, 2010). This would only require energization of one proton moving to the lumen. If this uses cyclic electron flow with four protons per absorbed photon, the photon requirement is 0.25 in addition to the nine photons per CO_2 converted to carbohydrate in the PCRC, at total of 9.25 photons required per CO_2 (Table 2). If the free energy difference for protons across the thylakoid membrane is 18 kJ/mol (but it may only be 15 kJ/mol, as discussed below), the maximum CO_2 concentration gradient between the lumen and the medium would be up to 1,000-fold, with a HCO_3^- concentration in the stroma equal to that in the medium. Such equality of HCO_3^- concentration in the stroma and the medium could not be achieved without further expenditure of energy, so the CO_2 concentration factor would be lower than 1,000.

The energization of HCO_3^- entry could come about from the efflux of the OH^- generated in the production of CO_2 from HCO_3^-, written above as H^+ consumption. The homeostasis of pH and charge balance requires that the OH^- is lost to the medium. If this is carried out by 1 HCO_3^- :1 OH^- antiport at both the plasmalemma and the inner chloroplast envelope membrane, even with the external pH equal to the stromal pH, the OH^- efflux could be coupled to HCO_3^- entry down a tenfold concentration gradient equivalent to 6 kJ/mol. This would require a stroma HCO_3^- concentration of 1/10 that in the medium; the maximum CO_2 concentration ratio is thus decreased to 100. There should be no effect of external pH, in air-equilibrated solutions, since a 1 pH unit decrease increases the OH^- gradient by 6 kJ/mol, while decreasing the HCO_3^- gradient by 6 kJ/mol, and vice versa for a 1 pH unit increase. It should be noted that the mechanism described here is not known to act as the sole active transport component in the CCM of any organism. *Chlamydomonas* not only has the *Cah3*-based mechanism, but also has other potential active transport steps for inorganic carbon species (Moroney and Ynalvez 2007; Raven 2010). Putative HCO_3^- transporters in *Chlamydomonas* include HLA3 at the plasmalemma and LC1A at the inner plastid envelope membrane, with a diversity of carbonic anhydrases in different compartments (Moroney and Ynalvez 2007; Spalding 2008; Raven 2010; Moroney et al. 2011; Wang et al. 2011; Meyer and Griffiths 2013). Even the operation of one of these transporters with a ATP:HCO_3^- ratio of only 0.5 and ATP production in cyclic electron transport would require an additional 0.5 photons for CO_2 assimilated, giving a total of 9.75 photons for conversion of one mol of inorganic carbon into carbohydrate.

What of the pH increase in the diffusion boundary layer during photosynthesis (Flynn et al. 2012)? Photosynthesis removes CO_2 from the medium, regardless of whether CO_2 is taken up directly or there is HCO_3^- influx with OH^- efflux. The increase of OH^- with increasing pH is marked by an increase in HCO_3^- to an extent which decreases as the pH approaches the pK_{a2} of the inorganic carbon system, which in turn decreases the CO_2 accumulation. Problems would also occur at very low external pH values when, assuming a constant pH in intracellular compartments, even an equilibrium acid zone concentration of HCO_3^- might be so low as to pose a problem for the kinetics of the transporter.

A further point can be made about this CCM with minimal energy requirements for operation: an inverse relation exists between the rate at which the reaction occurs and the energetic efficiency of the process. Here, the rate of the process catalysed by a proteinaceous integral membrane transporter, in units of mol substrate moved per unit time per unit protein mass, has an inverse relation to the

energetic efficiency of the process. In this case, there may be a greater requirement for transporter protein in the plasmalemma and the inner chloroplast envelope with an energy input of not more than 18 kJ/mol external HCO_3^- converted to CO_2 in the lumen of thylakoids penetrating the pyrenoid, than would be the case for a greater energy input (Falkowski and Raven 2007). An electrochemical potential difference across the thylakoid membrane during photosynthesis of 18 kJ/mol protons is likely to be an upper limit, during (non-photoinhibited: Raven 2011) steady-state photosynthesis. This is because, with a four proton per ATP stoichiometry of the CF_OCF_1 ATP synthase, ATP could be synthesised when the free energy of ATP hydrolysis in the stroma is 55 kJ/mol (Raven 1984; Falkowski and Raven 2007). With a proton electrochemical potential gradient of only 15 kJ/mol (Raven 2010), this could still yield a total energy input to ATP synthesis from 4 mol protons of up to 60 kJ/mol ATP.

