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CO$_2$ perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations

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Abstract

Increasing atmospheric carbon dioxide (CO_2) through human activities and invasion of anthropogenic CO_2 into the surface ocean alters the seawater carbonate chemistry, increasing CO_2 and bicarbonate (HCO_3^-) at the expense of carbonate ion (CO_3^{2-}) con-
centrations. This redistribution in the dissolved inorganic carbon (DIC) pool decreases pH and carbonate saturation state (Ω). Several of these components are considered potential key variables influencing calcium carbonate precipitation in marine calcifiers such as coccolithophores, foraminifera, corals, mollusks and echinoderms. In need for improved understanding of the sensitivities of marine organisms and ecosystems to
CO_2 induced ocean acidification (OA), experimental CO_2 perturbation studies will continue to be a backbone in future OA research. This requires accurate carbonate system manipulations and well-controlled experimental setups. Here we describe and analyse the chemical changes involved in the two basic approaches for carbonate chemistry manipulation, i.e. changing DIC at constant total alkalinity (TA) and changing TA at
constant DIC. Furthermore, we briefly introduce several methods to experimentally manipulate DIC and TA. Finally, we examine responses obtained with both approaches using published results for the coccolithophore *Emiliania huxleyi*. We conclude that under most experimental conditions DIC or TA manipulations yield similar changes in all parameters of the carbonate system, which implies direct comparability of data ob-
tained with the two basic approaches for CO_2 perturbation.

1 Introduction

With the beginning of the industrial revolution and the increasing utilisation of fossil fuels such as coal, oil and gas, atmospheric CO_2 levels started to increase from usual interglacial values of about 280 to about 380 ppmv at present day. As the demand for
fossil fuels is likely to further intensify, atmospheric CO_2 is projected to almost double within the next 100 years (see Fig. 1 and references therein). This has not only

profound impacts on global climate (IPCC, 2007), but also on the world's oceans.

As a result of air-sea gas exchange dissolved CO_2 in the surface ocean is increasing in concert with its atmospheric counterpart. This forces redistributions in the marine carbonate system, most importantly, decreasing pH and carbonate ion (CO_3^{2-}) con-
5   centrations together with calcite and aragonite saturation states, often referred to as ocean acidification. Ocean carbonation, on the other hand, refers to the concomitant increase in dissolved inorganic carbon, namely CO_2 and HCO_3^-. Experimental assessment of possible sensitivities of marine organisms to ocean acidification and carbonation requires an understanding of the chemical background of CO_2-induced changes
10 in carbonate chemistry, the design of suitable CO_2 perturbation experiments for which a variety of manipulation approaches are available (see the "Guide for Best Practise in Ocean Acidification Research"), and the monitoring and measurement of various carbonate chemistry parameters.

Here we provide the chemical background of ocean acidification, necessary to un-
15 derstand the various possibilities to experimentally manipulate the carbonate system. While naturally occurring ocean acidification can conceptually be understood as changing dissolved inorganic carbon (DIC) at constant total alkalinity (TA), the concomitant increases in CO_2 and HCO_3^- concentrations at decreasing pH and carbonate ion concentrations can also be brought about by changing TA at constant DIC. We highlight
20 the differences and similarities of these two fundamental concepts and assess their potential to simulate ongoing ocean acidification for various oceanographic settings characterised by different temperatures and salinities. Furthermore, we briefly address several experimental approaches to manipulate DIC at constant TA and vice versa and discuss their advantages and disadvantages for different experimental setups. Finally,
25 we compare various CO_2 perturbation studies with the coccolithophore *Emiliania huxleyi* with respect to the manipulation approach chosen.

2 Methods

2.1 The seawater carbonate system

The fundamental basis for understanding ongoing ocean acidification is the seawater carbonate system (for details see Zeebe and Wolf-Gladrow, 2001; Dickson et al., 2007). It is characterised by several acid-base equilibria, most importantly by those of carbonic acid (H_2CO_3)

