Review

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Inorganic Carbon Acquisition and Photosynthetic Metabolism in Marine Photoautotrophs: A Summary

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Abstract: The diffusive availability of CO_2 for photosynthesis is orders of magnitude lower in water than in air. This, and the low affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) for CO_2, implies that most marine photoautotrophs (cyanobacteria, microalgae, macroalgae, and marine angiosperms or seagrasses) would be severely restricted were they to rely only on dissolved CO_2 for their photosynthetic performance. On the other hand, the ~120 times higher concentration of bicarbonate (HCO_3^-) makes this inorganic carbon (Ci) form more available for utilisation by marine photosynthesisers. The most common way in marine macrophytes to utilise HCO_3^- is to convert it to CO_2 within acidic micro-zones of diffusion boundary layers, including the cell walls, as catalysed by an outwardly acting carbonic anhydrase (CA). This would then generate an intra-chloroplastic (or for cyanobacteria intra-carboxysomal) CO_2-concentrating mechanism (CCM). Some algae (e.g. the common macroalgae *Ulva* spp.) and most cyanobacteria and microalgae feature direct HCO_3^- uptake as the most efficient CCM, while others (e.g. some red algae growing under low-light conditions) may rely on CO_2 diffusion only. We will in this contribution summarise our current understanding of photosynthetic carbon assimilation of submerged marine photoautotrophs, and in particular how their 'biophysical' CCMs differ from the 'biochemical' CCMs of terrestrial C_4 and Crassulacean acid metabolism (CAM) plants (for which there is very limited evidence in cyanobacteria, algae and seagrasses).

Keywords: marine; photoautotrophs; cyanobacteria; algae; seagrasses; inorganic carbon (Ci); bicarbonate (HCO_3^-); CO_2 concentrating mechanism (CCM)

1. Introduction

Although oceans have supported oxygenic photosynthesis of cyanobacteria for close to 3.5 billion years, and about half of earth's primary (photosynthetic) production today occurs in our oceans [1], the study of the mechanisms involved in marine photosynthesis lags far behind that of terrestrial plants, which colonized land 'only' some 400 million years ago. This is despite the fact that the discovery and elucidation of the (almost) universal pathway of CO_2 fixation and reduction, the Calvin-Benson-Bassham cycle (or the photosynthetic carbon reduction cycle, PCRC), was based on work using an, albeit freshwater, unicellular alga.

Two distinct differences between photosynthesis in terrestrial and aquatic environments are that 1) the diffusivity of solutes, including CO_2, is orders of magnitude lower in water than in air, and 2) irradiance is exponentially attenuated with depth such that the euphotic zone often extends only a few tens of metres down along a depth gradient [2]. Regarding photosynthetically active radiation (PAR), the dogma has been that net photosynthesis (photosynthetic activity corrected for mitochondrial respiration and other, minor, carbon losses) on a diel cycle is positive (and thus supports growth) down to a depth where 1% of surface irradiance remains. However, it is now apparent that there are many exceptions to this, where net photoautotrophic growth occurs at much

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lower light levels [3,4]. Marine angiosperms (seagrasses, which colonised the seas secondarily only less than 100 million years ago) must support an often-extensive rhizome and root biomass and, thus, need up to 10% of surface light in order to grow (Chapter 9 in [5]). While adaptations to various environments, including the various and varying irradiances, are a must in both terrestrial and marine photoautotrophs (see [1,2,5]), we will summarise here our current understanding of what we see as the main difference between the two groups, i.e. how the marine species in general, as well as using specific examples, cope with the scarce supply of CO_2 in seawater while also featuring a generally low CO_2-affinity of their ultimate carboxylating enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco).

2. Marine Inorganic Carbon Sources for Carbon Assimilation

When in equilibrium with today's atmospheric CO_2 concentration of ~410 ppm (or 18 μM), and rising, the marine dissolved CO_2 concentration at 20°C and a salinity of 35 is some 25% lower (a value which depends on temperature and salinity). In addition, the diffusivity of solutes (including CO_2) in water is orders of magnitude slower than in air. Therefore, and given the generally low CO_2-affinity of Rubisco in marine photoautotrophs (see below), photosynthetic rates of the latter would be severely limited were they to depend on dissolved CO_2 only. However, atmospheric CO_2 (CO_{2air}) reacts with water molecules to form carbonic acid (H_2CO_3), which dissociates to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions according to Equation 1.

$CO_{2air} \leftrightarrow CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+$ (Eqn. 1)

18 μM 14 μM CA <1 μM ~1,700 μM ~300 μM

Air │ Seawater (at pH 8.1, 20°C and a salinity of 35)

As can be seen, the ionic concentrations in equilibrium with dissolved CO_2 are strongly dependent on pH (H^+), and for most submerged marine photoautotrophs the most available Ci form (in terms of concentration) is HCO_3^-, which e.g. in today's air-equilibrated seawater pH of 8.1 is 120 times higher than that of CO_2. (CO_3^{2-} is not used in marine photosynthesis.) Secondly, the hydration of CO_2 to form H_2CO_3 and vice versa is a slow process with half-times approaching a minute. However, it becomes virtually instantaneous when catalysed by the enzyme carbonic anhydrase (CA), and the importance of this enzyme in marine photosynthetic Ci acquisition will become apparent below.

