



# Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions

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**ABSTRACT:** Despite their microscopic size, marine phytoplankton are responsible for about half of the global primary production and represent the basis of the marine food web. This diverse group of organisms drives important biogeochemical cycles, exporting massive amounts of carbon to deep waters and sediments, and strongly influencing ocean–atmosphere gas exchanges. Anthropogenic climate change will result in significant alterations in the marine environment over the next 100 yr and beyond. The increase in atmospheric CO<sub>2</sub> has already caused significantly higher aquatic CO<sub>2</sub> concentrations and lower pH values ('ocean acidification') than in pre-industrial times. Rising temperatures will also impact surface ocean stratification, which in turn will affect the surface-water light regime and nutrient input from deeper layers. Phytoplankton will be affected by these environmental changes in many ways. In this article we assess the possible responses of different phytoplankton groups with regard to the expected physico-chemical changes. In addition to summarizing laboratory and field studies, we outline the current understanding of the underlying mechanisms that cause processes such as photosynthesis, calcification, and nitrogen fixation to be sensitive to ocean acidification. We describe different approaches to manipulate carbonate chemistry (e.g. acid/base or CO<sub>2</sub> addition), discuss their potential to simulate future ocean acidification, and allude to common problems in experiments caused, for instance, by high biomass or the use of buffers. In addition to guidelines for CO<sub>2</sub> perturbation experiments, we argue that it is essential to look at multiple environmental factors in combination with CO<sub>2</sub>, to aim for process-understanding rather than correlation, and to assess a wider diversity of phytoplankton species both in laboratory and field studies.

**KEY WORDS:** Ocean acidification · CO<sub>2</sub> manipulations · Photosynthesis · Carbon acquisition · Calcification · Nitrogen fixation

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## GLOBAL CHANGE IN THE MARINE ENVIRONMENT

The Earth's climate has undergone major changes over geological time scales, shaping the structure and productivity of ecosystems and the proliferation or disappearance of species. Biological activity has in turn directly affected climate by driving many of the global elemental cycles. Phytoplankton has played a central role in mitigating and amplifying climate change in the past and may have contributed to stabilizing the climate by influencing the partitioning of climate-relevant gases between the ocean and atmosphere (Schlesinger 2005).

Changes in environmental conditions are presently occurring at an unprecedented rate due to large-scale perturbations induced by human activities. For the past 10 million yr the atmospheric partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) has most probably remained <300 µatm (Berner 1990, Pearson & Palmer 2000) and fluctuated between 180 µatm in glacial and 300 µatm in interglacial times over the last 800 000 yr (Petit et al. 1999, Lüthi et al. 2008). With the beginning of the industrial revolution, CO<sub>2</sub> emissions from the burning of fossil fuel and changes in land use led to atmospheric CO<sub>2</sub> concentrations well above the upper limit of the last several million years. At present, the pCO<sub>2</sub> has reached about

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380  $\mu\text{atm}$  and is expected to rise to 750  $\mu\text{atm}$  by the end of this century (IPCC Scenario IS92a; Houghton et al. 2001) or even values  $>1000$   $\mu\text{atm}$  (Raven et al. 2005, Raupach et al. 2007).

Such changes are altering the physico-chemical conditions in the marine environment. Changes in atmospheric  $p\text{CO}_2$  will directly affect the carbonate system of the surface ocean, since atmosphere and surface ocean exchange  $\text{CO}_2$  on time scales of several months (Zeebe & Wolf-Gladrow 2001). As  $\text{CO}_2$  dissolves in the surface ocean it reacts with water to form carbonic acid ( $\text{H}_2\text{CO}_3$ ), which dissociates to bicarbonate ( $\text{HCO}_3^-$ ), carbonate ions ( $\text{CO}_3^{2-}$ ), and 'protons' ( $\text{H}^+$ ). As a consequence of this chemical reaction, the ocean can take up large amounts of  $\text{CO}_2$  and store it as dissolved inorganic carbon (DIC), which is the sum of the concentrations of these carbon compounds. Currently,  $<1\%$  of DIC remains in the form of dissolved  $\text{CO}_2$  (including tiny amounts of  $\text{H}_2\text{CO}_3$ ), while the rest is in the form of  $\text{HCO}_3^-$  ( $\sim 90\%$ ) or  $\text{CO}_3^{2-}$  ( $\sim 9\%$ ). With increasing atmospheric  $p\text{CO}_2$ , DIC will increase and the equilibrium of the carbonate system will shift to higher  $\text{CO}_2$  and  $\text{HCO}_3^-$  levels, while  $\text{CO}_3^{2-}$  concentration and pH will decrease. These changes in carbonate chemistry, often referred to as 'ocean acidification', are already occurring and are expected to intensify in the future. The projected increase in atmospheric  $p\text{CO}_2$  to about 750  $\mu\text{atm}$  by the end of this century is estimated to almost triple surface water  $\text{CO}_2$  concentrations relative to preindustrial values. Concomitantly, seawater  $\text{CO}_3^{2-}$  concentrations and pH will drop by 50% and 0.4 units, respectively (Fig. 1; Wolf-Gladrow et al. 1999, Caldeira & Wickett 2003). It should be noted that this change in pH corresponds to a 2.5-fold increase in the  $\text{H}^+$  concentration. The lower  $\text{CO}_3^{2-}$  concentration will lead to a reduction of the saturation level for carbonates such as calcite or aragonite. These changes in carbonate chemistry will affect phytoplankton in general and certain processes in particular. Depending on the underlying process, the sensitivity to carbonate chemistry may be strongly modified by temperature, light, and nutrient availability. This is important to consider because other environmental conditions are likely to also change within the framework of global change.

