Photosynthesis and Calcification in aquatic environments a review about the role and the behaviour of Cyanobacteria and Coccolithophores

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Abstract

The objective of this review is to synthesize the present knowledge of the photosynthetic physiology of two organisms considered to be among the main players of oceanic primary production: cyanobacteria and coccolithophores. Furthermore, these two organisms represent interesting examples of calcifying phytoplankton groups with a completely different manner of using calcium: cyanobacteria are responsible for non-structured calcite and coccolithophorides for obligate synthesized calcite tests.

Biological calcification, whether induced as a by-product of biological activity (as in cyanobacteria) or highly controlled (i.e. formation of an exo-skeleton as in coccolithophores), is necessarily linked to cellular metabolism. The difference in the carbon uptake strategy developed by these two primary producers will be discussed, as well as the potential environmental triggering mechanisms of microbial calcification.

Finally, the acidification of oceans and the effect of climate change with the ongoing increase in atmospheric CO_2 concentration will be re-examined, especially with regard to the possible impact on microorganisms with calcareous tests for which a “grim future” was predicted.

The global carbon cycle in the Oceans

The carbon fixation by phytoplankton in the oceans plays a key role in the global carbon cycle. Nevertheless, all parameters controlling the primary production and its behaviour in the water column are not fully understood.

Phytoplankton are the primary producers of the oceans, consisting of nearly half of the total photosynthesis activity on the planet, while the remainder of the global production comes from terrestrial sources. This total primary production (photoautotrophic) in the ocean was estimated to be around 45-50 Gt C yr^{-1} (Falkowski et al., 1998). This carbon flux is driven by a phytoplankton biomass of ≈ 1 Pg C, which suggests a very fast C turnover resulting in dynamic organic carbon fluxes. This phytoplankton biomass represents only 0.2% of the photosynthetically active C biomass on Earth (Field et al., 1998).

Phytoplankton and primary production

The primary production is performed by microscopic organisms called phytoplankton[1]. Phytoplankton are autotrophic prokaryotic or eukaryotic algae that form the base of the food chain in the oceans. They live in the top 30-meter layer of the sea called the euphotic zone (where light can penetrate into the seawater), where they can use the energy of the sunlight to grow. Phytoplankton in today's oceans consist mostly of Cyanobacteria, Diatoms, Dinoflagellates and Coccolithophores. They include photoautotrophs from a variety of groups of organisms, prokaryotic bacteria (both eubacteria and archaea) and three eukaryote categories: green, brown and red algae. Photosynthetic phytoplankton vary in size between large (> 2–5 μm) and small (< 2μm). The phytoplankton biomass accounts for less than 1% of the total biomass living freely in the open sea.

Cyanobacteria (also called blue-green algae) in the genera *Prochlorococcus* and *Synechococcus* are picophytoplankton (the smallest phytoplankton). Like all bacteria, cyanobacteria are prokaryotes, which can be distinguished from eukaryotes by the absence of a cell nucleus and mitochondrion. In the case of photosynthetic organisms, prokaryotes are also distinguished from eukaryotes by the absence of chloroplasts. Cyanobacteria can be subdivided into two varieties: those that can fix nitrogen and those that cannot fix nitrogen. Cyanobacteria that can fix nitrogen have an advantage over other bacteria in nutrient poor waters, particularly those which are depleted in nitrogen sources (mainly nitrate or ammonia).

[1] Plankton is a general term given to describe all the small free living plants (phytoplankton) and animals (zooplankton) in the marine environment.

The eukaryotic phytoplankton community has long been a "black box" in terms of its composition as well as its contribution to the global carbon fixation. It includes several major lineages of chlorophyll- containing algae, including Heterokontophyta, Haptophyta and Chromalveolata. Diatoms constitute a known group of algae belonging to the phylum Heterokontophyta. The diatoms produce a silica test (frustule) and have chloroplasts. Dinoflagellates belong to the phylum Chromalveolata. About half of Dinoflagellates are photosynthetic, and these make up the largest group of eukaryotic algae after the diatoms. Coccolithophores are among the best known microorganism of the phylum Haptophyta. They have an exoskeleton of calcareous plates called coccoliths.

The quantification of this phytoplankton primary production is difficult because its activity varies greatly (in longitude and latitude) over the three oceans, depending strongly on various parameters such as sunlight intensity, water temperature, nutrient concentrations, etc. Until now, cyanobacteria have been considered to be the major contributors to carbon fixation in the open ocean. Eukaryotic phytoplankton are also important primary producers in the photic zone, even though they appear to be much less abundant than cyanobacteria. The eukaryotic phytoplankton could also contribute to almost half of the ocean's carbon fixation by phytoplankton. For example the Prymnesiophytes, among the phylum Haptophyta, could account for up to 38% of the total primary production in the subtropical and tropical northeast Atlantic Ocean (Jardillier et *al.*, 2010).

Carbonate production

These primary producers are not only responsible for biomass production, but some are also responsible for marine carbonate production (calcite and aragonite) through the formation of calcareous tests and non-structured carbonate. Many species of micro-organisms capable of $CaCO_3$ precipitation are found in both freshwaters and marine environments. Carbonate deposits throughout Earth's history are a striking demonstration of the biological mechanisms contributing to the huge precipitation of calcium carbonate in the oceans, and consequently to long term storage of carbon dioxide (CO_2). Cyanobacteria, especially in the Precambrian Period, are responsible for stromatolite formations covering wide shelf basins (Riding, 2006), and coccolithophores, during the late Cretaceous Period, have produced the voluminous chalk deposits that gave the period its name.

