

# CO<sub>2</sub> perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations

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**Abstract.** Increasing atmospheric carbon dioxide (CO<sub>2</sub>) through human activities and invasion of anthropogenic CO<sub>2</sub> into the surface ocean alters the seawater carbonate chemistry, increasing CO<sub>2</sub> and bicarbonate (HCO<sub>3</sub><sup>-</sup>) at the expense of carbonate ion (CO<sub>3</sub><sup>2-</sup>) concentrations. This redistribution in the dissolved inorganic carbon (DIC) pool decreases pH and carbonate saturation state ( $\Omega$ ). Several components of the carbonate system are considered potential key variables influencing for instance calcium carbonate precipitation in marine calcifiers such as coccolithophores, foraminifera, corals, mollusks and echinoderms. Unravelling the sensitivities of marine organisms and ecosystems to CO<sub>2</sub> induced ocean acidification (OA) requires well-controlled experimental setups and accurate carbonate system manipulations. 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 *Emiliana huxleyi*. We conclude that under most experimental conditions in the context of ocean acidification DIC and TA manipulations yield similar changes in all parameters of the carbonate system, which implies direct comparability of data obtained with the two basic approaches for CO<sub>2</sub> 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<sub>2</sub> levels started to increase from usual interglacial values of about 280 to about 390 ppmv at present day. As the demand for fossil fuels is likely to further intensify, atmospheric CO<sub>2</sub> 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<sub>2</sub> 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<sub>3</sub><sup>2-</sup>) concentrations 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<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>. Experimental assessment of possible sensitivities of marine organisms to ocean acidification and carbonation requires an understanding of the chemical background of CO<sub>2</sub>-induced changes in carbonate chemistry, the design of suitable CO<sub>2</sub> perturbation experiments for which a variety of manipulation approaches are available (see Riebesell et al., 2009), and the monitoring and measurement of various carbonate chemistry parameters.

Here we provide the chemical background of ocean acidification, necessary to understand 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<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> concentrations at decreasing pH and carbonate ion concentrations can also be brought about by changing TA at constant DIC. We highlight the differences and



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similarities of these two fundamental approaches 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 methods to manipulate DIC at constant TA and vice versa and discuss their advantages and disadvantages for different experimental setups. Finally, we compare various CO<sub>2</sub> 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<sub>2</sub>CO<sub>3</sub>)



When carbon dioxide (CO<sub>2</sub>) dissolves in water (H<sub>2</sub>O) carbonic acid is formed which readily dissociates into bicarbonate (HCO<sub>3</sub><sup>-</sup>), releasing protons (H<sup>+</sup>). Depending on pH (see below), HCO<sub>3</sub><sup>-</sup> is also formed by protons combining with carbonate ions (CO<sub>3</sub><sup>2-</sup>). It is in the nature of this equilibrium that any change in the concentration of one of the individual components will force the others to re-adjust as well. Hence, increasing CO<sub>2</sub> concentrations in the surface ocean, driven by the built-up of anthropogenic CO<sub>2</sub> in the atmosphere, will also change the concentrations of HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and H<sup>+</sup>. 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<sub>2</sub> and H<sup>+</sup>) or simply cannot be determined analytically (HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>). 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

$$\text{DIC} = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (2)$$

and

$$\begin{aligned} \text{TA} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] \\ & + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_3\text{SiO}_4^-] + [\text{NH}_3] \\ & + [\text{HS}^-] - [\text{H}_3\text{PO}_4] - [\text{H}^+]_{\text{F}} - [\text{HSO}_4^-] - [\text{HF}] \quad (3) \end{aligned}$$

where [H<sup>+</sup>]<sub>F</sub> is the free hydrogen ion, [HSO<sub>4</sub><sup>-</sup>] 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

$$\text{pH}_{\text{T}} = -\log([\text{H}^+]_{\text{F}} + [\text{HSO}_4^-]) \quad (4)$$

The use of pH<sub>T</sub> 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<sup>+</sup>]<sub>F</sub> alone rather than by a combination of protons and bisulfate ions, we are also reporting pH values on the so-called free scale with

$$\text{pH}_{\text{F}} = -\log[\text{H}^+]_{\text{F}} \quad (5)$$

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<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, H<sup>+</sup>, 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

$$\Omega = \left( [\text{Ca}^{2+}]_{\text{sw}} [\text{CO}_3^{2-}]_{\text{sw}} \right) / K_{\text{sp}}^* \quad (6)$$

where [Ca<sup>2+</sup>]<sub>sw</sub> and [CO<sub>3</sub><sup>2-</sup>]<sub>sw</sub> denote the seawater concentrations of calcium and carbonate ions, respectively and K<sub>sp</sub><sup>\*</sup> 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<sub>calc</sub> and Ω<sub>arg</sub>, 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<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and H<sup>+</sup>, governed by its acid-base equilibria (see Eq. 1). A perturbation could be, for instance, the addition of the gas CO<sub>2</sub>, the salts NaHCO<sub>3</sub> or Na<sub>2</sub>CO<sub>3</sub> dissociating initially into HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, respectively, or a strong acid releasing H<sup>+</sup>. 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<sub>2</sub> in gas form will increase DIC leaving TA constant while additions of NaHCO<sub>3</sub> will increase both DIC and TA by the same amount (compare Eqs. 2 and 3). As we shall see, the concentrations of CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and H<sup>+</sup> can change in a very similar fashion regardless whether DIC or TA is manipulated (compare Table 1).

