



Species-specific responses of calcifying algae to changing seawater carbonate chemistry

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[1] Uptake of half of the fossil fuel CO₂ into the ocean causes gradual seawater acidification. This has been shown to slow down calcification of major calcifying groups, such as corals, foraminifera, and coccolithophores. Here we show that two of the most productive marine calcifying species, the coccolithophores *Coccolithus pelagicus* and *Calcidiscus leptoporus*, do not follow the CO₂-related calcification response previously found. In batch culture experiments, particulate inorganic carbon (PIC) of *C. leptoporus* changes with increasing CO₂ concentration in a nonlinear relationship. A PIC optimum curve is obtained, with a maximum value at present-day surface ocean pCO₂ levels (~360 ppm CO₂). With particulate organic carbon (POC) remaining constant over the range of CO₂ concentrations, the PIC/POC ratio also shows an optimum curve. In the *C. pelagicus* cultures, neither PIC nor POC changes significantly over the CO₂ range tested, yielding a stable PIC/POC ratio. Since growth rate in both species did not change with pCO₂, POC and PIC production show the same pattern as POC and PIC. The two investigated species respond differently to changes in the seawater carbonate chemistry, highlighting the need to consider species-specific effects when evaluating whole ecosystem responses. Changes of calcification rate (PIC production) were highly correlated to changes in coccolith morphology. Since our experimental results suggest altered coccolith morphology (at least in the case of *C. leptoporus*) in the geological past, coccoliths originating from sedimentary records of periods with different CO₂ levels were analyzed. Analysis of sediment samples was performed on six cores obtained from locations well above the lysocline and covering a range of latitudes throughout the Atlantic Ocean. Scanning electron micrograph analysis of coccolith morphologies did not reveal any evidence for significant numbers of incomplete or malformed coccoliths of *C. pelagicus* and *C. leptoporus* in last glacial maximum and Holocene sediments. The discrepancy between experimental and geological results might be explained by adaptation to changing carbonate chemistry.

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1. Introduction

[2] Since the mid 19th century, fossil fuel burning has released more than 250 billion tons of carbon in the form of CO₂ into the atmosphere [Houghton *et al.*, 2001]. Nearly 50% of the fossil fuel CO₂ emitted into the atmosphere was subsequently taken up by the ocean, 80% of which is stored in the upper 200 m of the water column [Sabine *et al.*, 2004]. While ocean uptake of anthropogenic CO₂ provides an invaluable service by mitigating CO₂-related global warming, it leads to a continuous acidification of surface ocean seawater. Unabated CO₂ emissions will cause a doubling in surface ocean pCO₂ levels over their preindustrial values by the middle of this century, accompanied by a decrease in surface ocean pH three times greater than that experienced during the transition from glacial to interglacial periods [Wolf-Gladrow *et al.*, 1999]. Ocean acidification and the related changes in seawater chemistry may directly impact marine organisms and ecosystems.

[3] Recent laboratory and field studies have revealed a profound effect of seawater acidification and the related decrease in carbonate saturation state on the calcification rates of individual species and communities in both planktic and benthic habitats (Bijma *et al.* [1999] (I); Delille *et al.* [2005] (II); Kleypas *et al.* [1999] (III); Riebesell *et al.* [2000] (II)). Foraminifera (I), coccolithophores (II), and corals (III) each showed a decrease in calcification with increasing CO₂ concentration. A CO₂ doubling from preindustrial values of 280 ppm pCO₂ to 560 ppm pCO₂ caused a decreased in calcium carbonate precipitation by 5–25% in all calcifying organisms investigated to date [Feely *et al.*, 2004]. This rather uniform response appears enigmatic considering that major differences exist between the calcifying groups with regard to (1) the mineral phase of CaCO₃ produced, (2) the location of CaCO₃ precipitation, and (3) the pathways of

the associated ion transport. Whereas the aragonitic structures of corals are formed at external surfaces providing microenvironments favoring CaCO₃ precipitation, the calcitic shells and platelets of foraminifera and coccolithophores are produced internally in calcifying compartments through vacuolization of seawater [Erez, 2003] and via transmembrane ion transport [Brownlee and Taylor, 2004].

[4] Despite the apparent consistency in the observed CO₂ effects on biogenic calcification, predictions about the fate of calcifying organisms in an increasingly acidified ocean are problematic. Most results presently available are based on short-term experiments with calcifying organisms dominant in the present ocean. This may have created a bias for species and clones best adapted to modern CO₂ levels and raises questions regarding the general validity of the observed responses. It is also unknown whether calcifiers are capable to adapt to decreasing carbonate saturation states and at what rate. Aside from testing a wider range of calcifying species, there is obviously a need to examine long-term effects of CO₂-related changes in seawater chemistry.