The increase in atmospheric greenhouse gases like  $\text{CO}_2$  has caused global average temperatures to increase over the last century, especially in the past

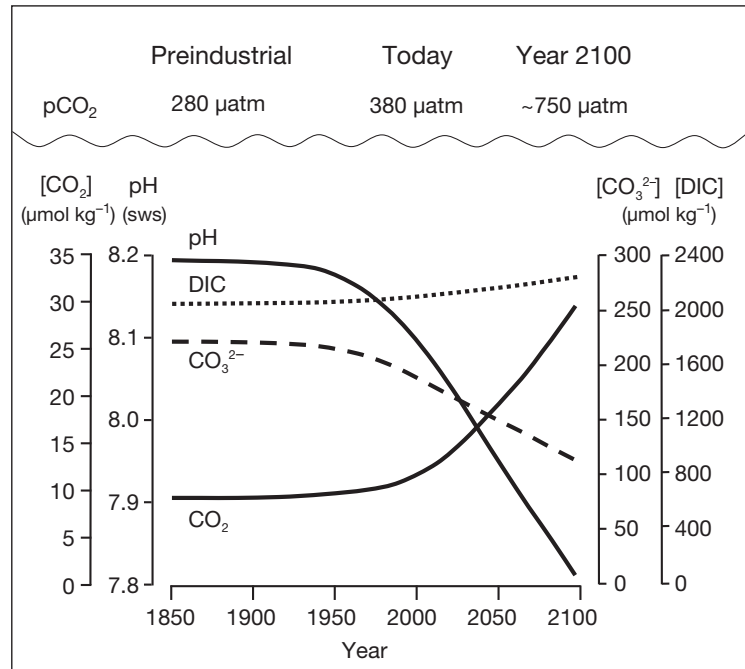


Fig. 1. Predicted changes in the surface ocean carbonate system in response to changes in atmospheric  $p\text{CO}_2$  assuming the IS92a Scenario. Modified after Wolf-Gladrow et al. (1999)

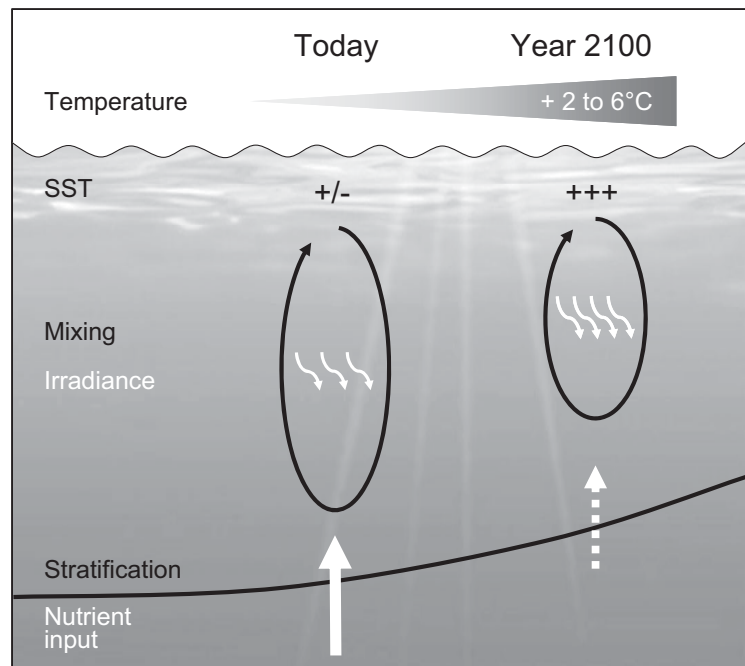


Fig. 2. Putative changes in sea surface temperatures (SST) in response to global warming and its effect on stratification, nutrient and light availability. Modified after Rost & Riebesell (2004)

few decades. The predictions for the future temperature rise range from 2 to 6°C until the end of this century, and, independent of uncertainties in magnitude, the changes will be most pronounced at high latitudes (IPCC 2007). The temperature increase will increase stratification of the surface ocean and this can, in turn affect phytoplankton. Enhanced upper ocean stratification reduces nutrient supply from deeper layers and increases light availability due to shoaling of the upper mixed layer (Fig. 2; Sarmiento et al. 2004). Such changes have opposing impacts on the productivity of phytoplankton, and the overall effect will vary in different oceanic provinces: further extension of nutrient-limited, low-productivity regions such as subtropical gyres (Bopp et al. 2001, Behrenfeld et al. 2006), and increases in productivity in high-latitude regions currently light-limited owing to pronounced vertical mixing can be expected (Bopp et al. 2001, Doney 2006).

