

Direct effects of CO₂ concentration on growth and isotopic composition of marine plankton

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ABSTRACT

The assessment of direct effects of anthropogenic CO₂ increase on the marine biota has received relatively little attention compared to the intense research on CO₂-related responses of the terrestrial biosphere. Yet, due to the rapid air–sea gas exchange, the observed past and predicted future rise in atmospheric CO₂ causes a corresponding increase in seawater CO₂ concentrations, [CO₂], in upper ocean waters. Increasing [CO₂] leads to considerable changes in the surface ocean carbonate system, resulting in decreases in pH and the carbonate concentration, [CO₃²⁻]. These changes can be shown to have strong impacts on the marine biota. Here we will distinguish between CO₂-related responses of the marine biota which (a) potentially affect the ocean's biological carbon pumps and (b) are relevant to the interpretation of diagnostic tools (proxies) used to assess climate change on geological times scales. With regard to the former, three direct effects of increasing [CO₂] on marine plankton have been recognized: enhanced phytoplankton growth rate, changing elemental composition of primary produced organic matter, and reduced biogenic calcification. Although quantitative estimates of their impacts on the oceanic carbon cycle are not yet feasible, all three effects increase the ocean's capacity to take up and store atmospheric CO₂ and hence, can serve as negative feedbacks to anthropogenic CO₂ increase. With respect to proxies used in paleo-reconstructions, CO₂-sensitivity is found in carbon isotope fractionation by phytoplankton and foraminifera. While CO₂-dependent isotope fractionation by phytoplankton may be of potential use in reconstructing surface ocean pCO₂ at ancient times, CO₂-related effects on the isotopic composition of foraminiferal shells confounds the use of the difference in isotopic signals between planktonic and benthic shells as a measure for the strength of marine primary production. The latter effect also offers an alternative explanation for the large negative swings in δ¹³C of foraminiferal calcite between glacial and interglacial periods. Changes in [CO₃²⁻] affect the δ¹⁸O in foraminiferal shells. Taking this into account brings sea surface temperature estimates for the glacial tropics closer to those obtained from other geochemical proxies.

1. Introduction

The increasing partial pressure of CO₂ in the atmosphere leads to a corresponding increase in dissolved molecular CO₂ concentrations, [CO₂], in the surface ocean on time scales of less than

one year. Thus, higher atmospheric CO₂ levels can almost immediately affect marine plankton. In order to distinguish such fast effects from the influences associated with dissolution of calcium carbonate in sediments or CO₂-related changes in ocean circulation, they will be called “direct effects”. Research on direct effects of increasing atmospheric CO₂ on land plants and ecosystems is well established. The bibliography by Strain

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and Cure (1994) contains approximately 800 references on this topic published between 1980 and 1994. In comparison, very few investigations have dealt with CO₂-related effects in the marine environment.

Until recently, carbon has been mostly ignored as a factor influencing the growth and elementary composition of marine plankton because dissolved inorganic carbon (DIC) is always available in excess of carbon demand by plankton. RUBISCO, the dominant carboxylating enzyme in marine phytoplankton, however, requires molecular CO₂ as inorganic carbon source (Cooper et al., 1969). Although CO₂ is replenished by conversion from HCO₃⁻, this process is too slow relative to photosynthetic carbon fixation in order to satisfy cellular carbon demand (Wolf-Gladrow and Riebesell, 1997). As a result, only a small fraction of DIC is actually available for carbon uptake by an algal cell on short time scales. Although the importance of the enzyme carbonic anhydrase to speed up the slow conversion between HCO₃⁻ and CO₂ is still unresolved, the kinetic inertia of the carbonate system with respect to resupply of CO₂ from the large DIC pool implies that phytoplankton may in fact be sensitive to changes in seawater CO₂ concentrations.

The isotopic compositions ($\delta^{11}\text{B}$, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$) of foraminiferal calcite are used as paleo proxies for the reconstruction of pH, primary productivity, and seawater temperature in ancient oceans. Only recently it has been discovered that carbon and oxygen isotope compositions of foraminiferal shells vary with the carbonate (CO₃²⁻) concentration in seawater (Spero et al., 1997). In view of the considerable changes in the surface water carbonate system with a related change in CO₃²⁻ concentrations between glacial and interglacial times, these findings require reinterpretation of foraminiferal isotopic signals from the geological record.

The intention of this paper is to summarize recent evidence for direct effects of CO₂ on plankton in combination with new findings, to discuss the mechanisms involved and their interrelations, and to estimate their possible impact on carbon uptake by the oceans and on the interpretation of paleo proxies.

2. The carbonate system in the sea

The carbonate system in the sea encompasses three species of dissolved inorganic carbon (DIC),

namely molecular dissolved CO₂ (or aqueous carbon dioxide, CO₂(aq), which will be referred to simply as CO₂), HCO₃⁻ (bicarbonate) and CO₃²⁻ (carbonate), as well as H⁺, OH⁻ and some minor components such as B(OH)₃ and B(OH)₄⁻. The relative amounts of the three DIC species are a function of pH. At typical seawater pH of 8.2, 90% is present in the form of HCO₃⁻, 9% as CO₃²⁻, and less than 1% as CO₂.

2.1. Anthropogenic effects on surface ocean pH

Since 1800 the pH in surface waters has decreased by ca. 0.1 units due to increased atmospheric CO₂ levels (Fig. 1; compare Brewer, 1997). In the IS92a scenario of the IPCC report (1995), it is assumed that anthropogenic CO₂ emissions increase almost linearly up to 20 GtC yr⁻¹ in the year 2100. The future atmospheric CO₂ concentrations can be estimated by a linear response model (Hasselmann et al., 1996) in which parameters are based on global carbon cycle models by Maier-Reimer and Hasselmann (1987) and Maier-Reimer (1993). The atmospheric CO₂ concentration is expected to triple compared to preindustrial time shortly after the year 2100, and accordingly, the pH in the surface ocean will approach 7.8.

2.2. Algal blooms

During blooms of non-calcifying microalgae, the DIC concentration decreases and the pH increases appreciably. These changes of the carbonate system are independent of the carbon source (CO₂, HCO₃⁻ or both) of the algae. The decrease in DIC is limited by other nutrients (phosphate, nitrate) or other factors (light, grazing, micronutrients) to less than 15% (Codispoti et al., 1982). When DIC decreases from 2000 mmol m⁻³ to 1800 mmol m⁻³ at total alkalinity 2300 mequiv m⁻³, $T = 15^\circ\text{C}$, $S = 35$, the pH increases from 8.2 to 8.5 and CO₂ decreases from 10 mmol m⁻³ to 4 mmol m⁻³.

