Journal of Oceanography, Vol. 60, pp. 719 to 729, 2004

Review

Effects of CO_2 Enrichment on Marine Phytoplankton

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(Received 14 October 2003; in revised form 5 May 2004; accepted 5 May 2004)

Rising atmospheric CO_2 and deliberate CO_2 sequestration in the ocean change seawater carbonate chemistry in a similar way, lowering seawater pH, carbonate ion concentration and carbonate saturation state and increasing dissolved CO_2 concentration. These changes affect marine plankton in various ways. On the organismal level, a moderate increase in CO_2 facilitates photosynthetic carbon fixation of some phytoplankton groups. It also enhances the release of dissolved carbohydrates, most notably during the decline of nutrient-limited phytoplankton blooms. A decrease in the carbonate saturation state represses biogenic calcification of the predominant marine calcifying organisms, foraminifera and coccolithophorids. On the ecosystem level these responses influence phytoplankton species composition and succession, favouring algal species which predominantly rely on CO_2 utilization. Increased phytoplankton exudation promotes particle aggregation and marine snow formation, enhancing the vertical flux of biogenic material. A decrease in calcification may affect the competitive advantage of calcifying organisms, with possible impacts on their distribution and abundance. On the biogeochemical level, biological responses to CO_2 enrichment and the related changes in carbonate chemistry can strongly alter the cycling of carbon and other bio-active elements in the ocean. Both decreasing calcification and enhanced carbon overproduction due to release of extracellular carbohydrates have the potential to increase the CO_2 storage capacity of the ocean. Although the significance of such biological responses to CO_2 enrichment becomes increasingly evident, our ability to make reliable predictions of their future developments and to quantify their potential ecological and biogeochemical impacts is still in its infancy.

Keywords:
- CO_2 effects,
- carbonate chemistry,
- phytoplankton,
- biocalcification,
- photosynthesis,
- ecosystem responses,
- biological carbon pump,
- biogeochemical feedbacks.

1. Introduction

The gas composition of the atmosphere and the climate of planet earth are products of the co-evolution of the biospheric and climatic systems. Over earth's history these have evolved gradually as a result of external forcings, such as solar irradiance and orbital patterns, and internal feedbacks between the atmosphere and biosphere. During its latest phase this co-evolution settled into a persistent pattern of glacial-interglacial cycles, with atmospheric CO_2 fluctuating between 180 μatm in glacial and 280 μatm in interglacial times (Petit *et al.*, 1999). For about the last 20 million years atmospheric pCO_2 has never exceeded 300 μatm (Berner *et al.*, 1990). While the periodic cycle of glacial-interglacial changes is clearly driven by earth's orbital patterns, the extent of change in climate, CO_2 and other climate-relevant gases is controlled by a variety of mechanisms, the exact balance of which is still unknown. Among these mechanisms, biologically-driven reactions and feedbacks, involving both terrestrial and marine ecosystems, are bound to play a critical role.

In the past 200 years, man has become a distinct factor of the earth system by influencing the carbon cycle, mainly through the injection of carbon dioxide by the burning of fossil fuels and by changes in land use. The current period, frequently termed the "anthropocene", has no direct analogue in the geological past. Although human activities impact most strongly on the carbon cycle, this has repercussions for the earth system as a whole, since the carbon cycle is coupled with climate, the water cycle, nutrient cycles and photosynthesis on land and in the oceans. It is also central to human society as it is directly linked to the production of energy. Growing world

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population and changing life styles put an increasing pressure on energy production and, hence, man-made changes in the carbon cycle proceed at an unprecedented rate.

While the impact of human existence on the carbon cycle may have been unintended, attempts to regulate or manage it, however, will require an intentional global effort on an unprecedented scale. No single measure is likely to provide the capacity to stabilize atmospheric CO_2 concentration in the presence of continued CO_2 emissions at the present-day level. Among the wide range of possible mitigation strategies ocean CO_2 sequestration, either through an enhancement of biological carbon fixation or through direct CO_2 injection, is receiving increasing attention. Obviously, any responsible effort aiming to mitigate the green-house problem through purposeful CO_2 sequestration in the ocean requires a solid understanding of marine ecosystem regulation and biogeochemical cycling. While the study of terrestrial ecosystem responses to man-made environmental changes, including CO_2 enrichment, has been a topic of intense research over more than a decade, research of this kind in the marine biosphere is developing only recently. The limited information presently available indicates, however, that marine ecosystems are indeed sensitive to CO_2 enrichment. This paper provides examples of marine planktonic responses to increased seawater CO_2 levels and the related changes in seawater carbonate chemistry. While the initial response typically occurs at the organismal level, the effects can carry on to the ecosystem level, which in turn can impact marine biogeochemical cycling. Corresponding changes in biologically-mediated carbon cycling can have both positive and negative feedbacks on oceanic CO_2 storage and air/sea CO_2 exchange.

How does this relate to deep ocean CO_2 sequestration? Ideally, it should not, at least not on centennial time scales. If properly conducted, the sequestered CO_2 would be cut off from exchange with the surface layer for at least several hundred years. Phytoplankton and the bulk of zooplankton thrives in or close to the sunlit surface layer of the ocean, i.e. outside the waters which would be considered for deep sea CO_2 injection. Hence, the effect of deep ocean CO_2 sequestration on the upper ocean plankton would be—if anything—to contribute to mitigating adverse effects of a human-induced increase in atmospheric CO_2 on marine plankton. The surface ocean, on the other hand, experiences an unintentional, gradual and global CO_2 enrichment due to the continuous rise of atmospheric CO_2. Changes in atmospheric pCO_2 are mirrored by corresponding changes in the carbonate system of surface seawater with a time lag of less than one year (Zeebe and Wolf-Gladrow, 2001). Responses of the marine plankton to CO_2 enrichment therefore can be expected to occur in phase with the present increase in atmospheric CO_2. The focus of this paper will therefore be on the effect of CO_2-related changes in seawater carbonate chemistry caused by the uptake of anthropogenic CO_2 by the surface ocean.