The physico-chemical changes described above will inevitably affect phytoplankton in numerous ways. Despite the complexity of these responses, they can be divided into physiological and ecological aspects, i.e. changes in the rates of processes and shifts in the dominance of species (Falkowski et al. 1998, Boyd & Doney 2002). Depending on which species or groups are affected in what manner, these changes have the potential to alter productivity and to feedback on biogeochemical cycles. With respect to the latter, phytoplankton can be distinguished into phytoplankton functional types. *Silicifiers* (mainly diatoms) play a major role in determining the vertical fluxes of silicate and organic carbon. *Calcifiers* (mainly coccolithophores), on the other hand, affect the carbon cycle through the production of calcium carbonate and its impact on seawater alkalinity. *Diazotrophs* ( $N_2$ -fixing cyanobacteria) influence marine productivity by altering the availability of reactive nitrogen. Several recent studies have found that key species from these groups are in fact sensitive to changes in carbonate chemistry.

In the following we describe the different responses of these phytoplankton groups to changes in carbonate chemistry and illustrate our current (or lack of) knowledge of the underlying mechanisms causing the sensitivity in key processes such as photosynthesis, calcification, or nitrogen fixation. Our aim is not to compare the results of the individual studies in detail, but rather to point out general observations and apparently contradictory results. Furthermore, we will describe common approaches to simulate ocean acidification in experiments and discuss potential problems of these manipulations as we believe much of the controversy in the literature might be caused by different protocols.

## SENSITIVITY OF PHYTOPLANKTON TO CARBONATE CHEMISTRY

### Photosynthesis and carbon acquisition

Assessing effects of rising atmospheric  $CO_2$  on phytoplankton requires an understanding of the photosynthetic processes that provide energy for growth and any other downstream process. Photosynthesis involves a series of reactions that start with capturing light energy, converting it into ATP and the reductant NADPH, and using these compounds to fix  $CO_2$  in the Calvin-Benson cycle (Falkowski & Raven 2007). As a consequence, photosynthesis and subsequent processes are primarily affected by light, but also by  $CO_2$  availability. The inherent  $CO_2$  sensitivity in photosynthesis is largely the result of the primary carboxylating enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This ancient and highly conserved enzyme, which evolved during times of elevated atmospheric  $CO_2$  and low  $O_2$  levels (Falkowski & Raven 2007), is characterized by low affinities for its substrate  $CO_2$  and a susceptibility to a competing reaction with  $O_2$ .

Despite differences in these catalytic properties of RubisCO, the generally poor substrate affinities for  $CO_2$  (i.e. high half-saturation constants [ $K_M$ ] with values from 20 to 185  $\mu\text{mol l}^{-1}$ ; Badger et al. 1998) impose constraints on carbon assimilation under the low  $CO_2$  concentrations present in seawater (5 to 25  $\mu\text{mol l}^{-1}$ ). To alleviate the risk of carbon limitation, most microalgae have thus developed different mechanisms that enhance  $CO_2$  concentration in the close vicinity of RubisCO (Badger et al. 1998, Thoms et al. 2001). Over the past 2 decades, significant progress has been made towards understanding  $CO_2$ -concentrating mechanisms (CCMs). Many microalgae have been shown to possess complex CCMs that involve the uptake of  $CO_2$  and/or  $HCO_3^-$ , as well as various isoforms of the enzyme carbonic anhydrase (CA), which accelerate the otherwise slow interconversion between these carbon species. Processes that minimize  $CO_2$  efflux from the cell are also important components of an efficient CCM. For details on the different CCMs we refer to reviews by Giordano et al. (2005), Price et al. (2007), and Roberts et al. (2007).

The extent to which various species operate these CCMs is still poorly understood, but the few existing studies on marine phytoplankton suggest that species differ in efficiency and regulation of their CCMs (e.g. Burkhardt et al. 2001, Tortell & Morel 2002, Rost et al. 2003, Trimborn et al. 2008). In general, species relying on diffusive  $CO_2$  uptake or those with inefficient CCMs (i.e. low apparent affinities for inorganic carbon) are highly  $CO_2$  sensitive in photosynthesis and

thus may directly benefit from the increase in CO<sub>2</sub>. Those species operating highly efficient CCMs are at, or close to, rate-saturation under present-day CO<sub>2</sub> concentrations. The latter species can nevertheless benefit in the future, since a down-regulation of the CCM under elevated CO<sub>2</sub> levels may allow for optimized energy and resource allocation. The capability for regulation is generally important to consider, as it permits phytoplankton to adjust CCM activity to the actual demand, which also explains the strong modulation of CO<sub>2</sub> sensitivity by light or nutrient availability. The observed species-specific differences in CCMs imply that changes in the carbonate chemistry may have profound effects on phytoplankton communities, e.g. by directly affecting the productivity of ecosystems or influencing the species assemblage and succession. In fact, several laboratory and field studies have observed CO<sub>2</sub> effects on photosynthesis and downstream processes in various phytoplankton taxa.

### Coccolithophores

Coccolithophores have been in the focus of discussion about the consequences of ocean acidification research because of their remarkable sensitivity in processes such as photosynthesis and calcification. In species such as *Emiliana huxleyi*, photosynthesis was found to be well below saturation under present-day carbonate chemistry and, hence, photosynthesis generally increases under elevated CO<sub>2</sub> levels (Paasche 1964, Nielsen 1995, Riebesell et al. 2000, Berry et al. 2002, Zondervan et al. 2002, Rost et al. 2003, Leonardos & Geider 2005, Iglesias-Rodriguez et al. 2008). The strong CO<sub>2</sub> sensitivity of photosynthesis is consistent with the low-affinity CCM observed in *E. huxleyi* (Rost et al. 2003). Apart from these laboratory studies, CO<sub>2</sub> effects on photosynthesis have also been observed in natural communities that were dominated by *E. huxleyi* (Engel et al. 2005, Riebesell et al. 2007, Schulz et al. 2008). It should be noted, however, that the dependency of photosynthesis on CO<sub>2</sub> concentration is not straightforward and seems to be modified by light and nutrient supply (for reviews see Rost & Riebesell 2004, Zondervan 2007).

