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Effects of CO₂-induced changes in seawater carbonate chemistry speciation on *Coccolithus braarudii*: a conceptual model of coccolithophorid sensitivities

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Abstract

Ocean acidification and associated shifts in carbonate chemistry speciation induced by increasing levels of atmospheric carbon dioxide (CO₂) have the potential to impact marine biota in various ways. The process of biogenic calcification, for instance, is usually shown to be negatively affected. In coccolithophores, an important group of pelagic calcifiers, changes in cellular calcification rates in response to changing ocean carbonate chemistry appear to differ among species. By applying a wider CO₂ range we show that a species previously reported insensitive to seawater acidification, *Coccolithus braarudii*, responds both in terms of calcification and photosynthesis, although higher thresholds. Thus, observed differences between species seem to be related to individual sensitivities while the underlying mechanisms could be the same. On this basis we develop a conceptual model of coccolithophorid calcification and photosynthesis in response to CO₂-induced changes in seawater carbonate chemistry speciation.

1 Introduction

Following the industrial revolution in the late 18th century, atmospheric carbon dioxide levels continuously increased from about 280 to presently 395 μatm , and are currently increasing further at a rate of 0.4% per year (IPCC, 2007). The magnitude of this change would have been considerably larger if the surface oceans had not absorbed approximately 50% of the fossil fuel emissions to date (Sabine et al., 2004). The dissolution of anthropogenic CO₂ in water is followed by several chemical reactions leading to shifts in carbonate chemistry speciation resulting in decreasing pH, carbonate ion concentration, and saturation state (ocean acidification) and concomitant increases in the overall dissolved inorganic carbon inventory (ocean carbonation). This conceptual separation is of potential interest for understanding the basis of any physiological response to changes in seawater carbonate chemistry.

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Coccolithophores are significant pelagic calcifiers, contributing about half to calcium carbonates preserved in open ocean sediments (Broecker and Clark, 2009). They are among the best examined organisms with respect to their response to ocean acidification/carbonation (e.g. Riebesell et al., 2000; Zondervan et al., 2002; Sciandra et al., 2003; Langer et al., 2006, 2009; Feng et al., 2008; Shi et al., 2009; Ramos et al., 2010). Confusingly, the species analyzed so far have different response patterns for calcification and photosynthesis in response to changing carbonate chemistry speciation. While *Emiliana huxleyi* and *Gephyrocapsa oceanica* usually decreased calcification rates between a partial pressure of CO₂ (*p*CO₂) from about 180 to 800 μ atm (Riebesell et al., 2000; Zondervan et al., 2002; Sciandra et al., 2003; Ramos et al., 2010), *Calcidiscus quadriperforatus* showed an optimum curve response, with a maximum at current *p*CO₂, and *Coccolithus braarudii* was not significantly changing calcification rates in this range (Langer et al., 2006). Similarly, there were also differences regarding organic carbon fixation rates by photosynthesis, questioning the operation of common cellular mechanisms for calcification and photosynthesis within the group of coccolithophores.

Here we investigate the response of *Coccolithus braarudii* to a broader *p*CO₂ range in two experiments, allowing to draw conclusions on the origin of observed differences between species.

2 Methods

The response of *Coccolithus braarudii* to changing seawater carbonate chemistry was analysed in two experiments. In the first experiment the *p*CO₂ was varied from 500 to 3500 μ atm and in the second from 400 to 1700 μ atm (compare Table 1).

Monospecific cultures of *Coccolithus braarudii* (strain AC-400, isolated in the South Atlantic off Namibia and kindly provided by the microalgal culture collection ALGO-BANK <http://www.unicaen.fr/ufr/ibfa/algobank/>) were incubated in triplicates as dilute batch cultures.

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Cells were grown in sterile-filtered natural North Sea water in the first experiment, and in artificial seawater (Kester et al., 1967) in the second experiment, both at a temperature of 17° C and a salinity of 34. To ensure exponential growth without nutrient limitation 80 μ mol kg⁻¹ of nitrate and 5 μ mol kg⁻¹ of phosphate, trace metals, selenium and vitamins according to the *f*/2-medium (Guillard, 1975) were added. The incident photon flux density (PFD) was 130 μ mol m⁻² s⁻¹ at a 16/8 light/dark cycle.