Although the *Cah3*-dependent CCM in *Chlamydomonas* involves the pyrenoid (Table 1), it must not be assumed that all pyrenoids are associated with such a CCM, e.g., those pyrenoids with few or no thylakoid membranes penetrating them (Badger et al. 1998).

CCM: passive CO_2 entry followed by energised conversion of CO_2 to HCO_3^- and active influx of HCO_3^-

A category of CCMs used by most cyanobacteria potentially has an energy cost as low as that based on carbonic anhydrase in the thylakoid lumen considered above (Kaplan and Reinhold 1999; Shibata et al. 2002; Badger and Price 2003). Here, CO_2 enters the cells by passive diffusion across the plasma membrane involving selective protein channels; CO_2 is then converted into HCO_3^- in the cytosol, using a specific NAD(P)H–PQ oxidoreductase in the thylakoid membrane (Kaplan and Reinhold 1999; Ogawa and Kaplan 2003; Badger and Price 2003). The energised step allows the accumulation of HCO_3^- in the cytosol to higher concentrations than are found in the bulk medium. It is believed that movement of a proton from the cytosol side of the thylakoid membrane to the lumenal side during electron flow energised by light generates an alkaline pocket on the cytosol side in which one CO_2 is converted to HCO_3^-. In *Gloeobacter*, the closest living relative of the ancestral, freshwater, cyanobacterium (Blank and Sanchez-Baracaldo 2010), there are no thylakoids and the CO_2 to HCO_3^- conversion, like other photosynthetic redox and acid–base functions, occurs in patches on the plasma membrane (Rexroth et al. 2011). The hydroxyl ion consumed in the CO_2 to HCO_3^- conversion is regenerated when the HCO_3^- moves from the cytosol into a carboxysome. There a carbonic anhydrase accelerates the conversion of the HCO_3^- to CO_2, the inorganic carbon substrate of Rubisco, which also occurs in the carboxysomes.

If a single photon moves a single electron in cyclic electron flow round photosystem I, each electron moves two protons in NADH dehydrogenase for each electron, and each proton converts one CO_2 to HCO_3^-, the cost is 0.5 photons per HCO_3^- produced; if only one of the two protons converts a CO_2 to HCO_3^-, the cost is one photon per HCO_3^-. Making these assumptions, the total photon cost of photosynthesis with this CCM is 9.5 per external CO_2 converted to carbohydrate (Table 2). The CO_2 produced from HCO_3^- may leak out of carboxysomes; conversion of this CO_2 into HCO_3^- at the cytosolic surface of the thylakoid (or plasma membrane in *Gloeobacter*) costs additional photons. Assuming that the energy available from a single electron–proton coupling site in NADH dehydrogenase is 18 kJ/mol, an appropriate mechanism could yield a CO_2 concentration in the carboxysome that is up to 1,000 times that in the external medium. If, as discussed above for the thylakoid lumen *Cah3* mechanism, the energy available is only 15 kJ, there could still be an accumulation of CO_2 up to several hundred fold.

Furthermore, the non-energised CO_2 entry and energised thylakoid surface conversion of CO_2 to HCO_3^- mechanism is not known to operate alone in any cyanobacterium (Badger and Price 2003). Cyanobacteria invariably have one or more HCO_3^- pumps in the plasma membrane which are either coupled directly to ATP hydrolysis in primary active transport, or to sodium entry in secondary active transport that uses a sodium gradient ultimately generated from ATP hydrolysis, probably via active proton efflux and proton-sodium exchange (Shibata et al. 2002; Badger and Price 2003). It is important to note that sodium dependence of HCO_3^- transport does not always indicate energization by sodium-HCO_3^- co-transport, since in *Synechococcus* R2 (PCC 7942) active HCO_3^- entry is associated with hyperpolarisation of the membrane potential, indicating primary active transport presumably coupled to ATP hydrolysis (Ritchie et al. 1996). Such mechanisms would have a total photon cost of photosynthesis plus CCM of 9.5 or, more likely, 10 photons for CO_2 assimilated into carbohydrate, depending on whether each ATP used directly or indirectly in powering active HCO_3^- influx transports two, or only one, HCO_3^- (Table 2). Eukaryotic algae with CCMs may also have active HCO_3^- entry at the plasmalemma and/or the inner plastid envelope membrane (Giordano et al. 2005; Hopkinson et al. 2011).