Calcifying organisms such as coccolithophorids, foraminifera or corals change both DIC and total alkalinity. Total alkalinity (TA) is defined by:

$$\begin{aligned} \text{TA} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+] \\ & + [\text{B(OH)}_4^-] \\ & + \text{minor constituents} \end{aligned}$$

(compare Dickson, 1981, for details). Calcification

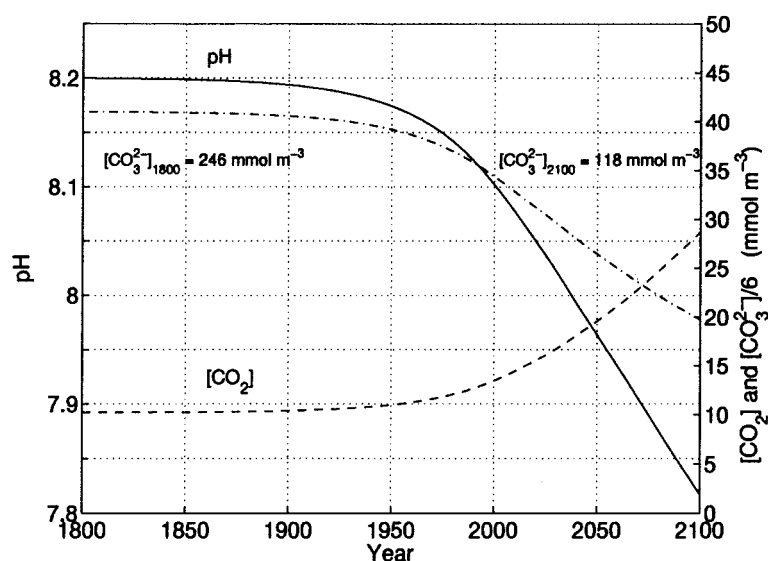
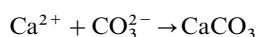
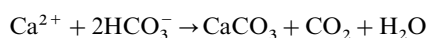


Fig. 1. Changes in the surface ocean carbonate system in response to an atmospheric CO_2 increase according to IS92a scenario of the IPCC report 1995 (approximated by a linear increase of CO_2 emissions from 6.3 GtC yr^{-1} to 20 GtC yr^{-1} in the year 2100): pH (solid line), CO_2 concentration (dashed line) and CO_3^{2-} concentration (dash-dotted line; please note the different scales for CO_2 and CO_3^{2-}).

according to



or



changes DIC and TA in the ratio 1:2. A calcifying alga with a rate of photosynthesis α and a rate of calcification β changes DIC and TA in the ratio $(\alpha + \beta)/2\beta$. The rain ratio parameter

$$\gamma = \frac{1}{2} \frac{d[\text{TA}]}{d[\text{DIC}]} \approx \frac{\beta}{\alpha + \beta}$$

thus varies between zero (photosynthesis alone) and one (calcification alone). The Revelle or buffer factor, RF, is defined as the relative change in CO_2 divided by the relative change in DIC

$$\text{RF} := \frac{d[\text{CO}_2]}{[\text{CO}_2]} \bigg/ \frac{d[\text{DIC}]}{[\text{DIC}]}$$

DIC decreases due to photosynthesis, calcification, and any combination of those two processes, the change in CO_2 can be positive or negative depending on the rain ratio parameter γ . In non-calcifying blooms ($\gamma \approx 0$) the RF is approximately 10 which means that reduction of CO_2 to half its

original value is associated with a decrease of (only) 5% in DIC. On the other hand, a doubling of CO_2 due to higher atmospheric $p\text{CO}_2$ results in (only) a 10% increase in DIC. In calcifying blooms RF is smaller and for $\gamma > 0.6$ the Revelle factor becomes negative which means that CO_2 increases while DIC decreases: calcification leads to an increase in CO_2 (a counter-intuitive result!). Such high values of γ can occur, for example, in intense blooms of coccolithophorids (Robertson et al., 1994).

2.3. Kinetics and carbonic anhydrase

The reactions of the various compounds of the carbonate system are very fast (diffusion controlled) with one exception: the conversion between CO_2 and HCO_3^- is slow ($\tau \approx 30 \text{ s}$ based on a rate constant of 0.036 s^{-1} , Johnson, 1982). Many organisms (from microalgae to mammals) use the zinc-containing enzyme carbonic anhydrase (CA) to speed up this conversion. Please note that catalysis by CA provides a net conversion in one or the other direction only when the system is out of chemical equilibrium such as, for example, in the diffusive boundary layer around an alga or

inside the cell where metabolic processes maintain non-equilibrium states. Thus, the net rate of conversion from HCO_3^- to CO_2 cannot be estimated from measurements of carbonic anhydrase activity (no net conversion at high CA activity in an equilibrium situation).

3. Direct effects

The distribution of dissolved inorganic carbon in the oceans shows relatively low values in the mixed layer and higher levels in the deep ocean which increase from the North Atlantic to the Southern Ocean and the North Pacific (Takahashi, 1989). This distribution is due to DIC pumps which are defined as processes that deplete the ocean surface of DIC relative to the deep-water DIC (Volk and Hoffert, 1985). Three pumps are distinguished: the carbonate pump, the soft-tissue pump, and the solubility (or physical) pump. The first two result from the flux of biogenically precipitated CaCO_3 and of photosynthetically produced organic material from the ocean's surface. The physical pump results from increased CO_2 solubility in downwelling cold water. The transport of CaCO_3 out of the surface ocean changes DIC and TA in the ratio 1:2 ($\gamma = 1$) and thereby increases CO_2 in surface water. Therefore the CaCO_3 pump is sometimes referred to as a counter pump because of its effect on CO_2 . Several authors have analyzed the strengths of the different DIC pumps. Volk and Hoffert (1985) find that results of an abiotic box model cannot explain observed DIC profiles. Sarmiento et al. (1995) compare GEOSECS observations with results of a three-dimensional ocean carbon cycle model. They conclude that 75% of the vertical gradients in DIC are due to the biological pump (soft-tissue plus carbonate pump). The influence of the biological pump is shown by a model experiment performed by Maier-Reimer et al. (1996) in which marine biological production was stopped completely at one instant resulting in nearly a doubling of the atmospheric CO_2 concentration. Based on these findings it is important to investigate whether the strength of the biological carbon pump is affected by anthropogenic perturbations. Three of the direct effects discussed here (increase in growth rate, shift in elementary composition, and decrease in calcification rate) are of potential importance

for the strengths of the soft-tissue and the carbonate pumps.

