Carbon Concentrating Mechanisms in Eukaryotic Marine Phytoplankton

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Abstract

The accumulation of inorganic carbon from seawater by eukaryotic marine phytoplankton is limited by the diffusion of carbon dioxide (CO₂) in water and the dehydration kinetics of bicarbonate to CO₂ and by ribulose-1,5-bisphosphate carboxylase/oxygenase’s (RubisCO) low affinity for its inorganic carbon substrate, CO₂. Nearly all marine phytoplankton have adapted to these limitations and evolved inorganic carbon (or CO₂) concentrating mechanisms (CCMs) to support photosynthetic carbon fixation at the concentrations of CO₂ present in ocean surface waters (<10–30 µM). The biophysics and biochemistry of CCMs vary within and among the three dominant groups of eukaryotic marine phytoplankton and may involve the activity of external or intracellular carbonic anhydrase, HCO₃⁻ transport, and perhaps a C₄ carbon pump. In general, coccolithophores have low-efficiency CCMs, and diatoms and the haptophyte genus Phaeocystis have high-efficiency CCMs. Dinoflagellates appear to possess moderately efficient CCMs, which may be necessitated by the very low CO₂ affinity of their form II RubisCO. The energetic and nutrient costs of CCMs may modulate how variable CO₂ affects primary production, element composition, and species composition of phytoplankton in the ocean.
Euphotic zone: the upper layer of the ocean or other aquatic system in which photosynthetically active solar radiation is present and can support primary production; often defined as the depth at which solar radiation is attenuated to 1% of the intensity at the surface.

THE CARBON PROBLEM FOR PHYTOPLANKTON IN THE OCEAN

Compared with the macronutrients nitrogen and phosphorus, which are present in the photosynthetically active surface layers of the ocean at nanomolar concentrations ($10^{-9}$), and the essential micronutrients iron, zinc, and manganese, which are present at picomolar concentrations ($10^{-12}$), inorganic carbon is relatively abundant in surface seawater at millimolar concentrations ($10^{-3}$) and is not considered limiting to marine primary production. And yet, the distribution of inorganic carbon in the ocean resembles that of a nutrient in some respects, including surface depletion relative to the deep ocean (Millero 2006) and the drawdown of dissolved carbon dioxide (CO$_2$) during intense phytoplankton blooms (Codispoti et al. 1982, Karl et al. 1991, Murata et al. 2002). Transient patches of CO$_2$-depleted surface seawater may persist for several days to weeks, but physical exchange with the vast reservoir of dissolved inorganic carbon (DIC) in the deep sea and the much smaller reservoir of gaseous CO$_2$ in the atmosphere eventually replenishes what has been removed by the phytoplankton. These reservoirs accumulate CO$_2$ from either the decay of organic matter or the geological transfer of sedimentary carbon to the atmosphere as part of the short- and long-term carbon cycles on earth.

In seawater, DIC represents the sum of the concentrations of bicarbonate (HCO$_3^-$), carbonate (CO$_3^{2-}$), and dissolved aqueous carbon dioxide (CO$_2$aq). Marine chemists measure CO$_2$ in the ocean as the equilibrium partial pressure of gaseous CO$_2$ in seawater (pCO$_2$) and use this value to estimate air-sea CO$_2$ exchange (Takahashi et al. 2009). From a biological perspective, however, CO$_2$aq is the chemical form of inorganic carbon used by the ultimate carboxylase in cyanobacteria and all eukaryotic phytoplankton, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) (Cooper et al. 1969), and the physical form of inorganic carbon to which marine phytoplankton respond. Since pCO$_2$ and CO$_2$aq are directly related by the temperature and salinity (S)-dependent solubility of gaseous CO$_2$, they will be referred to interchangeably as CO$_2$, except when specification of the physical species is needed for clarity.

In the surface ocean, CO$_2$aq is much lower and more variable than DIC (Figure 1). Thus, although DIC in the surface ocean varies by less than 10%, CO$_2$aq varies by a factor of three. A combination of physical and biological processes set the distribution of CO$_2$aq in ocean surface waters. CO$_2$aq is generally lower in the ocean’s euphotic zone than in deeper waters as a result of photosynthetic consumption at the surface and microbial remineralization of organic matter at depth. However, CO$_2$aq is relatively high in upwelling areas, where CO$_2$-rich deep waters mix with low-CO$_2$ surface waters (polar regions, equatorial Pacific), and lower where deep water masses form (northern North Atlantic) or where vertical mixing is limited (central gyres). The solubility of gaseous CO$_2$ in water and the concentration of CO$_2$aq in equilibrium with HCO$_3^-$ are inversely related to temperature, resulting in higher concentrations of CO$_2$aq in polar waters than in the tropics and higher concentrations in winter than in summer (Figure 1).

The accumulation of inorganic carbon from seawater by marine phytoplankton is limited by the relatively slow diffusion of CO$_2$aq in water (more than 5,000 times slower than in air), the sluggish dehydration kinetics of bicarbonate to CO$_2$aq, and the electrochemical potential gradient across the cell’s plasma membrane (negative inside) that opposes the passive transport of HCO$_3^-$ (Figure 2). These physical limitations are compounded by RubisCO’s low affinity for CO$_2$aq (Badger et al. 1998) and its oxygenase (photorespiration) activity that competes with carboxylation and leads to the loss of fixed carbon and the dissipation of photochemical energy (Bowes 1989, Ogren 1994).

Four phylogenetic forms of RubisCO have been identified, but only forms I and II are present in marine phytoplankton. The form I enzyme, which is present in cyanobacteria, green algae, diatoms, haptophytes, and higher plants, consists of four dimers of large catalytic subunits...
Figure 1

Global distributions of surface ocean \( \text{CO}_2 \text{aq} \) concentrations (\( \mu \text{M} \)) for January and July of the reference year 2000. Spatially averaged (4° latitude \( \times \) 5° longitude) concentrations of \( \text{CO}_2 \text{aq} \) were determined from \( p\text{CO}_2 \) values (converted to fugacities) and solubilities reported in the Global Ocean Surface Water \( p\text{CO}_2 \) database (Takahashi et al. 2009). Note that, for July, much of the area of high \( \text{CO}_2 \text{aq} \) in the Southern Ocean south of 60°S is under ice.

The relative carboxylation and oxygenation activities of RubisCO depend on the specificity factor \((=V_{\text{maxCO}_2}K_m\text{O}_2/V_{\text{maxO}_2}K_m\text{CO}_2)\) of the enzyme and the concentrations of \( \text{CO}_2 \text{aq} \) and dissolved \( \text{O}_2 \). RubisCO specificity factors have been measured for only a few marine diatoms and only one species each of coccolithophore and dinoflagellate (Badger et al. 1998). The form II RubisCO of dinoflagellates has a significantly lower carboxylation:oxygenation specificity factor compared with the form ID RubisCOs in diatoms and coccolithophores. Assuming these specificity factors...
Figure 2
Schematic representation of the physical (CO$_2$aq diffusion), chemical (HCO$_3^-$ dehydration), and biological (affinity of RubisCO for CO$_2$aq and O$_2$) limits of carbon acquisition in a phytoplankton cell. The uncatalyzed half-time for HCO$_3^-$ dehydration at 15°C, S = 35, and pH 8.1 was estimated as ln2/k' (Wolf-Gladrow & Riebesell 1997), where k' is the overall rate constant for the hydration of CO$_2$. Substrate affinity constants (K$_m$) are for diatoms as reported in Badger et al. (1998). Abbreviations: RuBP, ribulose-1,5-bisphosphate; PGA, phosphoglycerate; GLL, glycolate.