CCM: HCO_3^- use related to localised acidic zones on the surface of the organism

A related mechanism to that discussed above for catalysed conversion of HCO_3^- to CO_2 in the thylakoid lumen is that of external bicarbonate use in photosynthesis using

localised extracellular acidification. This acidification involves the active efflux of protons, i.e., a movement of protons in the direction opposite to that predicted by the prevailing electrochemical potential gradient and thus requiring external energy input, as proposed by Walker et al. (1980) and Ferrier (1980). Walker et al. (1980) used the observation that for every unit decrease in pH: (1) the CO_2/HCO_3^- ratio at equilibrium increases by a factor of 10, and (2) the uncatalysed rate of conversion of HCO_3^- to CO_2 also increases by a factor of 10. In its simplest form, the localised acidification of the surface speeds up the conversion of HCO_3^- to the equilibrium (at that pH) concentration of CO_2. This increases the CO_2 concentration in the acid zones; some of this CO_2 could enter the cells and provide a concentration of CO_2 at the site of Rubisco activity in steady-state photosynthesis which is higher than that in the bulk medium, i.e., acts as a CCM (Walker et al. 1980). The occurrence of external carbonic anhydrase speeds up the HCO_3^- to CO_2 conversion, but cannot replace active proton efflux, which is what sets the equilibrium CO_2 to HCO_3^- ratio at a higher value than occurs in the bulk medium.

This mechanism could apply to freshwater aquatic macrophytes where large areas (one mm^2 or larger) of acidification of the surface are balanced, in terms of intracellular acid–base regulation and charge balance, by large alkaline areas (Ferrier 1980; Walker et al. 1980; Price and Badger 1985; Smith 1985; Maberly and Madsen 2002). These acid and alkaline areas are found in giant intermodal cells of ecorticate characeans such as *Chara corallina*, and on the abaxial surface of leaves of submerged aquatic flowering plant of the elodeid life-form flowering plants such as *Elodea* and *Potamogeton* (Walker et al. 1980; Price and Badger 1985; Smith 1985; Maberly and Madsen 2002). The operation of this well-investigated mechanism is inhibited by the presence of pH buffers of an appropriate pK_a to eliminate the acid and alkaline areas (Price and Badger 1985). In seagrasses and many marine macroalgae, photosynthetic use of external bicarbonate is inhibited by buffers (Hellblom et al. 2001; Beer et al. 2002; Moulin et al. 2011). By the reverse of the argument used for freshwater aquatic macrophytes, it has been argued that such bicarbonate use depends on localised acidification of the surface (Moulin et al. 2011). While the effects of pH buffers and inhibitors of active proton efflux are consistent with a role for the localised efflux of protons into the generally alkaline diffusion boundary layer in many marine macrophytes (macroalgae and seagrasses), there is clearly a need for direct investigation of the occurrence of the acidic zones using, for example, fluorescent pH indicators (Raven and Hurd 2012; Raven 2013b). In several freshwater macrophytes, e.g., ecorticate giant intermodal cells of characean green algae, and such submerged flowering plants as *Elodea* and *Potamogeton*, the occurrence of calcium carbonate deposits identifies the larger (mm^2 or more) alkaline areas, with the acid zones identified by the absorption properties of pH indicators, while quantification is best performed using pH microelectrodes. However, the use of these methods has not so far allowed the identification of acid zones on photosynthesising marine macrophytes. An important aspect of the comparison of marine and freshwater macrophytes is the effect of salinity in decreasing the pK_a values of the inorganic carbon system (Falkowski and Raven 2007). The most significant effect for the acid zone hypothesis is that the pK_{a1} value in seawater is about 0.5 units lower than in freshwater at the same temperature, so that an acid zone on a marine macrophyte must be 0.5 pH units more acid than that on a freshwater macrophyte to achieve the same CO_2:HCO_3^- ratio (Falkowski and Raven 2007).