[5] In the present study we have combined experimental results of two previously untested coccolithophore species, *Calcidiscus leptoporus* and *Coccolithus pelagicus*, with the examination of coccolith morphology in glacial and preindustrial marine sediments as well as from laboratory assay. *C. leptoporus* and *C. pelagicus* are two of the heaviest calcifying extant coccolithophores and, alongside with the bloom forming cosmopolitan *Emiliania huxleyi*, the most important species in terms of calcite export to the sediment [Broerse *et al.*, 2000]. Comparison of short-term responses of calcification to abrupt CO₂ perturbations as observed in the laboratory with coccoliths originating from sedimentary records of periods with different CO₂ levels provides first evidence for adaptation

Table 1. Data Set Derived From the Experiments

	<i>C. leptoporus</i> AC365 Experiment Number						<i>C. pelagicus</i> AC400 Experiment Number		
	1	2	3	4	5	6	7	8	9
<i>Medium Chemistry</i>									
pCO ₂ , μatm	98	216	345	477	768	920	149	345	915
CO ₂ , μmol kg ⁻¹	3.2	7.1	11.3	15.7	25.3	30.3	5.4	12.4	32.8
HCO ₃ ⁻ , μmol kg ⁻¹	1614	1807	1901	1941	1987	1973	1692	1813	1871
CO ₃ ²⁻ , μmol kg ⁻¹	516	306	203	160	104	82	340	169	68
DIC, μmol kg ⁻¹	2133	2121	2115	2116	2116	2086	2038	1994	1971
TA, μmol kg ⁻¹	2827	2548	2393	2336	2248	2179	2517	2234	2047
pH, NBS	8.74	8.44	8.27	8.13	7.93	7.86	8.56	8.22	7.81
omega	12.8	7.5	5.0	3.9	2.6	2.0	8.3	4.1	1.7
<i>Sample Parameters</i>									
pg POC cell ⁻¹	74	63	66	63	69	67	184	198	218
pg PIC cell ⁻¹	93	122	146	130	118	101	276	338	354
PIC/POC	1.25	1.94	2.22	2.05	1.71	1.55	1.50	1.70	1.63
P (μ) POC, pg POC cell ⁻¹ day ⁻¹	36	41	42	39	44	40	143	144	158
P (μ) PIC, pg PIC cell ⁻¹ day ⁻¹	45	78	94	80	75	62	214	245	256
Growth rate μ (per day)	0.48	0.64	0.64	0.62	0.63	0.62	0.78	0.73	0.73
<i>Coccolith Morphology</i>									
Sphere size, μm	12.6		14.7				13.2		
Coccoliths/cell	15	14	14	14	14	16			
(1) Normal coccoliths, %	11	45	52	47	19	4	50	54	53
(2) Malformed coccoliths, %	2	3	25	11	6	8	5	9	18
(3) Incompletely grown coccoliths, %	41	33	6	9	5	2	22	12	14
(4) Malformed and incompletely grown coccoliths, %	46	18	17	32	69	86	23	25	16

of coccolithophore calcification to changing CO₂ concentration.

2. Material and Methods

2.1. Experimental Design

[6] Monospecific cultures of *Calcidiscus leptoporus* (strain AC365, from South Atlantic off South Africa, CODENET culture collection, ALGO-BANK http://www.nhm.ac.uk/hosted_sites/ina/CODENET/caencultures.htm) and *Coccolithus pelagicus* (strain AC400, from South Atlantic off Namibia, CODENET culture collection, ALGO-BANK) were grown in sterile filtered (0.2 μm) seawater enriched with 100 μM nitrate and 6.25 μM phosphate and with trace metals and vitamins according to f/2 [Guillard and Ryther, 1962]. The incident photon flux density was 350 μmol/m²*s and a 16/8 hours light/dark cycle was applied. Experiments were carried out at optimum growth temperature, i.e., 20°C for *C. leptoporus* and 17°C for *C. pelagicus*. Cells were acclimated to experimental conditions for approximately 10 generations and grown in dilute batch cultures in triplicate [Zondervan et al., 2002]. For a summary

of all measured parameters see Table 1. Low cell density at harvest (in general less than 6000 cells per ml) resulted in less than 8% DIC (dissolved inorganic carbon) consumption (i.e., DIC consumed by the cells at the end of experiment) and a shift in pH of no more than 0.06 units. CO₂ levels were adjusted by adding calculated amounts of 1 M HCl or 1 M NaOH to the medium. The carbonate system of seawater can be manipulated in various ways. In an open system changes in atmospheric pCO₂ cause changes in [CO₂], pH and DIC, while TA (total alkalinity) remains constant. In an experiment this method requires bubbling with CO₂ gas. The addition of HCl or NaOH to a closed system affects [CO₂], pH, and TA, while DIC remains constant. Precise manipulations of the carbonate system are easier to achieve applying the latter method, which is the reason why it was applied in this study. Changes in DIC or TA with respect to the CO₂ range tested are on the order of 10% and therewith not likely to produce an effect on the measured parameters. Experiments in which DIC was altered should thus be comparable to experiments in which TA was altered. In order to prevent gas exchange with the atmosphere 2.4 l borosilicate flasks were filled without headspace and closed with Teflon lined screw caps. Determination of