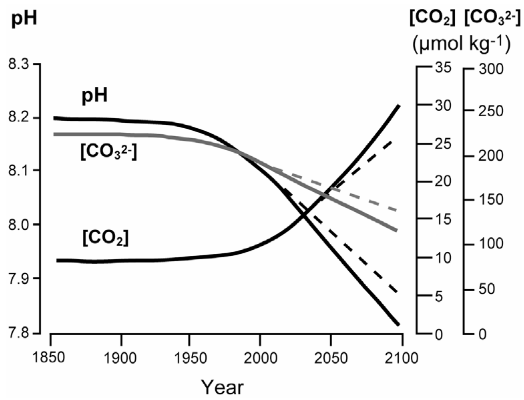

Fig. 1. Seawater pH and the dissolved carbon dioxide (CO_2) and carbonate ion (CO_3^{2-}) concentrations in the surface layer of the ocean assuming a "business as usual" (IS92a) anthropogenic CO_2 emission scenario (Houghton *et al.*, 1995). Dashed lines represent the predicted changes in carbonate chemistry if CO_2 emissions are reduced according to the Kyoto Protocol (modified after Wolf-Gladrow *et al.*, 1999).

2. Effects of CO_2 Enrichment on Seawater Carbonate Chemistry

The present rise in atmospheric CO_2 and the resulting net flux of CO_2 into the surface ocean causes a continuous change in seawater carbonate chemistry. The dissolved CO_2 reacts with water according to:

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}.$$

At a typical surface ocean pH value of 8.2, less than 1% of the added CO_2 remains as dissolved CO_2, while the rest is converted into HCO_3^- (ca. 90%) and CO_3^{3-} (ca. 9%). The reaction of CO_2 with water generates one proton for each HCO_3^- and two protons for each CO_3^{3-} formed. This acidification causes a shift of the pH-dependent equilibrium between CO_2, HCO_3^- and CO_3^{3-} towards higher CO_2 levels and lower carbonate ion concentrations, $[CO_3^{3-}]$. The net result of CO_2 addition therefore is an increase in the concentration of dissolved CO_2, $[CO_2]$, a decrease in $[CO_3^{3-}]$ and seawater pH (Fig. 1), and a slight increase in the concentrations of HCO_3^- and dissolved inorganic carbon, DIC.

The recent increase in atmospheric CO_2 from a pre-industrial level of 280 μatm to the present level of 370 μatm has decreased surface ocean pH values by approximately 0.12 units. At atmospheric CO_2 levels of 700 μatm, as expected for the end of this century in a "business as usual" scenario (IS92a, Houghton *et al.*, 1995, 2001), seawater pH will decrease by an additional 0.3 units. While the concentration of dissolved CO_2 will have tripled by this time, the carbonate ion concentration will decrease by nearly 50%. The projected pH shift covers the entire range of pH variations between 7.7–8.3 presently observed in open ocean surface waters. The projected increase in $[CO_2]$ and decrease in $[CO_3^{3-}]$ is outside the natural range of the past 20 Million years.

Changes in surface ocean carbonate chemistry caused by the present rise in atmospheric CO_2 are slow and modest compared to those occurring in the vicinity of CO_2 injection plumes. Depending on the method of injection and the surface area of the hydrate formed, seawater pH in close vicinity of the injected CO_2 can decrease by as much 2–3 pH units (Brewer *et al.*, 2000). With the formation of a thin hydrate film after slow injection of liquid CO_2, on the other hand, pH values became indistinguishable from the *in situ* background level. While these short-term changes in seawater chemistry occur on very small scale, from an impact assessment point of view the longer-term and larger-scale effects of CO_2 injection, including the risk of re-surfacing of sequestered CO_2, need to be considered.

Following sections outline potential effects of CO_2 enrichment and the related changes in seawater carbonate chemistry on the marine phytoplankton from the organismal (Section 3) to the ecosystem level (Section 4) and will give examples of possible implications for marine biogeochemical cycling (Section 5).

3. Effects on the Organismal Level

3.1 Photosynthesis

Photosynthesis involves a series of reactions that start with capturing light energy, transferring it into the energy-conserving compounds NADPH and ATP, and using these compounds to fix CO_2 in the Calvin cycle. Actively growing algae allocate about 50% of recent photosynthate to protein synthesis, resulting in a competition for NADPH and ATP between CO_2 fixation, transport processes, nitrate reduction and protein formation (Geider and MacIntyre, 2001). Thus photosynthesis and downstream processes leading to cellular growth are primarily light-dependent processes. Photosynthesis-irradiance response curves are commonly used to assess the photosynthetic performance.

In the dark reaction of photosynthesis CO_2 is fixed by the carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). RUBISCO uses CO_2 as the only carbon substrate and has a half-saturation constant of 20 to 70 μmol kg^{-1} of CO_2, depending on the species (Badger *et al.*, 1998). Typical seawater CO_2 concentrations, ranging between 10 and 25 μmol kg^{-1}, are therefore insufficient to ensure effective operation of RUBISCO carboxylation. To overcome the low CO_2 affinity of their main carboxylating enzyme, algae have developed mechanisms to actively increase the CO_2 concentration at the site of carboxylation. These include active uptake of CO_2 and HCO_3^- into the algal cell and/or into the chloroplasts. In concert with active carbon transport, algae catalyse the conversion between HCO_3^- and CO_2 intra- and extracellularly with the enzyme carbonic anhydrase. Which ever the pathways for this so-called carbon concentrating mechanism (CCM) may be, its operation requires additional energy. Since marine phytoplankton live in an environment in which light (energy) is one of the primary limiting resources, the energy available for active transport of inorganic carbon and other nutrients is often limited. Energy-limitation may be one of the reasons why phytoplankton do not rely entirely on the active uptake of inorganic carbon from the abundant pool of HCO_3^-, but are drawing part of their carbon from the small and variable pool of CO_2.