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + H^+ \qquad (1)$$

When carbon dioxide (CO_2) dissolves in water (H_2O) carbonic acid is formed which readily dissociates into bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}), releasing protons (H^+). It is in the nature of this equilibrium that any change in the concentration of one of the individual components will force the other three to re-adjust as well. Hence, increasing CO_2 concentrations in the surface ocean, driven by the built-up of anthropogenic CO_2 in the atmosphere, will also change the concentrations of HCO_3^-, CO_3^{2-}, and H^+. While the carbonate system can be understood in terms of the acid-base equilibria of carbonic acid, two additional concepts have proven very useful, those of dissolved inorganic carbon (DIC) and total alkalinity (TA). This is because they are relatively easy to measure accurately in comparison to the other parameters which either require considerable efforts (CO_2 and H^+) or simply cannot be determined analytically (HCO_3^- and CO_3^{2-}). Both DIC and TA are composite parameters describing the total amount of dissolved inorganic carbon and the charge balance of seawater (see Zeebe and Wolf-Gladrow, 2001; Wolf-Gladrow et al., 2007; Dickson et al., 2007 for details) as

$$DIC = [CO_2] + [HCO_3^-] + \left[CO_3^{2-}\right] \qquad (2)$$

and

$$TA = [HCO_3^-] + 2\left[CO_3^{2-}\right] + [B(OH)_4^-] + [OH^-] + \left[HPO_4^{2-}\right] + 2\left[PO_4^{3-}\right] +$$
$$[H_3SiO_4^-] + [NH_3] + [HS^-] - [H_3PO_4] - [H^+]_F - [HSO_4^-] - [HF] \qquad (3)$$

where $[H^+]_F$ is the free hydrogen ion, $[HSO_4^-]$ the bisulfate ion, and $[HF]$ the hydrogen fluoride concentration. Although confusing in the first place, seawater has several pH values, all valid on different scales (for details on pH scales see Zeebe and Wolf-Gladrow, 2001) with

$$pH_T = -\log([H^+]_F + [HSO_4^-]) \qquad (4)$$

The use of pH_T on the so-called total scale is recommended as meaningful measurements on the others are not possible (Dickson et al., 2007). Nevertheless, since organisms are most likely affected by changes in $[H^+]_F$ alone rather than by a combination of protons and bisulfate ions, we are also reporting pH values on the so-called free scale with

$$pH_F = -\log[H^+]_F \qquad (5)$$

From measurements of TA and DIC, all other carbonate system components can be calculated. An important feature of this system with its 6 parameters (CO_2, HCO_3^-, CO_3^{2-}, H^+, DIC and TA) is that if the ionic composition of the seawater is known by its salinity (note for instance borate and sulfate in Eq. 3), any two of the six parameters describe the entire acid-base equilibrium. In other words, if two parameters are known, the others can be calculated.

Another concept important in the context of ocean acidification is the saturation state for calcium carbonate (Ω). This parameter, potentially relevant for marine calcifiers, is defined as

$$\Omega = \left([Ca^{2+}]_{SW}[CO_3^{2-}]_{SW}\right)/K_{sp}^* \qquad (6)$$

where $[Ca^{2+}]_{SW}$ and $[CO_3^{2-}]_{SW}$ denote the seawater concentrations of calcium and carbonate ions, respectively and K_{sp}^* the stoichiometric solubility constant of calcium carbonate. The two major forms of calcium carbonate, calcite and aragonite, are characterised by individual solubility constants leading to distinct saturation states Ω_{calc} and Ω_{arg}, respectively. If saturation levels are below one, calcium carbonate, which is generally a stable mineral in present day surface waters, will start to dissolve.

2.2 Perturbations of the seawater carbonate system

The seawater carbonate system will react to any perturbation by redistributions of its individual components such as CO_2, HCO_3^-, CO_3^{2-}, and H^+, governed by its acid-base equilibria (see Eq. 1). A perturbation could be, for instance, the addition of the gas CO_2, the salts $NaHCO_3$ or Na_2CO_3 dissociating initially into HCO_3^- and CO_3^{2-}, respectively, or a strong acid releasing H^+. The resulting redistributions of carbonate system components can equally be understood as the consequence of changes in the total amount of dissolved inorganic carbon (DIC) and/or changes in the charge balance (TA) of seawater. For instance, additions of CO_2 in gas form will increase DIC leaving TA constant while additions of $NaHCO_3$ will increase both DIC and TA by the same amount (compare Eqs. 2 and 3). As we shall see, the concentrations of CO_2, HCO_3^-, CO_3^{2-} and H^+ can change in a very similar fashion regardless whether DIC or TA is manipulated.