3. Assimilation of Inorganic Carbon via Rubisco

The central enzyme of carbon assimilation in cyanobacteria, eukaryotic algae, marine (seagrasses) and terrestrial vascular plants, is Rubisco, which is the first step in the PCRC for net assimilation of inorganic carbon to organic matter. Although other pathways for the conversion of CO_2 to organic matter exist, those are found in non-oxygenic autotrophic bacteria [6]. The reactions of the PCRC and differences in its regulation among cyanobacteria and eukaryotic algae have been recently discussed in [7].

Rubisco will catalyse both the carboxylation of ribulose bisphosphate, to yield 2 molecules of phosphoglycerate), and its oxygenation to yield one phosphoglycolate plus one phosphoglycerate according to Equations 2 and 3 below:

$CO_2 + H_2O +$ D-ribulose-1,5-bisphosphate → 2 X 3-phosphoglycerate (Eqn. 2)

O_2 + D-ribulose-1,5-bisphosphate → 3-phosphoglycerate + 2-phosphoglycolate (Eqn. 3)

The phosphoglycolate compound) produced by the reaction shown in Equation 3 can be processed by the reactions of photorespiration, which sees the loss of one of the carbons as CO_2, with the other carbon being recouped as glycerate. It has been shown [8] that, at least in the cyanobacterium *Synechococcus*, this is achieved through three pathways, i.e. 1) a 'conventional' PCOC involving glycolate dehydrogenase, and regeneration of glycerate via glyoxylate, glycine, serine and hydroxypyruvate, 2) the glycerate via tartronic semialdehyde, or 3) conversion of glyoxylate via

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oxalate and formate, to CO_2. Further details of photorespiration in aquatic phototrophs can be found in [9].

Since the two reactions catalysed by Rubisco are competitive, using the same active site on the enzyme, the relative rate of the two reactions in a photosynthesising cell can be given by the specificity factor S_{rel} according to Equation 4,

$$S_{rel}=[K_{0.5}(O_2)\bullet k_{cat}(CO_2)]/[K_{0.5}(CO_2)\bullet K_{cat}(O_2)] \quad \text{(Eqn. 4)}$$

where $k_{cat}(CO_2)$ = CO_2 saturated specific rate of carboxylase activity of RUBISCO (mol CO_2 mol^{-1} active sites s^{-1}), $K_{0.5}(CO_2)$ = concentration of CO_2 at which the CO_2 fixation rate is half of $k_{cat}(CO_2)$, $k_{cat}(O_2)$ = O_2 saturated specific rate of oxygenase activity of RUBISCO (mol O_2 mol^{-1} active site s^{-1}) and $K_{0.5}(O_2)$ = concentration of O_2 at which the O_2 fixation rate is half of $k_{cat}(O_2)$. The lower the selectivity factor, the higher will be the impact of oxygenation on net photosynthetic CO_2 fixation and photorespiration. For organisms with the lowest S_{rel} values, the kinetics of Rubisco do not allow for net fixation of CO_2 at air-equilibrate seawater (or freshwater), irrespective of the pathway of glycolate metabolism used in photorespiration [9].

4. Kinetics of Rubisco vs. Whole Cell Photosynthesis

4.1. Cyanobacteria and Microalgae

The forms of Rubisco vary considerably across the cyanobacterial and microalgal groups and show different kinetic properties and selectivity towards CO_2 and O_2. The general trend across all photoautotrophs, though, is that a low $K_{0.5}(CO_2)$ and a high S_{rel} are correlated with a low $k_{cat}(CO_2)$ and vice versa. Cyanobacteria tend to possess Rubiscos with high $K_{0.5}(CO_2)$ and somewhat lower selectivity factors than the eukaryotic algae, which may reflect the cyanobacterial evolutionary origins when CO_2 levels were higher and O_2 concentrations lower than the present-day values [10,11]. Affinities of Rubiscos from eukaryotic algae tend to be higher (lower $K_{0.5}(CO_2)$) and S_{rel} values higher. Values of kinetic properties of Rubiscos from a range of cyanobacteria, and microalgae are given in Table 1. This table includes values from both marine and freshwater species, but differences in values reflect more the taxonomic position of the species than their salinity environments.