Direct effects on the isotopic fractionation in microalgae and foraminifera enable us to use isotopic composition (of parts) of these organisms as a diagnostic tool. Their main application is the reconstruction of various paleo-quantities from sedimentary material. At the same time, isotopic fractionation in microalgae can also help to understand the mechanisms involved in two of the other direct effects (increase in growth rate, shift in elementary composition).

3.1. Direct effects on the carbon pumps

3.1.1. Growth rate of phytoplankton. The potential of direct effects of CO_2 on marine phytoplankton growth in the natural environment is presently an issue of debate. Clearly, dissolved inorganic carbon concentrations, which are two to three orders of magnitude higher than those of other macronutrients (N and P) and are depleted by no more than 15% even by the most intense phytoplankton blooms (Codispoti et al., 1982), are always in ample supply. As outlined above, however, reactions within the carbonate system, particularly the interconversion between HCO_3^- and CO_2 , are slow compared to rates of carbon uptake by marine phytoplankton. Conditions in the diffusive boundary layer around a photosynthesizing cell therefore deviate significantly from those in the bulk medium (see Wolf-Gladrow and Riebesell, 1997). Still, even for a cell restricted to CO_2 uptake, the photosynthetic carbon demand in most cases will be met by the diffusive supply of CO_2 from its environment (Wolf-Gladrow and Riebesell, 1997). In fact, at cell sizes representative for marine diatoms (5–50 μm in radius) and growth rates typically observed in natural populations ($0.5\text{--}1\text{ d}^{-1}$), cellular carbon demand remains well below the potential CO_2 supply from the bulk medium to the cell surface (Fig. 2). In addition to CO_2 uptake, utilization of HCO_3^- , either by direct HCO_3^- uptake or catalyzed conversion of HCO_3^- to CO_2 via extracellular carbonic anhydrase, would tend to diminish or abolish potential effects of CO_2 limitation. Why then should CO_2 concentration have any effect on phytoplankton growth?

CO_2 -dependent growth was first reported for three species of marine diatoms, which showed a

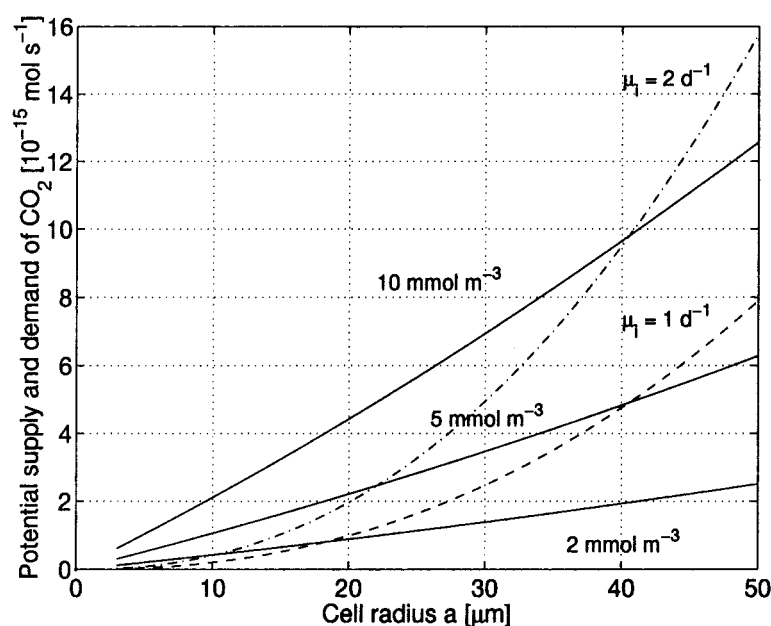


Fig. 2. Potential supply of CO_2 from the bulk medium to the outer surface of an algal cell assuming diffusional transport of CO_2 (solid lines) and demand of carbon by the cell for two instantaneous growth rates, μ_i (dashed lines) as a function of cell radius a . The carbon content, γ (in mol C) of diatoms is calculated from cell volume, V (in μm^3) by $\gamma = 3.154 \times 10^{-14} V^{0.758}$ (Strathmann, 1967). The potential supply is proportional to $[\text{CO}_2]_{\text{bulk}}$, whereas the actual CO_2 flux is proportional to $([\text{CO}_2]_{\text{bulk}} - [\text{CO}_2]_a)$ where $[\text{CO}_2]_{\text{bulk}}$ and $[\text{CO}_2]_a$ are the concentrations in the bulk medium and at the cell surface, respectively (Wolf-Gladrow and Riebesell, 1997).

decrease in cell division rate with decreasing CO_2 at concentrations below about 10 mmol m^{-3} (Riebesell et al., 1993; Fig. 3). These results were corroborated by mesocosm experiments showing similar CO_2 -dependent changes in growth rate for mixed populations of marine phytoplankton (Chen and Durbin, 1994). Recently, Hein and Sand-Jensen (1997) reported significant effects of CO_2 concentration on the rate of ^{14}C incorporation also in natural populations. During a transect in the North Atlantic, these authors changed the CO_2 concentrations in seawater samples through the addition of HCl or NaOH to 1/3, 3 times, and about 10 times the ambient levels (10 mmol m^{-3}), and found primary production to decrease by 25% at reduced levels and to increase by 15–20% under elevated CO_2 concentrations. How do these findings reconcile with CO_2 supply rates in excess of photosynthetic carbon demand under natural CO_2 concentrations and reports of the potential for direct HCO_3^- uptake or CA-catalyzed HCO_3^-

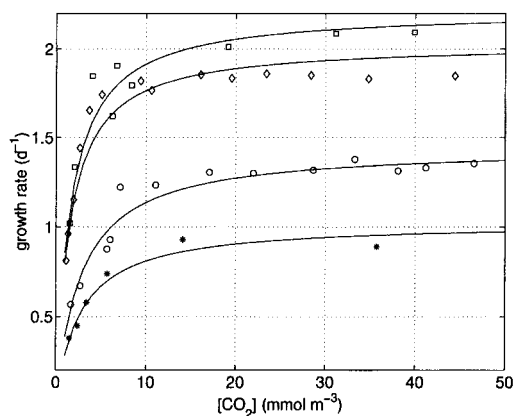


Fig. 3. The instantaneous growth rates μ_i of marine diatoms as a function of CO_2 -concentration: *Ditylum brightwellii* (\diamond), *Thalassiosira punctigera* (\square), *Rhizosolenia cf. alata* (\circ), *Coscinodiscus wailesii* (*) (data from Riebesell et al., 1993; Burkhardt, unpublished).

conversion in various groups of marine phytoplankton?