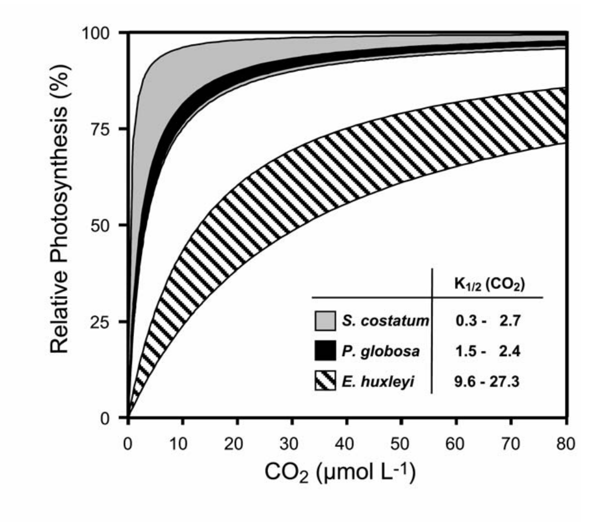

Fig. 2. Photosynthesis of phytoplankton species differs with respect to CO_2 sensitivity: While most species (here *Skeletonema costatum* and *Phaeocystis globosa*) are at or close to CO_2 saturation at present day CO_2 levels (8–20 μmol L^{-1}), coccolithophores such as *Emiliania huxleyi* have comparatively low affinities for inorganic carbon and appear to be carbon-limited in today's ocean. This raises the possibility that coccolithophores may benefit directly from the present increase in atmospheric CO_2. The range in half-saturation concentrations ($K_{1/2}$; in μmol L^{-1}) for photosynthesis shown here reflects the degree of regulation as a function of pCO_2 during growth (according to Rost *et al.*, 2003). Highest apparent affinities for CO_2 were generally observed in cells which were grown under low pCO_2.

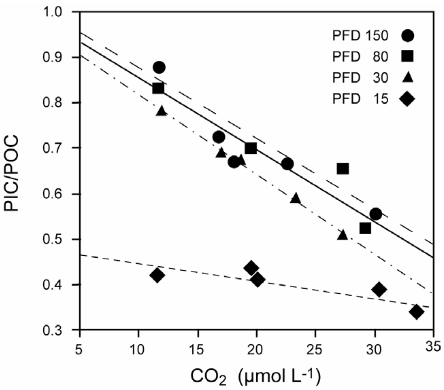

Fig. 3. Rising CO_2 decreases the ratio of calcification to organic carbon production (PIC/POC) in *E. huxleyi*. The decrease in PIC/POC is caused by enhanced photosynthetic carbon fixation and reduced or constant calcification. This trend is consistent over a range of photon flux densities (PFDs; in μmol photons $m^{-2}s^{-1}$), yet declines under severe light-limitation (modified after Zondervan *et al.*, 2002).

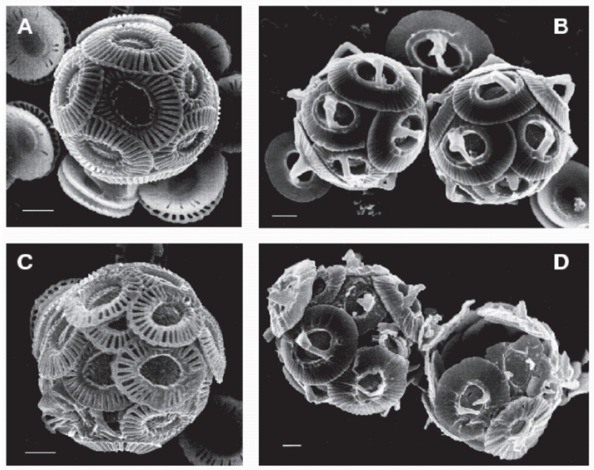

Fig. 4. Scanning electron microscopy (SEM) photographs of *Emiliania huxleyi* (A, C) and *Gephyrocapsa oceanica* (B, D) collected from cultures incubated at pCO$_2$ levels of ca. 300 μatm (upper panel) and 780–850 μatm (lower panel). Scale bars represent 1 μm. Note the difference in the coccolith structure (including distinct malformations) and in the degree of calcification of cells grown at normal and elevated CO_2 levels (adapted form Riebesell *et al.*, 2000).

Recent studies indicate that dominant phytoplankton species differ in their CO_2 requirement. Whereas some species preferably use CO_2 as a carbon source, others mainly draw their inorganic carbon from the large pool of HCO_3^- (e.g. Elzenga *et al.*, 2000). Group-specific differences in CO_2 sensitivity also exist in the carbon metabolism (Fig. 2). Most notably, the photosynthetic carbon fixation rate of all diatom species tested so far and of the prymnesiophyte *Phaeocystis globosa* are at or close to CO_2-saturation at present day CO_2 levels (Burkhardt *et al.*, 1999, 2001; Rost *et al.*, 2003). In contrast, the coccolithophorids *Emiliania huxleyi* and *Gephyrocapsa oceanica* are well below saturation at these levels (Riebesell *et al.*, 2000; Rost *et al.*, 2003). These findings suggest large differences in CO_2-sensitivity between major phytoplankton taxonomic groups. CO_2-sensitive taxa, such as the calcifying coccolithophorids, should therefore benefit more from the present increase in atmospheric CO_2 compared to the non-calcifying diatoms and *Phaeocystis*.