We note that the values of $K_{0.5}(CO_2)$ for Rubisco are in most cases higher than the air-equilibrium CO_2 concentrations generally found in aquatic environments (10-25 μM, depending on temperature, salinity, and the presence and density of various respiring and photosynthesising organisms, see Section 7). Consequently, air-equilibrium levels of CO_2 would, all other things being equal, be limiting to carbon assimilation by cyanobacteria and microalgae. Furthermore, most cyanobacteria and microalgae show much higher affinity for Ci in whole cell photosynthesis than is exhibited by the isolated Rubisco. To cite a few examples, values for $K_{0.5}(CO_2)$ in Ci-dependent photosynthesis of cells of *Phaeodactylum tricornutum* have been reported as 0.53-4.5 μM [12,13] whereas that for Rubisco is 28-41 μM (see [14] and references therein). Similarly, while [15] report that *Chaetoceros muelleri* cells has $K_{0.5}(CO_2)$ values of ~0.26 μM in photosynthesis, [16] quote an equivalent value for Rubisco from this species of 23 μM. The marine cyanobacterium *Synechococcus* PCC7002 with Form 1Bc Rubisco has a $K_{0.5}(CO_2)$ for Rubisco of 185 μM [14] and the equivalent value for photosynthesis of ~0.7 μM [17]. There are only a few exceptions to this trend, with the most rigorous examples coming from freshwater Synurophyceae, which have evolved Rubiscos with a high affinity for CO_2 ranging from 18-42 μM [18] compared to similar values of whole cell photosynthesis of 22-45 μM.

It is clear that cyanobacteria and most microalgae have evolved mechanisms to enhance CO_2 concentrations at the active site of Rubisco such that carbon assimilation is at, or close to, CO_2-saturation at air-equilibrium levels in the surrounding medium. These CO_2-concentrating mechanisms (CCM) are discussed below in Section 5.

Table 1. Kinetic properties of Rubiscos from different cyanobacterial and microalgal groups. Note these data include values from freshwater as well as marine species. After [9,14] and references therein.

Organisms	Rubisco	$K_{0.5}$ CO_2	S_{rel}	K_{cat}

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		(mM)	(mol mol^{-1})	(mol CO_2 mol^{-1} active sites s^{-1})
b-cyanobacteria	Form IBc	200-260 *	35-56	2.6 - 11.4
a-cyanobacterium *Prochlorococcus*	Form 1Ac	750		4.7
Green algae	Form 1B	29-38	61-83	
Diatoms	Form 1D	23-68	57-116	2.1-3.7
Synurophyceae	Form 1D	18.2-41.8		
Dinoflagellates#	Form II		~37	
Rhodophyta *Porphyridium purpureum*	Form 1D	22	144	2.6
Rhodophyta *Cyanidium*	Form 1D	6.6-6.7	224-238	1.3-1.6

* A minority of reports have lower values down to 105 mM. #Kinetic data on dinoflagellate Rubiscos is limited due to the instability of their Form II Rubiscos.

4.2. Macroalgae and Seagrasses

The $K_{0.5}(CO_2)$ of Rubiscos in macroalgae varies between 30 and 85 μM. This was established for several common macroalgae from all three phyla such as the green algae *Ulva expansa*, *Ulva fasciata*, *Enteromorpha* (now *Ulva*) *linza*, the brown *Fucus gardneii*, *Padina pavonia*, *Dictyota fasciola*, and the red algae *Polyneura latissima*, *lridaea cordata,* and *Gigartina latissimi* [19–24]. In all cases, we conclude that the CO_2 concentration giving half-maximal rates of Rubisco carboxylation in marine macroalgae are higher than the marine concentration of CO_2 and, so, they require a CCM in order to perform optimal rates of photosynthesis. These $K_{0.5}(CO_2)$ values when measured under low O_2 levels were not significantly higher than when measured in air [23], indicating a more efficient carboxylation via Rubisco than in seagrasses (see below) as well as a virtual lack of photorespiration [21].

While the different $K_{0.5}(CO_2)$ values can partly be due to the red and brown macroalgae possessing the Form 1D while the green algae and seagrasses contain Form 1B Rubiscos [25], the different values for species which have the same form is less understood. However, the link between the evolutionary occurrence of various Rubiscos and the evolution of marine photoautotrophs has been reviewed by [26] and has gained much recent interest [25].

Seagrasses are marine angiosperms that colonised shallow softbottom areas some 100 million years ago. Like their terrestrial counterparts from which they evolved, they possess roots and rhizomes as well as flowers and pollination (although meadows mostly grow clonally by rhizome extensions). The photosynthetic traits of these plants have been reviewed in [27–29] and, more recently, in [25]. $K_{0.5}(CO_2)$ values for Rubiscos in seagrasses have recently been measured to be around 40 μM with values being significantly lower in the absence of O_2 [25], indicating the presence of photorespiration. These include the Mediterranean species *Posidonia oceanica* ($K_m(CO_2)$ ~45 μM), *Cymodocea nodosa* (37 μM) and the temperate *Zostera marina* (32 μM) under air-equilibrium O_2 concentrations. As is the case for macroalgae, these plants must thus also possess CCMs.

We conclude in this section that the low availability of CO_2 in seawater and the low affinity of Rubiscos for CO_2 necessitates the presence of a CCM in almost all marine photoautotrophs. As follows, we will see that such CCMs are most often based on the use of HCO_3^- which, again, is present at a much higher concentration in seawater than CO_2.