2.1 Carbonate system manipulation

Manipulation of carbonate chemistry speciation to simulate ongoing ocean acidification can be achieved by either changing dissolved inorganic carbon (DIC) at constant total alkalinity (TA) or by varying TA at constant DIC (compare Schulz et al., 2009; Gattuso et al., 2010). The latter approach was chosen here for direct comparability with a previous study on *Coccolithus braarudii* (Langer et al., 2006). It is noted that there are differences in carbonate chemistry speciation between DIC and TA manipulation. In terms of magnitude and sign in concentration changes they are marginal for CO₂ ranges from 180 to 800 μ atm, while towards higher levels there are increasing differences in terms of bicarbonate (HCO₃⁻) concentration changes which start to decrease when manipulating TA instead of constantly increasing when manipulating DIC (Schulz et al., 2009). However, this was only the case towards higher *p*CO₂ levels above 3000 μ atm (compare Experiment I Table 1). There are also increasing differences in CO₃²⁻ ion concentration changes towards such high *p*CO₂ levels, but CO₃²⁻ concentrations steadily decrease in both manipulations.

TA was manipulated by adding suitable amounts of 1 M HCl or 1 M NaOH to the 0.2 μ m filtered seawater. Carbonate chemistry speciation was calculated from measured DIC and TA (see below) adopting the stoichiometric equilibrium constants for carbonic acid determined by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987) as a mean of initial and final values (compare Table 1).

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2.2 Experimental setup and measurements

For acclimation, pre-cultures were grown exponentially for 7 days under the experimental conditions before transfer to experimental bottles. All bottles were incubated in triplicates without headspace. To avoid pronounced variations in the carbonate system, maximum cell numbers at the end of incubations did not exceed 2600 in the first

5 and 4500 cells ml⁻¹ in the second experiment. This corresponds to a maximum DIC drawdown of less than 12%.
 Samples for DIC and TA were taken at the beginning and the end of both the acclimation and experimental phase. Suspended particulate inorganic carbon (PIC), otherwise interfering with the measurements, was removed by gentle pressure filtration (0.2 μm) from DIC and by vacuum filtration (GF/F, nominal pore size 0.7 μm) from TA samples. DIC was determined photometrically according to Stoll et al. (2001) with an auto-analyzer (QUAATRO, Bran & Lübbe) at a precision of ±20 μmol kg⁻¹. TA was determined by potentiometric titration in duplicate samples at 20 °C (Metrohm Basic
 10 Titrino 794) with 0.05 M HCl (Dickson, 1981; Dickson et al., 2003) at a precision of ±3 μmol kg⁻¹. Certified reference material (University of California, San Diego, Marine Physical Laboratory, A. G. Dickson) was measured alongside with the samples as an independent standard and used for corrections.

20 Samples for cell abundance were determined with a Z2™ COULTER COUNTER® cell and particle counter in the first and by light microscopy in the second experiment.

The cell abundances at the start (c_0) and at the end of incubations (c_1) were used to calculate daily growth rates (μ) during the experimental incubation period (Δt , incubation time in days) as:

$$\mu = \frac{\ln c_1 - \ln c_0}{\Delta t} \quad (1)$$

25 At the end of incubations duplicate samples for total particulate carbon (TPC) and nitrogen (TPN) and particulate organic carbon (POC) and nitrogen (PON) were filtered onto pre-combusted (450 °C for 8 h) GF/F (Whatman) filters and frozen at -20 °C until 8767

analysis. For POC determination, particulate inorganic carbon was removed by exposing the filters to fuming hydrochloric acid for 12 h. Before measurement all filters were dried at 60 °C. Afterwards they were folded and packed in tin cups and analysed in an elemental analyser with a heat conductivity detector (EuroVector EA) according to
 5 Sharp (1974).