3 Results

3.1 Changing DIC at constant TA

Invasion of anthropogenic CO_2 into the surface ocean increases seawater $[CO_2]$ and DIC without changing the charge balance and hence TA (compare Eqs. 2 and 3). Considering a pre-industrial surface ocean water mass with a typical open ocean TA of 2350 µmol kg^{-1} (no silicate, phosphate, ammonia or bisulfide present) and a CO_2 fugacity in equilibrium with the atmosphere of 280 µatm, DIC concentrations would have been about 2054 µmol kg^{-1} (calculated for 15°C and a salinity of 35, using the stoichiometric equilibrium constants for carbonic acid as determined by Roy et al., 1993). In this water mass, DIC has increased since then by about 60 µmol kg^{-1} at a fCO$_2$ of ~380 µatm in the year 2007 and will increase an additional ~100 µmol kg^{-1} when atmospheric CO_2 reaches 700 µatm (Fig. 2a). At the same time, TA is not influenced by the oceanic CO_2 uptake and stays constant (Fig. 2b).

As a consequence there are changes in carbonate system speciation. Increasing $[CO_2]$ (which is equivalent to increasing DIC at constant TA) lead to an increase in $[H^+]$ (decreasing pH) and $[HCO_3^-]$, and decreasing $[CO_3^{2-}]$. This re-equilibration can be thought to occur by CO_2 partly dissolving in seawater, producing bicarbonate and protons as

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \qquad (7)$$
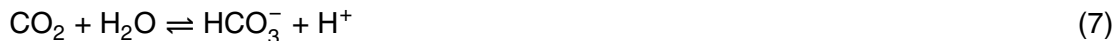
and partly by combining directly with carbonate ions forming bicarbonate as

$$CO_2 + CO_3^{2-} + H_2O \rightleftharpoons 2HCO_3^- \qquad (8)$$

Increasing $[CO_2]$ therefore leads to increasing $[HCO_3^-]$ and $[H^+]$ while $[CO_3^{2-}]$ and hence calcite and aragonite saturation states decrease (Fig. 2). It is noted that the magnitude of change and absolute values depend on temperature and salinity, the sign of change, however, is always the same.

3.2 Changing TA at constant DIC

While the ongoing increase in oceanic CO_2 changes DIC but not TA, the same CO_2 increase could be observed at constant DIC with variable TA. For example, in the parcel of seawater described above, rising TA from 2350 by about 70 µmol kg^{-1} at present day DIC of about 2114 µmol kg^{-1} would reduce seawater CO_2 from 380 to 280 µatm, its pre-industrial value. Similarly, decreasing TA by about 115 µmol kg^{-1} would increase oceanic CO_2 from 380 to 700 µatm (Fig. 2). Although conceptually different, the concomitant changes in carbonate chemistry speciation closely follow those occurring at constant TA and variable DIC (compare Fig. 2). The drop in pH, $[CO_3^{2-}]$, and calcite and aragonite saturation states in response to increasing $[CO_2]$ are very similar. An exception is $[HCO_3^-]$ which, in the 280 to 700 µatm CO_2 range, increase only about half as much at constant DIC and variable TA in comparison to constant TA and variable DIC. However, in relation to the other species of the carbonate system the absolute

change in $[HCO_3^-]$ is relatively small. While $[HCO_3^-]$ is increasing by about 12 and 5% (at changing DIC and constant TA and constant DIC at changing TA, respectively), the variations of $[CO_2]$ and $[CO_3^{2-}]$ are about an order of magnitude larger. Also $[H^+]$ is decreasing more than 50%.

3.3 Temperature, salinity and CO_2 range considerations

While in the variable DIC at constant TA scenario $[HCO_3^-]$ is progressively increasing in the 280 to 700 µatm CO_2 range, the variations in $[HCO_3^-]$ in response to changes in TA at constant DIC depend on temperature and salinity. This is because DIC remains constant and cannot compensate for the salinity and temperature dependent redistributions in the carbonate system. Those redistributions are such that colder and less saline seawater has a higher $[CO_2]$ and $[HCO_3^-]$ and lower $[CO_3^{2-}]$ at a given DIC and fCO_2 (compare Fig. 3a). Hence, for certain combinations of low salinity and low temperature $[HCO_3^-]$ is not progressively increasing with decreasing TA at constant DIC, in the 280 to 700 µatm CO_2 range. For instance, in seawater at typical DIC concentrations of 2100 µmol kg^{-1} and a salinity of 35 the temperature must be about 0°C or warmer in order to allow $[HCO_3^-]$ to steadily increase between 280 and 700 µatm in response to changes in TA (Fig. 3b).