5. Inorganic Carbon Acquisition and 'Biophysical' CCMs

Measurements of the kinetics of whole-cell photosynthesis vs. CO_2 in most marine phototrophs show $K_{0.5}(CO_2)$ values that are much lower than for the isolated Rubisco. This higher affinity of intact cells/tissues implies that the CO_2 concentration at the active site of Rubisco is higher than in the bulk medium, i.e. a CCM is present. This is supported by direct measurements of internal CO_2 pools with CO_{2in} vs. CO_{2out} ratios ranging from ~2 fold in symbiotic dinoflagellates, 20-fold in the macroalga *Ulva fasciata*, to 800-900 in cyanobacteria, though there is considerable variation depending on species and

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environmental conditions (see Table 1 in [9] and references therein). There is also other, indirect, evidence for CCM activity including carbon isotope discrimination values against ^{13}C being much less negative than for isolated Rubisco, low or absent inhibition of photosynthesis by O_2 and low CO_2 compensation points in photosynthesis vs. Ci experiments.

Inorganic carbon assimilation by marine phototrophs is, in most cases, driven by the use of HCO_3^-, the main Ci source in seawater. Bicarbonate acquisition can be demonstrated in several ways. One approach, which may also be the easiest one, is to measure final pH values in pH-drift experiments [30]. In short, cell suspensions, macroalgae or seagrasses (or parts thereof) are inserted into a closed illuminated chamber in which the rise in pH is recorded as they photosynthesise. If the final pH is around 9 or higher, then this is taken as evidence for HCO_3^- use since the CO_2 concentration is extremely low at the high pH values generated in closed systems. If, on the other hand, the final pH is 8.5 or less, then HCO_3^- is not used since this ion is present but obviously does not contribute to raise the pH by photosynthesis. Thus, those (albeit few) algae and plants would be CO_2-only users. Burns and Beardall [12] used the pH dependence of $K_{0.5}CO_2$ and independence of $K_{0.5}(HCO3^-)$ to infer HCO_3^- use in a number of marine microalgae. Another way to demonstrate HCO_3^- use is to determine the ^{13}C vs. ^{12}C contents in algal and plant material vs. those of the Ci sources in the seawater. These values are expressed as $\Delta^{13}C$ ($\delta^{13}C$ if the source ^{13}C:^{12}C ratio is not determined). Isolated Rubisco shows values close to -27‰ to-30‰ but HCO_3^- use and activity of a CCM is reflected in less negative values. A summary of the use of $\delta^{13}C$ in investigations of marine photoautotrophs may be found in [31]. Isotope disequilibrium techniques as described by [32,33] can also be used to indicate HCO_3^- vs. CO_2 use. Alternatively, membrane inlet mass spectroscopy as described for marine *Synechococcus* [17] can be used to directly determine rates of uptake of CO_2 and/or HCO_3^-.

5.1. Cyanobacteria and Microalgae

Use of HCO_3^- and its role in driving CCM activity has been demonstrated in a range of cyanobacteria and microalgae, and CO_2 can also cross the plasma membrane by diffusion, in some cases aided by aquaporins [7,9,34].

In cyanobacteria there are multiple systems for active Ci entry into cells and accumulation of CO_2 within the carboxysome, with some differences in the transporters between marine and freshwater species [35]. Most of these transport systems involve direct active transport of HCO_3^- or an energised conversion of CO_2 to HCO_3^- via a NAD(P)H dehydrogenase, which effectively acts like a Ci-pump, even though direct active transport of CO_2 has not occurred ([34] and references therein, [35]). Enhanced HCO_3^- supply to the carboxysome (the site of Rubisco activity in cyanobacteria) leads to increased CO_2 levels, involving the activity of a carboxysomal CA (see [34] and references therein).

The CCMs in eukaryotic microalgae are less well elucidated than those of cyanobacteria. Nonetheless, HCO_3^- transporters (SCL4-type transporters) associated with the plasma membrane have been identified in the marine diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* [36]. Energy for this process appears to be associated with linear electron flow involving both photosystems II and I [37]; in eustigmatophyte algae respiratory derived ATP is used [38]. There is also evidence, based on observations for some species, of active transport of CO_2 and HCO_3^- and CO_2 accumulation by isolated chloroplasts [39] and there is evidence for SCL4-type transporters at the chloroplast envelope of diatoms [36,40] and a range of transporters of inorganic carbon have been postulated for all four of the plastid envelope membranes [41].

In eukaryotic microalgae (but not cyanobacteria), Ci acquisition can be modulated by an extracellular CA associated with the cell wall. This converts HCO_3^- to CO_2 in the periplasmic space, thereby increasing CO_2 concentrations locally and assisting its diffusion across the cell wall and plasma membrane. A range of other CAs are present in various compartments within the eukaryotic algal cell and are involved in maintaining the equilibrium between CO_2 and HCO_3^- [34,42].