3.2 Calcification

The formation of calcareous skeletons is a widespread phenomenon in various groups of marine planktonic organisms. Their production and subsequent sinking generates a continuous rain of calcium carbonate to the deep ocean and the sediments. CO_2-related changes in seawater carbonate chemistry were recently shown to affect marine biogenic calcification. A doubling in present-day atmospheric CO_2 concentrations is predicted to cause a 20–40% reduction in biogenic calcification of the predominant calcifying organisms, the corals, coccolithophorids (Fig. 3), and foraminifera (Gattuso *et al.*, 1998; Kleypas *et al.*, 1999; Bijma *et al.*, 1999; Riebesell *et al.*, 2000). Diminished calcification in coccolithophorids was accompanied by an increased proportion of malformed coccoliths and incomplete coccospheres (Fig. 4) in the two bloom-forming species *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Riebesell *et al.*, 2000).

Although the sensitivity of the different groups of calcifying organisms to CO_2-related changes in carbonate chemistry is very similar, suggesting a common mechanism for the observed responses in each of these groups, it is not clear yet which is the physiologically relevant factor ultimately causing the slow down in biogenic calcification. While the pH value, carbonate ion concentration and carbonate saturation state each may directly impact the calcification process, a reasonable representation of the effect of carbonate chemistry on biologically-mediated $CaCO_3$ precipitation can be based on the carbonate saturation state of seawater, Ω, which is expressed as:

$$\Omega = \frac{\left[\mathrm{Ca}^{2+}\right]_{\mathrm{sw}} \times \left[\mathrm{CO}_3^{2-}\right]_{\mathrm{sw}}}{K_{\mathrm{sp}}^*}$$

where $[Ca^{2+}]_{sw}$ and $[CO_3^{2-}]_{sw}$ are the calcium and carbonate ion concentrations in seawater and K_{sp}^* is the stoichiometric solubility product, defined as $K_{sp}^* = [Ca^{2+}]_{sat} \times [CO_3^{2-}]_{sat}$. $[Ca^{2+}]_{sat}$ and $[CO_3^{2-}]_{sat}$ refer to the equilibrium ion concentrations in a seawater solution saturated with $CaCO_3$.

Concerning the question which is the relevant parameter determining the sensitivity of biogenic calcification to seawater carbonate chemistry, it may help to take a look at the geological past. Recent evidence from fluid inclusions suggests that calcium concentrations in the Cretaceous were more than twice as high as in the modern ocean (Horita *et al.*, 2002). As $CaCO_3$ compensation tends to restore the saturation state of the ocean towards $CaCO_3$ saturation, higher calcium concentrations would correspond to lower carbonate ion concentrations. In spite of lower expected CO_3^{2-} concentration, massive accumulation of biogenic carbonates occurred in the Cretaceous, suggesting that the carbonate saturation state may exert a stronger control on biogenic calcification than any of the other possible candidates, e.g. pH, CO_2, or CO_3^{2-} concentrations.

What effect reduced $CaCO_3$ production has for calcifying organisms ultimately depends on the role of calcification and calcareous skeletons in the cell physiology and ecology of these organisms. Despite intensive research on coccolithophores the function of calcification in this group is still not well understood (Young, 1994; Paasche, 2002). It has been hypothesized that the cell-covering layer of coccoliths, the coccosphere, serves to protect against grazing and/or viral attack and that coccoliths could bundle and modify the light intercepted by the algal cell (Young, 1994). While these hypotheses remain speculative, there is some support for the "trash-can function" of calcification, whereby $CaCO_3$ precipitation serves as a mechanism to facilitate the use of bicarbonate in photosynthesis. Provided that HCO_3^- is the carbon source used for calcification, one possible benefit for photosynthesis could be the release of CO_2 or protons in the course of calcification according to the following reactions:

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

or

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

Either CO_2 could be used directly in photosynthesis or protons could be used in the conversion of HCO_3^- to CO_2. In either case this would provide the calcifying cell with a mechanism to access HCO_3^-, the largest pool of inorganic carbon in seawater, for photosynthetic carbon fixation.

While compelling evidence for or against such a mechanism is still lacking, it appears that calcification in coccolithophores is neither a prerequisite for efficient photosynthesis nor is it particularly effective in mitigating CO_2 limitation. This is indicated, for example, by the fact that photosynthesis continues unaltered when calcification ceases, such as when cells are grown in calcium-free medium (Paasche, 1964). Likewise, non-calcifying cells can photosynthesise as efficient or even more efficiently than calcifying ones, as indicated by P vs. E relationships of non-calcifying compared to calcifying strains of *E. huxleyi* (Rost and Riebesell, 2004). Moreover, mass spectrometric measurements indicate that non-calcifying cells of *E. huxleyi* are capable of direct uptake of HCO_3^- (Rost and Riebesell, 2004), which implies that HCO_3^- utilisation is not tied to calcification. Further evidence that calcification is unsuited to prevent CO_2 limitation of coccolithophores comes from observations that the rate of photosynthesis decreases with decreasing CO_2 concentration despite a concomitant increase in calcification rate (Riebesell *et al.*, 2000; Zondervan *et al.*, 2002). In essence, if supply of CO_2 for photosynthesis is indeed a primary role of calcification, this mechanism is rather inefficient when compared to CO_2 concentrating mechanisms of non-calcifying phytoplankton such as diatoms and *Phaeocystis* (Burkhardt *et al.*, 2001; Rost *et al.*, 2003).

Whatever the purpose of calcification in coccolithophores, continued acidification of surface seawater due to rising atmospheric CO_2 will further deteriorate the chemical conditions for biogenic calcification. Despite a wealth of information on the effect of environmental conditions on coccolith production, the mechanism of calcification by coccolithophores is not completely understood. It is too early, therefore, to make any predictions regarding the physiological or ecological consequences of a CO_2-related slow down in biogenic calcification. Obviously, a better mechanistic understanding of the process of biogenic calcification will help to better constrain the effects of global changes, including changes in temperature and light availability, on bio-calcification.