The mechanism proposed by Walker et al. (1980) and Ferrier (1980), i.e., extracellular conversion of HCO_3^- to CO_2 in acid zones without the need for carbonic anhydrase, followed by CO_2 entry, could have acted as the original variant of acid zone HCO_3^- use, and may well persist today in some organisms. However, there is a widespread occurrence of extracellular carbonic anhydrase in organisms using acid zones (Lucas 1985; Price et al. 1985; Raven et al. 2002; Moulin et al. 2011; Raven 2013a, b, c), and there are possibilities other than the mechanism suggested by Walker et al. (1980) for entry of inorganic carbon in the acid zones (Lucas 1985; Beer et al. 2002). For the analysis of the minimum photon costs of the localised acid zone mechanism, it is assumed that the simple mechanism proposed by Walker et al. (1980) and Ferrier (1980) is appropriate.

Analysing the energy cost of the Walker et al. (1980) mechanism requires that we know the proton:ATP ratio of the plasmalemma proton pump responsible for active proton efflux. This ratio has been reported as either one or two protons per ATP hydrolysed (Raven 1984; Briskin and Reynolds-Nielman 1991; Miedena and Prins 1993). Taking the higher value of two protons per ATP, if the ATP was produced by cyclic electron transport around photosystem one then, as argued above, a maximum of one ADP could the phosphorylated per photon used in photosystem one for cyclic electron flow and proton pumping. No extra energy is consumed in moving the ATP from the stroma to the cytosol where it can be used by the plasmalemma proton ATPase, since this could be achieved by the combined activity of the 1:1 coupled triose phosphate export–phosphoglycerate import and the 1:1 coupled malate influx–oxaloacetate export (Falkowski and Raven 2007). This photon requirement of photon per ATP produced means that 0.5 photons are required to pump one proton into a surface acid zone. The total photon requirement for

photosynthesis using this mechanism of carbon dioxide accumulation is 9.5 absorbed photons per carbon dioxide entering and accumulated (Table 2).

This photon requirement is a minimal value, since some of the exported protons may not be used in converting HCO_3^- to CO_2, and some of the CO_2 produced might escape back to the medium rather than entering the cell followed by fixation by Rubisco. Such leakage could be minimised if CO_2 channels, such as are hypothesised for the cyanobacteria, were in close association with the proton ATPase and carbonic anhydrase at the outer surface of the plasmalemma. At least this mechanism, unlike the thylakoid lumen-based *Cah3* system, does not suffer from CO_2 leakage from an internal accumulated pool, since the CO_2 diffusion gradient in the case of external acid zones is directed inwards. As well as leakage there could be increased photon costs if mitochondrial ATP production (Carr and Axelsson 2008) rather than cyclic photophosphorylation supplies the ATP. We assume that oxidation of each external (cytosolic) NADH and each reduced FMN oxidises pumps six protons, and each internal NADH pumps ten protons, out of the mitochondrial matrix, and that the phosphorylation of each external (cytosolic) ADP to ATP required four protons (three for ADP phosphorylation in the matrix, one for transport of ADP and phosphate into the matrix and ATP out of the matrix). This means that oxidation of photosynthate via glycolysis and mitochondrial reactions produces five ATP per carbon oxidised. Generation of one carbon in carbohydrate by photosynthesis (ignoring the energy needed for the CCM) costs nine photons, so each ATP produced by this pathway uses 9/5 or 1, 8 photons. With a requirement of 0.5 ATP per proton transported into an extracellular acid zone, the photon cost of photosynthesis using a respiratory ATP source for the CCM is 9.9 photons (Table 2).

These minimum energy costs assume that only a mechanism such as that suggested by Walker et al. (1980), or HCO_3^-:proton co-transport (Lucas et al. 1983; Neer et al. 2002) is operating. If additional energised processes are involved (Brechignac and Lucas 1987), the energy cost will increase.

A problem for this low energy cost CCM mechanism is that of the size of organism in which it can operate. Presumably, there is a minimum surface area of organism needed for the generation and maintenance of the acid and alkaline zones. For a very small unicell, even with just one acid and one alkaline area on the surface, there are two constraints on maintaining the acid zone. One constraint is the decreasing diffusion boundary layer thickness with decreasing equivalent spherical diameter of the cells; this would increase the radial loss of protons from the acid zone to the higher-pH bulk medium (Raven 1998). The other constraint is occurrence in smaller organisms of a greater length of the junction between acid and alkaline surface zones over which tangential loss of protons from the acid zone to the alkaline zone can occur for a given area of acid zone in which active proton efflux produces the acid zone.