5.2. Macroalgae and Seagrasses

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The macroalgal photosynthetic traits studied the most are for various species of *Ulva* (reviewed in [43]), and this genus will therefore be used here as an example applicable for many others that have been studied, as well as what will most probably be found in future studies. So, among other genera of red, brown and green algae, *Ulva* spp. (including what was once named *Enteromorpha*) stand out as being extreme HCO_3^- users, i.e. a macroalga in which additional CO_2 or Ci beyond the normal seawater Ci composition will not significantly increase photosynthetic rates ([44,45] and Figure 7.5 in [5]). Species of this genus can use HCO_3^- in two different ways (see below) and the Rubisco $K_{0.5}(CO_2)$ for subtropical *Ulva fasciata* was found to be 70 µM [24]. In comparison with today's open-water CO_2 concentration of 14 µM, at the salinity and temperature used, it can be ascertained that a diffusive CO_2-equilibrium could yield only ~5% of this alga's photosynthetic potential as based on its Rubisco – if assuming that the CO_2 concentration inside the photosynthesising cells would be in equilibrium with that of the seawater (which is an overestimation). Thus, like most macroalgae, *Ulva* must possess a CCM.

A spin-off from pH-drift experiments is to actively vary the pH with HCl or NaOH within an illuminated closed system containing algae or seagrasses (or, usually, parts thereof) while measuring both pH and photosynthetic rates, usually by O_2 evolution. Given the total Ci in natural seawater (~2.2 mM) and the pH, the Ci composition can then be calculated. The results of experiments using such systems showed already early on that the common macroalgal genus *Ulva* used HCO_3^- in such an efficient way that this Ci form saturated photosynthesis without any need for supplemental Ci ([45,46] and Figure 7.5 in [5]). This was not the case for seagrasses where both subtropical [47] and, later, temperate and Mediterranean [48–50] species could use HCO_3^- under normal seawater Ci conditions, but increased their photosynthetic rates, often more than doubling them, as CO_2 was released by lowering the pH to <8 (see Figure 7.12 in [5]). Our conclusion of the above-mentioned approaches is that marine macrophytes in general use HCO_3^- as their external Ci source for photosynthesis, but that seagrasses do so less efficiently and, thus, are Ci-limited in today's air-equilibrated seawater and will benefit from future increases in atmospheric and, consequently, dissolved CO_2 levels. This general conclusion was somewhat challenged recently [11] as being an underestimation of the capacity of seagrasses to photosynthesise at close to the HCO_3^- concentration of today's oceans (see also Section 7 below).

Two cautionary notes before we continue: Most experiments on photosynthetic properties of marine photoautotrophs are done in laboratories. For the macrophytes, this usually means that parts of thalli or seagrass leaves are cut into sections so as to fit into small O_2-electrode vials. Such (mis)handlings may show results that are different from those measured in situ. This was shown for the shallow-growing tropical seagrasses *Halophila ovalis* and *Cymodocea serrulata*, in which results of previous laboratory experiments showed that they were Ci-limited in the ~2.2 mM-Ci normal seawater [51,52]. However, when the measurements were done in situ on intact plants, both species were Ci-saturated [47]. Secondly, buffers have often been used in order to keep a certain pH while measuring O_2 evolution. However, it was shown that TRIS buffer itself lowered photosynthetic rates even at normal seawater pH and Ci conditions [53]. This led to the conclusion that e.g. the common temperate seagrass *Zostera marina* was highly dependent on active trans-membrane pumps producing protons (H^+) toward the DBL which, when using buffers, were neutralised.

As mentioned before, the most common way for marine photoautotrophs to utilise HCO_3^- is to convert it to CO_2 within their diffusion boundary layers (DBL, including the cell walls) as catalysed by membrane-bound, extracellularly acting, CA (Figure 2 in [43]). If the pH within the DBL where CA activity is present is lower than that of the surrounding seawater (e.g. in acid zones [29], then this will account for an efficient conversion of HCO_3^- to high concentrations of CO_2 that can diffuse through the plasma membrane into the photosynthesising cells. This way of Ci utilisation in macroalgae and, especially, in most seagrasses, is today common knowledge that-has been detailed in e.g. Chapter 7 in textbook [5] and reviews [11,27–29]. The easiest way to detect this form of HCO_3^- use is to add the membrane-impermeable CA inhibitor acetazolamide (AZ) while measuring

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photosynthesis; if rates cease, then this is a strong indication of this mechanism being used. If not, then either another way to use HCO_3^- (see the following) is in effect, or CO_2 only (Section 6) is used.