Transfer of the energy produced by the phytoplankton through photosynthesis into the ocean ecosystem: the biological pump

The traditional view of organic carbon fluxes for the transfer of the energy produced by the phytoplankton through photosynthesis into the ocean ecosystem, has three main pathways. The first route is the direct consumption by zooplankton (grazing). The second pathway is the rapid re-mineralization by bacteria and micro-zooplankton, the so-called "microbial loop" that occurs in surface and intermediate waters. The third pathway is the transport from the surface water through the whole water column to the bottom (ocean floor), fuelling the benthic production. Additionally, tests of dead organisms (phytoplankton such as coccolithophores, zooplancton such as foraminifera, etc) reach the sea floor and contribute to the carbon sink. This non-remineralized exportation, although very minor in comparison to the primary production, contributes to carbon sequestration on the ocean floors and is currently estimated to be 0.4 to 0.5 Pg y^{-1}.

This view is obviously simplified; the reality is much more complicated. Indeed, the evaluation of the steady state phytoplankton biomass remains a challenge, since it is simultaneously the carbon source for zooplankton in the food chain (grazing), very susceptible to viral attack, and subject to coagulation forming organic aggregates that sink to deeper waters. Of these, the organic carbon exportation is probably the most difficult phenomenon to understand. The primary producers are responsible for a rain of particulate matter in the water column (known as sea snow), where the tests of phytoplankton join with zooplankton (i.e. pelagic tunicates, pteropods) act as ballast. High-biomass diatom blooms (that occur only intermittently in the open oceans) have conventionally been thought to be the main contributors to the sink of organic matter in the seawater column. However, it has recently been postulated that a background food web based on very small unicellular plants (picoplankton with a diameter <5 μm) could also contribute to organic matter exportation (Richardson and Jackson, 2007). These small phytoplankton are present in all the open oceans and are continuously active throughout the year (as opposed to the intermittant blooms).

The exportation to deep waters of a part of the primary photosynthetic production severely impacts the chemical composition of both the surface and deep waters in the three major oceans. In surface water, the carbonate concentration is affected by two opposing phenomena: it increases due to photosynthesis and it decreases due to (bio)calcification and

CO_2 exchange (see below). In deep waters, both Total Alkalinity (TA)[2] and Dissolved Inorganic Carbon (DIC) increase with the influx of part of the primary photosynthetic production. As the imported production is re-mineralized in the deep waters, it contributes to the release of CO_2 (acidification of the water) and consequently enhances the calcium carbonate dissolution. Today, the DIC and TA[3] in the deep waters are respectively about 220 $\mu mol.kg^{-1}$ and 50 to 150 $\mu mol\ kg^{-1}$ higher than in the surface waters (Feely *et al.*, 2004).

Biological impact of anthropogenic CO_2 oceanic uptake

Rising atmospheric carbon dioxide (CO_2) concentrations over the past two centuries have led to greater CO_2 uptake by the oceans. This CO_2 uptake results in acidification of the oceans due to the change in the carbonate system. This pH change occurs in the upper zone of the ocean that actively participates to the CO_2 exchange with the atmosphere, and can potentially significantly impact the biological systems in the oceans. We have presented in the previous chapters the strong involvement of the phytoplankton activity in bio-geochemical cycles, particularly the carbon cycle, through the primary production (Boyce and *al.*, 2010). It is of prime importance to understand macro-ecological changes in the ocean resulting from acidification (i.e pCO_2) and temperature changes. A decline of the global phytoplankton population by 1% each year in eight out of ten ocean regions has be reported (Boyce et al, 2010). Nevertheless, we are only beginning to understand some of the future impacts on the biological systems in the oceans due to these macro-ecological changes.

Among the most clearly observed phenomenon, the acidification of the seawater surface (via CO_2 trapping from the atmosphere) modifies the *in-situ* carbonate concentration curve and contributes to the upward migration of the calcite and aragonite saturation horizons. This phenomenon could have an effect on the carbon sink (i.e. sequestration of $CaCO_3$ through incorporation into the sediments) by rapidly increasing the dissolution of $CaCO_3$ during exportation to deep waters (when Ω is <1).

As for the biological impact, the acidification of the oceans possibly has an impact on the delicate balance of marine planktonic species, especially on microorganisms with calcareous tests for which a "grim future" was predicted. Barker and Elderfield (2002) have highlighted a relation between calcification and carbonate ion concentration in these coccolithophore and foraminiferan calcareous contributors. The reduction of the carbonate

[2] Total Alkalinity : $[HCO_3^-]+ 2[CO_3^{2-}]$

[3] During calcification, $CaCO_3$ is precipitated by using one CO_3^{2-}, thereby reducing dissolved organic carbon and total alkalinity in a molar ratio of 1:2. Carbonate dissolution causes the reverse reaction.

saturation state below a threshold value (due to carbonate concentration decrease) will lead to large decreases in biological calcification rates, even when calcite saturation (Ω) is greater than one.

Such a decrease in the $CaCO_3$ production would possibly affect carbon sequestration via incorporation in the sediments (carbon sink), if the process of $CaCO_3$ ballasting of organic carbon is essential, as is commonly believed. Nevertheless, this question of biological matter exportation is not fully resolved. It appears to depend not only on ballast with pelagic tunicates, such as pteropods (therefore phytoplankton balance is important), but also on the participation of very small unicellular plants (see Richardson and Jackson (2007) commented by Barber (2007) about the evocated complicated picoplankton food web).

Finally, rising CO_2 concentrations affect the water surface temperature, resulting in upper-ocean stratification in the next 50 years (Rost et al.., 2008). This will lead to a reduced nutrient supply at the surface, to a modified light transmission to deeper water zones, and perhaps to a change in the respective contribution to the NPP (Net Primary Production) by pico- and microplankton.