The CCM based on surface acidification occurs in organisms that have neither pyrenoids nor carboxysomes, i.e., *Chara, Nitella, Elodea, Potamogeton,* seagrasses and a number of marine macroalgae (Walker et al. 1980; Beer et al. 2002; Maberly and Madsen 2002; Moulin et al. 2011; Table 2). In this respect, it differs from the other two CCMs considered above, since the CCM based on HCO_3^- conversion to CO_2 in the thylakoid lumen involves pyrenoids, at least in *Chlamydomonas* (Raven 1997; Ratti et al. 2007), and the cyanobacterial CCM based on the energised conversion of CO_2 to HCO_3^- at the surface of thylakoids involves carboxysomes (Table 2). There are also CCMs in eukaryotic algae in cases where it is unlikely that extracellular acid zones are involved (Badger et al. 1998; Ratti et al. 2007).

CCMs based on C_4 photosynthetic metabolism

C_4 metabolism was discovered in terrestrial flowering plants; it comprises the sole component of the CCM of these plants (Sage and Zhu 2011; Sage et al. 2012). C_4 photosynthetic metabolism also occurs in a number of aquatic photolithotrophs, in some cases, in series with membrane-associated CCMs (Bowes et al. 2002; Maberly and Madsen 2002). The occurrence of C_4 metabolism in diatoms is still a matter of controversy (Beardall et al. 1976; Reinfelder et al. 2000; Johnston et al. 2001; Morel et al. 2002; Reinfelder et al. 2004; Granum et al. 2005; Cassar and Laws 2007; Roberts et al. 2007a, b; Kroth et al. 2008; McGinn and Morel 2008; Granum et al. 2009; Raven 2010; Reinfelder 2011; Haimovich-Dayan et al. 2013). In contrast, it is well established in the ulvophycean green alga *Udotea flabellum* (Reiskind et al. 1988; Reiskind and Bowes 1991; Koch et al.2013) and in some freshwater flowering plants and seagrasses (Holaday and Bowes 1980; Bowes et al. 2002; Maberly and Madsen 2002; Koch et al. 2013). In *Udotea*, phosphoenolpyruvate carboxykinase (PEPCK) is the carboxylase (Reiskind et al. 1988; Reiskind and Bowes 1991); PEPCK may not have a lower half-saturation concentration for carbon dioxide than do the highest-affinity Rubiscos (Chen et al. 2002); thus, C_4 photosynthesis with PEPCK as the carboxylase may not act alone as a CCM. In the submerged aquatic flowering plants *Egeria densa* and *Hydrilla verticillata*, C_4 metabolism has been demonstrated to take place concomitantly with external HCO_3^- use (Maberly and Madsen 2002).

Assuming that C_4 photosynthesis can be the sole component of a CCM in an aquatic plant, what is the photon cost of such a CCM? For the mechanism proposed by Reinfelder et al. (2000, 2004) and Morel et al. (2002),

phosphoenolpyruvate carboxylase (PEPC) is the carboxylase operating in the compartment to which external inorganic carbon is supplied, i.e., the cytosol, and PEPCK is the decarboxylase in the compartment containing Rubisco, i.e., the chloroplast or some sub-compartment thereof. We assume that oxaloacetate (the immediate product of PEPC) moves from the cytosol to the chloroplast, and PEP (phosphoenolpyruvate, the product of decarboxylation by PEPCK) moves from the chloroplast to the cytosol. We further assume that the influx of inorganic carbon into the cytosol is by diffusion of CO_2 and is converted to HCO_3^-, the inorganic carbon substrate for PEPC, by a cytosolic carbonic anhydrase, just as occurs in terrestrial C_4 plants, and that the inter-compartmental fluxes of oxaloacetate and PEP are also independent of energy input other than the diffusion gradients set up by C_4 photosynthetic biochemistry.