growth rate, however, required regular sampling for cell counts, thereby creating a maximum headspace of 6 ml. The resultant air-water CO₂ equilibration of 3% shift in CO₂aq concentration is negligible. Samples for alkalinity measurements were filtered (approx. 0.6 μm), poisoned with 1 mL of a HgCl₂ solution (35 g/l) and stored in 300 mL borosilicate flasks at 0°C. DIC samples were sterile filtered (0.2 μm) and stored in 13 mL borosilicate flasks free of air bubbles at 0°C. Total alkalinity was calculated from linear Gran plots [Gran, 1952] after duplicate potentiometric titration [Bradshaw et al., 1981; Brewer et al., 1986] and DIC was measured photometrically [Stoll et al., 2001] in triplicate. Precision of the total alkalinity measurements is ~3 μmol/L and accuracy ~4 μmol/L. For dissolved inorganic carbon precision is ~4 μmol/L and accuracy ~5 μmol/L. The carbonate system was calculated from temperature, salinity, and the concentrations of DIC, total alkalinity and phosphate, using the program CO₂sys [Lewis and Wallace, 1998]. Equilibrium constants of Mehrbach et al. [1973] refit by Dickson and Millero [1987] were chosen. Samples for determination of total particulate carbon and particulate organic carbon were filtered on precombusted (12 hours, 500°C) GF/F-filters (approx. 0.6 μm) and stored at -20°C. Prior to analysis, the POC filters were fumed for two hours with a saturated HCl solution to remove all inorganic carbon. TPC and POC were subsequently measured on a Carlo Erba NA-1500 Analyzer. Particulate inorganic carbon (PIC) was calculated as the difference between TPC and POC. For determination of cell density samples were taken daily or every other day, stored at 0°C and counted within 3 hours after sampling using a Sedgwick Rafter counting cell. Cell density was plotted against time and growth rate (μ) was calculated by means of exponential regression. Inorganic and organic carbon production (P, pg C/cell*d) was calculated according to $P = \mu * (\text{cellular carbon content})$.

2.2. Coccolith Morphology

[7] Samples for scanning electron microscope analysis were filtered on cellulose nitrate filters (0.45 μm pore size), dried in a drying cabinet at 60°C for 24 hours, then sputter-coated with gold-palladium. Imaging was performed with a Philips XL-30 digital scanning field-emission electron microscope. Four categories were used to describe the morphology of *C. leptoporus*: normal, malformed, incomplete, and incomplete and malformed coccoliths (for reference images for the categories,

see Figure S3 in the auxiliary material¹). An average of approx. 500 coccoliths was analyzed per CO₂ treatment. The relative numbers of coccoliths in the four categories were constant when counting more than 50 coccoliths, so that the relative numbers obtained from counting 500 coccoliths can safely be regarded as statistically significant. In order to get a morphological index of reduced cellular calcite content we combined the two categories containing incompletely grown coccoliths, since an incompletely grown coccolith contains less calcite than a completely grown one (including the malformed coccoliths). The relative number of all incompletely grown coccoliths is given in Table 1.

2.3. Sediment Core Samples

[8] Six cores were selected for the geological control study representing a range of latitudes through the Atlantic Ocean; all cores were from well above the lysocline. The LGM sample set used here is mostly adopted from the GLAMAP-2000 study [Niebler et al., 2003; Pflaumann et al., 2003], which greatly benefits from a detailed AMS¹⁴C-stratigraphy or other high-resolution age control and careful selection of the LGM interval. In addition, we tried to investigate the topmost centimeter of the sediment to generate a modern reference data set. Although most of the samples were taken with a multicorer or a giant box corer, we cannot exclude that due to bioturbation and sampling disturbances the surface sediment samples represent up to 1,500 years in open ocean areas [Andruleit and Baumann, 1998]. As a consequence sedimentation predominantly took place under preindustrial CO₂ levels of roughly 280 ppmv [Indermöhle et al., 1999]. From each core one sample was taken from the last glacial maximum (LGM) when CO₂ levels are believed to have been ~180 ppmv [Petit et al., 1999], and a second from the sediment top (i.e., upper 1 cm of the core).

[9] For the counting of core top and LGM core samples the same procedure of dividing the coccoliths into categories and determining the relative numbers of coccoliths in the respective categories was applied. This allowed a quantitative comparison of coccolith morphology.

3. Results

[10] The laboratory assay consisted of batch culture incubations of *C. leptoporus* and *C. pelagicus*

¹Auxiliary material is available at <ftp://ftp.agu.org/apend/gc/2005gc001227>.