The most efficient "other way" to use HCO_3^- from seawater is by its direct uptake (Figure 3 in [43]). Being an ion, HCO_3^- cannot easily diffuse through plasma membranes and, so, needs to be transported. For subtropical *Ulva* sp. it was found that photosynthesis was fully inhibited by 4,4'-diisothiocyanostilbene-2,2'disulphonate (DIDS) [54] which, till then, had been used as a classical inhibitor of HCO_3^- transport in red blood cells (RBC) via a membrane-bound anion exchange (AE) protein. It was further found that the AE from *Ulva* and from RBCs shared very similar properties [54,55] with the main difference that RBCs exchanged HCO_3^- for chloride (Cl^-) while *Ulva* exchanged it for hydroxyl ions (OH^-) [56]. Interestingly, when AZ-sensitive temperate *Ulva lactuca* was exposed to pH 9.8 for some 10 h, it converted to a direct, DIDS-sensitive, HCO_3^--uptake alga similar to those *Ulva* spp. growing in subtropical waters of the Mediterranean [57]. When in this state, *Ulva* had a 10 times higher affinity for HCO_3^- than the one growing in temperate waters. It was concluded that, ultimately, the pH near the plasma membrane was the trigger for the alternative ways in which subtropical *Ulva fasciata* and temperate *Ulva lactuca* used HCO_3^-. The addition of DIDS was subsequently used by several researchers as a way to indicate direct uptake of HCO_3^- so, besides *Ulva*, *Chaetomorpha melagonium* [58], *Macrocystis pyrifera* [59] and *Posidonia oceanica* [60] could also transport HCO_3^- into their photosynthesising cells.

There is, of course, an energy cost for CCMs [61] which obviously is met by the irradiances where CCM-requiring algae and seagrasses grow. At lower light (at depts) and in colder waters, some macroalgae may not need CCMs and can, like terrestrial C_3 plants, use the, albeit slow, diffusional supply of CO_2 for their lower photosynthetic rates. These algae include several rhodophytes [62], but they still represent an exception among macroalgae which, again, use HCO_3^-. The same goes for algae growing in subarctic or Arctic areas [63] where photosynthetic rates are restricted by low temperatures (see however a report on two polar red algae being HCO_3^- users and possessing CCMs [64].

Several parameters found in macroalgae form strong evidence that they a) use HCO_3^- as their external Ci source under today's marine Ci composition [30], and b) that they possess CCMs. These include low CO_2-compensation points for many macroalgae [30,65] including the common species *Enteromorpha* (now *Ulva*) *intestinalis*, *Ulva lactuca*, *Porphyra umbicalis*, *Palmaria* (then *Rhodymenia*) *palmata*), *Fucus serratus*, and *Pelvetia canaliculata*; in all cases were the CO_2 compensation concentrations well below ~10 µM, showing that virtually no CO_2 is leaked outward even at low CO_2 concentrations and, thus, indicating an efficient Ci utilisation system (a CCM) that keeps all CO_2 within the photosynthesising cells to be used by photosynthesis. There is, however, a lack of direct evidence that intracellular CO_2 concentrations surrounding Rubisco are higher than that of the surrounding seawater. As far as we know, such estimates are limited to, again, *Ulva fasciata* where intracellular Ci concentrations within the photosynthesising cells were found to be 220 µM [66]. With a Rubisco $K_m(CO_2)$ of 70 µM, and assuming an intracellular pH of 7.2 [67] and that ~200 µM CO_2 was present also within the chloroplasts, this meant that this alga could concentrate CO_2 so as to fully saturate its Rubisco.

A positive correlation between deep- and shallower-growing (<10 m) macrophytes (macroalgae and seagrasses), as based on $\delta^{13}C$ values and end-pH values of pH-drift experiments indicates that the deep-growing ones are less in need for CCMs (see Section 6 below).

Seagrasses in general increase their rates of photosynthesis when CO_2 is allowed to increase above air-equilibrated seawater concentrations (~14 µM today). This can be done by lowering the pH or adding Ci in a closed system [52,54], or by adding Ci above the natural seawater concentration ([11,48,49,68], see Figure 7.12 in [5] for an early example). There is a whole range of affinities to Ci in various species; some seem to be severely limited in their HCO_3^- utilisation capacity (e.g. *Thallasia testudinum*) while others are more efficient (e.g. *Halodule wrightii*, *Syringodium filiforme* [48], *Posidonia oceanica* and are less affected by additional Ci or CO_2 [49,50].

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Rubisco originally evolved when CO_2 levels were very much higher, and O_2 levels lower, than in the present day; most probably during periods when CO_2 fell to 2–16 times the present atmospheric level, depending on Rubisco kinetics [10,26]. Adaptations of Rubisco's capacities, and how it co-evolved with photoautotrophs, was recently thoroughly reviewed [11] and will not be treated further here.

There have been a few additional reviews on Ci utilisation and CCMs specifically in seagrasses [28,29,69], and, lately, [11], all of which generally agree on HCO_3^- being the main external Ci form used.

Some variations on the theme of external-CA mediated HCO_3^- use in some macroalgae and seagrasses have also been reported. One originated in 2001 from experiments in which buffers (usually TRIS) were (mis)used for keeping certain pH values within O_2 electrode systems. It was then found that the TRIS buffer itself at the natural seawater pH had a negative effect on photosynthetic rates of the seagrass *Zostera marina* [53]. This led to the assumption that H^+ release was an alternative way for this seagrass to utilise HCO_3^- by neutralising H^+ efflux, which otherwise would co-transport HCO_3^- into the photosynthesising cells [69] (see (b) in Figure 7b in [5]. Another way of using HCO_3^- was also suggested for the Indo-Pacific seagrass *Halophila stipulacea* in which both TRIS and AZ together could impede CO_2 formation and fluxes of CO_2 into the cells ((c) in Figure 7.13 in [5]). This was also found for several warmer-water species such as *Halodule wrightii*, *Halophila ovalis*, and *Cymodocea rotundata* [70]. However, most works points to the external CA-mediated conversion of HCO_3^- to CO_2 as the commonest way of Ci acquisition in seagrasses (Figure 7.7 and (a) in Figure 7.13 in [5]).