Consequenly, some concepts about oceanic bio-geochemical cycling will need to be revisited, such as our general view of the food web, which is continuously changing,

General strategies of inorganic carbon uptake

Bicarbonate and CO_2 are the two possible mineral carbon sources for aquatic photosynthesis activities. The effective selection of the carbon source by the photosynthetic organisms is dependant on environmental conditions. Most known algae can take up both HCO_3^- and CO_2, although there are some exceptions. No matter which carbon source is used, all the photosynthetic organisms ultimately assimilate the CO_2 using a universal and essential enzyme called Rubisco, which is short for ribulose 1,5-bisphosphate carboxylase/oxygenase. Rubisco is the first and the key enzyme in the photosynthetic assimilation of inorganic carbon into organic carbon compounds through the Calvin-Benson cycle (the C3 pathway). In addition to the carboxylating reaction, Rubisco is used in photorespiration, acting as an oxygenase. This highly conserved enzyme has a low affinity for CO_2. Consequently, cyanobacteria and eukaryotic microalgae have developed various strategies to overcome constraints on carbon assimilation under the low CO_2 concentrations present in modern seawater (≈10 μmol l^{-1}). All these strategies have the same objective, namely to elevate CO_2 concentration in the vicinity of the Rubisco. It is commonly mentioned that Cyanobacteria,

thanks to their CO_2 Concentrating Mechanisms (CCM), can increase CO_2 concentration close to Rubisco in the carboxysome by 10 up to 1000-fold. The major categories of CCM in terrestrial and aquatic phototrophs have been extensively studied and well reviewed by Giodano et *al.* (2005), Price et *al.* (2008), and Roberts et *al.* (2007). As opposed to cyanobacteria, the microalgae do not possess a carboxysome structure and have instead developed another strategy, also sometimes confusingly referred to as CCM, to overcome the CO_2 limitation and to saturate Rubisco. Stable isotope measurements ($^{13}C/^{12}C$ ratio) of organic cellular material can be used to indicate the presence of the CCM capacity of algae. Isolated eukaryote Rubisco discriminate against ^{13}C to the extent of ~30‰. Consequently, species without CCM may show isotope discrimination ratios approaching this value. However, CCM tends to reduce this discrimination.

Among CCM variants, active membrane transport of HCO_3^- and/or CO_2 is identified. This requires that the membrane across which active transport occurs has a low permeability for the DIC species delivered to the side of membrane closer to Rubisco, otherwise active transport becomes short-circuited (Raven and Beardall, 2003). This rules out two plastid membranes in eukaryotes, as well as in the gram-negative cyanobacteria outer membrane, which have high porin densities. These porins are membrane proteins which allow non-selective membrane crossing for molecules with a molecular mass below 800. Moreover, a range of carbonic anhydrases (CAs), including external (extracelullar) CA, is involved for inter-conversion between the two carbon species CO_2 and HCO_3^- in the various compartments (cytosol, chloroplast).

Photosynthesis and Calcification with Cyanobacteria

Cyanobacteria probably have the most effective biological system to assimilate inorganic carbon (Ci), regardless of the dissolved CO_2 concentration, in various habitats, such as benthic microbial mats or planktonic blooms. In cyanobacteria, the CCM uptake system includes different Ci transport systems associated with a carboxysome. The Ci transport can be based on either CO_2 or HCO_3^-, either at the plasmalemma or the thylakoid membrane (Kaplan and Reinhold, 1999; Klughammer et *al.*, 1999; Omata et *al.*, 1999; Raven and Beardall, 2003; Ritchie et *al.*, 1996). The CO_2 concentration takes place in the carboxysome inside the cytosol of the cell.

Carboxysome in cyanobacteria

A carboxysome is a micro-compartment that contains enzymes involved in carbon fixation. HCO_3^- diffuses from the cytosol into the carboxysome (Lane et *al.*, 2000; Price et *al.*, 2002; Smith and Ferry, 2000; Sültemeyer, 1998) and is cleaved into CO_2 by carbonic anhydrase (CA), an enzyme which is confined to the carboxysome and has never been found in the cytosol of cyanobacteria. Other enzymes of the Calvin cycle are located outside the carboxysome. A carboxysome spatial structure has been proposed in which CA is positioned in the centre of the carboxysome, allowing an efficient catalytic action of the Rubisco. In this dense packing of Rubisco and CA, the generated CO_2 is used up before it can diffuse across this thick intracarboxysomal protein arrangement. This system acts as a substantial barrier to CO_2 diffusion and leakage from the carboxysome, increasing the CO_2 concentration inside this structure in a higher steady-state value than in the bulk medium. This proximity of the CA and the Rubisco compensates for the low CO_2 affinity of Rubisco (depression of the photorespiration) (Kaplan and Reinhold, 1999; Ludwig et *al.*, 2000).

CO_2 and HCO_3^--uptake systems in cyanobacteria

Cyanobacteria are known to use bicarbonate and CO_2 as mineral carbon sources. A number of CO_2 and HCO_3^- transporters contribute to the accumulation of HCO_3^- in the cytosol. The various transporters move (HCO_3^- from the periplasm to the cytosol regardless of the DIC species (CO_2 or HCO_3^-). The HCO_3^- accumulated in the cytosol then diffuses into the carboxysome.

HCO_3^- is moved via an active transport mechanism using HCO_3^-/Na^+ symports (sodium-dependant transporters) or ATP-driven uniports (an ABC-type high affinity transporter), both of which function at low CO_2 levels. However, the symports are probably absent in all marine strains. Both of these active transport enzymes are localized on the cytoplasmic membrane (plasmalemma) and present a K_m (the concentration of DIC which allows one half of the maximum velocity of photosynthesis) value of 12~15 μM for HCO_3^-.

CO_2 enters a cell by diffusion through aquaporins and is converted into HCO_3^- by NADPH dehydrogenase (NDH) complexes on the thylakoid and plasma membranes. No active transport systems have been found in the plasmalemma of cyanobacteria, however two CO_2 uptake systems have been reported. One is induced by low CO_2 concentration, and the other one is constitutive. The K_m value of the low-inducible CO_2 transporter is low (0.8 μM)

and remains constant when cells are transferred from high to low CO_2 conditions. Nevertheless, the V_{max} of uptake can increase significantly with environmental changes, suggesting increased synthesis of the enzyme (Price and *al.*, 2008).