are representative, RubisCO carboxylation:oxygenation ratios ($V_c/V_o$) for these three groups as a function of CO$_2$aq can be compared (Figure 3). At a dissolved O$_2$ concentration of 250 μM, $V_c/V_o$ ratios for diatom and coccolithophore RubisCOs are above the level (2.5) at which 50% of photosynthetic energy is lost to photorespiration for CO$_2$aq concentrations ≥6–8 μM. However,

Figure 3
RubisCO carboxylation:oxygenation activity ratios (specificity factors times [CO$_2$]:[O$_2$]) as a function of CO$_2$aq for diatoms, coccolithophores, and dinoflagellates at a dissolved O$_2$ concentration of 250 μM. Dashed line indicates the carboxylation:oxygenation ratio (2.5) at which 50% of photosynthetic energy is lost to photorespiration (Ogren 1994). RubisCO specificity factors (=$V_{\text{maxCO}_2}K_{\text{mCO}_2}/V_{\text{maxO}_2}K_{\text{mO}_2}$) from Badger et al. (1998).
REACTION-DIFFUSION KINETICS OF CO$_2$ IN A CELL’S BOUNDARY LAYER

Physical limits of the supply of CO$_{2aq}$ to the surface of a cell are set by the reaction-diffusion kinetics of dissolved inorganic carbon in water (Gavis & Ferguson 1975, Wolf-Gladrow & Riebesell 1997). The diffusion rate of CO$_2$ from the bulk solution to the surface of a sphere ($Q_D$, mol cell h$^{-1}$) is given by

$$Q_D = 14.4\pi R D (C_{CO2bulk} - C_{CO2R})$$

where $R$ is the cell radius (cm), $D$ is the diffusivity of CO$_{2aq}$ in water (cm$^2$ s$^{-1}$), $C_{CO2bulk}$ and $C_{CO2R}$ are the bulk and cell-surface concentrations of CO$_{2aq}$ (M), and $14.4 = 4 \times 3,600$ s h$^{-1} \times 10^{-3}$ L cm$^{-3}$. Assuming (a) negligible depletion of HCO$_3^-$ at the cell surface and (b) near equilibrium in the bulk phase ($dC_{CO2bulk}/dt = 0$), the rate of formation of CO$_2$ from the dehydration of HCO$_3^-$ is

$$\frac{dC_{CO2}}{dt} = (k_1 + k_4 [OH^-])(C_{CO2bulk} - C_{CO2})$$

where $k_1$ is the rate constant for the hydration of CO$_2$ by reaction with H$_2$O and $k_4$ is the rate constant for the hydration of CO$_2$ by reaction with OH$^-$. Integrating the reaction and diffusion rates of CO$_2$ supply over spherical coordinates yields the total reaction-diffusion rate at the surface of the cell (mol cell$^{-1}$ h$^{-1}$):

$$Q_T = 14.4\pi R D (R(k'/D)^{1/2} + 1)[(C_{CO2bulk} - C_{CO2})k]$$

At 2/3 of the maximum diffusion flux, i.e., $C_{CO2cell\, surface} = 1/3 C_{CO2bulk}$ (Milligan et al. 2009), the reaction-diffusion rate becomes

$$Q_T = 14.4\pi R D (R(k'/D)^{1/2} + 1)[2/3(C_{CO2bulk})]$$

The fraction of the total reaction-diffusion flux due to chemical dehydration is then $R(k'/D)^{1/2}/(R(k'/D)^{1/2} + 1)$. For surface seawater with a temperature of 15°C, $S = 35$, and pH = 8.1, $D = 1.45 \times 10^{-5}$ cm$^2$ s$^{-1}$, and $k' = 0.018$ s$^{-1}$ (Wolf-Gladrow & Riebesell 1997).

Even at a CO$_{2aq}$ concentration of 30 μM (nearly triple the current average surface-ocean level), the carboxylation:oxygenation ratio of dinoflagellate RubisCO is below the 50% energy loss level.

Concentrations of CO$_{2aq}$ in surface seawater (10–30 μM) are subsaturating with respect to the forms of RubisCO in marine phytoplankton (Badger et al. 1998), and slow reaction-diffusion kinetics of CO$_{2aq}$ (see the sidebar Reaction-Diffusion Kinetics of CO$_2$ in a Cell’s Boundary Layer, above) might result in the depletion of CO$_2$ in a phytoplankton cell’s boundary layer and inorganic carbon limitation of phytoplankton growth. Diffusion limitation of solute transport to cells from the bulk solution may be overcome by cell motility such as that characteristic of dinoflagellates (Gavis & Ferguson 1975, Munk & Riley 1952), but the extent to which cell motion relieves CO$_2$ diffusion limitation in marine phytoplankton has not been quantified.

Based on laboratory pH manipulation experiments, and extending the work of Gavis and Ferguson (Gavis & Ferguson 1975), Riebesell et al. (1993) argued that, depending on cell size and seawater pH, the supply of CO$_{2aq}$ at a cell’s surface from CO$_{2aq}$ diffusion and HCO$_3^-$ dehydration (reaction-diffusion supply) could limit photosynthesis and growth in marine phytoplankton (Riebesell et al. 1993). Theoretical relationships between cell size, bulk seawater concentration of CO$_{2aq}$, and cell carbon-specific reaction-diffusion CO$_2$ supply rate are plotted in Figure 4. Also plotted is the percent contribution of HCO$_3^-$ dehydration to the total reaction-diffusion CO$_2$ supply rate, which is less than 12% for cell radii up to 40 μm. For cells with radii smaller...
Figure 4

Reaction-diffusion supply rate of CO₂ to the surface of a cell as a function of equivalent cell radius for bulk CO₂aq concentrations ranging from 3 to 30 μM (colored lines) and percent contribution of HCO₃⁻ dehydration (gray line). CO₂ supply rates were calculated for spherical cells using Equation 4, assuming steady state at 2/3 of the maximum diffusion flux (CO₂cellsurface = 1/3CO₂bulk). Temperature (15°C), salinity (35), and pH-dependent (8.1) diffusivities and kinetic constants were calculated according to Wolf-Gladrow & Riebesell (1997). CO₂ supply rates were normalized to cell carbon assuming 20 fmol C μm⁻³.

than approximately 10 μm, CO₂ reaction-diffusion could support high specific carbon fixation rates (> 1 d⁻¹) at bulk seawater CO₂aq concentrations > 10 μM. At CO₂aq concentrations from 3 to 20 μM, reaction-diffusion-supported specific carbon fixation rates drop sharply as cell radius increases from 10 μm to 20 μm.

To overcome potential limitations of an inefficient RubisCO and maintain high rates of photosynthesis at low concentrations of CO₂, nearly all marine phytoplankton have evolved inorganic carbon (or CO₂) concentrating mechanisms (CCMs) (Giordano et al. 2005). In some species of marine phytoplankton, increased production of RubisCO at low CO₂ (Tortell et al. 2000) or the evolution of high-CO₂-affinity RubisCO (Tchernov et al. 2008) may support photosynthesis. Although the diffusion of CO₂ and RubisCO activity help cells concentrate inorganic carbon, the term CCM is commonly applied to other biophysical or biochemical means of carbon acquisition.