Photorespiration (the beginning stage of which is given in Equation 3) can be present in plants lacking efficient CCMs. This is true for e.g. the seagrass *Zostera marina* and, often seen as a wasteful process, can lower photosynthetic rates significantly, especially under conditions of low Ci and high O_2 levels such as generated when growing together with the highly efficient photosynthesiser *Ulva* ([71] and see Section 7). On the other hand, photorespiration can also protect the seagrass from photodamage by dissipating solar energy at high irradiances. The presence of photorespiration was indicated by a lower gross O_2 evolution rate under natural O_2 conditions than when O_2 was reduced.

6. Alternative 'Biochemical' Modes of Inorganic Carbon Utilisation in Some Marine Photoautotrophs

The CCMs described above are sometimes referred to as 'biophysical' CCMs because they involve active or facilitated transport of Ci into the photosynthesising cells. Although uncommon, there have been reports of phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate carboxykinase (PEPCK) being primary carboxylases present in micro- and macro-algae and leading to 'biochemical' CCMs similar to those found in terrestrial C_4 and CAM plants.

6.1. Cyanobacteria and Microalgae

Despite early reports of C_4-like photosynthesis in a marine diatom [72], later work [73] ascribed the observed labelling patterns and enzyme activities to high rates of anaplerotic β-carboxylation reactions, necessary to 'top up' intermediates of the TCA cycle. While single-cell C_4 photosynthesis has been more recently reported by [74,75], it is now more generally accepted that among the microalgae this is only present in the diatom *Thalassiosira weissflogii*, which shows C_3–C_4 intermediate Ci assimilation [9,76–78]. All other cyanobacteria and microalgae examined possess a standard form of the PCRC, though there are several phylogenetic variations in the regulation of the pathway (see [7] for a recent review).

6.2. Macroalgae and Seagrasses

According to early works, it was claimed that the common macroalga *Ulva* must be a C_4 alga based on its low Ci-compensation point and O_2-insensitive photosynthesis, as well as its high

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activities of PEPC and PEPCK relative to Rubisco. However, that was decided before 'biophysical' CCMs became known and accepted, and marine C_4 macroalgae were later seen as rare exceptions (e.g. we don't know of any except *Udotea flabellum* [79]. Rather, from the mid-70s, ^{14}C-pulse/^{12}C-chase experiments showed that several macroalgal species including *Ulva lactuca* [80], another *Ulva* sp. [24], and what was then called *Enteromorpha* (now *Ulva*) *compressa* [82] featured a typical C_3 incorporation pattern with phosphoglycerate (a 3-carbon compound, thus C_3-metabolism plants) formed as the first compound in the PCRC cycle (Equation 2), they were all C_3 algae. Thus, it may be that also other macroalgae previously considered as C_4 are C_3 with additional 'biophysical' CCMs. While some workers found high levels of PEPC and PEPCK in *Ulva prolifera* [83], they still agree that this species is basically of the C_3 type. Another caution: the genus *Ulva* has at least 100 separate species [84], but they are often hard to tell apart. In our experience, however, all those *Ulva* forms (or alleged species) we have investigated perform similarly in terms of photosynthetic characteristics, including the feature of switching between external CA-mediated and the direct-HCO_3^- uptake mode. Low-level CAM has also occasionally been reported for members of the Fucales (see review by [85]); however, that too is a very rare exception.

Some macroalgae can live with diffusional CO_2 acquisition only. These include some red algae [62,63] growing in low-light and low-temperature environments where, apparently, photosynthetic rates are so low as not to require a CCM. On the other hand, this would also alleviate the need for energy to drive a CCM.

We conclude from parts 5 and 6 that marine photosynthesisers in general possess CCMs that are different from the 'biochemical' ones of terrestrial C_4 and CAM plants and, rather, are 'biophysical' as based on HCO_3^- utilisation.

7. Inorganic Carbon Acquisition in Various Marine Environments

Active acquisition of Ci via CCM activity is downregulated by elevated CO_2 supply where CO_2 supply by diffusion is sufficient to meet the needs of growth. CCMs appear also to be downregulated under low light. This seems to be the case for a number of red macroalgae grown at low light [86] as well as for the freshwater cyanobacterium *Anabaena variabilis* [87] where light supply is insufficient to drive CCM activity. Energy constraints on CCM activity are also seen under conditions of P-limitation (see [88] and references therein). In the marine diatom *Chaetoceros muelleri*, external CA and CCMs were up-regulated as cell density in cultures increased to a point where uncatalyzed rates of CO_2 supply from HCO_3^- were insufficient to meet the demands of carbon fixation [15]. It has been suggested that CCMs allow increased nutrient use efficiencies under nutrient limiting conditions, but evidence for N-limitation and Fe-limitation is mixed and a detailed discussion of environmental effects on carbon acquisition and CCM regulation is provided by [88].