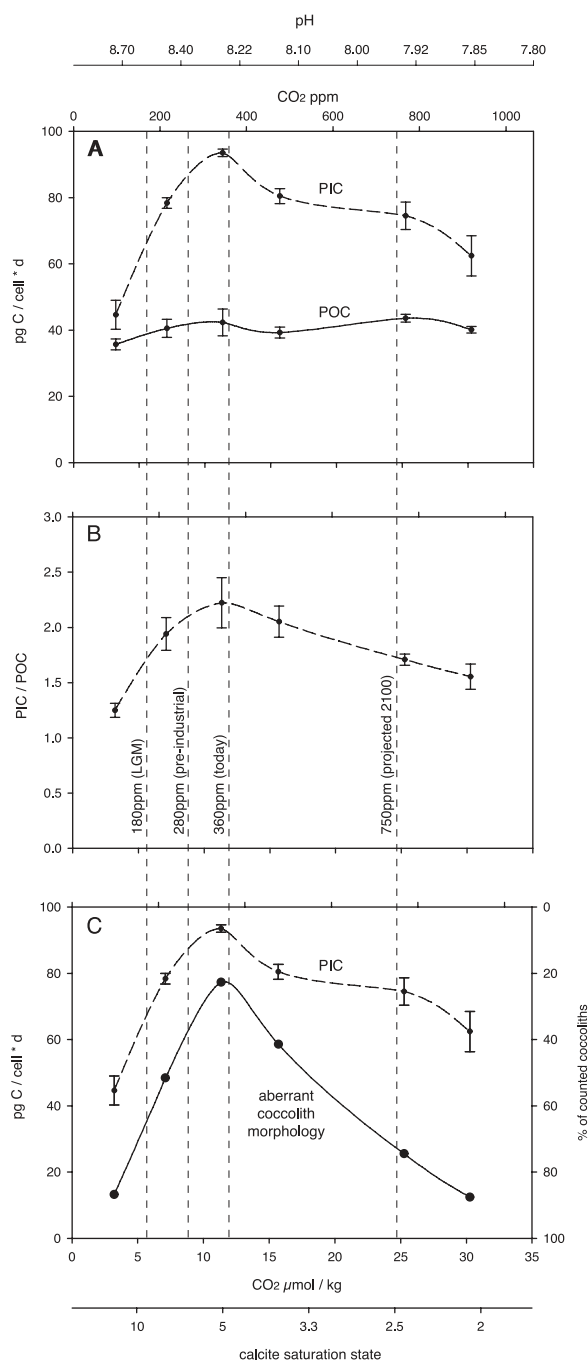


Figure 1. Data from *C. leptoporus* culture experiments plotted against carbonate chemistry in the culture medium (pH scale is NBS). (a) Production of inorganic (dashed line) and organic (solid line) carbon per cell and day. (b) Ratio of inorganic to organic carbon content. (c) Production of inorganic carbon per cell and day (as in Figure 1a, dashed line) and relative number of incompletely grown and incompletely grown/malformed coccoliths (solid line).

grown over a range of CO₂ concentrations (Table 1) in which cellular particulate inorganic carbon (PIC), particulate organic carbon (POC), growth rate, and coccolith morphology were monitored. In *C. leptoporus* the cellular inorganic carbon content changes with increasing CO₂ concentration in a nonlinear relationship (Figure 1). An optimum curve is obtained, with a maximum value at modern surface ocean pCO₂ levels (~360 ppm CO₂). With the cellular organic carbon content remaining constant over the range of CO₂ concentrations, the PIC/POC ratio also shows an optimum curve. Since the growth rate does not change with pCO₂, rates of POC and PIC production show the same pattern as POC and PIC content per cell (Table 1). Scanning electron microscopy analysis reveals highly variable degrees of coccolith malformation and proportions of incomplete coccoliths between CO₂ treatments (Figure 3). At 360 ppm CO₂ most coccoliths show normal morphology, at higher and lower CO₂ concentrations the proportion of coccoliths showing incomplete growth and malformation increases notably.

[11] In *C. leptoporus* changes of calcification rate are highly correlated to changes in coccolith morphology. This raises the question whether decreasing calcite content per cell is due to a lowering of the calcite content per coccolith or to a reduction in the number of coccoliths per cell. Incomplete growth implies less calcite per coccolith. Plotting the relative number of incomplete coccoliths against CO₂ concentration yields an optimum curve of similar shape to the inorganic carbon content per cell curve. Therefore it is likely that the changing inorganic carbon content per cell is due to altered calcite content per coccolith. This interpretation is supported by the fact that no differences in the number of visible coccoliths per cell could be observed (Table 1).

[12] In the *Coccolithus pelagicus* cultures neither PIC nor POC content per cell changes significantly over the CO₂ range tested (Figure 2, Table 1), yielding a stable PIC/POC ratio. The significance was determined using the Student's *t* test at a confidence level of 0.05. Because growth rate does not change, PIC and POC production also remain constant with CO₂. Scanning electron microscope analysis of *C. pelagicus* coccoliths showed no significant change in the proportions of malformed or incomplete coccoliths over the range of CO₂ concentrations (Figure 2 and Figure 3).

[13] Analysis of sediment samples was performed on six cores obtained from location well above the