Apart from temperature and salinity which can modify the response of $[HCO_3^-]$ changes towards increasing CO_2 levels in the varying TA at constant DIC scenario in comparison to ongoing ocean acidification (constant TA at varying DIC), there is another factor to be considered. Extending the CO_2 range towards relatively high levels, considerably exceeding those expected for the end of this century (about 700 µatm), will amplify the differences regarding $[HCO_3^-]$ changes between the two fundamental approaches. While $[HCO_3^-]$ also continues to increase progressively in the changing DIC at constant TA scenario with CO_2 levels beyond 700 µatm, they are eventually decreasing again in the changing TA at constant DIC scenario (Fig. 4e). For all other carbonate chemistry parameters ($[CO_2]$, $[CO_3^{2-}]$, pH, Ω_{calc} and Ω_{arg}), however, the

two approaches give basically the same result both in terms of trend and magnitude (Fig. 4).

3.4 Experimental manipulation methods

As we have shown both fundamental carbonate chemistry manipulation approaches (changing DIC at constant TA and changing TA at constant DIC) generally give similar results in terms of variations in $[CO_2]$, $[HCO_3^-]$, $[CO_3^{2-}]$, $[H^+]$ (pH), Ω_{calc} and Ω_{arg}. Experimentally, DIC or TA can be manipulated in different ways, depending on whether initial concentrations are to be increased or decreased. While TA can be manipulated by additions of a strong acid or base, a variety of methods exist for increasing or decreasing DIC (practical aspects and potential pitfalls can also be found in Rost et al., 2008 and the "Guide for Best Practise in Ocean Acidification Research").

3.4.1 Increasing DIC at constant TA

DIC can be increased to target values in three ways which, although different in practice, give basically the same results. First, seawater can be aerated with air at target CO_2 levels (aka bubbling). By equilibration of the gas bubbles with the water phase, CO_2 and hence DIC is adjusted to desired values. Seawater CO_2 and DIC will increase if initial values are below those in the aeration gas. This procedure will have no impact on TA. Second, DIC can be increased by injection of certain amounts of CO_2 enriched seawater. Such seawater can easily be prepared by aeration with pure CO_2 gas. Again, only DIC will be modified if the water used for enrichment had the same TA as that used in the experiment . And third, additions of certain salts of carbonic acid such as $NaHCO_3$ or Na_2CO_3, will also increase DIC. However, both salts $NaHCO_3$ and Na_2CO_3 also introduce alkalinity in form of HCO_3^- and CO_3^{2-}, respectively (compare Eqs. 2 and 3). Hence, additions of a strong acid, which can be regarded negative alkalinity, such as HCl will counterbalance the otherwise unavoidable increase in TA. Here, one has to keep in mind that for the same DIC increase Na_2CO_3 increases TA

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twice as much as $NaHCO_3$ additions which has therefore to be neutralised by twice as much acid. In this way it is possible to change DIC without concomitant changes in TA.

3.4.2 Decreasing DIC at constant TA

Depending on the experimental setup it might be necessary to decrease DIC and CO_2
5 levels, for instance when initial seawater values are higher than intended. This can be achieved again by aeration with air at target CO_2 (aka bubbling) which would decrease DIC when CO_2 levels prior to aeration were higher. Another possibility is the injection of CO_2 free seawater which, in analogy to the CO_2 enriched seawater, can be prepared by aeration with CO_2 free air. A third option could be a combination of TA and DIC
10 manipulation where in a first step TA is increased (see below) to values corresponding to the lowest of the desired CO_2 levels and then, in a second step, combined additions of $NaHCO_3$ or Na_2CO_3 and HCl are used to adjust DIC and CO_2 to the higher levels. Although this would result in an overall higher TA to salinity relationship, TA would still be the same in all CO_2 treatments.

15 ### 3.4.3 Increasing and decreasing TA at constant DIC

In contrast to different experimental methods to manipulate DIC, TA is basically modified by additions of strong acids or bases such as HCl or NaOH, respectively. Here, additions of acid and hence H^+ reduce TA while bases and hence OH^- increase TA. (compare Eq. 3).

20 ### 4 Discussion and summary

In practice there are several considerations relevant for the choice of the carbonate chemistry manipulation method, each of which having certain advantages and drawbacks (compare Table 1). For instance, seawater manipulation by acid/base addition is very quick, precise and simple in comparison to aeration (aka bubbling) or injection of

CO$_2$ enriched/free seawater. Important considerations are the experimental setup with the organisms to be studied (autotrophs, heterotrophs or mixed communities), and the size (liters or cubic meters of seawater) and duration (hours, days or weeks) of the experiment. Furthermore, any biological activity is bound to change carbonate chemistry speciation with photosynthesis and respiration impacting DIC in opposite directions and calcification decreasing both DIC and TA. Hence, careful planning and most importantly monitoring of at least two carbonate system parameters is vital to ensure that initial and final carbonate chemistry is as intended.