3.3 Elemental composition

One of the few canonical numbers in marine biogeochemical sciences is the Redfield ratio, the ratio of C:N:P in marine particulate matter. When Redfield discovered an unexpected congruence of this value in numerous regions of the world's ocean (Redfield *et al.*, 1963), it soon became one of the cornerstones in biological oceanography and geochemistry, commonly used to infer about the large-scale operation of the marine biogeochemical system. Recent studies have challenged the concept of a constant Redfield ratio and have highlighted the importance to better constrain the factors determining phytoplankton stoichiometry (for review see

Sterner and Elser, 2002). Phytoplankton indeed show a high degree of variability in their stoichiometric composition C:N:P. While a large body of literature exists on the role of nutrients, light and temperature on algal stoichiometry (Sterner and Elser, 2002), comparatively little is known about the effects of inorganic carbon and CO_2 availability on phytoplankton elemental composition.

Initial studies by Burkhardt and Riebesell (1997) and Burkhardt *et al.* (1999) showed considerable changes in C:N:P composition in response to increasing $p\mathrm{CO_2}$ but also revealed large species-specific differences. Whereas in some microalgae cellular C:N and C:P increased with increasing $p\mathrm{CO_2}$, other species responded with lower C:nutrient ratios under increased CO_2 availability. Moreover, algal C:N:P mainly responded to altered CO_2 at $p\mathrm{CO_2}$ levels lower than current values and remained more or less stable at higher than present $p\mathrm{CO_2}$ values. It should be noted, however, that these experiments were conducted under nutrient replete conditions. Strongest deviations from Redfield proportions typically occur in nutrient limited phytoplankton and under conditions of unbalanced nutrient supply. Clearly, more work is needed to further examine the effect of CO_2 availability on phytoplankton stoichiometry.

Research on microalgal stoichiometry has commonly focussed on the *cellular* composition of phytoplankton. Although it is well-recognized that the proportion of primary produced organic matter lost from the cell through exudation can be substantial, particularly under nutrient-limitation, this fraction of primary production is frequently neglected in biogeochemical analyses. As dissolved organic matter can be subject to rapid bacterial degradation, this material is thought to contribute little to vertical carbon transport in the ocean. However, recent work demonstrated the spontaneous assembly of dissolved organic matter into polymer gels (Chin *et al.*, 1998). Colloidal organic matter is an important precursor for the formation of transparent exopolymer particles (TEP) which are shown to accelerate particle aggregation and thereby enhance vertical particle flux (Passow, 2002). This process may therefore provide an efficient pathway to channel dissolved and colloidal organic matter into the particulate pool. Analyses on the variability of Redfield proportions should therefore include dissolved organic components.

That the production of dissolved organic matter may be sensitive to CO_2 availability is indicated in a recent study by Engel (2002), showing that in a natural plankton assemblage TEP production increases with increasing $p\mathrm{CO_2}$. A similar response was also observed in monospecific cultures of the diatom *Thalassiosira weissflogii* and the coccolithophorid *Emiliania huxleyi* (Heemann, 2002). Since transparent exopolymer particles primarily form from exuded polymeric carbohydrates they have a molar C:N:P ratio much higher than the Redfield ratio (Engel and Passow, 2001). CO_2 sensitivity of TEP production may therefore cause significant shifts in elemental composition of particulate matter towards higher than Redfield ratios.

4. Effects on the Ecosystem Level

The ecological success of a phytoplankton species is ultimately determined by its ability to optimise the balance between growth and loss processes. While the former is largely controlled by the availability and optimal utilisation of essential resources, such as light and nutrients, the latter includes factors such as cell sinking, cell mortality due to grazing, viral and parasite infection, as well as autolysis. Any investment successfully reducing mortality therefore weighs equally strong as cellular measures capable of increasing cell growth by the same magnitude. Although the role of $CaCO_3$ production in coccolithophores is not clear, reduced calcification is likely to affect both resource utilization and cellular protection with possible adverse effects on the competitive fitness of this group. Although it would be premature to speculate on possible implications of reduced calcification for coccolithophore distribution and productivity, a likely scenario would be a shift in the relative distribution and productivity of the calcareous primary producers relative to their siliceous counterpart, the diatoms. Both groups are important components of the pelagic food web and major drivers of biogeochemical cycling in the ocean. Due to their specific roles in plankton ecosystem regulation, a shift in the ratio of coccolithophores to diatoms is likely to also affect higher trophic levels. A basin-wide shift in the opal:carbonate ratio of sedimenting particles has in fact occurred across the entire North Atlantic and is attributed to large-scale changes in climatic forcing (Antia *et al.*, 2001). The observed steady decrease in this ratio since the early eighties may reflect an increase in the abundance and productivity of coccolithophores relative to diatoms.

To examine the direct effect of CO_2 enrichment on the ecosystem level, CO_2 perturbation experiments were conducted in outdoor mesocosms with natural plankton communities, in Raunafjorden south of Bergen, Norway. Nine 11 m^3 enclosures moored to a floating raft were aerated in triplicate with normal air, CO_2-enriched and CO_2-depleted air to achieve $p\mathrm{CO_2}$ levels of 190 μatm, 370 μatm, and 710 μatm, simulating glacial, present day, and predicted year 2100 conditions (assuming IPCC scenario 92a; Houghton *et al.*, 1995), respectively (Fig. 5). The enclosures were covered with gas tight tents made of ETFE foil (Foiltec, Bremen, Germany), which allowed for 95% light transmission of the complete spectrum of sunlight. To allow the seawater carbonate system to freely change in response to biological activities, aeration of the