CCMs IN MARINE PHYTOPLANKTON

How marine autotrophs accumulate inorganic carbon for photosynthesis was not an important subject of inquiry during much of the history of oceanography. However, interest in the effects of CO₂ on marine photosynthesis began to develop in the 1930s (Raven 1970). Emerson & Green's (1934) examination of the effects of CO₂ on photosynthesis in the red macroalga Gigartina is perhaps the first evidence of bicarbonate use by a marine organism, and the effects of CO₂ on photosynthesis in marine diatoms were examined by Barker (Barker 1935). Interest in inorganic carbon accumulation by marine autotrophs increased following the discovery of the C₄ pathway of carbon assimilation (a terrestrial CO₂ concentrating mechanism) in certain grasses (Hatch & Slack 1966). Kinetic photosynthesis experiments conducted under conditions of controlled inorganic carbon concentration showed that marine eukaryotic (Beardsall & Morris 1975, Zenvirth & Kaplan 1981) and prokaryotic (Badger & Andrews 1982) phytoplankton have the ability to concentrate inorganic carbon for photosynthesis from their environment in excess of the diffusion supply.
At the same time and partly as a consequence of these studies, the notion began to emerge that the ubiquitous enzyme carbonic anhydrase (CA) plays a role in CCMs of marine phytoplankton (Litchfield & Hood 1964, Steemann Nielsen 1966). CA catalyzes the reversible dehydration of $\text{HCO}_3^{-}$ to $\text{CO}_2\text{aq}$, potentially raising the concentration of $\text{CO}_2\text{aq}$ at a phytoplankton cell’s surface or at the site of carbon fixation by RubisCO in the cell’s chloroplast. CA activity has been measured in diatoms (Morel et al. 1994, Colman & Rotatore 1995), coccolithophores (Sikes & Wheeler 1982, Rost et al. 2003), and dinoflagellates (Nimer et al. 1999, Dason et al. 2004, Lapointe et al. 2008), but the precise role of CA in the CCMs of these organisms remains unclear. Since CA requires a metal cofactor [usually zinc (Zn) but also cobalt or cadmium; Xu et al. 2008] for activity, the possibility of Zn-C colimitation was proposed (Morel et al. 1994).

CA activity alone, which can only maintain equilibrium proportions of $\text{CO}_2\text{aq}$ and $\text{HCO}_3^{-}$ as determined by cellular pH, is unlikely to provide a concentration of $\text{CO}_2\text{aq}$ at the site of carbon fixation in the chloroplast (pH 8) that would saturate RubisCO. A concentration of $\text{HCO}_3^{-}$ in excess of that outside the cell must be maintained near CA and RubisCO, as in the cyanobacterial carboxysome (Price et al. 2008).

In addition to observations of CA activity, laboratory studies showed that diverse marine phytoplankton have CCMs (Burns & Beardall 1987, Colman & Rotatore 1995, Nimer & Merrett 1996). CCMs were described for even tiny picoeukaryotic marine phytoplankton (<2 μm) in which the diffusion of CO$_2$ is not expected to limit photosynthesis (Iglesias-Rodriguez et al. 1998, Giordano et al. 2005). A critical finding in this research was that phytoplankton leak substantial CO$_2$ and DIC to seawater in a light-dependent manner (Tchernov et al. 1998, 2003), confirming that the operation of CCMs concentrates DIC inside cells in excess of seawater concentrations and that CCM activity is an active, energy-consuming process. Based on these and other studies, the presence of CCMs in marine phytoplankton is principally supported by three lines of evidence: (a) faster rates of photosynthesis or growth than reaction-diffusion supply of $\text{CO}_2\text{aq}$ to the cell’s surface, (b) increased affinity for DIC in cells acclimated to low DIC, and (c) higher internal than external concentrations of $\text{CO}_2\text{aq}$ or DIC.

An example of the first type of evidence is shown in Figure 5. Here, inorganic carbon-dependent photosynthesis in the coastal marine diatom *Thalassiosira weissflogii* acclimated to 12 μM $\text{CO}_2\text{aq}$ exceeds the rate of $\text{CO}_2$ supply by chemical reaction or diffusion. The estimated half-saturation concentrations of $\text{CO}_2\text{aq}$, with respect to photosynthesis ($K_{\text{1/2-CO}_2}$) in this and other experiments (2–5 μM), are lower than the $K_m$ of RubisCO (Rost et al. 2003, Trimborn et al. 2009). These results provide a strong indication, but not necessarily proof, of a CCM, as RubisCO could be present at sufficient levels to support maximum rates of photosynthesis while the enzyme is undersaturated with respect to $\text{CO}_2$, although this would likely require unsustainable investments in RubisCO protein. Moreover, these results demonstrate the capacity for inorganic carbon concentration at DIC levels typical of the ocean but not of the mechanism or the extent to which a CCM supports growth.

Experimental evidence supports two types of biophysical CCMs. In the first model, $\text{CO}_2$ is produced at the cell surface by the CA-catalyzed dehydration of $\text{HCO}_3^{-}$ and enters the cell by passive diffusion. In the second model, $\text{HCO}_3^{-}$ is transported across one or more cell membranes and then converted to $\text{CO}_2$ by internal CA. Biochemical models analogous to CCMs in terrestrial C$_4$ plants have also been proposed in which transported inorganic carbon is stored as an organic C$_4$ carbon compound prior to fixation by RubisCO.

Because of the resource and energy costs of building cells with specific biochemical compositions, CCMs are more efficient with respect to short-term photosynthesis than growth. Thus, $\text{CO}_2$ and DIC-specific half-saturation concentrations for photosynthesis inform our understanding
Figure 5
Dissolved inorganic carbon (DIC)-dependent photosynthesis (O₂ evolution) in the marine diatom *Thalassiosira weissflogii* acclimated to 12 μM CO₂aq (blue circles). Cells were transferred to pH-buffered, low-DIC media (350 mM sorbitol, 10 mM bicine, pH 8.1), illuminated at 450 μmol photon m⁻² s⁻¹ and brought to the DIC compensation point (DIC < 2 μM) before the addition of various amounts of NaHCO₃ (pH 8.3). DIC-dependent photosynthesis was fit to a saturation model (Hanes-Woolf linearization) with \( K_{DIC} = 210 \) μM and \( P_{\text{max}} = 1,370 \) fmol cell⁻¹ h⁻¹ (black line). Also shown is the uncatalyzed rate of CO₂ production from the dehydration of HCO₃⁻ in the bulk media (orange line), i.e., the maximum abiotic rate of CO₂ supply to the cells.

of the physiology of carbon acquisition but do not necessarily correspond to CO₂ or inorganic carbon levels that would limit growth in the environment.

Despite a growing body of laboratory evidence for CCMs in marine phytoplankton, how natural phytoplankton assemblages respond to variable CO₂ was largely unknown until the mid-1990s. Laboratory studies were often carried out at conditions very unlike the natural habitats of marine phytoplankton, with respect to DIC concentrations, which are often much lower, and cell densities, which are often much higher than those found in surface seawater. Field incubation experiments showed that primary production was significantly stimulated in oceanic surface and deep chlorophyll maximum water from 18 locations in the eastern North Atlantic in which the concentration of CO₂aq was increased from 10 to 36 μM by lowering pH (Hein & Sand-Jensen 1997); this result suggested that marine phytoplankton could be carbon-limited. The response to elevated CO₂ was variable, however, and some stations showed no response to increased CO₂, presumably because of differences in phytoplankton community composition or acclimation history. A similar study found little stimulation of growth by elevated CO₂ in coastal California or Delaware Bay diatoms incubated in seawater in which CO₂ was increased by gas equilibration (Tortell et al. 1997). (For a description of experimental CO₂ manipulation methods, see Schulz et al. 2009, Shi et al. 2009.)