All useful carbonate chemistry manipulation methods fall into two categories of basic experimental approaches, i.e. changing DIC at constant TA and changing TA at constant DIC. Naturally occurring ocean acidification increases seawater DIC at constant TA. However, under conditions typical for most experimental setups TA manipulation at constant DIC changes carbonate chemistry in a similar way, both in terms of trend and magnitude (compare Figs. 2 and 5).

In this context it is important to remember that both DIC and TA are composite parameters. Organisms will sense changes in individual carbonate chemistry species such as H$^+$, CO$_2$, HCO$_3^-$ or possibly CO$_3^{2-}$ but not in DIC or TA. Nevertheless, those changes can equally be understood as driven by DIC and/or TA (see Materials and Methods for details). Whichever species of the carbonate system is ultimately responsible for an observed biological response, the response would be the same independent of whether the carbonate system manipulation was achieved by changing TA or DIC. The one parameter where TA and DIC manipulations generate a noticeable difference, i.e. [HCO$_3^-$], is also the parameter which experiences the smallest relative change due to ocean acidification and is the far most abundant form of dissolved inorganic carbon in seawater. But even if the relatively small change in [HCO$_3^-$] should be responsible for any of the observed biological responses, under most experimental conditions (see Fig. 3) they should still have the same trend since [HCO$_3^-$] changes in the same direction (Figs. 2 and 5). A comparison of experimental results on sensitivities of the coccolithophore *Emiliania huxleyi* to ocean acidification indeed indicates no system-

atic differences in observed responses between the two approaches (Table 2). With the exception of cultures grown under low light levels, calcification of *Emiliania huxleyi* decreases with increasing CO_2 and decreasing pH in experiments employing both TA and DIC manipulation. The apparent discrepancy between earlier work and a recent report by Iglesias-Rodriguez et al. (2008b) therefore cannot be explained by differences in the approach used for carbonate system manipulation. This is confirmed by a study experimentally comparing manipulations by acid (TA) with CO_2 aeration (DIC), indicating that reported differences might apply to that particular strain of *Emiliania huxleyi* only (Shi et al., 2009).

In summary, for most experimental conditions both CO_2 manipulation methods (variations in TA or DIC) change carbonate chemistry in a similar way. Exceptions are low salinity brackish water at low temperatures where there might be differences in terms of [HCO_3^-] changes, and CO_2 levels substantially higher than 700 µatm, when especially [HCO_3^-] start to diverge significantly between both approaches (compare Figs. 3, 2 and 4, respectively). However, also under these circumstances differences in biological responses between the two manipulation approaches are expected only where the underlying processes are sensitive to the small relative changes in [HCO_3^-]. While carbonate system manipulation by changing DIC at constant TA best mimics ongoing ocean acidification and therefore may be regarded the preferred approach for sensitivity studies, changing TA at constant DIC may sometimes be more practical and/or cost effective (compare Table 1). With the exceptions outlined above, no systematic differences in the biological responses are expected between the different approaches.

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Table 1. Comparison of experimental carbonate system manipulation approaches in terms of practical aspects and feasibility.

Method	Feasibility	Practical aspects
TA manipulation at constant DIC		
• Acid/base additions	• Equally suitable for small and large scale manipulations • Does not require sophisticated infrastructure • Quick and simple, intensively used in OA research	• May not be suitable for certain oceanographic settings characterized by low salinities and temperatures as well as CO$_2$ ranges considerably exceeding 700 µatm
DIC manipulation at constant TA		
• Aeration at target CO$_2$ (aka bubbling)	• Relatively easy to adjust • Equilibration might take a long time • Simultaneous supply of oxygen during aeration	• Direct seawater aeration might disturb organisms and/or impact dissolved organic matter pool • CO$_2$ and pH change gradually during aeration
• Injection of CO$_2$ enriched/free seawater	• Ideal for large volumes • Difficult to precisely adjust carbonate chemistry	• About one liter of CO$_2$ enriched seawater per cubic meter reduces pH by about 0.2 pH units, while about fifty liters of CO$_2$ free seawater decrease pH by about 0.1
• Combined NaHCO$_3$/Na$_2$CO$_3$ and HCl additions	• Ideal for small scale bottle experiments • Manipulation can be extremely precise	

Table 2. Comparison of cellular responses to elevated CO$_2$ levels in the coccolithophore *Emiliania huxleyi*, such as division rate (d^{-1}), POC and PIC production rates (percent change) in various perturbation studies at different light intensity (PAR, µmol m^{2}s^{-1}), light/dark cycle (L/D, h/h), temperature (°C), salinity (psu) and nutrient (NO$_3^-$, PO$_4^{3-}$) conditions. A dash represents unreported values. Also compare Zondervan (2007).