3 Results

Manipulation of TA by about 300 μmol kg⁻¹ at constant DIC created a $p\text{CO}_2$ gradient from about 500 to 3500 μatm in the first experiment and from about 400 to 1700 μatm in the second experiment. Considering the combined $p\text{CO}_2$ range [CO_2], [CO_3^{2-}] and
 10 [H^+] changed relatively proportional by a factor of about 9. While [CO_2] was rising from 14 to 124 μmol kg⁻¹ and [H^+] from 6.9 to 63 nmol kg⁻¹, [CO_3^{2-}] dropped from 181 to 21 μmol kg⁻¹. [HCO_3^-] was much less affected within the same range and varied by a factor less than 1.06 between treatments (compare Table 1). Two out of ten triplicate treatments were clearly under-saturated with respect to calcite ($\Omega_{\text{calcite}} < 1$)
 15 while another two were only slightly above one.

POC production rates of *Coccolithus braarudii* were highest at intermediate $p\text{CO}_2$ (300 pg C cell⁻¹ d⁻¹) and declined towards lower and higher levels (200 and less than 100 pg C cell⁻¹ d⁻¹, respectively), although more pronounced in case of the latter (Fig. 1a). Calcification rates, although quite noisy, clearly decreased towards
 20 higher $p\text{CO}_2$ levels from about 300–400 pg C cell⁻¹ d⁻¹ at the lower end to less than 25 pg C cell⁻¹ d⁻¹ at the higher end of the $p\text{CO}_2$ range (Fig. 1b). The considerably stronger decrease in PIC compared to POC production led to a pronounced drop in PIC/POC (Fig. 1c). The variations in POC production, however, were not reflected in POC/PON, which generally fluctuated slightly above the Redfield ratio of 6.6 in all
 25 treatments (Fig. 1d).

Growth rates ranged between 0.6 and 0.9 d⁻¹ at lower pCO₂ and decreased to less than 0.2 d⁻¹ towards the higher levels (Fig. 2a), while cell diameter decreased almost by a factor of two (Fig. 2b).

4 Discussion

5 The response of coccolithophorid calcification and photosynthetic carbon fixation rates to changing carbonate chemistry speciation, more specifically to a pCO₂ range from about 180 to 800 μatm, has been shown to differ between species, challenging the concept of common cellular mechanisms for these two processes in coccolithophores. While calcification rates were found to decrease with increasing pCO₂ in *Emiliana huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al., 2000; Zondervan et al., 2002; Scian-
10 dra et al., 2003; Ramos et al., 2010), *Calcidiscus quadriperforatus* showed an optimum curve response, and *Coccolithus braarudii* no sign of change (Langer et al., 2006). Similarly, photosynthetic carbon fixation rates were found to increase in *Emiliana huxleyi* and *Gephyrocapsa oceanica*, while not being significantly affected in *Calcidiscus quadriperforatus* and *Coccolithus braarudii*. Extending the pCO₂ range towards higher values, however, revealed that the processes of calcification and photosynthesis in *Coccolithus braarudii* are indeed prone to changes in carbonate chemistry speciation (Fig. 1a, b). Calcification rates clearly decreased with increasing pCO₂ like in *Emiliana huxleyi* and *Gephyrocapsa oceanica*, although at higher levels, while photosynthetic carbon fixation rate was highest at intermediate pCO₂, suggesting an optimum curve response like seen for calcification in *Calcidiscus quadriperforatus*. When extending the pCO₂ range towards lower levels calcification and photosynthetic carbon fixation rates have been shown to decrease with decreasing pCO₂ in *Emiliana huxleyi*, although seemingly stronger in case of the latter (Buitenhuis et al., 1999).

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4.1 A conceptual model of carbonate chemistry dependant photosynthesis and calcification in coccolithophores

Putting all pieces of information together we propose that changes in calcification and photosynthetic carbon fixation rates in response to changing carbonate chemistry are likely to have the same underlying mechanisms in all coccolithophores, with both processes characterized by an optimum curve response (compare Fig. 3). The observed species specific differences would reflect species specific carbonate chemistry sensitivities rather than different cellular mechanisms. This concept could also explain strain-specific differences observed for *Emiliana huxleyi* (Langer et al., 2009).