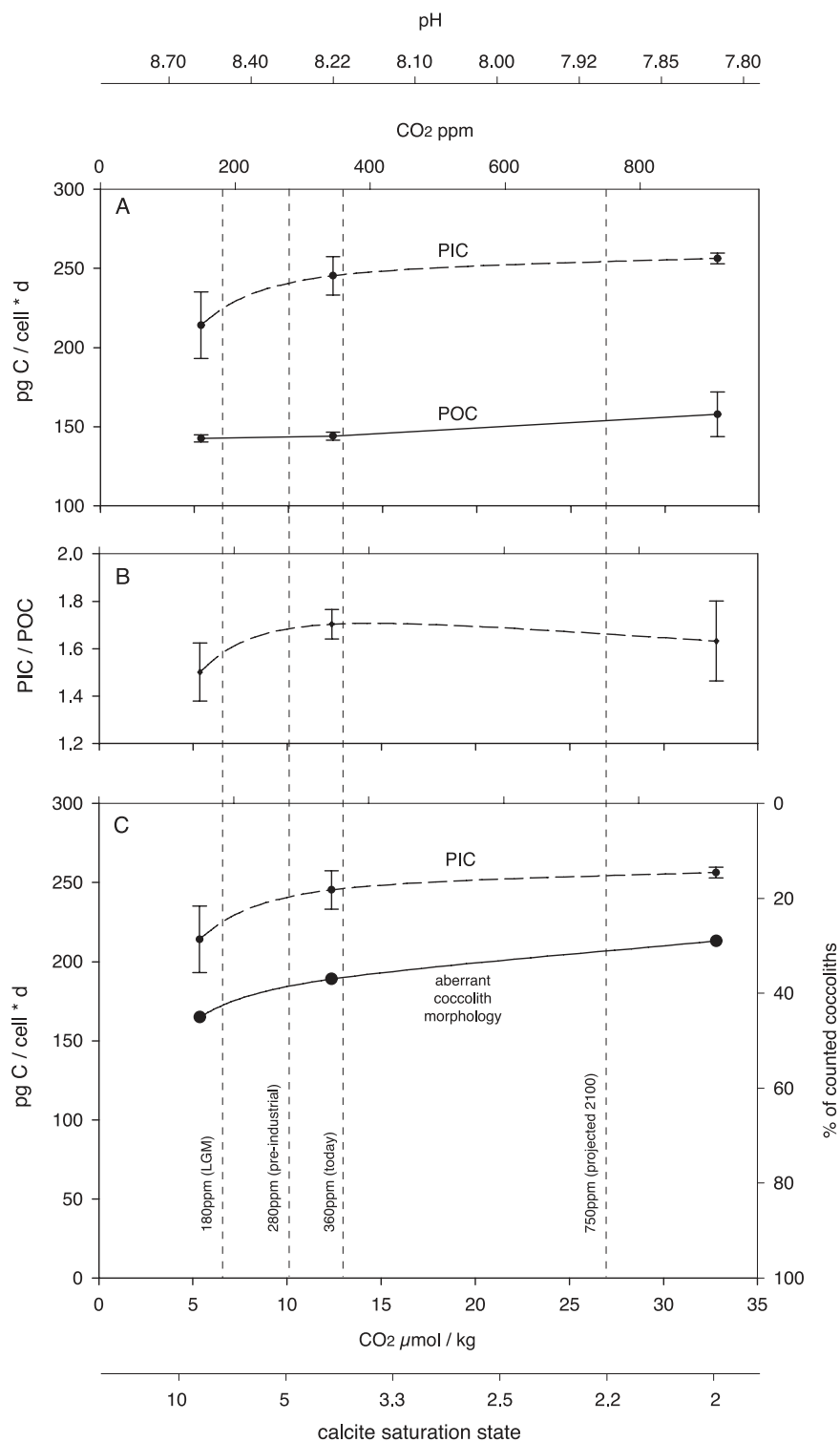


Figure 2. Data from *C. pelagicus* laboratory culture experiments plotted against carbonate chemistry in the culture medium. (a) Production of inorganic (dashed line) and organic (solid line) carbon per cell and day. (b) Inorganic to organic carbon ratio. (c) Production of inorganic carbon per cell and day (as in Figure 2a, dashed line) and relative number of incompletely grown and incompletely grown/malformed coccoliths (solid line).

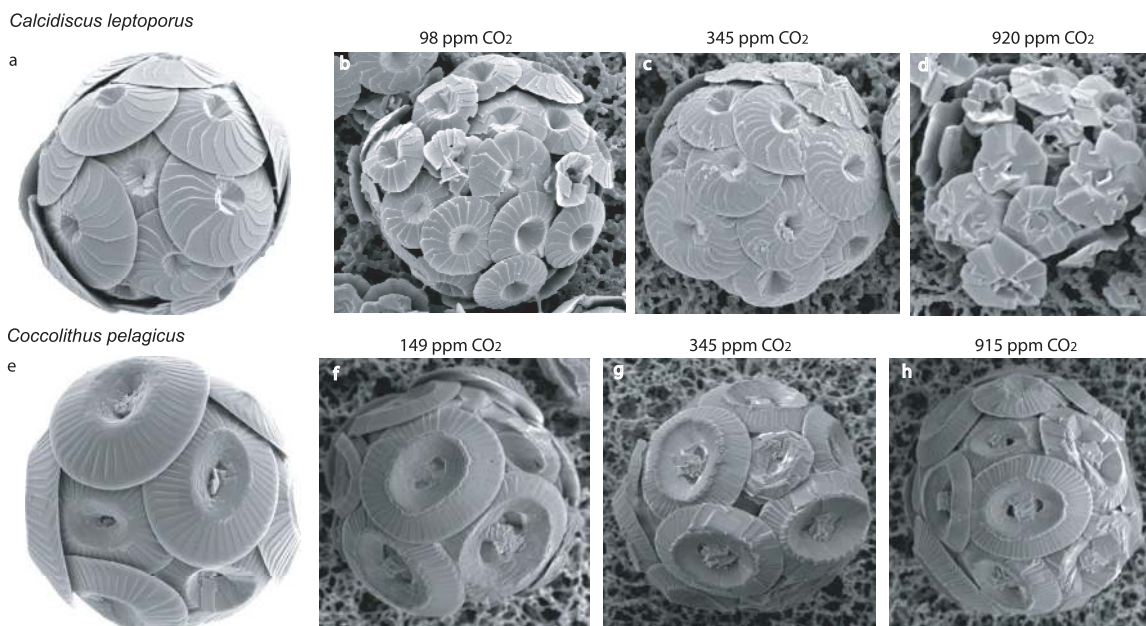


Figure 3. Representative specimens of (a–d) *C. leptoporos* and (e–h) *C. pelagicus* from (a and e) natural samples and (b–d and f–h) laboratory culture experiments grown at different CO₂ concentrations. Note the increase in malformation and/or incomplete growth (narrow rims) with elevated or reduced pCO₂ in *C. leptoporos*, where incomplete growth dominates samples from lower than present-day pCO₂ values, whereas malformation and incomplete growth dominated the sample with higher pCO₂ values.