Laboratory experiments (Riebesell et al. 2000, Zondervan et al. 2002, Sciandra et al. 2003), as well as mesocosm studies (Dellile et al. 2005), suggest that calcification by coccolithophores will be reduced in response to ocean acidification. The changes in calcification rates under elevated pCO<sub>2</sub> have been related to the concomitant decrease in carbonate ion concentration and thus calcite saturation levels, but other entities of the carbonate system such as the pH may also be responsible for the observed relationship. A reduction

in the degree of calcification is assumed to put coccolithophores at an ecological disadvantage, suggesting a rather 'grim future' for this group of phytoplankton. Although this view is widely accepted, there are also other lines of evidence and many open questions.

First of all, most of our current understanding on the process and sensitivity of calcification, as well as photosynthesis, stems predominantly from studies on *Emiliana huxleyi* and the closely related *Gephyrocapsa oceanica*. Both species belong to a lineage of rather atypical coccolithophores in terms of structure, physiology, and ecology (Sáez et al. 2003). A study by Langer et al. (2006) with the globally important CaCO<sub>3</sub> producers *Coccolithus pelagicus* and *Calcidiscus leptoporus* showed that species-specific differences in the sensitivity to carbonate chemistry do exist. While in *C. leptoporus* an optimum curve was observed with maximum calcification rates at present-day CO<sub>2</sub> levels, calcification rates did not vary significantly with pCO<sub>2</sub> in *C. pelagicus*. In both species, photosynthetic carbon fixation rates remained constant at CO<sub>2</sub> levels ranging between 150 and 920 μatm. Also challenging the general view are the recent findings by Iglesias-Rodriguez et al. (2008), who observed a stimulation in calcification rate of *E. huxleyi* under elevated CO<sub>2</sub>.

In view of these apparently contradictory findings it is essential to unravel the process of calcification, which is not completely understood (for review see Brownlee & Taylor 2004). Moreover, the fate of coccolithophores can only adequately be predicted when we have revealed the function(s) of calcification and understand the consequences of different degrees of calcification. The latter remain enigmatic, since reduced calcification rates do not alter growth or photosynthesis in *Emiliana huxleyi* (Herfort et al. 2004, Rost & Riebesell 2004, Trimbom et al. 2007). Independent of the discussed changes in process rates, coccolithophores may benefit from increasing stratification, since they favor moderately stratified conditions (for review see Tyrrell & Merico 2004). Floristic shifts are already occurring (Smyth et al. 2004) and will be important to consider when assessing the fate of coccolithophores.

### Diatoms

Regarding this important group of phytoplankton, many studies have investigated the influence of light or nutrients, but very few have focused on the potential effect of ocean acidification. While earlier studies suggested that large diatoms were limited by CO<sub>2</sub> supply in the contemporary ocean (Riebesell et al. 1993), subsequent studies found that many diatoms, especially bloom-forming ones, were capable of compensating

for low CO<sub>2</sub> supply through the use of highly regulated and efficient CCMs (Burkhardt et al. 2001, Rost et al. 2003, Trimborn et al. 2008). With respect to the process of silification, diatoms also do not appear to be particularly CO<sub>2</sub> sensitive (Milligan et al. 2004). Field studies have demonstrated, however, that different CO<sub>2</sub> levels caused shifts in the dominance of diatom species in the phytoplankton assemblage of the Equatorial Pacific (Tortell et al. 2002), as well as of the Southern Ocean (Tortell et al. 2008). In the latter study, elevated CO<sub>2</sub> concentrations led to an increase in phytoplankton productivity and promoted the growth of larger chain-forming diatoms.

While the effect of CO<sub>2</sub> on photosynthesis and growth may yet be small in diatoms, at least when compared to other taxa, the predicted changes in stratification and, thus, light and nutrient availability (Fig. 2) will certainly affect this group strongly. Thriving in turbulent waters with high nutrient concentrations, diatoms will possibly suffer under enhanced stratification in most regions. In higher latitudes, however, diatoms may benefit, since the projected reduction in mixing may alleviate light limitation and thereby increase the productivity. Future studies on diatoms should therefore investigate carbonate chemistry effects in combination with nutrient and light availability.

### Cyanobacteria

N<sub>2</sub>-fixing cyanobacteria support a large fraction of total biological productivity in tropical and subtropical areas and exert, over long time scales, a significant influence on the global carbon cycle by providing a major source of reactive N to the water column (Falkowski 1997, Gruber & Sarmiento 1997). A number of recent studies (Barcelos e Ramos et al. 2007, Hutchins et al. 2007, Levitan et al. 2007) have investigated the effect of elevated CO<sub>2</sub> on the bloom-forming cyanobacterium *Trichodesmium*, species of this genus are responsible for much of the marine N<sub>2</sub> fixation (Capone et al. 1997). All 3 studies observed a strong increase in photosynthesis, N<sub>2</sub> fixation, and even division rates under elevated CO<sub>2</sub> levels. The magnitude of these CO<sub>2</sub> effects exceeds those previously seen in other photoautotrophs and would, if representative for the natural environment, have large implications for the future ocean. The processes responsible for the strong CO<sub>2</sub> sensitivity are currently unknown.