With these assumptions, the only energy input is one ATP per oxaloacetate decarboxylated in the chloroplast to produce CO_2, the substrate for Rubisco, and PEP, a co-substrate with HCO_3^- for PEPC. The production of this one ATP from cyclic photophosphorylation would require one photon making a total of 10 photons for overall photosynthetic fixation of one external inorganic carbon into carbohydrate (Table 2). The same photon cost occurs for the use of PEPCK as the carboxylase in the cytosol using PEP, ADP and CO_2 as substrate, and ATP and oxaloacetate as the products. The oxaloacetate is then converted into malate using malate dehydrogenase; malate then moves into the chloroplast, where it is converted to CO_2 and pyruvate using NAD-malic enzyme or NADP-malic enzyme. The CO_2 is assimilated by Rubisco, while the pyruvate moves to cytosol where it is converted to PEP using pyruvate, phosphate dikinase, using 2 ATP per PEP produced. Considering that one ATP is produced in the PEPCK carboxylase reaction, one net ATP is used to make one inorganic carbon from the medium available to Rubisco as CO_2. We have already seen that PEPCK as a carboxylase does not have a higher affinity for CO_2 than do the highest-affinity Rubiscos; it is therefore not as good a candidate as PEPC for the carboxylase of a C_4 mechanism acting as the sole energised component of a CCM. By contrast, PEPC, as used in the first C_4 CCM scheme mentioned above, has a high affinity for HCO_3^-, which translates into an equilibrium CO_2 concentration at cytosol pH values that is higher than that of the highest-affinity Rubiscos. Neither PEPCK nor PEP have O_2 as an alternative substrate as is the case for Rubisco. However, there are a number of problems with the PEPC carboxylase–PEPCK decarboxylase scheme, using only one ATP per external inorganic carbon made available as CO_2 to Rubisco, as the sole component of a CCMs in algae (Johnston et al. 2001; Granum et al. 2005; Cassar and Laws 2007; Roberts et al. 2007a, b; Kroth et al. 2008; Granum et al. 2009; Haimovich-Dayan et al. 2013). However, it appears that terrestrial flowering plant C_4 metabolism with PEPCK as the decarboxylase can operate with only one ATP per CO_2 pumped into the compartment containing Rubisco (Sage and Zhu 2011; Sage et al. 2012; Sage 2013). Furthermore, there are also problems with using the other low energy input CCMs listed in Table 2.

These predictions of the energetics of CCMs ignore leakage of carbon dioxide from the pool accumulated by the CCM. It is difficult to estimate the extent of leakage (Raven 1984; Badger et al. 1985; Raven and Lucas 1985; Giordano et al. 2005; Hopkinson et al. 2011). We assume that the leakage of carbon dioxide is equal to the rate of carbon assimilation in photosynthesis. The predicted photon costs are given in square brackets in column 2 of Table 2 for the entries involving CCMs. It would be expected that the fraction of the inorganic carbon entering the cells would, as discussed below, increase with decreasing PAR.

Measured photon yields in relation to photorespiratory and CCM activity

Many measurements of cyanobacterial and microalgal photon yields using gas exchange (not just fluorescence) have been carried out on organisms grown on high CO_2. However, some measurements were made on cultures of marine microalgae, bubbled with air, that are expressing CCMs, and experimented on without CO_2 enrichment (Welschmeyer and Lorenzen 1981; Geider et al. 1985, 1986) (Table 3). There are also data on marine macroalgae grown and experimented on with air-equilibrium carbon dioxide concentrations (Lüning and Dring 1995; Markager 1993). Lüning and Dring (1995) used marine macroalgae expressing, or not possessing, CCMs, while Markager (1993) worked on marine macroalgae which all expressed CCMs (Table 3). However, at low PAR, the leakage of carbon dioxide from the pool accumulated by the CCM is a larger fraction of the inorganic carbon transported into the cells by the CCM (e.g., Beardall 1991, Raven et al. 2000). This might favour down-regulation of the CCM and reliance on a purely diffusive supply of carbon dioxide from the medium to Rubisco, remembering that this purely diffusive carbon dioxide entry cannot co-exists with a CCM (Hepburn et al. 2011; Raven et al. 2012). Beardall (1991) showed that the cyanobacterium *Anabaena variabilis* grown at low inorganic carbon and low PAR has a decreased inorganic carbon affinity and maximum rate of the CCM than when grown at low inorganic carbon and higher PAR. However, there is little effect of low PAR on the capacity for the CCM to accumulate inorganic carbon in the cells. It could be argued that cyanobacteria (and