lysocline and covering a range of latitudes throughout the Atlantic Ocean (for details, see auxiliary material). From each core one sample was taken from the last glacial maximum (LGM) when CO₂ levels are believed to have been ~180 ppmv [Petit *et al.*, 1999], and a second from the sediment top (i.e., upper 1 cm of the core), representing preindustrial times. Scanning electron micrograph analysis of coccolith morphologies did not reveal any evidence for significant numbers of incomplete or malformed coccoliths of *C. pelagicus* and *C. leptoporos* in LGM and Holocene sediments (Table 2). In general, carbonate overgrowth or early diagenetic alterations cannot be found in any of the samples, examples are given in Figure 4.

4. Discussion

[14] The assessment of the CO₂ sensitivity of marine biogenic calcification is primarily based on short-term experimental studies using single clones of selected species. The observed deterioration of coccolith production above as well as below present-day CO₂ concentrations in *C. leptoporos* and the lack of a CO₂ sensitivity of calcification in *C. pelagicus* refute the notion of a linear relationship of calcification with the carbonate ion concentration and carbonate saturation state, as

previously suggested for corals [e.g., Feely *et al.*, 2004]. Consequently, species-specific effects have to be taken into account when assessing whole ecosystem responses to changing carbonate chemistry.

[15] In case of *C. leptoporos*, changes in coccolith morphology as occurring in response to the abrupt CO₂ perturbation applied in the experimental treatments are not mirrored in the sedimentary record. This suggests that in the natural environment *C. leptoporos* has adjusted to the 80 ppm CO₂ and 180 ppm CO₂ difference between present, preindustrial and glacial times, respectively.

[16] An alternative explanation for observing only normal coccoliths in the fossil record would be selective removal through dissolution of incomplete or malformed coccoliths. This is not inconceivable, considering water column dissolution of coccoliths and assuming that malformed morphotypes may be more vulnerable to dissolution. Since dissolution is a continuous process [Hay, 2004], however, complete removal of incomplete and malformed coccoliths cannot occur without inflicting visible changes to normal coccoliths. Such changes were not observed. This reasoning was confirmed in a thorough field study in the Indonesian Seas, where the coccolith morphology of a

Table 2. Sample Locations and Data Set Derived From the Geological Samples^a

Sediment Core	Latitude	Longitude	Water Depth, m	Age	<i>C. leptoporus</i>				<i>C. pelagicus</i>			
					Total Observations	Normal in %	Malformed in %	Incomplete in %	Total Observations	Normal in %	Malformed in %	Incomplete in %
GeoB11105	1°40'N	12°26'W	3232	surface	42	98	2	0				
GeoB22204	8°32'S	34°02'W	2085	surface LGM	21 53	100 100	0 0	0 0				
GeoB1214	24°41'S	7°14'E	3220	surface LGM	121 231	99 100	1 0	0 0				
GeoB1710	23°26'S	11°42'E	2983	surface LGM					34 54	94 96	3 4	3 0
GeoB3603	35°07'S	17°33'E	2851	surface LGM	100 100	99 96	1 4	0 0				
GeoB5546	27°32'N	13°44'W	1071	surface LGM	26 57	100 96	0 4	0 0	31 36	97 97	0 3	3 0
ODP site 980B	55°30'N	15°42'W	2172	surface	154	97	2	1	71	99	0	1
ODP site 982A	57°51'N	15°52'W	1135	LGM	111	99	1	0	32	97	3	0
K 708-7	53°56'N	24°05'W	3502	surface					43	100	0	0
GIK 23062	68°43'N	0°11'E	2243	surface					32	100	0	0
GIK 23199	68°23'N	5°19'E	1968	LGM surface					14 68	93 99	7 1	0 0

^aThe data displayed are showing the morphology of *C. leptoporus* and *C. pelagicus* coccoliths in samples from Holocene age (surface) and from the last glacial maximum (LGM). Note the low number of abnormally formed coccoliths in all samples. Samples are from the University of Bremen (GeoB) and the Ocean Drilling Program (both at the ODP core repository at University Bremen), core K 708-7 is from a 1970 cruise of R/V *Kane* (subsamples are at the University Bremen), and cores designated GIK are from the geological institute of the University Kiel.