Clearly, direct effects of CO_2 on phytoplankton growth under natural conditions are to be expected only in cases where (1) CO_2 is a significant or dominant source of inorganic carbon and (2) ambient levels of surface water CO_2 concentrations are subsaturating photosynthetic CO_2 fixation.

Surprisingly, the question of which form of inorganic carbon is predominantly utilized by natural marine phytoplankton is still open. Of the few species of marine microalgae examined so far, several showed the potential for direct uptake of HCO_3^- (Burns and Beardall, 1987; Colman and Rotatore, 1995; Korb et al., 1997). In addition, the majority of marine phytoplankton species tested were found to have extracellular CA activity (Burns and Beardall, 1987; Morel et al., 1994; Roberts et al., 1997). In a recent study on mixed coastal diatom assemblages, in fact, Tortell et al. (1997) suggested that "bloom-forming diatoms use HCO_3^- as a source of DIC ... and are not limited by CO_2 availability." On the other hand, studies on stable carbon isotopes of marine microalgae (compare Subsection 3.2.1) suggest CO_2 -dependence of photosynthetic isotope fractionation, even in those species for which extracellular CA activity or the ability for direct HCO_3^- uptake were demonstrated (Hinga et al., 1994; Laws et al., 1995 and 1997). CO_2 -sensitivity was also observed in two strains of the coastal marine diatom *Skeletonema costatum*, for which the elemental composition C:N:P was found to change as a function of ambient CO_2 concentration (Burkhardt and Riebesell, 1997; see Subsection 3.1.2).

With respect to the dominant carboxylating enzyme in marine diatoms, ribulose 1,5-bisphosphate carboxylase-oxygenase (RUBISCO), point 2 above can easily be answered. With CO_2 as the form of inorganic carbon fixed by RUBISCO and with its half-saturation concentration for CO_2 ranging between 12 and 240 mmol m^{-3} (see review by Raven (1997)), seawater CO_2 concentrations are far too low to saturate RUBISCO carboxylase activity. In order to have RUBISCO operate efficiently and cost effectively it appears essential for an algal cell to actively increase CO_2 concentrations at the site of carboxylation (Raven, 1997). Although the func-

tioning and location of such a carbon concentrating mechanism (CCM) is still unclear, its induction and regulation as a function of external CO_2 concentrations has been demonstrated in various studies (for review, see Falkowski and Raven (1996)).

Obviously, the key to the question of how seemingly contradictory findings such as sufficient CO_2 supply from the bulk medium, direct utilization of HCO_3^- , and CO_2 -sensitivity of phytoplankton growth rate and elemental composition can be reconciled lies in the energy-dependence of active carbon acquisition. Since living in a potentially CO_2 -limiting environment due to the low CO_2 affinity of RUBISCO, microalgae are obliged to spend energy in order to fuel their carbon concentrating mechanism(s). Whatever carbon source and pathway of carbon acquisition an alga uses, the increasing energetic cost of satisfying cellular carbon demand under low CO_2 conditions is bound to affect the cell's overall performance. Thus, CO_2 sensitivity of marine phytoplankton may be best viewed as co-limitation of CO_2 in concert with energy availability and other factors controlling operation of the carbon concentrating mechanism.

Whereas one can easily imagine that biomass limitation will influence the strength of the soft-tissue pump, this is not necessarily the case for growth rate limitation. Faster growth may lead, however, to higher algal concentrations for short periods and thereby enhance the physical aggregation of phytoplankton which depends nonlinearly on the concentrations. Larger aggregates sink at speeds of about 100 m d^{-1} and can penetrate the winter mixed layer before being remineralized.

3.1.2. Effect of CO_2 on the elemental composition (C:P) of marine phytoplankton. Drawdown of atmospheric CO_2 by the soft-tissue pump is driven by photosynthetic carbon fixation of marine phytoplankton and its subsequent export from the euphotic zone to deeper waters. In biogeochemical models of oceanic carbon flux, a constant C:P ratio is commonly used to estimate particulate organic carbon production in the surface layer from phosphate concentrations (e.g., Six and Maier-Reimer, 1996). Variable C:P ratios would alter the strength of the soft-tissue pump. As an example, Heinze et al. (1991) demonstrated that a 30% global increase in C:P could explain most

of the 80 μatm reduction of atmospheric $p\text{CO}_2$ during the last glaciation.

A constant elemental proportionality in the utilization of primary nutrients by marine plankton during synthesis and in the subsequent remineralization of organic matter was first suggested by Redfield (1934). The "Redfield ratio" of C:P = 106:1 (by atoms, Redfield et al., 1963) represents an average value for the elemental composition of marine phytoplankton (108:1) and zooplankton (103:1). Since the chemical composition is likely to differ between species, and species composition is variable in both time and space, Redfield et al. (1963) concluded that "statistical uniformity in composition is probably approached only in large masses of water, where deviations of this sort are averaged out". In marine phytoplankton, elemental ratios of a given species also vary with environmental conditions such as nutrient availability. Large deviations from the Redfield ratio, for example, occur upon N-limited or P-limited growth in chemostats (Perry, 1976; Sakshaug and Holm-Hansen, 1977).

High ambient concentrations of DIC relative to N and P led Redfield et al. (1963) to conclude that carbon was not limiting phytoplankton growth in the ocean. Even when gradients in nutrient concentrations between the bulk medium and the boundary layer of an algal cell are considered, diffusive supply of CO_2 appears to meet cellular carbon demand in most cases (Wolf-Gladrow and Riebesell, 1997), as was outlined in the previous section. Nevertheless, variable $[\text{CO}_2]$ has been demonstrated to affect not only growth rate (see above) but also elemental composition of an algal cell. In batch culture experiments with two strains of the marine diatom *Skeletonema costatum*, Burkhardt and Riebesell (1997) observed variation of C:P with $[\text{CO}_2]$ (Fig. 4). Minimum C:P ratios were only 41 to 55% of maximum values over the range of CO_2 concentrations ($<2 \text{ mmol m}^{-3}$ to $>30 \text{ mmol m}^{-3}$) tested.