Both carbon uptake and fixation and nitrogen fixation are key processes, which compete for energy and reductive power (Berman-Frank et al. 2001). Since cyanobacteria possess RubisCOs with very low CO<sub>2</sub> affinities ( $K_M$  values from 105 to 185  $\mu\text{mol l}^{-1}$ ; Badger et

al. 1998), increasing CO<sub>2</sub> levels could favor this group, either by directly increasing the carboxylation efficiency of RubisCO or, indirectly, by reducing the energy costs of their CCMs. Recent results revealed changes in CCM efficiency under elevated CO<sub>2</sub> and point to improved resource allocation between photosynthesis, carbon acquisition, and N<sub>2</sub> fixation (Kranz et al. 2009). Significant uncertainties remain, however, as to the degree of sensitivity for CO<sub>2</sub>, the modulation by other environmental factors (light, P, or Fe), and whether the observed responses can be generalized to other important diazotrophic species (Montoya et al. 2004). In addition to the CO<sub>2</sub> stimulation, the magnitude of marine N<sub>2</sub> fixation may also increase due to the expansion of oligotrophic regions to higher latitudes as a consequence of increased warming, stratification, and the concomitant changes in nutrient levels (Boyd & Doney 2002). On the other hand, Breitbarth et al. (2007) predict the overall N<sub>2</sub> fixation to decrease, despite wider distribution of *Trichodesmium* spp., because temperatures will rise above the optimum for N<sub>2</sub> fixation in some areas.

Although CO<sub>2</sub> effects have also been investigated in a number of non-diazotrophic cyanobacteria (for review see Price et al. 2007), most of these studies compare unnaturally high (2 to 5% CO<sub>2</sub>) with ambient CO<sub>2</sub> levels. Currently, there is little information available on the sensitivity of this group to more realistic CO<sub>2</sub> scenarios. A recent study by Fu et al. (2007) observed higher rates of growth and photosynthesis in *Synechococcus* spp. when grown at 750  $\mu\text{atm}$  CO<sub>2</sub>. *Prochlorococcus* spp. remained unaffected by elevated CO<sub>2</sub> in the present study. Such species-specific difference in CO<sub>2</sub>/pH sensitivity could lead to shifts in community structure. Our current knowledge is, however, based on too few studies (and species). In view of the potential ecological and biogeochemical implications, investigation of diazotrophic and other cyanobacteria is clearly a research priority.

### EXPERIMENTAL DESIGN

Despite addressing similar questions and working with the same species, studies often have yielded different and sometimes even contradictory results. While some of the discrepancy might reflect the variability in physiology, a significant part most probably results from differences in the experimental design regarding growth conditions, as well as methodology, specifically, how the response is measured. Here, we describe the most common ways to manipulate the carbonate chemistry, explain how it is affected by physiological processes, and point to pitfalls associated with CO<sub>2</sub> perturbation experiments.

The marine carbonate system comprises  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{H}^+$ ,  $\text{OH}^-$ , and several weak acid–base systems of which borate-boric acid [ $\text{B}(\text{OH})_3$ ,  $\text{B}(\text{OH})_4^-$ ] is the most important. For an accurate description, 2 different components are of particular interest because they are conservative in the sense that their concentrations do not change with temperature or pressure. As introduced earlier, DIC is the sum of all dissolved inorganic carbon species, while total alkalinity (TA) equals  $[\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-]$  + minor components and reflects the excess of proton acceptors over proton donors with respect to a zero level of protons (for details see Dickson 1981, Wolf-Gladrow et al. 2007). Analytically, TA is determined by the titration of seawater with a strong acid and thus can also be regarded as a measure for the buffering capacity of seawater. If 2 components of the carbonate system are known (for example,  $\text{CO}_2$  and  $\text{H}^+$  or DIC and TA), all other components can be calculated for seawater with typical nutrient concentrations for a given temperature, salinity, and pressure (more information is necessary for solutions with different compositions, e.g. high nutrient concentrations, unusual ionic compositions, organic buffers). Changes in any single component due to physical or biogeochemical processes lead to changes in several if not all other components. In other words, it is impossible to vary a single component of the carbonate system while keeping all other components constant. This interdependency in the carbonate system is important to consider when performing  $\text{CO}_2$  perturbation experiments.

### Carbonate chemistry manipulations

To adjust different  $\text{pCO}_2$  values, the carbonate system can be manipulated in various ways, which are depicted in Fig. 3. Like in the natural system, the carbonate chemistry can be altered by equilibrating seawater with gas mixtures of different  $\text{pCO}_2$ . The  $\text{CO}_2$