Experiment	Strain	culture type	PAR	L/D cycle	Temp., Sal.	Nutrients	CO$_2$ range (µatm)	division rate	POC prod. rate	PIC prod. rate
TA manipulation at constant DIC										
Riebesell et al. (2000)	PML B92/11	dilute batch	150	16/8	15, 31	replete	180–750	const.	~10% ⇑	~10% ⇓
Zondervan et al. (2001)	PML B92/11	dilute batch	150	24/0	15, –	replete	280–800	const.	~20% ⇑	~25% ⇓
Zondervan et al. (2002)	PML B92/11	dilute batch	15–80	24/0, 16/8	15, –	replete	150–850	0.5–1.1[a]	up to 50% ⇑	const. ⇔
DIC manipulation at constant TA										
Riebesell et al. (2000)	nat. assembl.	North Pacific	–	–	13, –	–	250, 800	–	const. ⇔	~50–80 % ⇓
Sciandra et al. (2003)	TW1	chemostat	170	14/10	17, –	NO$_3^-$ lim.	400, 700	0.5	~15% ⇓	~10–25 % ⇓
Delille et al. (2005)	nat. assembl.	mesocosm	150–650	16/8	~10, 30	final lim.	190–710	–	const. ⇔	~40% ⇓
Leonardos & Geider (2005)	PML 92A,	chemostat	80	14/10	18, –	PO$_4^{3-}$ lim.	380, 2000	0.3	const. ⇔	–
	(non calcifying)	chemostat	80	14/10	18, –	NO$_3^-$ lim.	380, 2000	0.3	~30% ⇓	–
		chemostat	500	14/10	18, –	P/N lim.	380, 2000	0.3	~30% ⇑	–
Feng et al. (2008)	CCMP 371	semi-cont.	50	12/12	20/24, –	replete	375,700	0.28–0.7[b]	⇓,⇑[b]	⇑, ⇓[b]
		semi-cont.	400	12/12	20/24, –	replete	375,700	0.58–0.73[b]	⇑[b]	⇓[b]
Igl.-Rodriguez et al. (2008b)	CAWPO6	batch [c]	150	12/12	19,34	replete[c]	280–780	0.8–0.5[d]	~100% ⇑	~100% ⇑

[a] Differences in growth rate are primarily due to variations in light intensities.

[b] Relative change was estimated from reported division rates and cellular quotas. Differences in growth rate are primarily due to differences in temperature.

[c] Compare Riebesell et al. (2008); Iglesias-Rodriguez et al. (2008a).

[d] The variability in growth rate at a certain CO$_2$ level is higher than the overall reduction trend observed, for instance growth rates at 300 µatm range from 0.5 to 0.9.
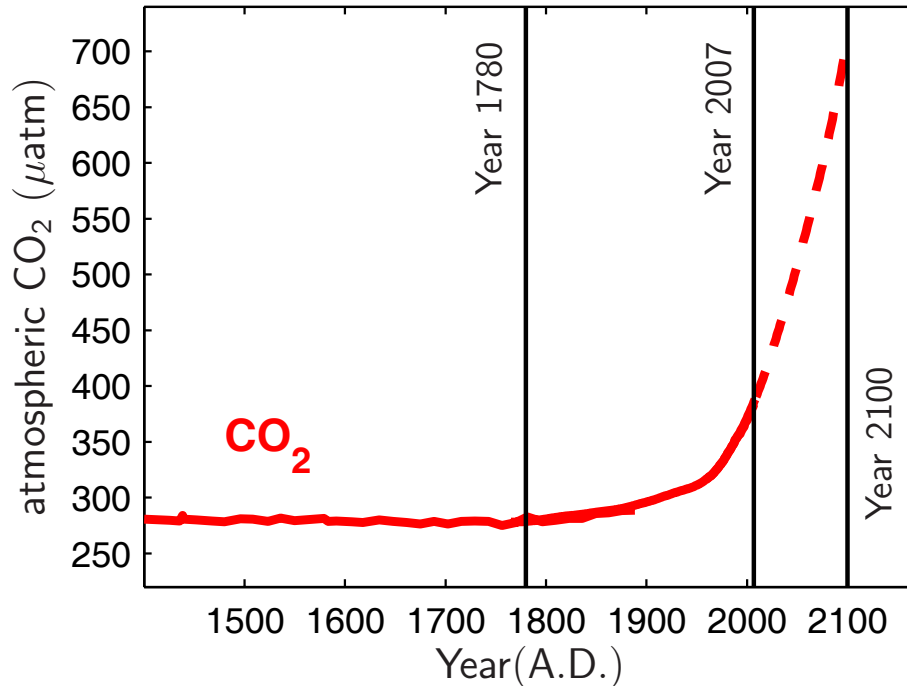
Fig. 1. Atmospheric CO$_2$ evolution of the last couple of hundred years together with future projections. The solid line comprises historical data from Siegenthaler et al. (2005); Enting et al. (1994) and Tans (2007), while the dashed line represents the IS92a emission scenario according to Schimel et al. (1994).