10 Identifying the carbonate chemistry parameters responsible for calcification and photosynthetic carbon fixation rate changes towards both ends of the optima of the proposed model is difficult. While towards the lower end concentrations of CO₂, HCO₃⁻ and H⁺ are decreasing and pH, CO₃⁻² concentration and calcite saturation state (Ω_{calc}) are increasing, these trends are reversed towards the higher end (compare Fig. 3).
15 This poses the question for the optimum conditions in terms of carbonate chemistry speciation for calcification and photosynthesis.

Maximizing any biological rate requires the availability of saturating substrate concentrations and optimal ambient conditions. During photosynthetic carbon fixation CO₂ is fixed by the enzyme Ribulose-1,5-Bisphosphat-Carboxylase/Oxygenase (RuBisCO) in the chloroplast, while calcification is sustained by inorganic precipitation of Ca²⁺ and CO₃⁻² ions forming calcite in the coccolith producing vesicle. *Emiliana huxleyi*, like probably most phytoplankton species, is known to fuel photosynthesis by active uptake of both CO₂ and HCO₃⁻ from the surrounding seawater medium (Rost et al., 2003; Schulz et al., 2007), however simultaneous CO₃⁻² ion uptake by HCO₃⁻ transporters can also not be ruled out (Mackinder et al., 2010). Although there is no convincing proof for the actual substrate taken up for calcification it is difficult to conceive how cells would be able to operate an internal carbon pool for calcification separated from that used in photosynthesis. Therefore it seems reasonable to state that calcification is sustained

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by the uptake of some form of dissolved inorganic carbon, probably a mixture like for photosynthesis. This means that there should be thresholds for the concentrations of at least two of the dissolved inorganic carbon species in seawater (CO_2 , HCO_3^-) below which uptake and ultimately supply to photosynthesis and calcification becomes sub-saturating. Furthermore, the concentration of CO_2 in seawater influences the operation of carbon concentrating mechanisms (CCMs) in marine phytoplankton additionally. Decreasing concentrations can be thought to increase the gradient between seawater and cytosol, resulting in enhanced net diffusive loss of CO_2 out of the cytosol, and, if not compensated for, decreasing internal CO_2 concentrations (compare Schulz et al., 2007). This implies that if cytosolic pH is not simultaneously increasing there would be less dissolved inorganic carbon ultimately available as substrate, eventually resulting in reduced rates of photosynthesis and calcification. The enhanced leakiness at low seawater CO_2 concentrations can also be thought to increase metabolic costs of carbon supply to photosynthesis and calcification.

Concerning optimal conditions for these two processes cellular pH, or H^+ concentration, is certainly a factor. Photosynthetic carbon fixation is mediated by a pH dependant enzymatic reaction (Portis et al., 1986) and calcium carbonate precipitation rate is depending on calcium carbonate saturation state (Morse et al., 2007), highly influenced by pH. Decreasing seawater pH has been shown to result in lower intracellular pH levels in *Emiliana huxleyi* (Suffrian et al., 2010). Changes in seawater pH therefore have the potential to directly impact rates of photosynthesis and calcification.

In the proposed model, changes in carbonate chemistry speciation such as increasing DIC at decreasing pH could be thought to enhance calcification and photosynthesis by supplying increasing amounts of substrate until a certain threshold beyond which pH levels become unfavourable (compare Fig. 3). Ocean carbonation (increasing DIC, but most importantly CO_2 and HCO_3^-) and acidification (decreasing pH) could therefore act differently on these two processes. However, at this point contributions of pH to the shape of the optimum curve on the left side of the optimum and of CO_2 on the right side cannot be ruled out either. In any case, both photosynthesis and calcification

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are likely to have optimum concentrations for at least CO_2 , HCO_3^- and H^+ . Observed differences in coccolithophorid photosynthesis and calcification to changes in carbonate chemistry speciation could then be the result of different optimum concentrations for these ions. In other words, different sensitivities rather than different intra-cellular mechanisms for photosynthesis and calcification in species and maybe even strains can explain the differences described for *Emiliana huxleyi*/*Gephyrocapsa oceanica*, *Calcidiscus leptoporus* and *Coccolithus braarudii*.

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Table 1. Carbon chemistry speciation for experiment I and II, calculated as the mean of start and end values of measured DIC and TA. See materials and methods for details. It is noted that the higher variability of DIC concentrations in the second compared to the first experiment is due to inaccuracies in artificial seawater preparation and slightly higher variability in final cell densities.