Over the last decade, field experiments have demonstrated that marine diatoms and certain populations of the globally distributed genus *Phaeocystis* are capable of indirect or direct HCO₃⁻ uptake (Tortell et al. 2002, Tortell & Morel 2002, Cassar et al. 2004, Martin & Tortell 2006) and that, in general, their growth is not limited by carbon (Tortell et al. 1997, Goldman 1999). In contrast, coccolithophores and dinoflagellates may be limited by CO₂ under certain circumstances (Ratti et al. 2007, Riebesell et al. 2007).

How marine phytoplankton accumulate inorganic carbon, much less the constraints that carbon acquisition strategies place on phytoplankton ecology and marine biogeochemistry, is not entirely
Eukaryote: a single or multicellular organism in which cells have both an intracellular compartment for storing genetic material (the nucleus) and other membrane-bound compartments for carrying out cellular metabolism.
In laboratory experiments, activities of CA in diatoms are inversely related to the concentration of CO$_2$ in acclimation media (Morel et al. 1994, Burkhardt et al. 2001, McGinn & Morel 2008b), indicating a role for this enzyme in inorganic carbon accumulation. However, isotope disequilibrium studies with natural diatom assemblages suggest that extracellular CA activity is low in marine diatoms in the ocean (Martin & Tortell 2006) and may not always be related to ambient CO$_2$ (Tortell et al. 2006).

How CA might support inorganic carbon uptake depends on its intracellular location. External CA could support biophysical CCMs through the dehydration of HCO$_3^-$ to generate CO$_2$ at the cell surface. Chloroplastic CA could provide a direct supply of CO$_2$ to RubisCO from the dehydration of transported HCO$_3^-$, with cytoplasmic CA, CO$_2$ transported across the cell membrane would be hydrated to HCO$_3^-$ and effectively trapped in the cytoplasm. In this case, active transport of either CO$_2$ or HCO$_3^-$ would result in the delivery of HCO$_3^-$ to the cytoplasm. Since HCO$_3^-$ is the inorganic carbon substrate of the C$_4$ carboxylase phosphoenolpyruvate carboxylase (PEPCase), cytoplasmic CA would support a biochemical C$_4$ CCM (see below).

In the pennate marine diatom *Phaeodactylum tricornutum*, two of the nine putative CAs were localized to the chloroplast (Tanaka et al. 2005, Kitao et al. 2008). Intracellular localizations have not been established for the other seven CA genes that possess endoplasmic reticulum signal but no organelle transit peptide presequences (Montsant et al. 2005, Kroth et al. 2008). *P. tricornutum* may use a biophysical CCM analogous to that described for cyanobacteria and chlorophytes, in which HCO$_3^-$ is pumped into the cell and converted to CO$_2$ in the chloroplast, instead of (or in addition to) a biochemical CCM. None of the predicted CAs in the centric diatom *T. pseudonana* have chloroplast-targeting presequences (Armbrust et al. 2004), and at least one CA in *T. weissflogii* was reported to be localized in the cytoplasm (Morel et al. 2002).

### The C$_4$ CCM Hypothesis

The current disagreement concerning the physiology of the diatom CCM centers on the existence and relative importance of a biophysical CCM based on the transport of inorganic carbon across one or more cell membranes versus a biochemical C$_4$ CCM. Evidence supporting a C$_4$ CCM in marine diatoms was first provided by relatively high C$_4$ carboxylase activities and early $^{14}$C labeling of C$_4$ acids during photosynthesis (Beardall et al. 1976, Morris 1980). Further studies of the regulation of C$_4$ carboxylation by light and its relation to carbon isotopic fractionation, however, supported a primarily anaplerotic (replenishment of metabolic intermediates) role for C$_4$ carbon fixation in diatoms (Mortain-Bertrand et al. 1987, Descolas-Gros & Oriol 1992).

C$_4$ metabolism in marine diatoms was reexamined by Reinfelder et al. (2000, 2004), who demonstrated, working with the marine diatom *T. weissflogii*, the predominance of C$_4$ compounds in short-term $^{14}$C labeling experiments, transfer of $^{14}$C from C$_4$ to C$_i$ intermediates, regulation of PEPCase activity by CO$_2$, and a strongly negative effect of PEPCase inhibition on in vivo photosynthesis. Recently, major short-term $^{14}$C labeling (>60%) of C$_4$ metabolites was also observed in the pennate marine diatom *Haslea ostrearia* acclimated to growth-saturating, but not growth-limiting, light (Rech et al. 2008).

In the PEPCase inhibition experiments, addition of the PEPCase-specific inhibitor 3,3-dichloro-2-dihydroxyphosphinomethyl-2-propenoate (DCDP) to suspensions of *T. weissflogii* cells grown in air-equilibrated medium resulted in the complete cessation of photosynthetic O$_2$ evolution compared with uninhibited cells (Reinfelder et al. 2004). The restoration of photosynthetic O$_2$ evolution upon the addition of 150 $\mu$M CO$_2$ to DCDP-inhibited *T. weissflogii* cells confirmed that the inhibitor blocked inorganic carbon accumulation, not carbon fixation by RubisCO. The strong depression of in vivo photosynthesis by PEPCase inhibition has been
Table 1  Effect of PEPCase inhibition on in vivo photosynthesis in marine diatoms and marine chlorophytes

<table>
<thead>
<tr>
<th>Species</th>
<th>500 μM DCDP</th>
<th>20 μM quercetin&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
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<tr>
<td>Skeletonema costatum (CCMP 1332)</td>
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<tr>
<td>Thalassiosira pseudonana (CCMP 1335)</td>
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<td>67</td>
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<tr>
<td>T. weissflogii (CCMP 1336)</td>
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<td>55</td>
</tr>
<tr>
<td>Navicula incerta (CCMP 542)</td>
<td>82</td>
<td>—</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum (CCMP 632/2557)</td>
<td>50</td>
<td>62</td>
</tr>
<tr>
<td><strong>Marine chlorophytes</strong></td>
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<td>0</td>
</tr>
<tr>
<td>Dunaliella tertiolecta (CCMP 364)</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>PEPCase was inhibited by the addition of either 500 μM DCDP or 20 μM quercetin to photosynthesizing whole cells (the marine chlorophytes were treated with either 500 μM DCDP or 40 μM quercetin). Values are percent inhibition of uninhibited rates of photosynthetic O2 evolution.

<sup>b</sup>Data from McGinn & Morel 2008a.

Abbreviations: PEPCase, phosphoenolpyruvate carboxylase; DCDP, 3,3-dichloro-2-dihydroxyphosphinoylmethyl-2-propenoate.

observed in two additional centric diatoms, including *T. pseudonana*, and two pennate diatoms (Table 1). Similar results were found for *T. weissflogii* and *P. tricornutum* when PEPCase was inhibited with the flavonoid compound quercetin (McGinn & Morel 2008a). In diatoms acclimated to 12 μM CO2aq, PEPCase inhibition resulted in a 50–90% decrease in photosynthesis compared with uninhibited controls but had no effect in marine chlorophytes (Table 1). Photosynthesis in *P. tricornutum* was least sensitive to PEPC inhibition by DCDP, perhaps indicating that this species is less dependent on a C4 CCM than other diatoms. These results indicate that PEPCase is required for photosynthesis in four or five species of marine diatoms acclimated to present (low) surface-ocean concentrations of CO2 and that this strategy may be common among diatoms as a group.