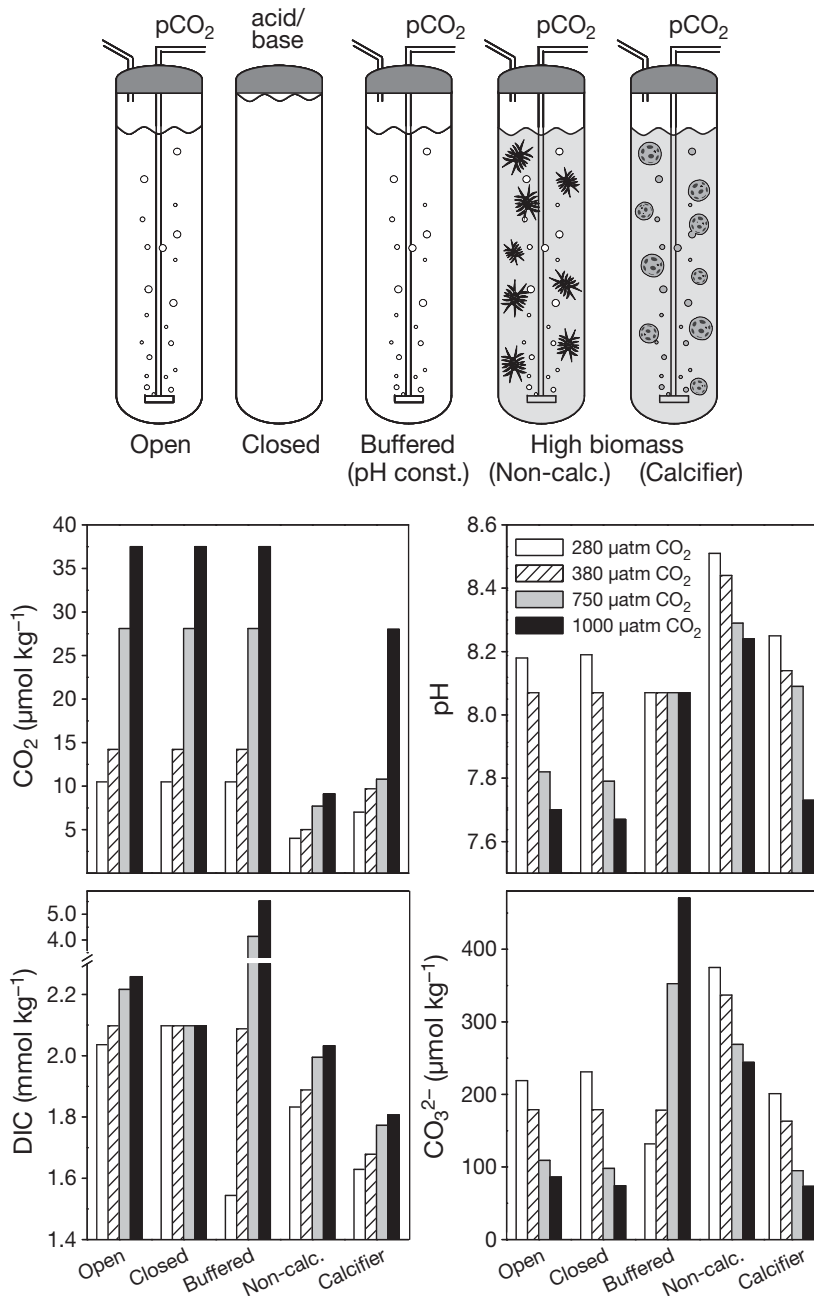


Fig. 3. Different carbonate chemistry manipulations and the effect of high biomass in laboratory experiments. Top panel illustrates the manipulation systems; bottom panels show the respective carbonate chemistry. The open system reflects the future scenario of ocean acidification, while, in the closed system, pH is altered by acid or base addition to closed bottles without headspace, where dissolved inorganic carbon (DIC) remains constant. In a buffered system, the pH is kept constant by means of organic buffers, which prevent the typical shifts in carbon speciation with changes in  $\text{pCO}_2$ . The example for high biomass of a non-calcifier reflects a situation in which organic matter production by phytoplankton exceeds the  $\text{CO}_2$  supply by bubbling, here causing DIC levels to be reduced by 10% with respect to the initial equilibrated conditions. In the case of a calcifier, we assume equal molar quantities of  $\text{CaCO}_3$  to be precipitated with the biomass. In both cases, biological activity causes strong deviations from the desired carbonate chemistry (compare with open system), which is discussed in the subsection 'Effect of biology'. For all calculations, temperature = 15°C and salinity = 35

exchange in such an 'open system' is driven by differences in atmospheric ( $p\text{CO}_2$ ) and aquatic partial pressure ( $\text{PCO}_2$ ) until an equilibrium is established. According to Henry's law, the equilibrium  $\text{CO}_2$  concentration is dependent on a temperature- and salinity-dependent solubility coefficient. Consequently,  $\text{CO}_2$  concentrations will increase with increasing atmospheric  $p\text{CO}_2$  but also with decreasing temperature or decreasing salinity through changes in solubility. When seawater is manipulated in this way, the carbon speciation, pH, and DIC are affected, while TA remains constant (Fig. 3). Note that the relative changes in DIC are an order of magnitude smaller than the relative changes in the aquatic  $\text{CO}_2$  or  $\text{CO}_3^{2-}$  concentrations. To investigate the effect of ocean acidification, manipulations with the 'open system' reflect future changes in the carbonate chemistry and thus will be used as a reference for further comparisons.