CO_2 -dependent changes in C:P of the same magnitude and in the same direction were also observed for the marine diatoms *Coscinodiscus wailesii* and *Asterionella glacialis* (Fig. 4; Burkhardt et al., in press). This correlation of C:P with $[\text{CO}_2]$ indicates that systematic changes in plankton stoichiometry might occur in response to the currently observed, rapid increase in atmospheric $p\text{CO}_2$. Elevated C:P ratios at high concen-

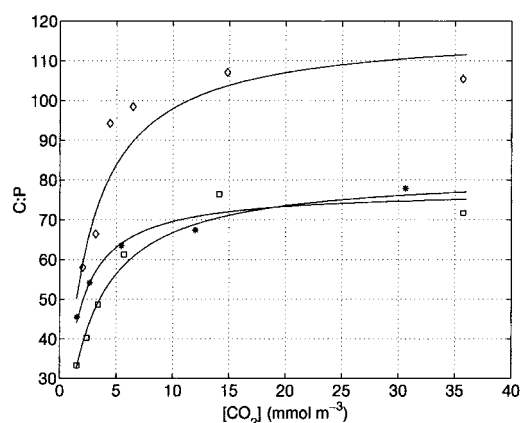


Fig. 4. The organic matter elementary composition (C:P) in marine diatoms *Seile tonema costatum* (*), *Asterionella glacialis* (\diamond) and *Coscinodiscus wailesii* (\square) as a function of $[\text{CO}_2]$ in the medium (Burkhardt and Riebesell, 1997; Burkhardt et al., in press).

trations of carbon dioxide would enhance the strength of the soft-tissue pump by increasing the amount of carbon fixed per unit phosphate and ultimately the carbon flux from the euphotic zone to deeper waters. However, the CO_2 concentration at which C:P approaches an upper limit in the diatom species investigated so far (between 5 and 10 mmol m^{-3}) is low and may not lead to an appreciable increase in carbon fixation under currently rising CO_2 concentrations. On the other hand, it needs to be considered that even within one algal strain, cells might respond differently to variation in CO_2 concentration depending on the time they have to acclimate to ambient conditions. In contrast to pre-adapted laboratory cultures, phytoplankton cells experience a rapid decline in $[\text{CO}_2]$ during bloom development and might then show a larger response with respect to their elemental composition.

In spite of open questions, the observation of variable phytoplankton stoichiometry in response to CO_2 supply challenges the commonly accepted notion of a CO_2 -independent Redfield ratio. Its relevance in the light of the current increase in CO_2 concentrations remains to be resolved. Because of the regional and seasonal heterogeneity of plankton stoichiometry it is unlikely that a basin-wide systematic trend in the elemental composition of marine phytoplankton could be detected by direct measurements of sedimented

particulate organic matter. On the other hand, long-term global trends in the Redfield ratio should be reflected in nutrient gradients in oceanic deep water.

3.1.3. Calcification. Increasing atmospheric CO_2 concentrations will lead to lower CO_3^{2-} concentrations in ocean surface waters (Fig. 1). Accordingly, the degree of oversaturation with respect to calcite and aragonite will decrease. This may have two consequences. First, CaCO_3 in sediments, especially the Mg^{2+} rich calcites in shelf regions, could dissolve (Wollast, 1994). Second, the rate of CaCO_3 production could decrease. Almost all CaCO_3 in the oceans is precipitated by organisms. The only exceptions are minor abiotic precipitations ("whitings") observed in the Persian Gulf (Morse and He, 1993). The response of calcifying marine organisms to changing carbonate concentrations has not yet been investigated in any detail. One can expect that the response depends on the carbon source used for calcification as well as the kind of mineral precipitated (aragonite, calcite, and the fraction of MgCO_3).

For coccolithophorids HCO_3^- has been suggested to be the carbon source used in calcification (Sikes and Wilbur, 1982). Since HCO_3^- concentrations remain more or less constant with CO_2 -related changes in the carbonate system of the magnitude discussed here, calcification in coccolithophorids should be unaffected by these changes. In recent laboratory experiments with the coccolithophorid *Emiliana huxleyi*, however, the rate of calcification was found to decrease by ca. 40% with CO_2 increasing from 5 to 35 mmol m^{-3} (Zondervan, personal communication). Over this CO_2 range the rate of organic matter production remained more or less constant. Since increasing CO_2 in these experiments was associated with a corresponding decrease in pH and in the CO_3^{2-} concentration, it is not possible to distinguish which of these factors ultimately caused calcification to subside. Nevertheless, as changes in $[\text{CO}_2]$, pH, and $[\text{CO}_3^{2-}]$ also go hand in hand under natural conditions, the observed CO_2 -related effect on the rate of calcite production by *E. huxleyi* is directly applicable to the natural environment.

Orbulina universa, a planktonic foraminifer, most probably uses CO_3^{2-} as substrate for shell formation. Laboratory experiments under controlled conditions show that the shell weight

decreases at CO_3^{2-} concentrations below 150 mmol m^{-3} (Fig. 5; Bijma et al., in press). Experimental results obtained by Langdon et al. (1997) and Gattuso et al. (1998) imply that a doubling of atmospheric CO_2 will result in a 10–20% reduction in coral and reef calcification. To the best of our knowledge the response of pteropods, the most important aragonite forming organisms for the open ocean, has not been investigated.

Reduced CaCO_3 production or increased dissolution of carbonates in shelf sediments leads to higher alkalinity in surface waters. The mean difference in alkalinity (normalized to a constant salinity of 35) between the surface ocean and the deep ocean is of the order of 100 mequiv m^{-3} (for example, Volk and Hoffert (1985), Sabine et al. (1995) measured a difference of 150 mequiv m^{-3} between the surface and 1000 m at the Hawaii Ocean Time series station). In order to estimate the potential impact of decreasing calcification rates on atmospheric CO_2 levels the change in $p\text{CO}_2$ due to a shutdown of the calcium carbonate pump has been calculated for a preindustrial situation. When the CaCO_3 pump is shut down, the vertical alkalinity gradient in the upper 500 to 1000 m will almost vanish (for the influence of minor components such as nitrate on alkalinity see, for example, Brewer and Goldman, 1976) and surface water alkalinity will attain the deep ocean value. For $\text{DIC} = 2000 \text{ mmol m}^{-3}$ and $\text{TA} = 2300 \text{ mequiv m}^{-3}$ a $p\text{CO}_2 = 279 \text{ } \mu\text{atm}$ is obtained

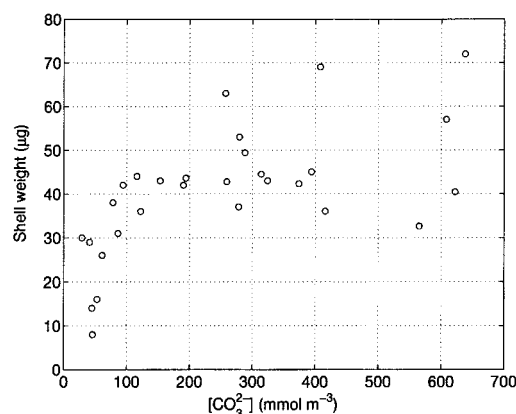


Fig. 5. The weight of CaCO_3 shells of *Orbulina universa* grown in a 12 h:12 h light–dark cycle as a function of CO_3^{2-} -concentration (data from Bijma et al., 1998).

at $T = 15^\circ\text{C}$ and $S = 35$. When TA and DIC are increased by $100 \text{ mequiv m}^{-3}$ and by 50 mmol m^{-3} , respectively, $p\text{CO}_2$ drops by almost $40 \text{ }\mu\text{atm}$. This back-of-an-envelope estimate is confirmed by a numerical experiment (Wischmeyer, personal communication) using the global carbon cycle model of Six and Maier-Reimer (1996). It is currently not known how marine CaCO_3 production is distributed among foraminifera, pteropods, coccolithophorids and corals (Milliman and Droxler, 1996; Holligan and Robertson, 1996) and how different groups of organisms are affected by lower CO_3^{2-} concentrations. Thus, a quantification of possible changes in CaCO_3 production and dissolution and their effect on the marine carbon cycle is presently not possible.