Experiment I									
	DIC $\mu\text{mol kg}^{-1}$	TA $\mu\text{mol kg}^{-1}$	$p\text{CO}_2$ μatm	$[\text{CO}_2]$ $\mu\text{mol kg}^{-1}$	$[\text{HCO}_3^-]$ $\mu\text{mol kg}^{-1}$	$[\text{CO}_3^{2-}]$ $\mu\text{mol kg}^{-1}$	Ω_{calcite}	pH_{total}	$[\text{H}^+]$ nmol kg^{-1}
1	2048	2240	505	17.8	1887.9	142.3	3.4	7.96	10.96
2	2058	2249	512	18.0	1898.0	142.0	3.4	7.96	10.96
3	2062	2238	555	19.5	1909.9	132.6	3.2	7.92	12.02
4	2058	2135	1015	35.7	1947.0	75.3	1.8	7.67	21.38
5	2022	2077	1166	41.0	1917.4	63.6	1.5	7.60	25.12
6	2057	2103	1273	44.8	1951.8	60.4	1.4	7.57	26.92
7	2005	2021	1566	55.1	1903.2	46.7	1.1	7.47	33.88
8	2048	2052	1755	61.7	1942.9	43.4	1.0	7.43	37.15
9	2058	2041	2075	73.0	1948.1	36.9	0.9	7.36	43.65
10	2032	2008	2170	76.3	1921.3	34.3	0.8	7.33	46.77
11	2065	2030	2384	83.8	1949.0	32.2	0.8	7.30	50.12
12	2060	2025	2380	83.7	1944.2	32.0	0.8	7.30	50.12
13	2026	1943	3311	116.5	1887.8	21.7	0.5	7.14	72.44
14	2035	1940	3581	126.0	1888.9	20.1	0.5	7.11	77.62
15	2042	1945	3631	127.7	1894.4	19.9	0.5	7.10	79.43
Experiment II									
	DIC $\mu\text{mol kg}^{-1}$	TA $\mu\text{mol kg}^{-1}$	$p\text{CO}_2$ μatm	$[\text{CO}_2]$ $\mu\text{mol kg}^{-1}$	$[\text{HCO}_3^-]$ $\mu\text{mol kg}^{-1}$	$[\text{CO}_3^{2-}]$ $\mu\text{mol kg}^{-1}$	Ω_{calcite}	pH_{total}	$[\text{H}^+]$ nmol kg^{-1}
1	2132	2385	407	14.3	1932.7	185.0	4.4	8.06	8.66
2	2122	2370	413	14.5	1926.2	181.3	4.3	8.06	8.80
3	2131	2375	423	14.9	1937.2	178.9	4.3	8.05	8.97
4	2048	2230	532	18.7	1893.3	136.0	3.2	7.94	11.54
5	2053	2235	534	18.8	1898.1	136.1	3.2	7.94	11.55
6	2068	2243	560	19.7	1916.1	132.2	3.2	7.92	12.01
7	2061	2162	863	30.4	1942.5	88.2	2.1	7.74	18.25
8	2055	2149	901	31.7	1939.1	84.2	2.0	7.72	19.08
9	2059	2152	910	32.0	1943.2	83.8	2.0	7.72	19.22
10	1973	2028	1133	39.9	1870.8	62.3	1.5	7.60	24.87
11	1974	2018	1235	43.4	1873.2	57.4	1.4	7.57	27.06
12	1976	2017	1265	44.5	1875.4	56.1	1.3	7.56	27.70
13	2094	2108	1656	58.2	1987.6	48.1	1.1	7.47	34.21
14	2102	2107	1781	62.6	1994.3	45.1	1.0	7.44	36.67
15	2080	2083	1792	63.0	1973.1	43.8	1.0	7.43	37.30