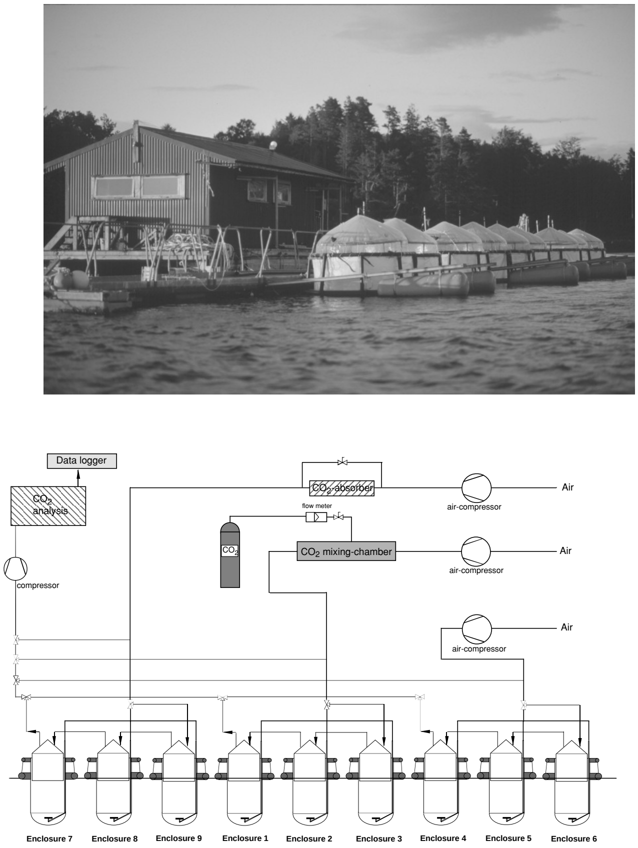

Fig. 5. Upper panel: Mesocosm facilities of the University of Bergen Large-Scale-Facilities in the Raunefjorden, 60.3°N, 5.2°E. Shown are the nine enclosures covered by tents made of gas-tight ETFE foil. Lower panel: Sketch of the aeration systems for CO_2 manipulation of the seawater inside the mesocosms. Ambient air was pumped either directly to the mesocosms (4–6), enriched with CO_2 in a mixing chamber (1–3), or depleted in CO_2 through adsorption onto Na_2CO_3 platelets (7–9).

enclosed water column was discontinued after target CO_2 values were achieved. At this point the nutrient depleted water was fertilized with nitrate and phosphate to initiate the development of a phytoplankton bloom. Aeration of the atmosphere underneath the tents at target CO_2 levels continued throughout the investigation.

During the course of this study the development and decline of a bloom consisting of a mixed phytoplankton community was followed. Significantly higher net community production (as measured by DIC drawdown) was observed under elevated CO_2 levels during the build-up the bloom (Bellerby *et al.*, unpubl.). CO_2-related differences in primary production continued after nutrient exhaustion, leading to higher production of transparent exopolymer particles (TEP) under high CO_2 conditions (Engel *et al.*, unpubl.). CO_2-dependent differences were also observed with respect to the phytoplankton composition. Whereas under glacial CO_2 conditions diatoms dominated at the end of the bloom, the community developing under CO_2 conditions as expected for the end of this century was dominated by *Emiliania huxleyi* (Huonic *et al.*, unpubl.). These findings are consistent with observed differences in carbon acquisition mechanisms between diatoms and coccolithophorids as outlined above (Subsection 3.1).

In principle, coccolithophores may benefit from the present increase in atmospheric pCO_2 and related changes in seawater carbonate chemistry. At pre-industrial CO_2 levels, rates of photosynthetic carbon fixation of *E. huxleyi* and *Gephyrocapsa oceanica* are well below CO_2 saturation. In comparison, photosynthesis of other bloom-forming phytoplankton groups such as diatoms and *Phaeocystis* are less CO_2-sensitive due to their efficient CCMs (Rost *et al.*, 2003). Although under natural conditions CO_2 limitation is likely to be of minor importance for the proliferation of *E. huxleyi* compared to other limiting resources and loss processes, increasing CO_2 availability may improve the overall resource utilisation of *E. huxleyi* and possibly of other fast-growing coccolithophore species. If this provides an ecological advantage for coccolithophores, rising atmospheric CO_2 could potentially increase the contribution of calcifying phytoplankton to overall primary production.

CO_2 effects on the taxonomic composition and nutrient utilization were also observed in a natural phytoplankton assemblage by Tortell *et al.* (2002). A decrease in the abundance of diatoms and an increase in that of *Phaeocystis* occurred when pCO_2 levels were raised from 150 μatm to 750 μatm. This CO_2-dependent shift was associated with a significant change in nutrient utilization, with higher ratios of nitrate:silicate and nitrate:phosphate consumption by phytoplankton in the low CO_2 treatment. Despite these changes, total biomass and primary productivity did not differ significantly between CO_2 treatments. This and the mesocosm study described above indicate that CO_2 sensitivity of microalgae is not limited to species relying entirely on CO_2 utilization. Even for species capable to access the large pool of HCO_3^-, either through direct uptake of HCO_3^- or its conversion to CO_2 via carbonic anhydrase, increased CO_2 availability can reduce the allocation of resources into carbon acquisition. In cases of resource (energy and/or nutrient) limitation, the ability to regulate resource allocation accordingly could provide these species a competitive advantage over those incapable to regulate in response to changes in CO_2 availability (Rost *et al.*, 2003).