The conclusion that *T. weissflogii* employs a C4 CCM has been largely corroborated (Roberts et al. 2007, Milligan et al. 2009), but evidence for a C4 CCM in *T. pseudonana* and other diatoms has been contradictory (Cassar & Laws 2007, Roberts et al. 2007, McGinn & Morel 2008a, Granum et al. 2009), and the C4 CCM hypothesis remains somewhat controversial. Differences in diatom C4 carbon fixation results may be due to different acclimation conditions or experimental protocols for in vitro C4 carboxylase [PEPCase and phosphoenolpyruvate carboxykinase (PEPCKase)] assays (Cassar & Laws 2007) and short-term 14C labeling experiments but may also reflect real physiological differences among the few diatom species that have been examined.

Based on an observed predominance of C3 labeling and minor changes in C4 gene transcript or protein levels in cells acclimated to ambient (380 ppm = 14 μM CO2aq) or low (100 ppm = 3.7 μM CO2aq) CO2, Granum et al. (2009) and Roberts et al. (2007) concluded that carbon fixed by *T. pseudonana* does not pass through a C4 intermediate and that *T. pseudonana* relies exclusively on a biophysical CCM. Working with the same strain, McGinn & Morel (2008a) measured two- to threefold higher levels of PEPCase transcripts in *T. pseudonana*, which through growth had lowered CO2aq to less than 2 μM compared with cells acclimated to high CO2aq > 40 μM. Differences in the levels and ranges of CO2 used in *T. pseudonana* cultures may partly account for observed differences in steady-state transcript levels in these studies. Interestingly,
steady-state levels of PEPCase and PEPCKase transcripts in *P. tricornutum* were relatively high and insensitive to CO₂ acclimation (McGinn & Morel 2008a), indicating constitutive transcription of these C₄ enzyme genes in this diatom. Recent short-term pH-shift experiments with *T. pseudonana* show a transient (within 20 min.), sixfold upregulation of PEPCase transcripts following an instantaneous shift from 60 μM CO₂aq to 7 μM CO₂aq by increasing pH from 7.6 to 8.5 (Kustka et al. 2008), demonstrating a rapid upregulation of PEPCase by CO₂ at the level of gene transcription.

In vitro measurements of RubisCO and C₄ carboxylase activities in field samples (Morris 1980, Smith et al. 1983, Tortell et al. 2006) and laboratory cultures of marine diatoms (Descolas-Gros & Oriol 1992, Cassar & Laws 2007, Trimborn et al. 2009) typically show much greater activities of RubisCO compared with PEPCase or PEPCKase, and C₄ carboxylase:RubisCO activity ratios have been used to argue for or against a C₄ CCM in diatoms. Difficulties in the optimization of assay conditions for the measurement of fully activated carboxylating enzymes have been noted (MacIntyre et al. 1997, Cassar & Laws 2007). PEPC activity is particularly difficult to measure, and separation of cell extracts may deactivate or remove PEPC or other enzymes or substrates necessary to convert the enzyme’s product, oxaloacetate, to a stable form (Ashton et al. 1990). Moreover, it is difficult to infer in vivo biochemistry from in vitro enzyme activities without knowledge of the concentrations of substrates in the intracellular compartments that each enzyme occupies.

Genomic analysis of *T. pseudonana* and *P. tricornutum* provides evidence, but not conclusive proof, of the operation of either a biophysical or C₄ CCM in these diatoms. Genes for the C₄ pathway enzymes PEPC, PEPCK, PPDK, ME, and MDH are present in the genomes of *T. pseudonana* and *P. tricornutum* (Armbrust et al. 2004, Montsant et al. 2005). However, the putative localizations of these enzymes based on gene product–targeting presequences indicate that the enzymes could constitute a possible futile cycle to dissipate excess energy (Kroth et al. 2008). In this case, such a system should be downregulated by low light, which is inconsistent with the diel transcript results of Roberts et al. (2007), which show constitutive or elevated levels of C₄ gene transcripts in *T. pseudonana* in the dark. In addition to differences in CA number, type, and localization, three genes coding for proteins potentially linked to HCO₃⁻ transport were identified in *P. tricornutum*, but only one such gene was found in *T. pseudonana* (Kroth et al. 2008).

Experimental evidence supports the conclusion that a C₄ CCM operates in at least one marine diatom (*T. weissflogii*) and perhaps others, although only a handful of species has been examined and methodological differences complicate direct comparisons. A critical missing piece to the C₄ CCM hypothesis is the enzyme responsible for C₄ decarboxylation to produce CO₂ and its intracellular localization. A resolution of this issue, at least in model species, is likely in the next 5 to 10 years through the application of molecular, cellular, and traditional biochemical techniques.

**CCMs IN COCCOLITHOPHORES AND PHAEOCYSTIS**

Coccolithophores and the bloom-forming genus *Phaeocystis* are globally distributed, photosynthetic marine protists of the phylum Haptophyta, class Prymnesiophyceae. Although the physiology of inorganic carbon acquisition in marine prymnesiophytes has received less attention than that of diatoms, the physiological response of coccolithophores to increasing CO₂ and lower pH, especially in the prominent species *Emiliania huxleyi*, has been rigorously examined over the past 10 years due to their role in the global carbon cycle through the production of organic matter, a CO₂ sink, and calcium carbonate, a CO₂ source in surface waters of the ocean (de Vargas et al. 2007). Production ratios of particulate inorganic carbon (PIC) to particulate organic carbon (POC) in coccolithophores vary from 0.2 to more than 1 (Engel et al. 2005; Riebesell et al. 2000;
As in diatoms, photosynthetic carbon fixation in *Phaeocystis* is presently near saturation with respect to current levels of CO$_2$ (Rost et al. 2003). In contrast, coccolithophores appear to lack an efficient DIC pump, and their growth may be undersaturated with respect to CO$_2$ in marine surface waters (Sekino et al. 1996, Riebesell et al. 2000, Rost et al. 2003), allowing for the possibility that future, elevated levels of CO$_2$ would favor expanded growth and blooms of these organisms (Riebesell et al. 2007). Coccolithophores are capable of utilizing HCO$_3^-$ as a source of inorganic carbon for photosynthesis (Rost et al. 2003, Trimborn et al. 2007), but unlike diatoms and *Phaeocystis*, coccolithophores have either undetectable (Nimer et al. 1997) or low activities of CA that when present are unregulated by CO$_2$ (Sikes & Wheeler 1982, Rost et al. 2003).