Another commonly used perturbation approach is the addition of strong acid (HCl) or base (NaOH) to a 'closed system', for instance a bottle filled without headspace. Such manipulation directly alters the pH and hence the DIC speciation, resulting in higher or lower  $\text{CO}_2$  concentrations (Fig. 3). Since these experiments are performed in gas-tight bottles, the shift in equilibrium concentrations and hence aquatic  $\text{PCO}_2$  cannot result in concomitant DIC changes by  $\text{CO}_2$  release or invasion. Addition of a strong acid or base is an easy way to realize the large relative changes in  $\text{H}^+$ ,  $\text{CO}_2$ , and  $\text{CO}_3^{2-}$  concentrations also typical for the natural system, while accepting small deviations in those quantities that show much smaller relative changes (DIC,  $\text{HCO}_3^-$ ) or no change at all (TA). The 'closed system' has advantages when working with phytoplankton that are sensitive to continuous bubbling. To combine this with the advantage of an 'open system' (i.e. more realistic simulation of ocean acidification), the media can be equilibrated with different  $p\text{CO}_2$  prior to the inoculation of cells. Alternatively, an increase of  $\text{CO}_2$  at constant TA can also be achieved without bubbling by adding equimolar amounts of HCl and  $\text{NaHCO}_3$ .

In order to elucidate particular mechanisms it can make sense to manipulate the carbonate system in a different way than that previously described. In order to separate  $\text{CO}_2$  effects from pH effects, for instance, one may change  $\text{CO}_2$  while keeping pH constant by addition of a certain ratio of  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$  (for details see Zeebe & Wolf-Gladrow 2001). More commonly, organic buffers (e.g. Tris, Bicine, HEPES) have been added to keep pH constant while changing  $\text{CO}_2$  concentrations (Fig. 3). Although often used in the attempt to keep the desired carbonate chemistry more stable, this approach causes large deviation from the 'natural' system and complicates the calculation of the

carbonate chemistry via TA. When buffered seawater is aerated over a range of  $p\text{CO}_2$ , the changes in DIC are much larger than in natural seawater and the  $\text{CO}_3^{2-}$  concentration increases with  $p\text{CO}_2$ . In terms of assessing the effects of ocean acidification this, buffered system is clearly the wrong approach.

Studies often present and compare their results on the basis of  $\text{CO}_2$  or pH, and take these quantities as a 'proxy' for the rest of the carbonate chemistry. This can be troublesome for 2 reasons: (1) investigations often do not report which of the 4 different pH scales (NBS, free, total, and seawater scale) has been used, and these can deviate by  $>0.1$  units. If these differences are ignored, the corresponding error in calculated  $p\text{CO}_2$  can easily be  $>100 \mu\text{atm}$  (for details see Zeebe & Wolf-Gladrow 2001); (2) in view of the different approaches used to manipulate pH or  $\text{CO}_2$ , it is not adequate to report only 1 quantity. Hence, for a thorough comparison of studies, the full description of the carbonate chemistry has to be provided. It is also advisable to over-constrain the carbonate chemistry (by measuring  $>2$  quantities of the carbonate system) to allow for cross-checks. In this respect, developing protocols for standardizing procedures is important.

### Effect of biology

As we examine the effects of carbonate chemistry on physiological processes, we have to be aware that these processes possibly alter the initial carbonate chemistry of the experiment significantly (Fig. 4). During photosynthetic carbon fixation, for instance,  $\text{CO}_2$  and DIC decrease while pH increases (respiration causes the reverse reaction). During calcification  $\text{CaCO}_3$  is precipitated, thereby reducing DIC and TA in a molar ratio of 1:2 (carbonate dissolution causes the reverse reaction). Since TA is more strongly affected than DIC, the chemical equilibrium shifts towards higher  $\text{CO}_2$  concentrations and a lower pH.

When working with high cell densities, these processes can severely shift experimental conditions (Fig. 3). The latter is especially true for closed systems, but, even working in an open system, the biological drawdown of inorganic carbon can quickly exceed the  $\text{CO}_2$  supply (taking into account the slow air-water gas exchange) and thus cause a shift in carbonate chemistry. This problem is more pronounced when processes such as calcification decrease TA, which cannot be restored by increasing the  $\text{CO}_2$  supply. It is important to mention here that, depending on the ratio of photosynthesis to calcification, this can elevate, decrease, or even maintain the desired  $p\text{CO}_2$ , but always at reduced concentrations of DIC. In view of the high cell densities reported in some studies, the poten-