When cells are exposed to Ci limited conditions (< 50 ppm), the inducible transport systems for both CO_2/HCO_3^- are activated and are accompanied with increased Rubisco activity in carboxysome content (Price and *al.*, 2008).

CO_2 leakage from the carboxysome is possible. Nevertheless, the CO_2 uptake activities associated with the NDH-1 CO_2 complexes on the thylakoid membrane may contribute to the recycling of this leaked CO_2 (Price and *al.*, 2008). So, there is no clear distinction between CO_2 and HCO_3^- for use as a mineral substrate.

All these transporters (bicarbonate and CO_2) appear to use photosynthetic energy, both in the form of ATP and of reducing equivalents, such as NADPH, to drive their reactions. Light energy is used to maintain the cytoplasmic concentration of CO_2 below chemical equilibrium by converting it to HCO_3^-. This release of HCO_3^- in the cytosol, where its concentration may exceed 50 mM, necessitates a unidirectional conversion of CO_2 to HCO_3^-. Consequently, a supply of OH^- ion is needed, which may be produced by the reduction of $NADP^+$ to NADPH. The OH^- could be also obtained through the NADH-1 complex via Zn-H_2O conversion to Zn-OH^- (Price et al., 2002). This conversion of CO_2 into HCO_3^- is accompanied by a proton production.

Moreover, in many situations, the amount of Ci taken up by the cells exceeds the quantity used in photosynthesis. This excess Ci leaks out of the cells and can be re-imported. This massive Ci cycling flux could be a protection strategy developed by the cyanobacteria against excess light conditions by dissipating excess light energy (Tchernov et *al.*, 2003).

Interestingly, the explanation as to why many cyanobacteria and eukaryotic microalgae have the ability to tolerate very high CO_2 concentrations, in some cases well above 50% CO_2 (Miyachi et *al.*, 2003; Gressel, 2008; Papazi et *al.*, 2008), might be found in the CCM. Inhibition of Rubisco through acidification under high CO_2 conditions is prevented by the CA reaction and by state II transition of Photosynthesis Electron Transport (PET) (rearrangement of the phycobilisomes to favour light absorption by PS I) (Miyachi et *al.*, 2003).

Figure 1 presents a model of the carbon-concentrating mechanism (CCM) in the cyanobacterial cell. This figure also shows the calcification process that can operate if calcium is present in the environment.

Photosynthesis and calcification

Although cyanobacteria calcification has been long recognized, its physiological function is still not clearly known. Calcification appears as a non-obligate process that is consecutive to photosynthetic growth; in particular, cyanobacteria can grow in calcium deprived environments. As presented above, the inorganic carbon import mechanism appears to rely on the two most important components: "cyanobacteria CCM" (an active uptake system for both CO_2 and HCO_3^-) and carboxysome structure.

As also mentioned, whatever the inorganic carbon source used for the photosynthesis, only bicarbonate is present in the cytosol. The bicarbonate then diffuses into the carboxysome structure, where the CA immediately generates CO_2 for Rubisco. When HCO_3^- is the predominant substrate, the conversion of HCO_3^- to CO_2 in the carboxysome will result in a net production of OH^- inside the cell that will either need to be excreted or neutralized by H^+ uptake from the external medium. This proton pump or OH^- expulsion may cause a rise in pH outside the cell, shifting the carbonate equilibrium toward an increase in carbonate (CO_3^{2-}), resulting in a nano-scale carbonate oversaturation condsition favourable for calcium carbonate precipitation. Nucleation could take place, and may be facilitated by the membrane surface that provides nucleation sites (Obst et *al.*, 2009). From then on, the crystal growth could proceed as a strictly chemical process. The ionic strength was shown to catalyze calcite nucleation (Bischoff, 1968b). Zuddas & Mucci (1998) postulated a two-step precipitation process of adsorption followed by ion incorporation into the crystal lattice. For example, in the case of $a[Ca^{2+})]>>a(CO_3^{2-})$ the precipitation rate is limited by the CO_3^{2-} adsorption rate.

Photosynthesis and calcification with coccolithophores

Most of the efforts in investigating carbon uptake in test-forming eukaryotic organisms are focused on diatoms and coccolithophores. These organisms are representative functional groups of marine phytoplankton. Diatoms which have siliceous tests are not treated in this review, which is focused on biological carbonate formation.

CO_2 and HCO_3^- and calcium uptake systems in Emiliania huxleyi

Although there are numerous existing coccolithophore species, the calcification studies have mainly been limited to those that are easily maintained in laboratory cultures, in particular the cosmopolitan coccolithophore species *Emiliania huxleyi*. It clearly appears from

the laboratory studies that the strain *Emiliania huxleyi* EH2 lacks efficient mechanism to facilitate a high DIC gradient between the external medium and the cytosol. This gradient is one to two orders of magnitude smaller than in cyanobacteria and several times smaller than in green algae (Tsuzuki and Miyachi, 1990). *Emiliania huxleyi* is a marine unicellular calcareous alga which can moderately concentrate DIC (~13-16 times) (Sekino and Shiraiwa, 1994) and presents a low affinity for CO_2 (apparent $K_{0.5DIC}$ equal to 55 µM for CO_2 at pH 8.0 and 25°C) and an apparent $K_{0.5DIC}$ of 5.5 mM for DIC ($K_{0.5DIC}$: the concentration of DIC which allows one half of the maximum velocity of photosynthesis) (Sekino and Shiraiwa, 1994). Consequently, the photosynthesis rate is not maximal at present-day marine bicarbonate concentration, and increases under elevated CO_2 levels (Herfort et *al.*, 2002). This low-affinity for DIC in *E. Huxleyi* has also been reported by many others authors (Paasche 1964, Nielsen 1995, Riebesell et al. 2000, Berry et al. 2002, Zondervan et al. 2002, Rost et al. 2003, Leonardos and Geider 2005, Iglesias-Rodriguez et *al.*, 2008). However, the state of the CA remains unclear. The lack of CA in *E. huxleyi* has been mentioned by many authors (Sikes and Wheeler, 1982; Nimer et al., 1994; Sekino and Shiraiwa, 1994). Conversely, CA induction has been reported at 0.5 mM DIC in *E. huxleyi* (Herfort et *al.*, 2002), but Quiroga and Gonzalez (1993) hypothesized the suppression of CA induction when growing under high external DIC concentration (above 1 mM).