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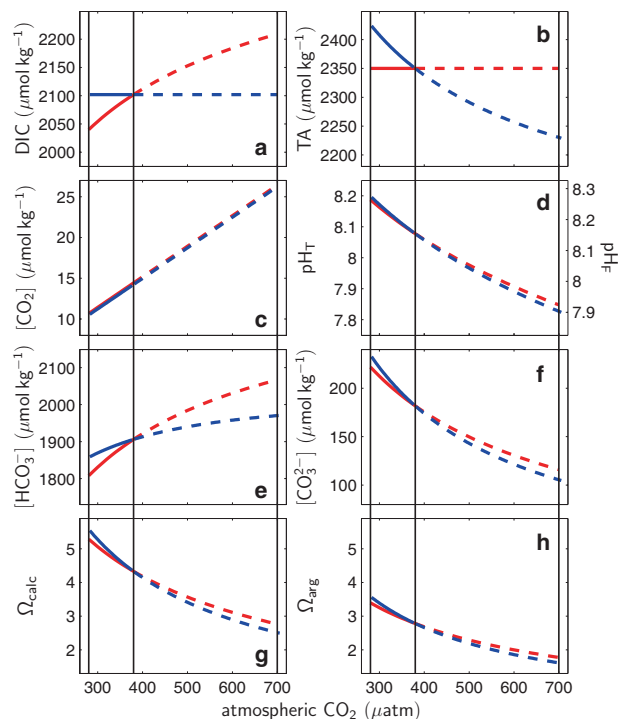
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Fig. 2. Redistributions in the seawater carbonate system in response to DIC changes at constant TA (red) and TA changes at constant DIC (blue). Shown are changes in surface seawater DIC **(a)**, TA **(b)**, [CO$_2$] **(c)**, pH on the total and free scale **(d)**, [HCO$_3^-$] **(e)**, [CO$_3^{2-}$] **(f)**, and calcite and aragonite saturation states (**g** and **h**, respectively). The black vertical lines denote atmospheric CO$_2$ values representative for pre-industrial ($\sim$280 µatm) and present day ($\sim$380 µatm), and projected values for the year 2100 ($\sim$700 µatm). The seawater carbonate system was calculated at a salinity of 35 and a temperature of 15°C using the stoichiometric stability constants for carbonic acid determined by Roy et al. (1993). For details see text.
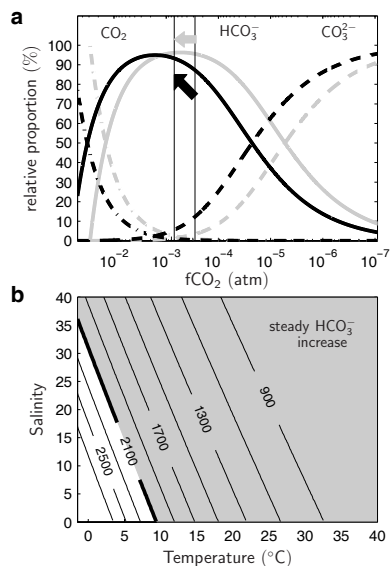
Fig. 3. The influence of salinity and temperature on dissolved inorganic carbon partitioning, in particular on HCO$_3^-$ concentration changes. **(a)** relative proportions of CO$_2$, HCO$_3^-$ and CO$_3^{2-}$ in response to changing fugacity of carbon dioxide (fCO$_2$) at constant DIC at salinities of 35 and 10, and temperatures of 20°C and 0°C (black and grey lines, respectively). The black vertical lines denote fCO$_2$ levels of 280 and 700 µatm, the black and grey arrows the change in [HCO$_3^-$] in this range. **(b)** DIC isopleths (concentrations given in µmol kg^{-1}) in response to salinity and temperature changes. The right hand side of an isopleth denotes the temperature-salinity space in which [HCO$_3^-$] are steadily increasing in the 280 to 700 µatm range in response to changing TA. This is exemplified for the 2100 µmol kg^{-1} DIC isopleth (thick black line) by the grey shaded area. See text for details.