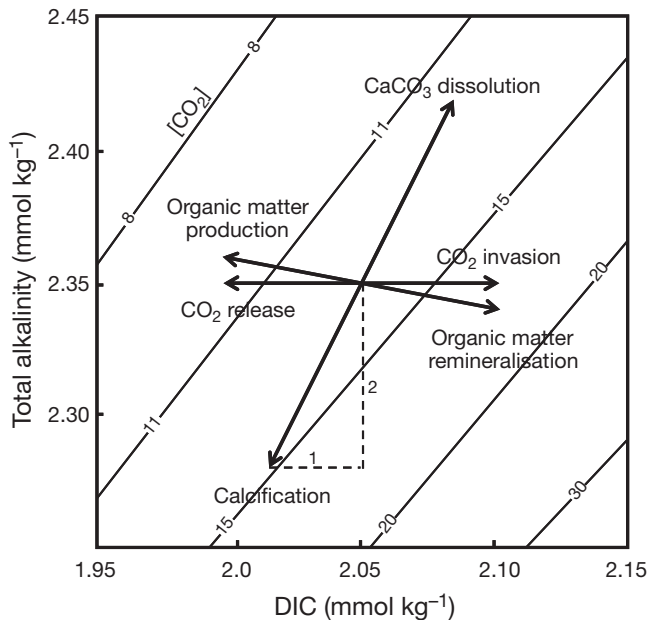


Fig. 4. Effect of various processes (arrows) on dissolved inorganic carbon (DIC) and total alkalinity (TA) that occur in culture experiments. Lines indicate levels of constant dissolved  $\text{CO}_2$  (in  $\mu\text{mol kg}^{-1}$ ) as a function of DIC and TA. Invasion of atmospheric  $\text{CO}_2$  into seawater (for instance by bubbling with elevated  $\text{pCO}_2$ ) increases DIC, while release of  $\text{CO}_2$  to the atmosphere has the opposite effect; TA remains constant in the 2 cases. Calcification reduces DIC by 1, and TA, by 2 units, thereby driving the system to higher  $\text{CO}_2$  levels and lower pH. DIC changes associated with organic matter production and remineralization are caused by photosynthesis and respiration, respectively. The small changes in TA reflect nitrate assimilation and remineralization assuming Redfield stoichiometry. Modified after Zeebe & Wolf-Gladrow (2001)

tial shifts in the experimental carbonate system may often be larger than described in our examples.

To ensure well-controlled experimental conditions, it is therefore crucial to work with low cell densities. This important canon of studying carbonate chemistry effects has often been overlooked, also due to other requirements such as the need for sufficient biomass for analysis. It should be mentioned that certain incubation techniques are better suited to work with low cell densities (e.g. semi-continuous or dilute batch) while others tend to have higher cell densities (e.g. chemostats or classical batch). Despite being more challenging, the goal of working with low biomass should be taken on more earnestly in future studies.

## CONCLUSIONS AND RESEARCH DIRECTIONS

While uncertainties regarding the magnitude of physico-chemical changes in the marine environment remain, we are only starting to understand how phyto-

plankton will respond. To answer the question regarding who will be the 'winners' and 'losers' of global change, future research must cover complementary issues on different processes and scales, ranging from the level of individual studies to community efforts in field research. In the following we summarize our main conclusions and outline future research priorities:

(1) Carbonate chemistry manipulations: future laboratory studies should aim to mimic environmental conditions as closely as possible. This relates to realistic  $\text{pCO}_2$  levels and manipulations, but also cell densities and their influence on carbonate chemistry. Few papers currently offer details on carbonate chemistry, irradiance, and other ancillary data. In order to be able to compare studies and to deal with controversial findings, it will therefore be critical to develop standard protocols.

(2) Multiple environmental factors: most experiments have examined  $\text{CO}_2/\text{pH}$  effects in isolation from other environmental factors, typically using saturating light and ample nutrient supply. Since light and/or nutrient availability have been shown to strongly modify the  $\text{CO}_2/\text{pH}$  sensitivity of phytoplankton (and these are also conditions predicted to change), future experiments should look at multiple variables in combination with  $\text{CO}_2$  and assess their interactive effects.

(3) Process-based understanding: an empirical relationship between growth conditions and response of a phytoplankton species is a necessary first step to estimate the potential impact of certain environmental variations on organisms, ecosystems, and the cycling of elements. However, growth conditions are often correlated or anti-correlated in experiments (e.g.  $\text{CO}_2$  and pH), and thus it is not possible to assign the observed response to a single environmental parameter. Future studies should therefore go beyond the descriptive level and unravel the underlying mechanism(s) for the observed responses. Such process-understanding will allow for extrapolation to other species or growth conditions and therefore significantly improve our predictive capabilities.

(4) Diversity in responses: thus far, studies have focused on a limited number of model species. With respect to coccolithophores, for example, only 4 species have been tested to date. Moreover, their different life cycle stages (haploid and diploid), which display different morphologies and modes of calcification, may differ completely in their responses to  $\text{CO}_2/\text{pH}$ . Regarding cyanobacteria, the mismatch between investigated species and overall diversity is even larger. Future studies should therefore acknowledge the diversity in phytoplankton groups and also include other relevant species.

(5) Acclimation versus adaptation: in all classical experimental work, the different growth conditions are



imposed rather quickly and experiments last days to weeks and perhaps months; hence, they only deal with the effect of *acclimation* (i.e. the plasticity of organisms to react to environmental conditions without genetic changes). Given that global change occurs gradually over decades, it is likely that evolution results in species that are genetically and phenotypically different from the contemporary population. Future studies should focus on this important aspect of *adaptation*, which has yet not adequately been addressed (however see Collins & Bell 2004, Collins et al. 2006).

(6) Community level: as laboratory studies with mono-specific cultures lack interactions within or between trophic levels, it will be difficult to draw robust conclusions for whole ecosystems. Possible ways to assess community responses are on-deck perturbations (Tortell & Morel 2002, Tortell et al. 2008) and mesocosms (Engel et al. 2005, Schulz et al. 2008). In addition to these perturbations, comparisons of phytoplankton communities in regions with differences in carbonate chemistry, noting that variations in the contemporary ocean are as high as the changes associated with the projected doubling of  $p\text{CO}_2$ , may improve our understanding of how phytoplankton will respond in the future at the ecosystem level.

(7) Quantitative predictions: models are critical for the integration of results from laboratory and field. Ecosystem models based on more detailed understanding of physiological and ecological responses to changes in  $\text{CO}_2$  and other relevant quantities coupled with general circulation models may lead to quantitative predictions of changes of the global carbon cycle and help to constrain the wide spectrum in future climate scenarios.

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