For calcifying coccolithophores, it has been proposed that CaCO$_3$ precipitation helps deliver CO$_2$ to RubisCO through the production of acid for the dehydration of HCO$_3^-$ in the chloroplast (Nimer & Merrett 1993). However, observations of lower rates of photosynthesis in coccolithophores acclimated to low CO$_2$ (pH manipulation) favor the argument that calcification, which usually increases at low CO$_2$, does not support DIC acquisition (Riebesell et al. 2000, Zondervan et al. 2002). Leonardos et al. (2009) concluded that calcification could not support photosynthetic carbon supply in *E. huxleyi* (strain PML B92/11A) based on observations that calcification, but not photosynthesis or growth, varied with Ca concentrations (Leonardos et al. 2009). Whereas these results demonstrate that *E. huxleyi* can accumulate sufficient inorganic carbon for photosynthesis in the absence of calcification when grown under nutrient- and light-replete conditions, they do not negate the possibility that calcification supports photosynthesis in *E. huxleyi* in the Ca-replete ocean, where nutrients and energy may be limiting. Relationships between calcification and CCM physiology depend on nutrient or light limitation (Sciandra et al. 2003, Schulz et al. 2007). For example, the calcification:photosynthesis ratio declined in N-replete, but not N-limited, cultures of *E. huxleyi* (strain TW1 = AC474) acclimated to 700 ppm CO$_2$ compared with 400 ppm (by bubbling) (Sciandra et al. 2003). At low light (≤80 μmol photon m$^{-2}$ s$^{-1}$), PIC production did not vary with CO$_2$ in *E. huxleyi* (strain PML B92/11) acclimated to 5–34 μM CO$_2$aq (pH manipulation) (Zondervan et al. 2002). Similarly, in DIC manipulation experiments (bubbling) with *E. huxleyi* (strain CCMP171), PIC production was not affected by CO$_2$ at low light (50 μmol photon m$^{-2}$ s$^{-1}$) but decreased when pCO$_2$ was increased from 375 ppm to 750 ppm at high light (400 μmol photon m$^{-2}$ s$^{-1}$) (Feng et al. 2008). Nutrient- or light-limited *E. huxleyi* cells may utilize calcification, even at elevated CO$_2$, as an inexpensive means to generate CO$_2$ for photosynthesis in the absence of more costly (N- or energy-requiring) DIC pumps. Resolution of this question will require a deeper understanding of carbon metabolism in coccolithophores at the cellular level.

The effects of past and future changes in surface-ocean CO$_2$ on coccolithophore growth and calcification is of fundamental interest in marine biogeochemistry (Ridgwell et al. 2009). Based on the results of laboratory and mesocosm experiments over the past 10 years, it has been shown that acclimation of coccolithophores to higher concentrations of CO$_2$ supports higher rates of photosynthesis but lower extents of calcification. Working with *E. huxleyi* (strain PLY B92/11A) and *Gephyrocapsa oceanica* (strain PC7/1), Riebesell et al. (2000) and Zondervan et al. (2002) showed a decrease in the production of PIC relative to POC as acclimation CO$_2$aq increased from 5 μM to 34 μM (150 ppm to 900 ppm, with pH manipulations; Riebesell et al. 2000, Zondervan et al. 2001). This was also observed in shipboard incubations of subarctic North Pacific phytoplankton assemblages (Riebesell et al. 2000) and in mesocosm experiments conducted near Bergen, Norway (Engel et al. 2005). In the Bergen mesocosms, the PIC:POC production ratio and net growth rate...
of *E. huxleyi* decreased at 410 ppm and 710 ppm CO₂ compared with 190 ppm (set by bubbling prior to the start of the experiment) (Engel et al. 2005), but primary production was unaffected (Delille et al. 2005). It was later shown that adaptation to elevated CO₂ (decreased calcification) in a strain of *E. huxleyi* isolated from the Bergen mesocosms occurs in less than 14 h after a shift from low to high CO₂ (Barcelos e Ramos et al. 2010).

Different responses of calcification to CO₂ may be due to differences among *E. huxleyi* strains or CO₂ control methods. In bubbled cultures, growth at high CO₂ resulted in higher production of POC and PIC on a per cell basis, lower growth rates, and declining but relatively invariant PIC:POC ratios in *E. huxleyi* strain PLY M219 (=NZEH) (Iglesias-Rodriguez et al. 2008). Similar results were obtained with the same *E. huxleyi* strain in experiments in which CO₂ concentrations were controlled by either pH manipulation or bubbling (Shi et al. 2009), indicating minor effects of the CO₂ control method. However, in pH manipulation experiments, the growth rate of *E. huxleyi* increased with increasing CO₂, whereas bubbling resulted in slightly lower growth rates at high CO₂ than did acidification (Shi et al. 2009). Physiological differences among *E. huxleyi* strains with respect to the response of calcification and growth to increasing CO₂ are likely important. For example, Langer et al. (2009) found that PIC:POC decreased as pCO₂ increased from 200 ppm to 750 ppm (pH manipulation) in three out of four *E. huxleyi* strains tested.

Inorganic carbon acquisition in the noncalcifying marine prymnesiophyte *Phaeocystis* appears to be equally or perhaps more efficient as that in diatoms. Results of ¹⁴C-isotope disequilibrium and membrane inlet mass spectrometry (MIMS) experiments show that extracellular CA in *Phaeocystis globosa* is regulated by CO₂ and that HCO₃⁻ is used as a source of carbon for photosynthesis (Elzenga et al. 2000, Rost et al. 2003). Half-saturation concentrations of CO₂ or HCO₃⁻ with respect to inorganic carbon accumulation were similar in *P. globosa* and the diatom *S. costatum* but were lower in *P. globosa* than in the diatom with respect to photosynthesis, indicating that *P. globosa* used pumped carbon more efficiently (Rost et al. 2003). Moreover, the affinity for inorganic carbon was not regulated by acclimation to CO₂ in *P. globosa* as it was in *S. costatum* and *E. huxleyi*, indicating a constitutive CCM in *P. globosa* (Rost et al. 2003). Rost et al. (2003) also estimated the ratio of internal:external CO₂ as the ratio of whole-cell, half-saturation concentrations for CO₂ with respect to photosynthesis and an assumed Kₘ of 30 μM for prymnesiophyte RubisCO. They found that internal CO₂ was up to 20 times higher than external CO₂ in *P. globosa*, whereas in *E. huxleyi*, the ratio was less than three.

**CCMs IN DINOFLAGELLATES**

With fossil dinoflagellate cysts dating to the early Triassic (240 Ma) and biochemicals of likely dinoflagellate origin extending into the Proterozoic (>570 Ma), dinoflagellates are perhaps the most ancient of the eukaryotic autotrophs that came to dominate the marine phytoplankton during the Mesozoic (Falkowski et al. 2004, Delwiche 2007). Dinoflagellates are unique among eukaryotic phytoplankton in that the plastids of some species were acquired via tertiary endosymbiosis involving chlorophyte, haptophyte, or diatom endosymbionts (Schnepp & Elbrachter 1999). Dinoflagellates are also the only oxygenic photosynthetic organisms with form II RubisCO (Morse et al. 1995), which was apparently acquired through lateral gene transfer from a prokaryotic anaerobe, most likely prior to the loss of endosymbiont RubisCOs (Delwiche & Palmer 1996). Form II RubisCO has the lowest carboxylation:oxygenation specificity factor among eukaryotic phytoplankton (Badger et al. 1998, Whitney & Andrews 1998), giving dinoflagellates a disadvantage with respect to carbon fixation in the high-O₂, low-CO₂ modern ocean. As a consequence, dinoflagellates likely require a CCM to sustain even their relatively slow rates of growth and photosynthesis.
Bblooms of free-living dinoflagellates occur under stratified hydrodynamic conditions, often succeeding those of diatoms or other phytoplankton (Hinga 1992). Thus, dinoflagellates have been observed to grow, generally at slower rates, in surface waters with high pH (≥ 9) (Hinga 1992, Hansen 2002) that persists as a result of the time lag between photosynthetic removal of CO₂ (an acid sink) by previous blooms and physical replenishment of CO₂ (an acid source) through air-sea exchange or upwelling. Growth at high pH provides indirect evidence that dinoflagellates possess a CCM. Direct experimental evidence for CCMs in marine dinoflagellates is limited, and results vary with species and experimental conditions.