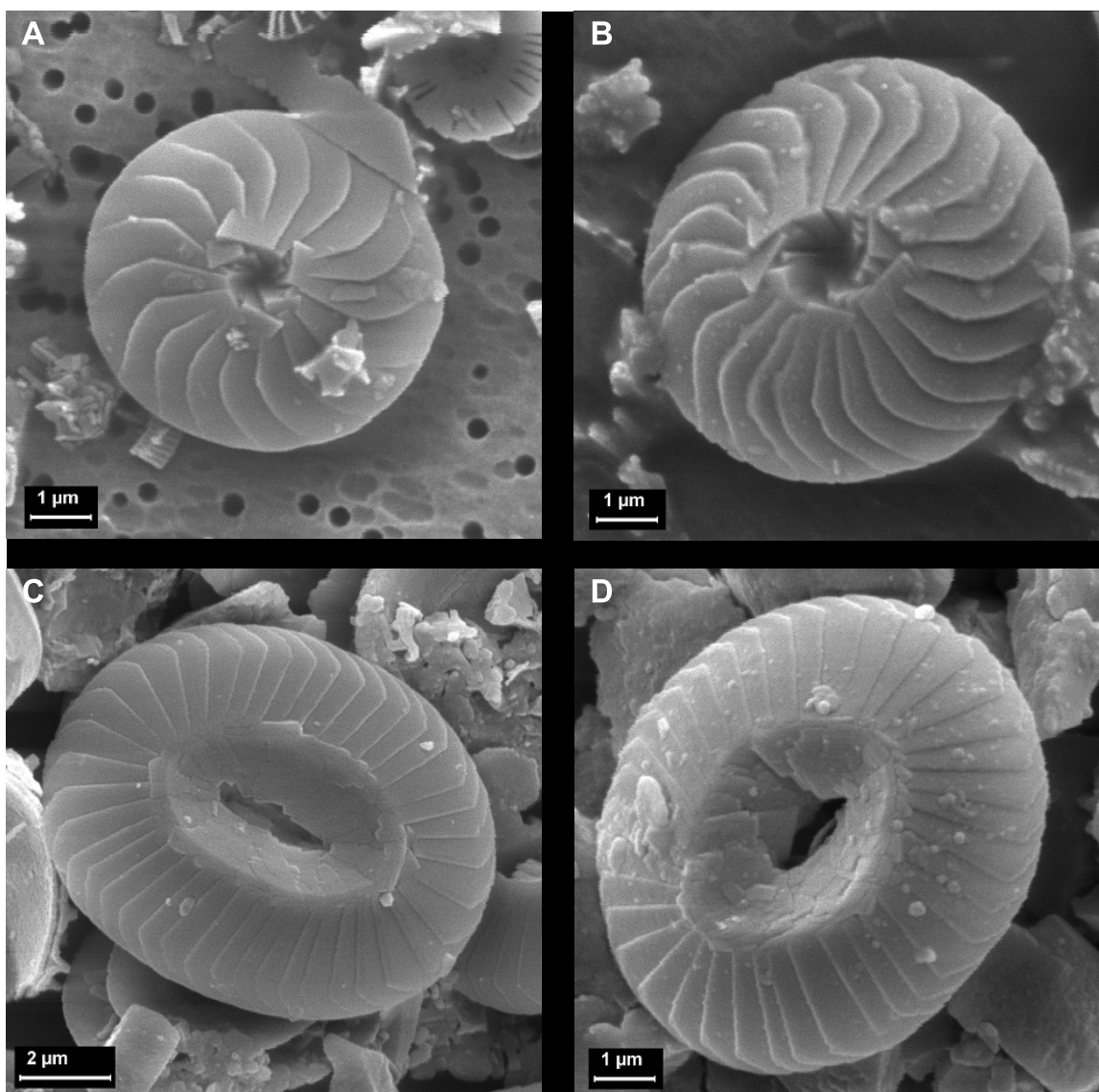


Figure 4. Representative images of coccolith morphology of the two species investigated taken from sediment samples. *C. leptoporus* (a) from surface sediment and (b) from the last glacial maximum. *C. pelagicus* (c) from surface sediment and (d) from the last glacial maximum. Images in Figures 4a and 4b were taken from samples from core GeoB 1214; images in Figures 4c and 4d were taken from samples taken from core GK 23062; refer to Table 2.

number of different species was investigated in both surface water- and sediment trap samples [Kleijne, 1990]. This study conclusively demonstrated that in sediment trap samples both malformed and normal coccoliths were subject to dissolution and hence should be observable in the fossil record. These findings support our hypothesis that selective dissolution cannot explain our results from fossil material.

[17] Thus it is reasonable to assume that *C. leptoporus* has adapted its calcification mechanism to the change in carbonate chemistry having occurred

since the last glacial maximum. A possible explanation for such adaptation is that the population is genetically diverse, containing strains with diverse physiological and genetic traits, as already demonstrated for *E. huxleyi* [Brand, 1981, 1982, 1984; Conte *et al.*, 1998; Medlin *et al.*, 1996; Paasche, 2002; Stolte *et al.*, 2000]. Our experimental data for *C. leptoporus* merely reflect the physiological response of one particular strain to short term changes of pCO₂. If genetic diversity is the basis of adaptation to changing CO₂ concentrations, this is not likely to be confined to *C. leptoporus* but can be assumed to play a role in other coccolithophore