Table 3 Photon requirements of photosynthesis in marine algae grown, and measured, at close to present day atmospheric concentrations of carbon dioxide

Organism, conditions of growth and measurements	Quantum requirement, absorbed mol photon per mol carbon or per mol oxygen	References[a]
Thalassiosira pseudonana and six other species not marine microalgae, all with CCMs	7.79–17.2 mol absorbed photons per mol carbon assimilated in gross photosynthesis at intervals during exponential growth, using the mean of three parallel batch cultures of *Thalassiosira pseudonana.* Mean value of 10.5 mol absorbed photons absorbed photons per mol carbon assimilated in gross photosynthesis from regression of mol absorbed photons per mol carbon assimilated, with r^2=0.994. 13.4–24.9 mol absorbed photons per mol carbon assimilated in gross photosynthesis during exponential growth in three parallel cultures of six other species of marine microalgae	Welschmeyer and Lorenzen (1981)
Phaeodactylum tricornutum (with CCM) grown with nitrate as nitrogen source; values on a carbon basis are obtained by assuming a Redfield Ratio for the C:N by atoms of 6,625. Oxygen initially 20 % of air equilibrium, but this alters rates by less than 10 %	7.41 ± 1.25 (standard deviation, $n = 9$) mol absorbed photon per mol oxygen produced in gross photosynthesis; approximately 9.6 ± 1.6 on a carbon basis	Geider et al. (1985, 1986)
Two green, two brown and two red marine macroalgal species, all with CCMs; two red marine macroalgal species lacking CCMs. Photon requirement values are reported on spectral basis; values cited are those at the wavelength giving the lowest photon requirement. Action spectra were based on measurements on at least five individual algae. Absorptance of whole thalli used in photon requirement estimates were measured on single thalli (replicate absorptances where measured had 55 % confidence limits of 5–10 %). Spectral photon requirements are cited with 95 % confidence limits of 5–10 %	7.8–18.0 mol photons absorbed by the thallus per mol oxygen produced for the six species with CCMs 9.7, 10.2 mol photons absorbed by the thallus per mol oxygen produced for the two species lacking CCMs With a C:N atomic ratio of 18, the values for mol photons absorbed by the thallus per mol carbon are 8.7–20 for the algae with CCMs, and 10.8 and 11.3 for the algae lacking CCMs	Lüning and Dring (1995), Maberly (1990), Maberly et al. (1992), Johnston et al. (2001), Raven et al. (2002), Gillies et al. (2012). Atkinson and Smith (1983) for C:N atomic ratio
One green, two brown and two red marine macroalgal species, all with CCMs. Values quoted are for photons absorbed by photosynthetic pigments, not total absorption by the thallus. In the cases where 95 % confidence limits are cited it appears that 10–14 thalli were used (p. 55 of Markager 1993)	All values in mol photons absorbed by photosynthetic pigments in the thallus per mol carbon assimilated in gross photosynthesis. Values with estimates of variance range from 8.4 ± 0.7 to 9.4 ± 2.3, where the variances are 95 % confidence limits. Individual values (so no variance) range from 9.4 to 12.7	Markager (1993), Maberly (1990), Johnston et al. (2001), Maberly et al. (1992), Raven et al. (2002)

[a] References to Maberly 1990, Maberly et al. (1992), Johnston et al. (1992), Raven et al. (2002), Marconi et al. (2011) and Gillies et al. (2012) provide evidence as to the presence or absence of CCMs in the macroalgae used for quantum yield measurements by Lüning and Dring (1995) and Markager (1993)

peridinin-containing dinoflagellates) are a special case as they could not carry out net inorganic carbon assimilation in air-equilibrated medium in the absence of a CCM (Raven 1984). In the absence of extensive relevant data sets, we assume, in considering measured photon yields, that CCMs are still expressed at low PAR.