Initial studies with *Amphidinium carterae* indicated that marine dinoflagellates have a high affinity for inorganic carbon (Caperon & Smith 1978, Burns & Beardall 1987). Nimer et al. (1997) measured extracellular CA activities in five species of marine dinoflagellates, including *A. carterae*, *Prorocentrum micans*, and *P. minimum*, and in *P. micans*, found tenfold higher concentrations of internal than external DIC (Nimer et al. 1999). Although external CA activity was observed in dinoflagellates acclimated to both 4 μM CO₂aq and 10 μM CO₂aq and was higher at low CO₂ (Nimer et al. 1997), such measurements may be due to the leakage of intracellular CA following cell breakage of fragile species (Lapointe et al. 2008). DIC affinity and total cellular CA were regulated by CO₂ in natural populations of the freshwater dinoflagellate *Peridinium gatunense* (Berman-Frank et al. 1998). In this case, although *P. gatunense* adapted its physiology to low CO₂, CO₂ limitation caused a decline in growth and primary production and ultimately contributed to the end of its bloom. The operation of a CCM has also been noted for symbiotic zooxanthellae dinoflagellates (Leggat et al. 2002).

Using MIMS and potentiometric CA assays, Dason et al. (2004) found that the marine dinoflagellates *A. carterae* and *Heterocapsa oceanica* possess internal but no external CA (Dason et al. 2004). They also found that these species selectively take up CO₂. Also using MIMS plus the ¹⁴C-disequilibrium technique, Rost et al. (2006) estimated that HCO₃⁻ uptake accounted for >80% of photosynthetic carbon fixation in three species of marine dinoflagellates (*P. minimum*, *H. triquetra*, and *Ceratium lineatum*). They also observed low or negligible extracellular CA in all three species and constitutive internal CA in two of three species (Rost et al. 2006). Rost et al. (2006) argued that the true extent of dinoflagellate CCMs may have been missed because rates of photosynthesis were low, perhaps as a result of photoinhibition. Low rates of photosynthesis and growth are expected to mask the full capacity for inorganic carbon accumulation due to a lower demand for CO₂, but this may be the natural condition of marine dinoflagellates.

In pH-buffered cultures of *Protoceratium reticulatum*, photosynthetic affinity for DIC and internal CA were suppressed by growth at very high CO₂ (5,000 ppm) compared with ambient CO₂ (370 ppm) (Ratti et al. 2007). *P. reticulatum* was also found to lack external CA, does not appear to utilize HCO₃⁻, and is expected to be DIC-limited at current levels of CO₂ in the surface ocean (Ratti et al. 2007). The planktonic marine dinoflagellate *Lingulodinium polyedrum* also does not appear to utilize HCO₃⁻ but appears to have an external CA (Lapointe et al. 2008). The contradictory evidence regarding external CA and HCO₃⁻ uptake in dinoflagellates supports either of two possible CCMs, one based on CO₂ uptake at the cell membrane, which may or may not be supplied via the external CA-catalyzed dehydration of HCO₃⁻ (*H. oceanica*, *L. polyedrum*, *P. reticulatum*, and perhaps *A. carterae*), and another based on HCO₃⁻ transport and intracellular CA (*P. micans*, *P. minimum*, *H. triquetra*, *C. lineatum*). Given the phylogenetic history of dinoflagellates, it would not be unexpected that different genera and even species express various modes and mechanisms of inorganic carbon acquisition. Methodological differences may bias the picture, and there is a need to understand the regulation of carbon accumulation physiologies by natural limitations on dinoflagellate growth.
ECOLOGICAL CONSEQUENCES OF CCMs IN MARINE PHYTOPLANKTON

In the sea, varying surface water conditions require phytoplankton to rely on reaction-diffusion supply of CO\textsubscript{2}\text{aq} (which is essentially free) when CO\textsubscript{2} is high or on carbon concentrating mechanisms (which have nutrient and/or energy costs) when CO\textsubscript{2} is low in order to grow faster than competitors and minimize losses to grazing (Raven & Johnston 1991, Beardall & Giordano 2002). Because of the costs of CCMs, light and nutrients may affect how CO\textsubscript{2} regulates photosynthesis in marine phytoplankton and, as a result, the species composition, timing, and duration of phytoplankton blooms. The interactions of CO\textsubscript{2}, nutrients, and light may also affect the geochemical cycles of elements in the sea because the C, N, and P contents and PIC:POC ratios of biogenic particles are modulated by CO\textsubscript{2}-driven changes in cell physiology and phytoplankton species composition.

CCM efficiency is regulated by light in freshwater phytoplankton (Shiraiwa & Miyachi 1983, Beardall 1991, Berman-Frank et al. 1998) and similar effects have been observed in marine diatoms. Subsaturating light caused a significant drop in photosynthetic carbon fixation in nutrient-replete cultures of the marine diatom *Skeletonema costatum* acclimated to low, but not high, CO\textsubscript{2} (Bartual & Galvez 2003, Chen & Gao 2004a). These observations and those showing that light is required for the induction of high-CO\textsubscript{2}-affinity photosynthesis in *S. costatum* (Chen & Gao 2004b) demonstrate a significant energetic cost for the diatom CCM. Likewise, higher carbon fixation and growth rates of nutrient-replete assemblages of marine diatoms and haptophytes incubated at higher partial pressures of CO\textsubscript{2} have been attributed to lower energetic costs of carbon assimilation and the downregulation of inorganic carbon transport at high CO\textsubscript{2} (Riebesell et al. 2007, Tortell et al. 2008b). In the coccolithophore *E. huxleyi*, however, photosynthesis is undersaturated by 2 mM DIC at a high (non-growth-limiting) light level but is saturated by only 1 mM at low light (Nimer & Merrett 1993), indicating that the carbon demand of growth at high light is not met by *E. huxleyi*’s CCM. In addition, adaptation to higher CO\textsubscript{2} may increase the sensitivity of marine phytoplankton to UV photoinhibition as cellular levels of light-capturing pigments decrease (Sobrino et al. 2008).

The effects of nutrient limitation on CCM efficiency in phytoplankton has been primarily addressed with respect to nitrogen. Theoretical estimates of N resource demands in microalgae suggest that increased CCM activity may affect N use efficiency (moles of carbon fixed per mole of cellular nitrogen) based on the balance between protein required to build RubisCO and CCM components (Beardall & Giordano 2002). Higher concentrations of CO\textsubscript{2} in the euphotic zone could increase the efficiency with which phytoplankton use available limiting nutrients to fix carbon, resulting in phytoplankton cells and sinking particulate organic matter with higher C:N or C:P ratios (Tortell et al. 2000, Engel et al. 2005). Increases in the C:N or C:P ratios of natural phytoplankton populations would increase the efficiency of the biological carbon pump in nutrient-limited areas of the ocean, provided phytoplankton could grow at all, and represents a potential negative feedback mechanism with respect to increasing CO\textsubscript{2}. In addition, by modulating the ratios and cellular distributions of C, N, and P in marine phytoplankton, increasing CO\textsubscript{2} may also affect the trophic transfer of essential elements in marine food webs.