The link between photosynthesis and calcification was suggested as early as 1962 (Paasche, 1964). From a metabolic point of view, growth can be performed on both CO_2 and bicarbonate, but bicarbonate is the only inorganic carbon source used for the calcium carbonate production. Nimer et al. (1995) proposed a growth model using HCO_3^- as substrate for both photosynthesis and calcification (Figure 2). In this model, calcification using bicarbonate as an inorganic carbon source supports the photosynthesis. HCO_3^- entry contributes to CO_2 supply at the Rubisco site, which in turn is consumed during photosynthesis through the action of the CA in the chloroplast compartment as presented in the following equation:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \rightarrow CH_2O + O_2.$$

The simultaneous linked calcification uses a second HCO_3^- molecule to provide the required proton (H^+) for the photosynthetic reaction:

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+$$

Furthermore, experiments with radiotracers have confirmed the global equation (Sikes et *al.*, 1980):

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H_2O + CO_2.$$

The protons produced during the calcification are used for the internal CO_2 production with an efficiency that has been estimated to be approximately 1 for *E. huxleyi* strain Ch 24-90. This value confirms the idea that there is a tight coupling between calcification and photosynthesis (Buitenhuis et *al.*, 1999). It should be noted that for HCO_3^- concentrations below 0.5 mM, the calcification rate is equal to zero.

This tight coupling between calcification and photosynthesis could be interpreted as an evolutionary mechanism to optimize the use of the dissolved inorganic carbon in low CO_2-concentration marine environments, and as an alternative to the CCM developed by the cyanobacteria. This mechanism offers the advantage of maintaining a pH homeostasis in the chloroplast and in the coccolith mineralizing vesicle. Intracellular calcification has also been interpreted as reducing the energy cost for transporting CO_2 inside the cells, thus enhancing photosynthetic carbon fixation (Anning et *al.*, 1996).

However, variations of this calcification/photosynthesis ratio (C/P ratio) have been obtained in laboratory conditions with constant HCO_3^- concentrations and CO_2 increases. The observed decreasing ratio suggested a photosynthetic growth on CO_2 which was decoupled from calcification process (Buitenhuis et *al.*, 1999). Using a strain of *E huxleyi* deficient in both internal and external CA, insights have been obtained on the specific role of CO_2 and HCO_3^- for photosynthesis and calcification by performing radiotracer pulse experiments adding $^{14}CO_2$ and ^{14}C-HCO_3^- separately. $^{14}CO_2$ was recovered mainly in the biomass and a DIC pool, but scarcely appeared in the $CaCO_3$ fraction. In contrast, ^{14}C-HCO_3^- was incorporated mainly into $CaCO_3$ and into a DIC pool and hardly any was recovered in the biomass (Sekino and Shiraiwa, 1994). Additionally, a growth on $^{14}CO_2$ with a photosynthesis inhibitor (like DCMU) allows the DIC pool to be incorporated into $CaCO_3$, but no incorporation of ^{14}C in DIC was found. Furthermore, the incorporation of ^{14}C-DIC into $CaCO_3$ was effective in darkness (Sekino and Shiraiwa, 1994, 1996). 1-hydroxyethylidene bisphosphonic acid (HEBP), an inhibitor for the growth of $CaCO_3$ crystals, partially suppresses incorporation of ^{14}C-DIC into the DIC-pool and totally suppresses its incorportation into the production of $CaCO_3$. These authors suggested a more complex model in which a "pre-formed $CaCO_3$ (HCO_3^- pool or DIC pool)" may be used as carbon source for photosynthetic fixation of CO_2, illustrated as "recycling of DIC" in Figure 3, possibly operating under DIC-limited or depleted conditions.

In fact, coccolithophorids show species-dependent variations in their photosynthetic DIC utilization and great variations in $CaCO_3$ production among algal cells obtained from

different ecosystems (Nimer and Merrett, 1992). The ratio between $CaCO_3$ production and photosynthetic fixation of CO_2 could vary from 1/20 to 1/1, according to the strain of *E. huxleyi* (Sekino and Shiraiwa, 1994). This species-dependent variation is a part of the great discrepancy observed in the coccolithophores world (Marsh, 2003). Indeed, "non-calcifying coccolithophores" have been mentioned and interpreted (perhaps wrongly) as either species which lost the ability to form calcified coccoliths or as unidentified coccolithophore species representing non-mineralizing phases (Edvardsen et *al.*, 2000; Fujiwara et *al.*, 2001). Considering such variation in the photosynthesis DIC utilisation, calcification and "calcifying and non-calcifying" coccolithophes, attention has been drawn to factors affecting the balance between photosynthesis and calcification.

Factors affecting the balance between growth and calcification: role of environmental variables

Since the work of Paasche (1964) and of Sekino and Shiraiwa (1994), environmental variables have been suspected to be responsible for unbalanced regulation between photosynthesis and calcification. Such an inverse relationship between algal growth and calcification has also been observed with nitrate or phosphate sufficient and deficient cultures (Paasche, 1998, Sorrosa et *al.*, 2005, Paasche and Brubak, 1994; Riegeman et *al.*, 2000). Moreover, these limitations in nitrogen and phosphorous have been considered as the prime reason for the decline of ocean blooms (Bratbak et *al.*, 1993; Egge and Heimdal, 1994; Van der Wal et *al.*, 1995). Microelements have also been shown to affect the growth of coccolithophores. For example, selenium is also effective at regulating coccolithophorid growth, although the relationship with calcification was not examined (Danbara and Shiraiwa, 1999). Sorrosa et *al.*, (2005) suggested that the growth could also be stimulated by iron enrichment, and the calcification excited by the temperature decrease.