5. Effects on the Biogeochemical Level

Despite a high degree of structural complexity, the energy flow and elemental cycling of many ecosystems

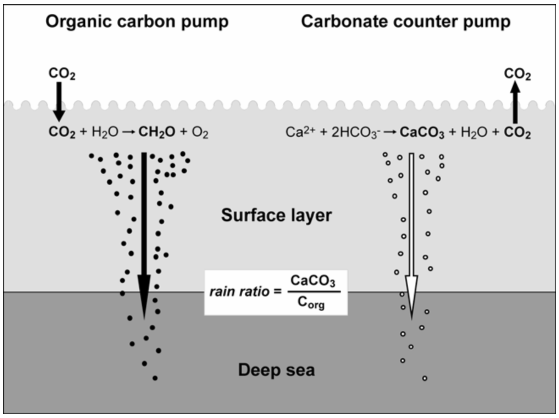

Fig. 6. Changes in the concentrations of DIC, total alkalinity, and CO_2 as a result of calcification at pre-industrial times (left vectors) and in year 2100 (right vectors). Numbers at the vectors are relative values. The difference in the effect of calcification on seawater carbonate chemistry in the two scenarios results from a CO_2-related change in the seawater buffer capacity (see text for details, modified after Zondervan *et al.*, 2001).

are dominated by a comparatively small number of species. A first step in understanding possible consequences of environmental change on ecosystem functioning, therefore, is to determine the relevant responses of these key species. This also holds true for the marine pelagic system, the largest ecosystem on our planet. While the base of its complex food web is formed by more than 5000 species of marine phytoplankton, only a few taxonomic groups of phytoplankton, and within these groups a relatively small number of species, are responsible for most of the system's primary production, the energy transfer to higher trophic levels and the vertical export of biogenic material to the deep ocean. These key species can be further distinguished into so-called "functional groups", i.e. phytoplankton building siliceous or calcareous shells, such as the diatoms and coccolithophores, respectively, flagellates forming organic plates or mucilaginous colonies, and cyanobacteria fixing atmospheric nitrogen. Each of these functional groups has a distinct effect on elemental fluxes, both between the surface and deep ocean as well as the overlying atmosphere.

With regard to the oceanic carbon cycle, the effect of calcifying plankton, including coccolithophores as the dominant calcifying phytoplankton, differs greatly from that of other primary producers. The fixation of inorganic carbon via photosynthesis in the sunlit upper mixed layer and the vertical export of part of this organic material cause a draw down of CO_2 in the surface ocean. Remineralisation of the particulate organic carbon on its way to depth releases organically-bound CO_2, which then accumulates in deeper layers (Fig. 6). This process, termed the *organic carbon pump*, thereby causes a net draw down of CO_2 from the atmosphere into the ocean. In contrast, the production and export of calcium carbonate not only binds dissolved carbon into particulate carbon and thereby reduces total dissolved inorganic carbon, it also lowers seawater alkalinity and changes the equilibrium between the different forms of dissolved inorganic carbon, causing a net release of CO_2 to the atmosphere. Due to its counteracting effect on CO_2 flux, this process is often referred to as the *carbonate counter pump*. The relative strength of the two biological carbon pumps, represented by the so-called *rain ratio* (the ratio of particulate inorganic to organic carbon in exported biogenic matter), determines to a large extent the flux of CO_2 between the surface ocean and the overlying atmosphere.

But how can a process transporting inorganic carbon from the surface to the deep ocean release CO_2 to the atmosphere? The formation of one unit of calcium carbonate according to

$$\mathrm{Ca}^{2+} + \mathrm{CO}_3^{2-} \rightarrow \mathrm{CaCO}_3 \tag{1}$$

lowers the dissolved inorganic carbon (DIC) concentration, with

$$\mathrm{DIC} = \left[\mathrm{CO}_2\right] + \left[\mathrm{HCO}_3^-\right] + \left[\mathrm{CO}_3^{2-}\right] \tag{2}$$

by one unit. Due to its double negative charge the concentration of carbonate ions carries the factor 2 in the expression of total alkalinity (T*Alk*), with

$$\mathrm{T}Alk = \left[\mathrm{HCO}_3^-\right] + 2\left[\mathrm{CO}_3^{2-}\right] + \left[B(\mathrm{OH})_4^-\right] + \left[\mathrm{OH}^-\right] - \left[\mathrm{H}^+\right]. \tag{3}$$

Thus, the precipitation of one unit of calcium carbonate lowers seawater alkalinity by two units. How this affects the concentration of dissolved CO_2 is best illustrated, following Zondervan *et al.* (2001), by plotting CO_2 concentration as a function of DIC and T*Alk* (Fig. 7). Starting at typical surface ocean conditions of DIC = 2.0 mmol kg^{-1} and T*Alk* = 2.3 mmol kg^{-1} (beginning of diagonal vector on left side), the corresponding CO_2 concentration, $[CO_2]$, is 10.5 μmol kg^{-1} (assuming T = 15°C, S = 35). Precipitating 50 μmol kg^{-1} of $CaCO_3$ lowers DIC and T*Alk* by 0.05 and 0.1 mmol kg^{-1}, respectively. With the new DIC and T*Alk* values of 1.95 mmol kg^{-1} and 2.2 mmol kg^{-1} (end of diagonal vector), the corresponding CO_2 concentration now is 12.4 μmol kg^{-1}. Assuming that the seawater initially was in equilibrium with the atmosphere with respect to CO_2, it is now CO_2 over-saturated. To restore equilibrium, 0.63 units of CO_2 need to be released for each unit of inorganic carbon precipitated via calcification. In our example, 50 μmol kg^{-1} $CaCO_3$ production

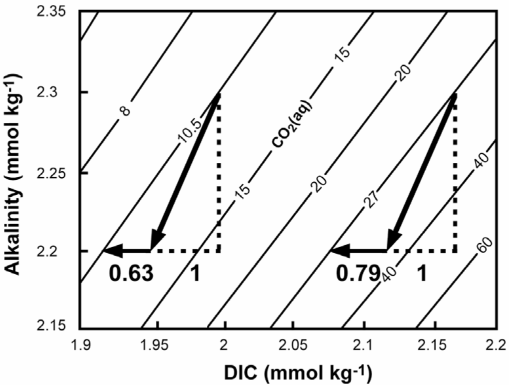

Fig. 7. The biological carbon pumps: Photosynthetic production of organic matter in the surface layer and its subsequent transport to depth, termed *organic carbon pump*, generates a CO_2 sink in the ocean. In contrast, calcium carbonate production and its transport to depth, referred to as the *carbonate counter pump*, releases CO_2 in the surface layer. The relative strengths of these two processes (*rain ratio*) largely determine the biologically-mediated ocean atmosphere CO_2 exchange.