In laboratory experiments, C:N ratios decreased in marine diatoms acclimated to low CO\textsubscript{2} (<10 μM CO\textsubscript{2}) but showed little variation in diatoms acclimated to 10–25 μM CO\textsubscript{2}, and some species showed no change in C:N ratio across all CO\textsubscript{2} levels (Burkhardt et al. 1999). In cultures of the dinoflagellate *P. reticulatum*, protein content was lower in cells acclimated to 12 μM CO\textsubscript{2}\text{aq} (380 ppm) than to 32 μM CO\textsubscript{2}\text{aq} (1,000 ppm) (Montechiaro & Giordano 2010), indicating a higher N demand at low CO\textsubscript{2}. Similarly, in 4.5-day incubations of coastal northeast Pacific phytoplankton (>60% diatoms), C:N and carbohydrate:protein ratios were lower (lower N-use efficiencies) in
cells maintained at a pCO$_2$ of 100 ppm compared with those at 350 ppm (Tortell et al. 2000). Interestingly, there was little to no difference in the C:N ratios of coastal Pacific phytoplankton incubated with a pCO$_2$ of 350 ppm or 800 ppm (Tortell et al. 2000). In contrast, higher cellular C:N ratios were observed in mesocosms of blooming *E. huxleyi* in which the initial pCO$_2$ was increased from 190 ppm to 410 ppm and to 710 ppm (Engel et al. 2005). In a mixed diatom and coccolithophore mesocosm, elevated initial pCO$_2$ (up to 700 ppm and 1,050 ppm) lead to higher DIC:NO$_3^-$/ drawdown ratios and an apparently higher sinking flux of biogenic particles, but C:N drawdown did not match particulate C:N ratios, because additional carbon fixed in the high-pCO$_2$ treatments was diverted to the production of DOC (Riebesell et al. 2007).

The ecological consequences of phytoplankton CCMs need to be evaluated in the context of the spatial and temporal ranges of CO$_2$, nutrients, and light in the modern ocean and the geologic past. At low CO$_2$, phytoplankton species with efficient CCMs will outcompete those with less efficient CCMs as long as nutrients and light support the additional biochemical and energy demands of pumping inorganic carbon. High CO$_2$ will support greater primary production by low-efficiency CCM species and potentially less nutrient or light limitation in high-efficiency CCM species (Raven & Johnston 1991, Beardall & Raven 2004).

Given the range of atmospheric CO$_2$ levels on earth during the last 250 Ma, 180 ppm to perhaps as high as 2,200 ppm (Royer 2006) CO$_2$ may have been a selective environmental factor in the evolution of marine phytoplankton (Tortell 2000, Raven et al. 2008). Based on fossil diversity, dinoflagellates and coccolithophores rose to dominance in the marine phytoplankton during a period of variable, but relatively high, atmospheric CO$_2$ in the Mesozoic (Falkowski et al. 2004). This evolutionary history may account for the low CO$_2$ affinities of dinoflagellate RubisCO and coccolithophore CCMs. Diatoms, in contrast, the youngest of the three major groups of eukaryotic marine phytoplankton, appeared in the fossil record during the early Cretaceous, and their diversity peaked at approximately 34 Ma (Rabosky & Sorhannus 2009). The ecological and environmental factors leading to increased diatom diversity and, presumably, abundance are not completely understood but, in addition to increased ocean turbulence and silicic acid concentrations (Falkowski et al. 2004), may also include declining concentrations of CO$_2$ during the Cenozoic (Katz et al. 2004, Kooistra et al. 2007).

In the modern ocean, variable CO$_2$ may lead to other short-term shifts in phytoplankton species composition. For example, incubations of equatorial Pacific phytoplankton assemblages at 150 ppm and 750 ppm pCO$_2$ resulted in a decrease in diatoms and an increase in *Phaeocystis* at low CO$_2$ (Tortell et al. 2002). Elevated CO$_2$ would also favor the growth of large cells more than small cells because larger cells are subject to greater reaction-diffusion limitation (Figure 4). Such a shift was observed in incubations of Ross Sea phytoplankton in which the proportion of large, chain-forming diatoms increased and that of small, pennate diatoms decreased as pCO$_2$ was increased from 100 ppm to 380 ppm and to 800 ppm (Tortell et al. 2008b). CO$_2$-driven shifts in phytoplankton species composition toward larger cells could increase the vertical flux of particulate organic carbon, which is usually dominated by large phytoplankton cells, particularly large diatoms (Dugdale & Wilkerson 1998, Goldman & McGillicuddy 2003).

Considering the response of marine phytoplankton to future elevated levels of atmospheric CO$_2$, it has been estimated that decreased calcification by coccolithophores in the surface ocean could lead to the oceanic removal of 5–20 Pg of atmospheric carbon over the next 100 years (Gehlen et al. 2007, Heinze 2004, Ridgwell et al. 2007). Unfortunately, this would be equivalent to only six months to two years of CO$_2$ emissions from fossil fuel combustion at current rates. Based on mesocosm experiments, however, it was estimated that by the year 2100, more than 100 Pg of carbon would be sequestered from the ocean-atmosphere system as a result of the additional carbon
fixation at increasing concentrations of CO₂ (Riebesell et al. 2007). Biogeochemical feedback of this kind is not accounted for in model simulations of the flux of CO₂ into the ocean, in which element ratios of primary producers are not affected by the concentration of CO₂aq.

SUMMARY POINTS

1. Marine eukaryotic phytoplankton utilize inorganic carbon (or CO₂) concentrating mechanisms (CCMs) to accumulate sufficient inorganic carbon for photosynthesis.

2. CCMs in the three dominant groups of eukaryotic marine phytoplankton, diatoms, haptophytes, and dinoflagellates appear to involve the activity of external or intracellular carbonic anhydrase and, in some cases, HCO₃⁻ transport.

3. A single-cell C₄ CCM is utilized by at least one marine diatom and perhaps others, although only a handful of species has been examined and contradictory results for some species have been reported.

4. Inorganic carbon acquisition in the noncalcifying marine haptophyte Phaeocystis is much more efficient than that in calcifying haptophytes (coccolithophores) and appears to be equally as, or perhaps more, efficient than that in diatoms.

5. The dinoflagellates that have been examined utilize one of two possible CCMs, one based on CO₂ uptake at the cell membrane, which may or may not be supplied via the external CA-catalyzed dehydration of HCO₃⁻, and another based on HCO₃⁻ transport and intracellular CA.

6. Variable CO₂ in the surface ocean may affect primary production, nutrient and light limitation, and species composition of marine phytoplankton as a consequence of the energetic and nutrient costs associated with phytoplankton CCMs.

FUTURE ISSUES

1. Studies of cell ultrastructure and the intracellular localization of key enzymes are needed to advance the understanding of CCM biophysics and biochemistry in eukaryotic marine phytoplankton.

2. The identification of the enzyme responsible for C₄ decarboxylation to produce CO₂ for RubisCO and its intracellular localization through the joint application of molecular and biochemical means is needed to clarify the biochemistry of the proposed diatom C₄ CCM.

3. Laboratory and field experiments are needed to understand the effects of CO₂ on phytoplankton growth and element stoichiometry under nutrient- and light-limited conditions.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.
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**RELATED RESOURCES**

4. European Project on OCean Acidification (EPOCA). [http://www.epoca-project.eu](http://www.epoca-project.eu)
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**Errata**

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