3.2. Direct effects as diagnostic tools

3.2.1. Carbon isotope fractionation in marine phytoplankton. The carbon isotopic composition ($\delta^{13}\text{C}$) of marine photosynthetic organisms can potentially provide important insights into the environmental conditions under which the organisms grew. Photosynthetic carbon isotope fractionation ($\varepsilon_p \approx \delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{POC}}$; please note that some authors define ε_p with respect to $\delta^{13}\text{C}_{\text{DIC}}$ instead of $\delta^{13}\text{C}_{\text{CO}_2}$) is thought to be a function of the ratio of carbon demand to carbon supply. A low demand (i.e., low growth rate, μ) and a high supply (e.g., high CO_2 concentration) favors high fractionation and vice versa. Based on theoretical considerations, an inverse linear relationship between ε_p and $\mu/[\text{CO}_2]$ is predicted in cases where diffusive CO_2 uptake is the dominant pathway of carbon acquisition (Laws et al., 1995; Rau et al. 1996). Such a relationship — if applicable to common marine phytoplankton — raises the possibility of reconstructing past CO_2 concentrations in the surface ocean from carbon isotope compositions of bulk organic matter or specific biomarkers in sedimentary material (Jasper and Hayes, 1990) and of estimating in situ phytoplankton growth rates from ε_p and $[\text{CO}_2]$ measurements (Laws et al., 1995).

A relationship between $[\text{CO}_2]$ and the carbon isotopic composition of marine phytoplankton has first been suggested by Degens et al. (1968) and has recently been confirmed in studies using monospecific marine phytoplankton cultures (e.g., Hinga et al., 1994; Laws et al., 1995 and 1997).

Significant correlations between $[\text{CO}_2]$ and $\delta^{13}\text{C}$ were also found for particulate organic matter (POM) in oceanic surface waters (e.g., Rau et al., 1989; Freeman and Hayes, 1992; Rau et al., 1992; Francois et al., 1993). However, as discussed by Goericke and Fry (1994), a large residual variance in surface ocean $\delta^{13}\text{C}_{\text{POM}}$ versus $[\text{CO}_2]$ relationships suggests that factors other than $[\text{CO}_2]$ also affect carbon isotope fractionation in natural populations.

The importance of cellular carbon demand on isotope fractionation, as suggested by Rau et al. (1992) and Francois et al. (1993), became evident in experiments by Laws et al. (1995), who obtained a linear relationship between ε_p and $\mu/[\text{CO}_2]$. This observation was consistent with CO_2 being the dominant carbon source and entering the cell via diffusive transport. Assuming diffusive CO_2 uptake, Rau et al. (1996) developed a model predicting isotope fractionation as a function of various environmental and biological variables, such as CO_2 concentration, temperature, salinity, pH, growth rate, cell size, and cell membrane permeability. Although comparison of model predictions to existing experimental (Rau et al., 1996) and field data (Rau et al., 1997) appeared to substantiate some of the model predictions, data on isotope fractionation obtained under well-constrained conditions presently available are insufficient to rigorously test all aspects of the model.

Most recently, deviations from the predicted linear relationship between ε_p and $\mu/[\text{CO}_2]$ were obtained in experiments in which the range of CO_2 concentrations has been extended to well below 5 mmol m^{-3} , concentrations generally not encountered in marine pelagic systems (Laws et al., 1997). This finding led Laws et al. (1997) to propose a non-linear relationship between ε_p and $\mu/[\text{CO}_2]$ over the entire range of CO_2 concentrations. A further complication resulted from observations of light-dependent differences in ε_p versus $\mu/[\text{CO}_2]$ relationships (Riebesell, unpublished data). Under otherwise identical conditions, growth rate-normalized isotope fractionation was higher at higher light intensities. In addition, differences in $\mu/[\text{CO}_2]$ responses in isotope fractionation were also obtained between different groups of marine phytoplankton (Popp et al., 1998). These findings suggest the importance of variables presently not constrained in the experi-

ments or of factors not accounted for in the models of isotope fractionation.

Some of the gaps in our understanding of photosynthetic carbon isotope fractionation relate to uncertainties regarding the specific mechanisms of inorganic carbon acquisition and fixation. As discussed in the previous section, uncertainties with respect to carbon acquisition pathways concern the source(s) of inorganic carbon used by marine phytoplankton (CO_2 and HCO_3^- differ in their isotopic signal by approximately 10‰), the operation and regulation of a carbon uptake mechanism, the cell membrane permeability to CO_2 , and the ratio of carbon uptake by the cell to carbon leakage out of the cell. Uncertainties regarding carbon fixation concern the contribution by β -carboxylases, the form of RUBISCO (form I or II) and its fractionation factor in various algal groups. Until we have a more complete understanding of carbon acquisition and fixation pathways, it will remain difficult to adequately interpret carbon isotope data or to make quantitative predictions of carbon isotope fractionation in phytoplankton.

3.2.2. Isotopic composition of calcite shells of foraminifera. Stable oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) isotope measurements on foraminiferal calcite are routinely used for reconstructing paleoceanographic and paleoclimatic change. Shell $\delta^{18}\text{O}$ values are a function of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and the calcification temperature, whereas shell $\delta^{13}\text{C}$ values reflect $\delta^{13}\text{C}_{\text{DIC}}$. These proxies have been used as a tool to reconstruct surface and deep water temperatures (Broecker, 1986; Labeyrie et al., 1987), glacial ice volumes (Shackleton and Opdyke, 1973), ocean circulation changes (Duplessy et al., 1988), and glacial–interglacial transfer between the terrestrial and oceanic carbon pools (Shackleton, 1977). Recently stable boron isotopes ($\delta^{11}\text{B}$) were introduced as a tracer for estimating glacial to interglacial changes in pH (Sanyal et al., 1995, 1996).