Regarding macrophytes, we can give three examples of how photosynthetic Ci-acquisition modes of the prolific algae *Ulva* spp. can influence other macrophytes. Firstly, the unique ability of *Ulva* spp. to switch between external-CA-mediated HCO_3^- use and the very efficient direct uptake of the ion (making the alga 10 times more efficient in HCO_3^- use than when in the external-CA mode, see Section 6) can be connected with the climates where they grow. For example, *Ulva fasciata* growing in the Eastern Mediterranean where conditions of high temperatures and high irradiances are conducive to high photosynthetic rates, its HCO_3^- exchange with OH^- [22] will generate high pH values in their DBL and in isolated surroundings, causing CO_2 concentrations to approach zero. If so, epiphytic or other algae will be unable to grow in its surroundings. However, when this *Ulva* is transferred to the temperate, lower irradiance, waters of the Swedish West Coast, it switches to the external-CA mode [58]. Secondly, *Ulva intestinalis* can generate its own high-pH surroundings by HCO_3^-/OH^- exchange in isolated rockpools where the conditions during summertime are also conducive to high photosynthetic rates. Thus, the photosynthetic mode of this alga can effectively hinder other genera from the nearby open waters, which feature external-CA mediated Ci acquisition, to grow there [89]. Thirdly, it was shown that the generally higher photosynthetic rates of *Ulva lactuca* than of the seagrass *Zostera marina* could increase the pH and, so, lower the Ci and CO_2

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and increase the O_2 concentrations to such values that they caused the seagrass to photorespire (71). Similarly, it was shown that ulvoid algae could deplete seawater conditions such that it affected the subtropical seagrass *Thalassia hemprichii* [90]. We estimate that future research will bring about more of these interactions based on photosynthetic capabilities.

8. Future Scenarios

Our planet is currently experiencing a period of rapid and intense environmental change which will result in elevated sea surface temperatures, higher CO_2 concentrations, lower ocean pH (ocean acidification, OA), and increased nutrient limitations due to enhanced stratification of the water column. All of these factors are likely to affect the physiological performance and population compositions of aquatic phototrophs. Consequences of these climate-induced changes have recently been reviewed [91].

At first sight, it would be predicted that aquatic photoautotrophs without a CCM, and thus showing a low affinity for CO_2 or HCO_3^- use in Ci acquisition, might show improved performance in the higher CO_2 environments of the future [92,93]. That said, such predictions have not always been borne out, at least in macroalgae [94] analysed reports on OA effects on a range of micro- and macroalgae and showed that in most species there was little or no influence of OA on performance, though in other cases there was a range of effects from decreases in photosynthesis and growth to large stimulatory effects. In the study of macroalgae by [95], of 55 macroalgae possessing CCMs, 21 showed no response to elevated CO_2, 15 exhibited increased growth and five had decreased growth relative to that in ambient seawater. Of the only five non-CCM species that were tested, three showed unaffected growth rates but enhanced growth in the other two species. However, it is clear that effects on growth are highly variable across taxa and not always predictable from the relationship between photosynthesis and Ci.

9. Summary

- Marine photoautotrophs in general use the 120 times higher HCO_3^- than CO_2 concentration in seawater for their photosynthetic needs;
- There are several ways in which cyanobacteria and microalgae can acquire Ci from seawater, including diffusion or active transport of CO_2. For many microalgae, however, as well as for macroalgae and seagrasses, the most common way is to convert HCO_3^- to CO_2 via membrane-bound CA activity associated with the periplasmic space. Another, more efficient way to acquire HCO_3^-, is by its direct uptake, mediated, at least in the macroalga *Ulva*, by an anion exchange protein bound to the plasma membrane;
- Because marine photosynthesisers contain Rubiscos with lower affinities for CO_2 than terrestrial C_3 plants, and given the slow diffusional supply of this Ci form in seawater, they are in need of (and typically possess) CCMs in order to partly or fully (depending on species) saturate Rubisco with CO_2 so as to optimise photosynthetic- and growth rates. Some algae, however, can under low irradiance utilise only CO_2 by diffusion;
- The 'biophysical' CCMs of marine photoautotrophs are different from the 'biochemical' CCMs of terrestrial C_4 and CAM plants as they rely on extracellular HCO_3^- supplying CO_2 to their Rubiscos;
- Photoautotrophs using C_4 and CAM pathways for inorganic carbon fixation are very rare in marine environments, but C_4 metabolism may in some cases have an anaplerotic carboxylation role;
- While many macroalgae and all seagrasses investigated in laboratory conditions require additional CO_2 to fully saturate carbon fixation, their performance in situ may be different such that they are closer to CO_2 saturation without additional CO_2 or Ci additions;

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- Responses to future changes in CO_2 levels would appear to be very species dependent and also influenced by modulation of CCM activity by other environmental conditions such as light and nutrient levels.

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