In the case of excess bicarbonate concentrations, as in marine environments, and without limitation of nutrients (nitrogen and phosphorous) or micronutrients (such as selenium, cobalt and zinc), the growth of coccolithophores will be not limited. This “favourable growth situation” has tentatively been reproduced in laboratory experiments with high bicarbonate concentrations (20 mM), largely exceeding concentrations encountered in marine environments. Under these conditions, Sorrosa et *al.*, (2005) reported that in a laboratory culture, the cell size of *E. huxleyi* EH2 increases, but not the number of cells. This high bicarbonate concentration results mainly in a strong stimulation of calcification, but does

not favour the cell growth. However, its mechanism is not yet fully understood. Conversely, the number of cells increases when *E. huxleyi* EH2 is cultivated in bicarbonate limitation (Fritz and Balch, 1996; Fritz, 1999). Calcification inhibition by HEBP also contributes to cell growth and to cell size decrease.

This inverse relationship between cellular division and calcification in experimental conditions with different limiting factors leads to a speculation about the occurrence of coccolithophore blooms in marine environments. Favourable growth conditions in marine water under unlimited inorganic carbon supply contribute to a progressive depletion of macro or micro nutrients in the seawater surrounding the bloom. The depletion in one of these parameters stops the cell division in the bloom and consequently favours calcification conditions.

The very efficient nitrate uptake found in *E. huxleyi* is also important to note (the half-saturation constant for nitrate uptake is very low: 100 nM) (Epply et *al.*, 1969, 1971). Indeed, this physiological characteristic may contribute to efficient growth in the bloom (practically logarithmic growth) until almost complete nitrate depletion, and consequently to a marked situation of limitation resulting in spectacular physiological change.

In conclusion, the calcification function(s) of coccolithophores remain mysterious because the physiology of this functional group is complicated. Paasche (2002) suggested that the high coccolith production at low temperatures may also increase the survival probability. The coccolith production might also have important cell protecting functions to limit photo-damage by consuming excess energy produced at low temperatures under strong light when photosynthesis is oversaturated because of the suppression of energy-consuming processes (photosynthesis for cell multiplication). In this situation, the production of coccoliths may greatly exceed the number necessary to make the overall test and this overproduction could shed in the culture medium. Sekino et *al.* (1996) suggested that the presence of coccoliths on the cell surface might present more ecological advantages than physiologic benefits. This could be deduced by the fact that artificially made protoplasts (naked cells) of *E. huxleyi* showed the same growth curve in a laboratory culture as did the wild strain.

Sensitivity of phytoplankton to ocean acidification

Biologically induced calcification processes, whether highly controlled (skeleton formation, as in coccolithophores) or induced as a by-product of biological activity (as in

cyanobacteria), are necessarily linked to cellular metabolism e.g. photosynthesis. We have reviewed in the previous paragraphs the specifics of the carbon uptake strategies developed for growth and calcification by cyanobacteria and coccolithophores.

The necessity to assess the effects of the rising atmospheric CO_2 concentration on phytoplankton has emerged at the end of 20th century, primarily to understand the observed impact on tropical coral reefs (Gatusso et *al.*, 1998; Leclercq et *al.*, 2000). Comprehensive knowledge of factors acting in the respective balance of organic (photosynthesis) and mineral (calcification) carbon fixation for these two types of organisms is of prime importance, in particular for a better understanding of their current behaviour in regard to the accelerated physico-chemical environmental changes with the present climate changes [temperature, light exposure, pH (bicarbonate/carbonate ratio), and variation of pCO_2].

Coccolithophore response

As the majority of biogenic carbonate precipitation (80%) is due to planktonic microorganisms, coccolithophores also came very quickly into the focus of interest. As previously mentioned, *Emiliania huxleyi* growth could be stimulated by CO_2 because of its low affinity for CO_2. Nevertheless, Riebesell et *al.* (2000) suggested that calcification by coccolithophores may decrease in response to ocean acidification and consequent elevated pCO_2. The range of CO_2 experimented is between 280 and 750 ppmv (up to 3 times the atmospheric concentration from the beginning of the 20th century). The same conclusion has been obtained by Dellile et *al.* (2005) in laboratory studies, but recent contradictory findings obtained by Iglesias-Rodriguez et *al.* (2008) showed a stimulation of the calcification rather than a reduction under elevated pCO_2.

Taking into account the knowledge of environmental variables suspected to be responsible for unbalanced regulation between photosynthesis and calcification and the great discrepancy observed in the coccolithopores world (Marsh, 2003), these contradictory findings could possibly be attributed to different coccolithophore species used in the experiments. In addition, *Emiliania* is not only atypical in its high rate of calcium secretion (as a coccolith), but also unusual in having arisen only 268 k.y. ago (Thierstein et *al.*, 1977). Nevertheless, *E huxleyi* is a predominant player in the oceanic CO_2 fixation with a mass involvement estimated at 30% (Milliman et *al.*, 1993).

Cyanobacterial response

Recent studies concerning the effect of elevated CO_2 have been reported on bloom-forming cyanobacteria. As mentioned previously, the cyanobacteria possess a Rubisco with very low CO_2 affinities (K_m varies from 105 to 185 µmol l^{-1}) (Badger et al. 1998). With increasing CO_2 pressure, a higher growth rate would be expected. Indeed some studies accredit high positive effects of CO_2 increase on the growth rate of cyanobacteria (Barcelos e Ramos et *al.*, 2007, Hutchins et *al.*, 2007, Levitan et *al.*, 2007).