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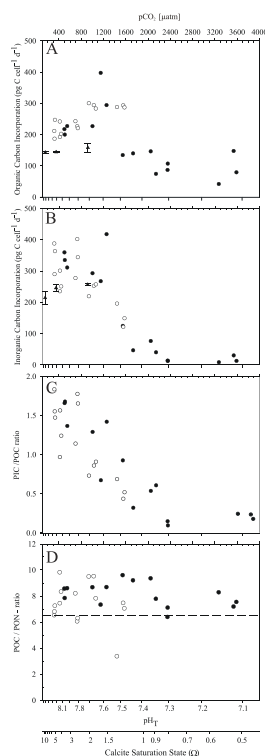


Fig. 1. Particulate organic and inorganic carbon production rates (**A** and **B**, respectively), together with ratios of particulate inorganic to organic carbon (**C**) and particulate organic carbon to nitrogen (**D**) in response to changes in carbon chemistry speciation. Filled circles represent results of the first and open circles of the second experiment, while triangles those of Langer et al. (2006) for comparison.

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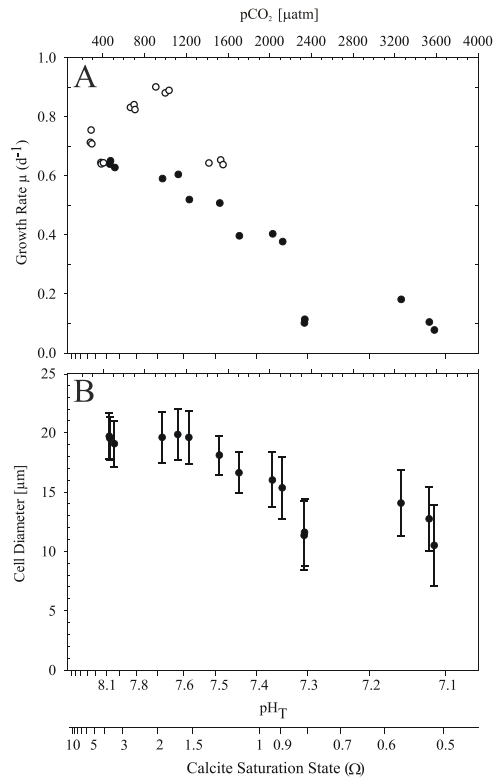


Fig. 2. Growth rates (μ) of *Coccolithus braarudii* in the first (filled circles) and second experiment (open circles) and together with cell diameter as measured in the first experiment in response to changing carbon chemistry speciation (**A** and **B**, respectively).

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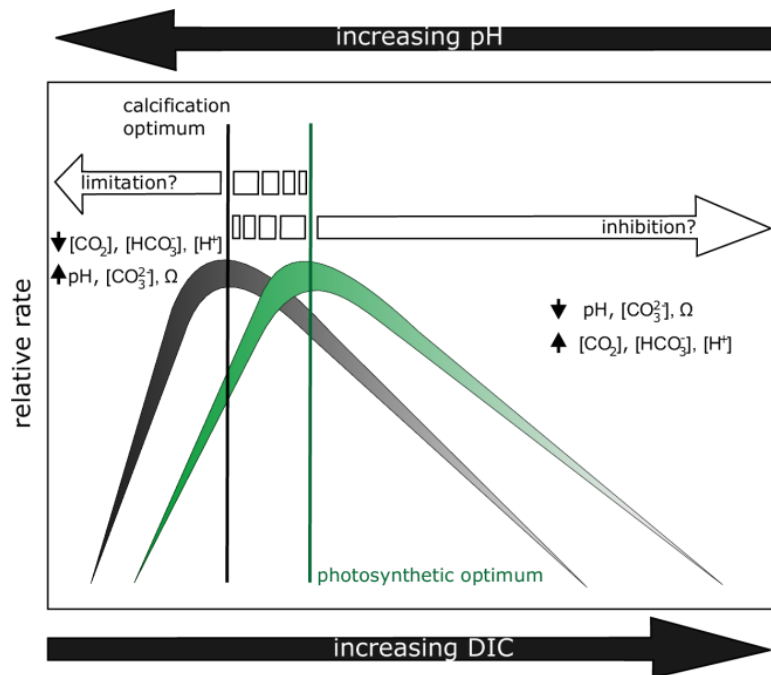


Fig. 3. Conceptual model of coccolithophorid photosynthesis and calcification in response to changes in carbonate chemistry speciation due to increasing DIC at decreasing pH.

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