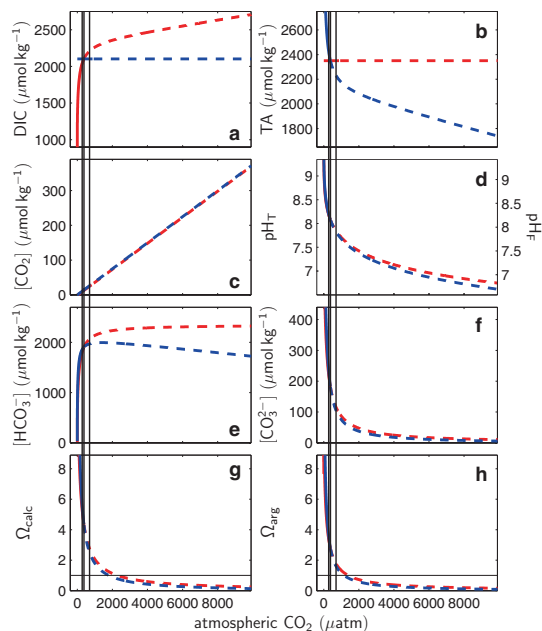
Fig. 4. Redistributions in the seawater carbonate system spanning a broad range of increasing atmospheric CO$_2$ levels, in response to varying DIC at constant TA (red) and varying TA at constant DIC (blue). Shown are changes in surface ocean DIC **(a)**, TA **(b)**, [CO$_2$] **(c)**, pH on the total and free scale **(d)**, [HCO$_3^-$] **(e)**, [CO$_3^{2-}$] **(f)**, and calcite and aragonite saturation states (**g** and **h**, respectively). Line style follows that of Fig. 2 where black vertical lines mark CO$_2$ levels of 280, 380 and 700 µatm.
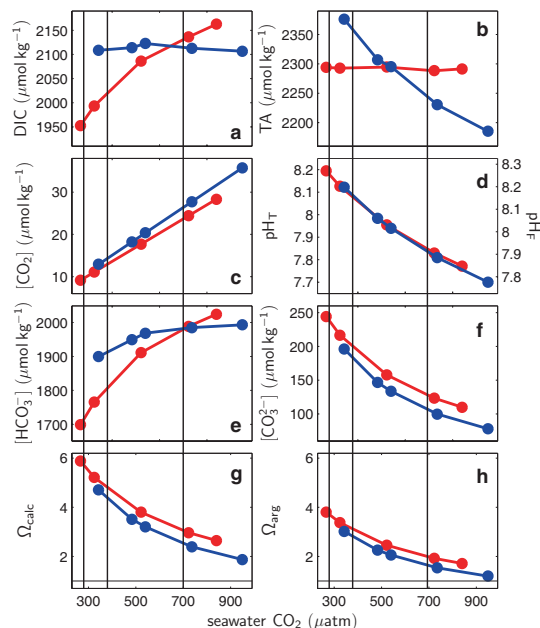
Fig. 5. Comparison of experimentally determined DIC **(a)** and TA **(b)** values of the two fundamental manipulation approaches, i.e. varying DIC at constant TA (red) and constant DIC at varying TA (blue). Red and blue symbols represent initial DIC and TA values from Iglesias-Rodriguez et al. (2008b) and Zondervan et al. (2001) (at a photon flux density of 150 µmol m^{2}s^{-1} and a 24/0 light/dark cycle), respectively. Resulting redistributions of the seawater carbonate system are shown in **(c)** ([CO$_2$]), **(d)** (pH on the total and free scale), **(e)** ([HCO$_3^-$]), **(f)** ([CO$_3^{2-}$]), and **(g)** and **(h)** (calcite and aragonite saturation states, respectively). Style and colour code follows that of Fig. 2. Note that the different seawater CO$_2$ slopes **(c)** are due to a 4°C difference in incubation temperature.

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Interactive Discussion