$\delta^{13}\text{C}$. The $\delta^{13}\text{C}_{\text{DIC}}$ in surface waters increases when organic material is formed by microalgae which discriminate against ^{13}C with a fractionation factor on the order of 20‰ (compare Subsection 3.2.1). While settling to the ocean floor part of this isotopically light material is remineralized, and thereby decreases the $\delta^{13}\text{C}_{\text{DIC}}$ of deeper waters. The difference of $\delta^{13}\text{C}_{\text{DIC}}$ between surface

and bottom thus varies with the amount of biological production. Consequently, if $\delta^{13}\text{C}$ of foraminiferal shells reflects $\delta^{13}\text{C}$ of DIC, the difference between benthic and planktonic foraminifera can be used as a proxy for biological production. However, already the pioneers of foraminiferal $\delta^{13}\text{C}$ (Duplessy et al., 1970; Shackleton, 1977; Berger, 1979; Kahn, 1979) observed that the carbon isotopic composition of the shells, $\delta^{13}\text{C}_s$, does not necessarily precisely reflect the isotopic composition of the dissolved inorganic carbon, $\delta^{13}\text{C}_{\text{DIC}}$. The species-specific and ontogenetic differences were attributed to (unspecified) “vital effects”. Using culture experiments, Spero and coworkers were the first to document the substantial impact of the symbionts on shell $\delta^{13}\text{C}$ (Spero and DeNiro, 1987; Spero, 1992) and the minor effect of host respiration (Spero and Lea, 1996). When applying $\delta^{13}\text{C}$ to the fossil record, the studies published to date assume that in addition to a shell-size dependent offset (Berger et al., 1978) the $\delta^{13}\text{C}_{\text{DIC}}$ can be derived from $\delta^{13}\text{C}_s$ plus a constant offset due to “vital effects” (Shackleton and Vincent, 1978).

The global mean value of $\delta^{13}\text{C}_{\text{DIC}}$ changes when isotopically light carbon from land biota ($\delta^{13}\text{C}_{\text{land biota}} \approx -25\text{‰}$) is deposited into the oceans. A compilation by Curry et al. (1988) suggests that mean ocean $\delta^{13}\text{C}$ was 0.4‰ more negative during the last glacial maximum. A 30% reduction of the present biospheric carbon reservoir (equivalent to 600 Gt carbon) is required to explain this shift by a transfer of carbon from the terrestrial biosphere to the oceans (Shackleton, 1977; Crowley, 1995).

However, laboratory experiments with living foraminifera under conditions of controlled CO_2 and alkalinity have shown that the carbon isotopic composition of the shells strongly depends on the chemical state of the carbonate system. Spero et al. (1997) and Bijma et al. (in press) demonstrated that the shells become isotopically lighter at higher CO_3^{2-} concentrations (Fig. 6). This finding provides an alternative hypothesis to the terrestrial biosphere-to-ocean transfer of carbon that has been put forward as an explanation for lower glacial shell $\delta^{13}\text{C}$ values. The 0.2 units higher pH of glacial surface waters, calculated by Sanyal et al. (1995) on the basis of boron isotopes, are evidence for a significantly higher carbonate ion concentration during the glacial. Calculations by

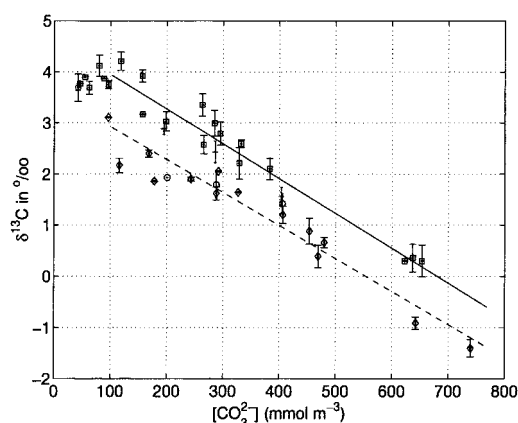


Fig. 6. Carbon isotopic composition, $\delta^{13}\text{C}$, of calcite shells of *Orbulina universa* as a function of CO_3^{2-} concentration (at constant alkalinity = ca. 2800 mequiv m^{-3}). Specimens were kept in a 12 h:12 h light–dark cycle (\diamond at 22°C; \circ at 17°C) or in darkness (\square at 22°C; $+$ at 17°C); data from Spero et al., 1997, and Bijma et al., in press.

Lea et al. (in press) show that to achieve equilibrium with a glacial atmospheric $p\text{CO}_2$ of 200 μatm , surface water $[\text{CO}_3^{2-}]$ must have increased by a minimum of 40 mmol m^{-3} . Combining this conservative estimate with the experimental relationships suggests that the glacial rise in $[\text{CO}_3^{2-}]$ can account for at least a 0.25 to 0.5‰ drop in shell $\delta^{13}\text{C}$ (depending on the species-specific response). However, depending on sea surface temperature and the actual mode of $p\text{CO}_2$ change, considerably larger changes are possible (Lea et al., in press). If part of the negative interglacial to glacial carbon isotope shift that is common to all species can be attributed to a $[\text{CO}_3^{2-}]$ increase, this reduces the inferred glacial–interglacial shift in mean ocean $\delta^{13}\text{C}_{\text{DIC}}$.

This new discovery also provides a novel explanation for the large, negative excursions in planktonic $\delta^{13}\text{C}$ observed in the glacial sections of southern ocean cores (Lea et al., in press) and confounds the interpretation of planktonic–benthic $\delta^{13}\text{C}$ differences ($\Delta\delta^{13}\text{C}$) as a measure of the efficiency of the biological production (Shackleton et al., 1983). Because a 0.1‰ increase in $\delta^{13}\text{C}$ translates to an approximate 10 μatm drop in $p\text{CO}_2$, a 0.25‰ correction on planktonic foraminifera, which is the minimum calculated, can explain an additional 25 μatm glacial–interglacial $p\text{CO}_2$

difference, implying a stronger glacial biological pump than previously indicated (Shackleton et al., 1992). However, this calculation does not take into account a potential $[\text{CO}_3^{2-}]$ influence on benthic shell $\delta^{13}\text{C}$ and depends on species-specific responses to $[\text{CO}_3^{2-}]$ for which experimental data are not available.

The variation of the carbon isotopic composition in *O. universa* under varying conditions of the carbonate system can be explained to a large degree by a reaction–diffusion model for the physiological micro-environment which takes into account respiration, calcification and photosynthesis by the symbionts (Wolf-Gladrow et al., 1999; Zeebe et al., 1999).