The nitrogen-fixing cyanobacteria are already strongly implicated in tropical and subtropical areas where they provide a nitrogen source to the food chain after their decomposition (Falkowski 1998, Gruber and Sarmiento 1997). A possible consequence of cyanobacterial growth stimulation by CO_2 would be the expansion of the marine nitrogen fixation, in particular in high latitude oligotrophic regions, concurrently with a warming climate, stratification, as well as nitrate and phosphorous limitations (Boyd & Doney 2002).

Nevertheless, as with coccolithophores, recently published studies raise numerous questions on the previous understanding of this functional phytoplankton group. For instance, it appeared that changes in CCM efficiency under elevated pCO_2 tended to improve resource allocation between photosynthesis, carbon acquisition and nitrogen-fixation (Kranz et *al.*, 2009).

Calcification in modern Oceans

Cyanobacteria and calcification in hard lake and marine waters

The question of calcification by cyanobacteria in modern oceans and lacustrine environments has been extensively discussed, but is still not fully understood. Numerous field studies have stimulated interrogation on biologically active precipitation with cyanobacteria. Two case studies are well reviewed: the Fayetteville Green Lake (FGL), a hard water lake (Thompson and Ferris, 1990, Thompson et *al.*, 1997) and the Great Bahamas Bank (GBB), a marine environment (Thompson, 2000). On the FGL site, sedimentation rates of carbonate precipitates have tentatively been estimated from field and experimental data. Thomson et *al.* (1997) measured up to 3.5 - 4.0 mg of calcite precipitation per litre in the open water column (between the water surface and a depth of 2 m) during the spring, and even more during the summer at depth of 8 m. This calcite precipitation occurs concurrently with a decrease of dissolved inorganic carbon (between 4 and 2.5 mM). The $\delta^{13}C$ value of the dissolved

inorganic carbon (mainly bicarbonate) increases at the same time and may be attributed to the selective uptake of $\delta^{12}C$ for photosynthetic CO_2 fixation by the cyanobacterial bloom. Conversely, the most recent carbonates (from bottom sediments) appeared to be highly enriched in $\delta^{13}C$ with respect to isotopic equilibrium with the lake water DIC. A similar $\delta^{13}C$ carbonate enrichment has been reproduced in laboratory microcosms using the water and the microflora from the site.

Despite the fact that a common mechanism might induce marine and lacustrine whitings, evidences of whiting in marine environment are not so easy to establish. Moreover, a major difference between carbonates from lacustrine and marine whitings is the inherent Mg/Ca ratio between fresh and marine waters (marine waters are richer in Mg). The source of whitings on the Great Bahama Bank has been the longest and most hotly debated topic in carbonate geochemistry. In Great Bahama Bank, *Synechococcus* appeared to be slightly more abundant within whitings than in the surrounding clear water. Nevertheless, the Bank waters are nutrient-poor (nitrate and phosphate are generally below 0.1 µM), and consequently are unfavourable for bloom formations. Any chemical shifts between whitings and the surrounding clear water in the Bahamas have never been observed (Morse et *al.*, 1984). Morse and Mackenzie (1990) suggested that these whiting events could be some pure inorganic process; they favoured the idea that suspended carbonate particles from a repetitive re-suspension process may act as nuclei for inorganic precipitation. Furthermore, estimated annual rates of sedimentation calculated from all of the material suspended in whitings were approximately 3 times the bank top Holocene observed (Robbins et *al.*,1997). Morse et *al.*, (2003) concluded that a slow calcium carbonate precipitation on re-suspended carbonate sediments, rather than new calcium carbonate precipitation in the water column, resulted in the formation of a whiting.

Coccolithophores and calcification in marine waters

The ability of coccolithophores to form blooms in coastal waters has been known since the beginning of last century (Gran, 1912). Thus this group of phytoplankton are thought to contribute to carbon deposits in the form of $CaCO_3$ on seafloors situated above the lysocline (Bramlette, 1958). However, their large-scale occurrence was only shown by ocean colour imagery through detection of high reflectance signal measured by satellites (Holligan et *al.*, 1983, Groom & Holligan 1987). NASA even provides a permanent satellite survey of their occurrence (Shutler et *al.*, 2010). Paasche (2002) estimated from field investigations that

the contribution of *E. huxleyi* to calcite mass and to total seafloor calcite is about 5% or less. Consequently, *E. huxleyi* has to be considered only as a minor contributor in the deposition of carbonate on the seafloor.

An extensive bloom (250 000 km^2) of the coccolithophore *Emiliania huxleyi* that developed in the northeast Atlantic in June 1991 was monitored over 15 days (Fernandez et *al.*, 1993). The particular organic carbon (POC[4]) and particular inorganic carbon (PIC) analysed in this gigantic bloom were in the order of 200 mg m^{-3} and 300 mg m^{-3} respectively. The coccoliths/cell ratio ranged between 20 and 40. In addition, photosynthetic rates as high as 40 mg C m^{-3} h^{-1} were measured over the top 35 m of the water column. This corresponds to an integrated daily organic carbon production rate of about 1 g C m^{-2} d^{-1}. Significant rates of inorganic carbon production were measurable only in surface waters and integrated daily rates of inorganic carbon production were estimated around 50 - 200 mg C m^{-2} d^{-1}. These values indicate that the percentage of carbon incorporated into coccoliths appeared to never be higher than 25%. This may be due to additional biomass production by non-coccolithophore phytoplankton. The production of calcite by this bloom in the North-East Atlantic has been estimated to be about 1.0×10^6 t of calcite-C. Ultimately, the significance of this coccolithophore bloom for CO_2 air-sea exchange depends on the PIC/POC ratio of the particulate material which will eventually be buried into the sediments.