would require a degassing of 32 μmol kg^{-1} of CO_2 in order to maintain CO_2 equilibrium between our parcel of seawater and the overlying atmosphere. Due to the decrease in the buffer capacity with increasing [CO_2] (decreasing pH) of seawater, the ratio of CO_2 released per carbonate precipitated increases with rising atmospheric CO_2 (Frankignoulle *et al.*, 1994). Under conditions expected for the year 2100 (see Fig. 1), the surface ocean equilibrium CO_2 concentration would be ca. 27 μmol kg^{-1}. Under these conditions, precipitation of 50 μmol kg^{-1} $CaCO_3$ would release ca. 40 μmol kg^{-1} of CO_2, corresponding to 0.79 units of CO_2 for each unit of $CaCO_3$ (right vectors). If global calcification were to remain constant over this time, increased calcification-induced CO_2 release results in a positive feedback to rising atmospheric CO_2 (Zondervan *et al.*, 2001). With the observed decrease of bio-calcification with increasing $p$$CO_2$ this positive feedback turns into a negative one, leading to an increase in the CO_2 storage capacity of the surface ocean (Riebesell *et al.*, 2000).

Obviously, the ratio of coccolithophore calcification to organic matter production, but even more so the ratio of calcareous to non-calcareous primary production, strongly determine the relative strengths of the two biological carbon pumps. $CaCO_3$ may also act as a “ballast” mineral that increases the transfer efficiency of POC from the surface to the deep sea (Armstrong *et al.*, 2002; Klaas and Archer, 2002). The presence of coccoliths could thus enhance the sedimentation of POC such as in faecal pellets (Buitenhuis *et al.*, 1996). On geological time-scales variation in coccolith abundance in the sedimentary record is often correlated with glacial-interglacial transitions, indicating a predominance of coccolithophores during interglacial periods (McIntyre *et al.*, 1972; Henrich, 1989). The presumably lower contribution of coccolithophores relative to non-calcifying phytoplankton may have contributed to the lower atmospheric CO_2 levels in glacial times (Harrison, 2000; Ridgwell *et al.*, 2002).

Estimates of global calcium carbonate production range from 0.64 to 2 Gt C per year (e.g. Milliman and Droxler, 1996). Recent estimates of global annual marine primary production are between 40 and 50 Gt C annually (Antoine *et al.*, 1996), of which about 10 Gt C are exported to the deep sea. The estimated export ratio of $CaCO_3$ to particulate organic carbon (rain ratio) is therefore relatively high, ranging from 0.17 to 0.4 (see Holligan and Robertson, 1996 for review). Since the export of $CaCO_3$ and POC have opposite effects on the surface ocean CO_2 partial pressure, a change in the rain ratio affects the partitioning of CO_2 between ocean and atmosphere. It has been suggested that the rain ratio is not constant through time and is an important factor in the regulation of global climate (Archer and Maier-Reimer, 1994).

Changes in marine production and phytoplankton species composition and succession will also impact other biogeochemical cycles, such as the nitrogen, opal, and sulphur cycles, which in turn is bound to feedback on climate. Despite the potential importance of global change-induced biogeochemical feedback, our understanding of these processes is still in its infancy. It is now becoming increasingly clear, however, that the assumption of a constant oceanic biosphere, implicitly used in many assessments of future global change, is no longer viable.

6. Outlook

In view of the rapid changes in environmental conditions presently occurring on a global scale, a major challenge in earth system sciences is to predict biospheric responses to global change. Any attempt to forecast biologically-mediated changes in marine biogeochemical cycling critically depends on our ability to explain the distribution and succession of the dominant plankton groups in relation to biotic and abiotic environmental conditions. The importance of a fundamental understanding of marine ecosystem regulation becomes even more pressing in view of recent considerations to manage or manipulate oceanic CO_2 sequestration. While the ocean’s capacity to store anthropogenic CO_2 is immense, its sensitivity to related changes in environmental conditions is likely to be high. In contrast to the terrestrial biosphere, marine ecosystems have evolved in a comparatively homogenous environment under conditions of relative stability over evolutionary timescales. Even subtle changes in the environmental conditions may therefore have strong effects on ecosystem functioning, with yet unforeseeable consequences for elemental cycling.

Considerable evidence is now accumulating and indicating that CO_2-related changes in seawater carbonate chemistry can directly affect the marine biosphere. CO_2-dependent effects on the marine plankton can range from physiological responses on the organismal level, changes in the ecosystem structure and regulation, to large scale shifts in biogeochemical cycling. Their net effect on carbon cycling ultimately depends on the relative importance and sensitivity of each of these processes to global change. In addition, possible changes at the level of primary producers, such as those outlined above, are likely to be amplified, compensated or reversed by interference from higher trophic levels, which themselves are bound to respond to environmental change. Much of the information presently available is based on single parameter perturbation studies. Global change, however, simultaneously modifies a multitude of environmental conditions, whose combined effects on the biosphere are largely unknown. Moreover, global change related responses of the marine plankton as described above have so far been observed only in short-term perturbation experiments. To what extent physiological acclimatization and genetic adaptation may counteract adverse effects related to environmental changes is presently unknown.

In view of the inherent complexity of biological systems, including marine pelagic systems, it seems impossible at this point to provide a comprehensive and reliable forecast of large-scale and long-term biological responses to global environmental change. Any responsible consideration aiming to regulate or manipulate the earth system in an attempt to mitigate the green-house problem is presently hindered by the large gaps in our understanding of earth system regulation. With regard to the marine pelagic system, understanding the complex structure and regulation of the marine plankton, their responses to global environmental changes and the consequences thereof for marine biogeochemical cycling will be a major challenge for marine sciences in the coming decades.

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