$\delta^{18}\text{O}$. Urey (1947) showed, from first principles, that the temperature at which calcium carbonate precipitates from a solution affects the ratio of the stable oxygen isotopes in the precipitate. In thermodynamic equilibrium, calcite and seawater differ in their $^{18}\text{O}/^{16}\text{O}$ ratios, and the difference decreases with increasing temperature. He proposed therefore that the $\delta^{18}\text{O}$ value of carbonate from the geologic record contains a paleotemperature signal. Foraminiferal shells precipitated in equilibrium are enriched in ^{18}O relative to seawater but less so with increasing temperature. As for $\delta^{13}\text{C}$, data from isotopic calibration studies suggested that a previously unidentified parameter also affects the oxygen isotope ratios in foraminiferal shells (Fairbanks et al., 1982; Spero and Lea, 1996). While observations of $\delta^{13}\text{C}$ -disequilibrium precipitation can be attributed to respiration or symbiont photosynthesis, shell $\delta^{18}\text{O}$ values should be insensitive to physiology. McCrea (1950) showed that $\delta^{18}\text{O}$ of inorganically precipitating calcite became depleted in ^{18}O as the carbonate ion concentration in the solution was increased. Spero et al. (1997) and Bijma et al. (in press) also find that $\delta^{18}\text{O}$ varies with CO_3^{2-} concentration (Fig. 7). The slope $d\delta^{18}\text{O}/d[\text{CO}_3^{2-}]$ differs by a factor of 2 between the two species *O. universa* and *G. bulloides*. Interestingly the slope $d\delta^{18}\text{O}/d\delta^{13}\text{C}$ is almost the same for both species and for the non-symbiotic coral *Tubastrea* sp., indicating that a similar mechanism is responsible for the observed variations.

Anomalies in $\delta^{18}\text{O}$ computed for an 80 μatm $p\text{CO}_2$ reduction, range from -0.1 to -0.3‰ , equivalent to a sea surface temperature (SST)

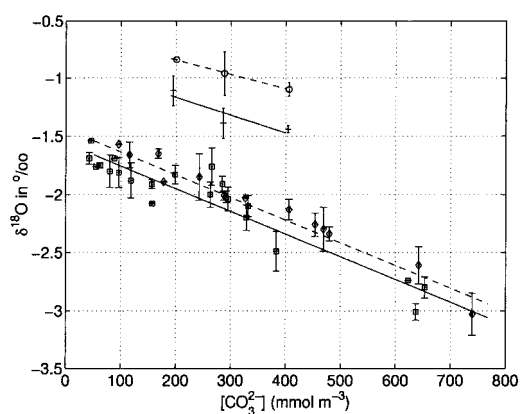


Fig. 7. Oxygen isotopic composition, $\delta^{18}\text{O}$, of calcite shells of *Orbulina universa* as a function of CO_3^{2-} concentration (at constant alkalinity = ca. 2800 mequiv m^{-3}). Specimens were kept in a 12 h:12 h light–dark cycle (\diamond at 22°C; \circ at 17°C) or in darkness (\square at 22°C; $+$ at 17°C); data from Spero et al. (1997) and Bijma et al., in press.

anomaly of approximately -0.5 to -1.5°C (Lea et al., in press). These anomalies in shell $\delta^{18}\text{O}$ translate into overestimates of paleotemperature during periods of low $p\text{CO}_2$ and thus bring oxygen isotope paleotemperatures closer to other marine SST proxies (e.g. coral Sr/Ca) and terrestrial indicators (e.g., snow-line and ice core $\delta^{18}\text{O}$) that suggest more intense tropical cooling at the last glacial maximum (Guilderson et al., 1994; Thompson et al., 1995).

$\delta^{11}\text{B}$. Hemming and Hanson (1992; see also Vengosh et al., 1991) found that the boron isotopic composition $\delta^{11}\text{B}$ in modern marine carbonates varies with pH. The isotopic composition of the two forms of borate, $\text{B}(\text{OH})_3$ and $\text{B}(\text{OH})_4^-$, differ by 20‰ and both vary with pH. Under the assumption that only the charged species ($\text{B}(\text{OH})_4^-$) is incorporated with a constant fractionation (0.8‰) into the shell the observations can be explained. Sanyal et al. (1995) calculated from boron isotope data that the pH of surface water of glacial age in the tropical Atlantic and Pacific oceans was $0.2 (\pm 0.1)$ pH units higher than that during the Holocene, and that the pH of glacial deep water in the equatorial Atlantic and Pacific oceans was $0.3 (\pm 0.1)$ pH units higher than during the Holocene.

4. Summary

The limited information presently available on direct effects of CO_2 -related changes in seawater chemistry on the marine biota is largely based on laboratory experiments. Until more field data become available extrapolation of these results to the natural environment remains hypothetical. Clearly, rising CO_2 concentrations will favor algal species with high carbon demand and a low surface area to volume ratio as well as species lacking a carbon concentrating mechanism. Thus, a shift in phytoplankton community structure towards larger cell sizes may occur in bottom-up controlled plankton communities. However, reliable predictions of future developments in plankton species composition are impossible as long as we are unable to fully understand the factors controlling composition and succession of present day plankton communities.

CO_2 -dependent responses of marine phytoplankton with regard to growth rate, elemental composition, and isotope fractionation are all reflections of the cell's nutritional status with respect to inorganic carbon. Thus, although a mechanistic explanation for the observed co-variation is still lacking, linkages between these responses are to be expected. Observations of changes in one factor may therefore be indicative for changes in the others. Recent findings of increased carbon isotope values in newly produced suspended and sedimented organic matter relative to material originating from preindustrial times (Bentaleb et al., 1996; Bentaleb and Fontugne, 1996; Fischer et al., 1997; Bentaleb et al., 1998; Fischer et al., 1998) suggest, that the increase in surface ocean CO_2 concentrations over the past century has affected carbon metabolism of natural marine phytoplankton. Whatever the corresponding effect on growth rate and elemental composition may be, it should not automatically be assumed that the ocean's biological carbon pumps are irresponsive to changes in atmospheric CO_2 . Enhanced growth rates and increasing C:P ratios of marine phytoplankton in response to rising CO_2 concentrations would strengthen the soft tissue pump. Reduced biogenic calcification, on the other hand, would weaken the CaCO_3 counter pump. Thus, all three effects would raise the ocean's capacity to store atmospheric CO_2 .

and hence represent negative feedbacks with respect to anthropogenic CO₂ increase. In view of the potential importance of these processes we wish to emphasize that future modeling of the global ocean carbon cycle should include the possibility of CO₂-sensitive biological carbon pumps.

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