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[4] POC for particulate organic carbon: by subtraction PIC from TPC. TPC and PIC were estimated from bloom material retained on filter. TPC was obtained with elemental analysis (C content). PIC was estimated from the measured calcium content, assuming that all of the particulate calcium was present as calcium carbonate.

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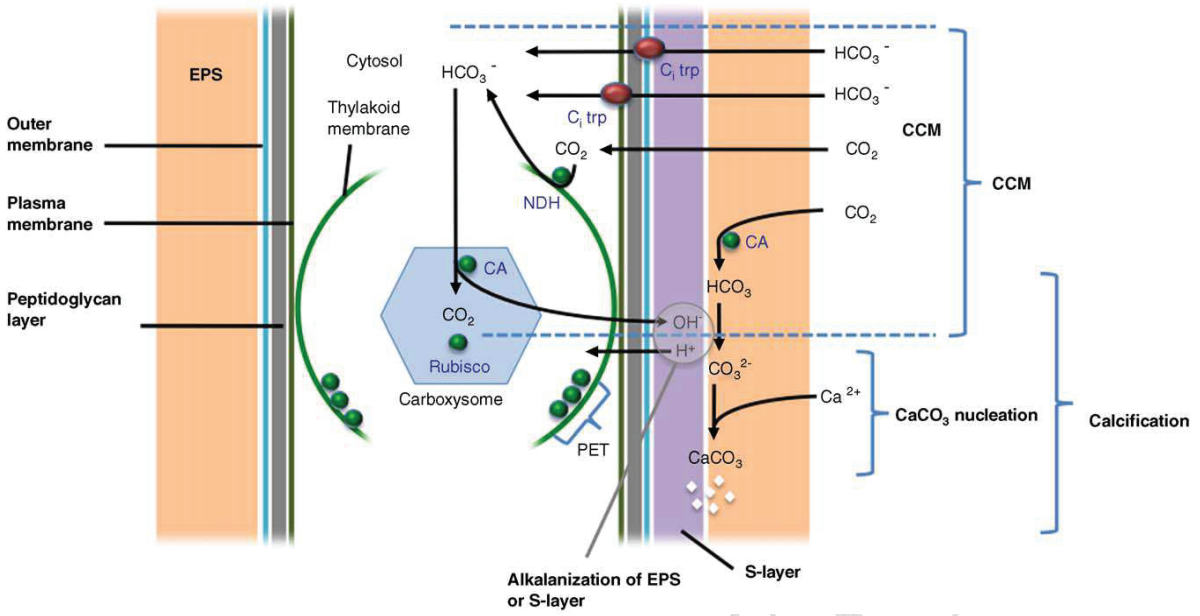
Figure Captions

Figure 1: Model of the carbon-concentrating mechanism (CCM) and calcification in cyanobacterial cell. EPS = exopolysaccharide sheath; NDH = NADPH dehydrogenase and PET = photosynthetic electron transport. (modified from Riding, 2006).

Figure 2: Inorganic carbon used by *Emiliania huxleyi* for photosynthesis and calcification. (according to Buitenhuis et *al.*, 1999).

Figure 3: Model of DIC utilization for photosynthesis and calcification in the coccolothophorid *E huxleyi* (Shiraiwa, 2003)



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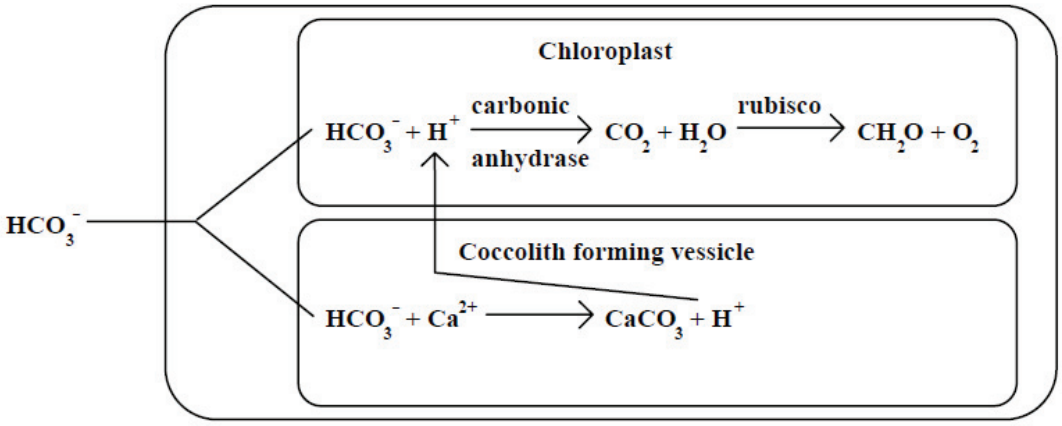

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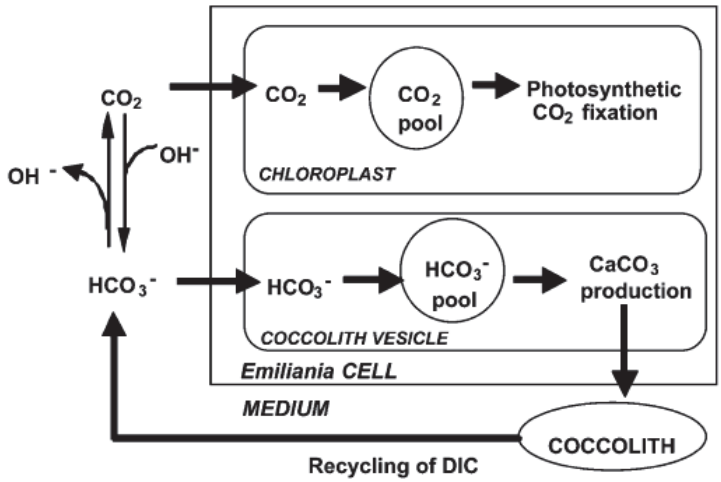

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