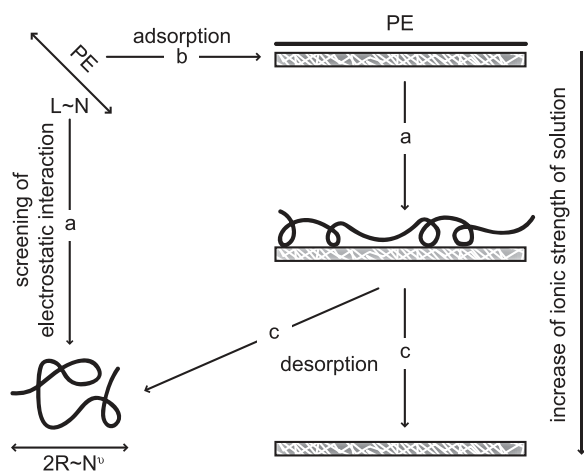


Figure 5. Behavior of polyelectrolytes (PEs) in relation to ionic strength of the solution. PEs are polymers that have ionizable groups (such as SO_3^- or CO_2^-). When dissolved in solutions, they dissociate into charged polymer chains (macroions) and free, mobile counterions. The delicate balance between chain configurational entropy and long range electrostatic monomer-monomer repulsion determines the structure of charged chains in dependence on the ionic strength of the solution. In the limit of low ionic strength, PEs are usually stretched and assume a rod-like conformation ($L \sim N$, N is the degree of polymerization). Increasing the ionic strength (a) can significantly reduce the rigidity of the chain, since the electrostatic interaction is screened over the Debye-Hückel screening length (which typically varies from less than 1 nm to more than 100 nm). At very high ionic strength, the distant parts of a charged chain do not interact, and the chain behaves as a random coil with short-ranged repulsive interactions ($R \sim N^\nu$, $0.5 < \nu < 1$). PEs can adsorb onto a charged surface (b) mainly caused by electrostatic effects. At low ionic strength, adsorption is very strong, and the PEs assume a conformation where the chain essentially lies flat on the surface. As the ionic strength is increased (a), the polymer chain can gain conformational entropy by extending somewhat into the solution. In the limit of high ionic strength, PEs can desorb from the surface (c).

species as well. The sexual life cycle of coccolithophores ensures extensive genetic recombination, resulting in natural populations with significant ecophysiological variability [Geisen et al., 2004; Paasche, 2002].

[18] On the cellular level the influence of changing carbonate chemistry can be interpreted in terms of a pH effect. It is well known that in many species changes in extracellular pH exert an influence on various physiological parameters, e.g., intracellular pH [Nimer et al., 1994], nitrate uptake [Thoresen et

al., 1984] and membrane potential [Felle, 1994]. A constant membrane potential is the result of a sophisticated ion balance mechanism. Hence it is likely that sudden external pH changes can upset this ion balance and consequently alter ionic composition and ionic strength of the cell. Intracellular ionic strength in turn determines the conformation of polyelectrolytes, e.g., cytoskeletal elements and coccolith-associated polysaccharides. According to polymer theory [Odijk, 1977; Skolnick and Fixman, 1977] changes in ionic strength can turn a rod-like polyelectrolyte into a coil (Figure 5), thereby disturbing its specific function. Both the cytoskeleton and the coccolith associated polysaccharides are thought to play an important role in coccolith morphogenesis [Henriksen et al., 2004; Marsh, 1994]. Conformational changes of these polyelectrolytes may have caused the observed coccolith malformation. On longer timescales however, the population may be able to evolve so that the optimal CO_2 level for calcification of the species tracks the environmental value.

[19] This study provides the first examination of possible effects of changing pCO_2 on coccolithophore calcification that does not entirely rely on experimental, i.e., very short, timescales. For planktic foraminifera there are both experimental [Bijma et al., 1999] and geological [Barker and Elderfield, 2002] observations suggesting a decline of calcification rates in response to carbonate ion concentration of seawater. Because these two studies use different species, a combination of the two approaches, as presented in this study, is not possible. Nonetheless, the foraminiferal data support the notion of a decrease in calcification on geological timescales whereas our coccolithophore data do not. It is generally accepted that in coccolithophores there is no obvious correlation between coccolith morphology, in terms of size and degree of calcification, and CO_2 levels (calcium carbonate saturation state) on geological timescales. The Pleistocene, for example, an epoch of low pCO_2 levels and slightly higher values of calcite saturation state compared to the Eocene and the Late Cretaceous [Demichco et al., 2003; Pearson and Palmer, 2000; Retallack, 2001; van Andel et al., 1977] is characterized by small lightly calcified coccoliths. In contrast, the Eocene and the late Cretaceous are times of large and heavily calcified coccoliths [Perch-Nielsen, 1985]. Further research is clearly warranted to elucidate the possible differences between the various groups of marine calcifiers with respect to the CO_2 sensitivity of their calcification processes.

[20] Our experimental data suggest a marked alteration in coccolith morphology of *C. leptoporus* with high levels of malformed and incomplete coccoliths when CO₂ levels are reduced to last glacial maximum values. A survey of *C. leptoporus* coccoliths from the last glacial maximum (LGM) at various sites in the Atlantic Ocean did not reveal altered morphology of *C. leptoporus* coccoliths. Since the LGM, CO₂ levels have risen from 180 to 370 ppm. The time interval of ~18000 years arguably provides ample time for adaptation via evolutionary processes. Malformed or incompletely grown specimens were also not observed in natural assemblages (screened prior to the industrial revolution and in the course of the last 150 years) as well as in Holocene samples (for details, see auxiliary material). Since the industrial revolution the rate of CO₂ increase was 10 and possibly 100 times faster than at any other time in the past 420,000 years [Falkowski et al., 2000]. The absence of a detectable alteration in coccolith morphology in natural samples suggests that adaptation to changing CO₂ levels occurred on much shorter timescale (i.e., hundreds of years). Whether adaptation to the projected future increase in CO₂-related ocean acidification will be possible, is presently unknown. The lack of CO₂ sensitivity observed in *C. pelagicus*, however, suggests that the dependence of calcification on carbonate saturation state is less uniform than previously thought. Genetic diversity, both between and within species, may allow calcifying organisms to prevail in a high CO₂ ocean as long as seawater remains supersaturated with respect to calcite and aragonite.

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