Table 2 shows values as low as 7.79–9.6 photons per carbon assimilated for two microalgae (both diatoms) examined in great detail, and 8.4 for the macroalgae expressing CCMs. Granted the quite large cited variances, and the sorts of inaccuracies inevitable in making photon yield measurements, the lowest photon requirements are

not significantly different from the predictions in Table 2 for the low energy cost CCMs, bearing in mind that the predictions may under-estimate carbon dioxide leakage and hence the photon requirement. The lowest observed photon yields of gross photosynthesis might also accommodate CCMs with higher energy costs, but not if they were very leaky and therefore needed additional energy input. The values for macroalgae lacking CCMs, again on a total adsorbed photon basis, are 11.8 and 12.4, and also not significantly different from the predictions in Table 2 for algae lacking CCMs.

Ecological implications

Terrestrial C_4 plants grow mainly in high irradiance environments, where the leakage of pumped CO_2 is small relative to the rate of pumping and assimilation, unlike at low light where CCMs are predicted to be increasingly short-circuited by leakage (Long 1999; Raven et al. 2000; Sage and Zhu 2011; Sage et al. 2012). CO_2 diffusion plus the PCOC involves a constant fraction of grow photosynthesis at low light (or even less at low light as CO_2 entry and O_2 loss become increasingly significant relative to the rate of the biochemical aspect of photosynthesis, Raven et al. 2000), unless adaptation or acclimation changes the capacity for diffusion and biochemistry in parallel. What evidence is available suggests that some aquatic photosynthetic organisms with CCMs occur in very low light habitats (Raven et al. 2000). This is not restricted to just cyanobacteria with Form IA or IB Rubiscos, or peridinin-containing dinoflagellates with Form II Rubiscos, with low CO_2 affinities and low CO_2–O_2 selectivities, which require CCMs unless very high CO_2 concentrations are supplied.

Terrestrial C_4 plants also occur mainly at higher temperatures, where the temperature-dependent CO_2–O_2 selectivity factor for their Form IB Rubiscos decreases to a value that energetically favours C_4 over C_3 plants (Long 1999; Tcherkez et al. 2006; Sage and Zhu 2011; Sage et al. 2012). However, cyanobacteria and algae with CCMs are also common in cold habitats—marine, freshwater and terrestrial (Raven 2013a). Although there are only two data sets for the temperature dependence of CO_2–O_2 selectivity for cyanobacteria and algae, the available data (Uemura et al. 1997; Haslam et al. 2005) and mechanistic modelling (Tcherkez et al. 2006) suggest that similar temperature dependence of selectivity to what is found in terrestrial plants (Form IB Rubisco) also occurs in a thermophilic red alga and four species of cryophilic diatoms (Form ID Rubiscos) (Uemura et al. 1997). Accordingly, the occurrence of CCMs in cyanobacteria and algae adapted to low temperatures (Raven 2013a) cannot be attributed to a different temperature dependence of CO_2–O_2 selectivity in Rubiscos from cyanobacteria and algae from what is found in flowering plants.

Conclusions

The predicted minimum energy (as photons) costs of photorespiratory metabolism in algae lacking CCMs, and of algae with various modes of CCM without allowance for leakage of CO_2 from the internal pool, are just compatible with the minimum photon costs of photosynthesis in microalgae and macroalgae expressing, or lacking, CCMs. The predictions of the photon cost of photosynthesis, photorespiration and CCM operation were made with optimistic assumptions about the energetic efficiency of the partial reactions of the core of photosynthesis, of photorespiration and of CCMs. This means that more energy-expensive photorespiration (e.g., with Rubiscos with lower CO_2–O_2 selectivity coefficients), more energy-expensive active transport in CCMs and CCMs with significant leakage, would be less readily accommodated by the measurements of the lowest photon costs of photosynthesis. Very high energetic efficiency may involve greater quantities of catalysts to achieve the same reaction rates, involving greater resource costs of growth. There is no obvious mechanistic interpretation of the occurrence of CCMs in cyanobacteria adapted to low light and low temperatures using the rationales adopted for the general restriction of C_4 photosynthesis in terrestrial C_4 flowering plants to well-illuminated and warmer habitats. There is an exception for cyanobacteria with low-selectivity Form IA or IB Rubiscos, and those dinoflagellates with low-selectivity Form II Rubiscos, for which very few natural environments, regardless of light or temperature conditions, have high enough CO_2:O_2 ratios to allow photosynthesis in the absence of CCMs.

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