**Table 1.** Carbonate chemistry speciation at variable dissolved inorganic carbon (DIC) and constant total alkalinity (TA), describing ongoing ocean acidification (OA), in comparison to four cases at constant DIC and variable TA where always one core parameter, i.e. 1)  $f\text{CO}_2$  and  $[\text{CO}_2]$ , 2)  $[\text{HCO}_3^-]$ , 3)  $[\text{CO}_3^{2-}]$ ,  $\Omega_{\text{calc}}$  and  $\Omega_{\text{arg}}$ , or 4)  $[\text{H}^+]$  and pH, is changing equally as in the OA scenario. The numbers in round brackets denote the fractional differences between the respective parameters at variable TA and variable DIC, and the dashes impossible carbonate chemistry parameter combinations. Concentrations are given in  $\mu\text{mol kg}^{-1}$  and  $f\text{CO}_2$  in  $\mu\text{atm}$ . The carbonate chemistry speciation was calculated for 15°C and a salinity of 35, using the stoichiometric equilibrium constants for carbonic acid as determined by Roy et al. (1993). See text for details and compare Fig. 2.

Method	DIC	TA	$f\text{CO}_2$	$[\text{CO}_2]$	$[\text{HCO}_3^-]$	$[\text{CO}_3^{2-}]$	$\Omega_{\text{calc}}$	$\Omega_{\text{arg}}$	$[\text{H}^+]_{\text{F}}$	pH <sub>F</sub>	pH <sub>T</sub>
var. DIC/const. TA	2040.0	2350.0	<b>280</b>	<b>10.5</b>	<b>1807.6</b>	<b>221.9</b>	<b>5.29</b>	<b>3.39</b>	<b>0.0054</b>	<b>8.264</b>	<b>8.188</b>
	2106.9	2350.0	<b>390</b>	<b>14.6</b>	<b>1913.7</b>	<b>178.6</b>	<b>4.25</b>	<b>2.73</b>	<b>0.0072</b>	<b>8.145</b>	<b>8.068</b>
	2209.0	2350.0	<b>700</b>	<b>26.2</b>	<b>2066.8</b>	<b>116.0</b>	<b>2.76</b>	<b>1.77</b>	<b>0.0119</b>	<b>7.924</b>	<b>7.848</b>
	2262.2	2350.0	<b>1000</b>	<b>37.5</b>	<b>2137.8</b>	<b>86.9</b>	<b>2.07</b>	<b>1.33</b>	<b>0.0164</b>	<b>7.784</b>	<b>7.708</b>
var. TA/const. DIC equal CO <sub>2</sub>	2106.9	2432.5	<b>280</b>	<b>10.5</b> (1.00)	1861.2 (1.03)	235.2 (1.06)	5.60	3.60	0.0053 (0.97)	8.277	8.200
	2106.9	2350.0	<b>390</b>	<b>14.6</b> (1.00)	1913.7 (1.00)	178.6 (1.00)	4.25	2.73	0.0072 (1.00)	8.145	8.068
	2106.9	2235.8	<b>700</b>	<b>26.2</b> (1.00)	1974.8 (0.96)	105.9 (0.91)	2.52	1.62	0.0125 (1.05)	7.904	7.828
	2106.9	2181.0	<b>1000</b>	<b>37.5</b> (1.00)	1993.8 (0.93)	75.6 (0.87)	1.80	1.16	0.0176 (1.07)	7.754	7.677
var. TA/const. DIC equal HCO <sub>3</sub> <sup>-</sup>	2106.9	2511.4	213	8.0 (0.76)	<b>1807.6</b> (1.00)	291.3 (1.31)	6.94	4.46	0.0042 (0.76)	8.382	8.306
	2106.9	2350.0	390	14.6 (1.00)	<b>1913.7</b> (1.00)	178.6 (1.00)	4.25	2.73	0.0072 (1.00)	8.145	8.068
	2106.9	–	–	–	<b>2066.8</b>	–	–	–	–	–	–
	2106.9	–	–	–	<b>2137.8</b>	–	–	–	–	–	–
var. TA/const. DIC equal CO <sub>3</sub> <sup>2-</sup>	2106.9	2413.4	301	11.3 (1.07)	1873.7 (1.04)	<b>221.9</b> (1.00)	<b>5.29</b>	<b>3.39</b>	0.0057 (1.04)	8.248	8.172
	2106.9	2350.0	390	14.6 (1.00)	1913.7 (1.00)	<b>178.6</b> (1.00)	<b>4.25</b>	<b>2.73</b>	0.0072 (1.00)	8.145	8.068
	2106.9	2252.6	634	23.8 (0.91)	1967.1 (0.95)	<b>116.0</b> (1.00)	<b>2.76</b>	<b>1.77</b>	0.0113 (0.95)	7.946	7.869
	2106.9	2202.4	864	32.4 (0.87)	1987.6 (0.93)	<b>86.9</b> (1.00)	<b>2.07</b>	<b>1.33</b>	0.0153 (0.93)	7.816	7.739
var. TA / const. DIC equal pH	2106.9	2423.9	289	10.8 (1.03)	1866.9 (1.03)	229.2 (1.03)	5.46	3.51	<b>0.0054</b> (1.00)	<b>8.264</b>	<b>8.188</b>
	2106.9	2350.0	390	14.6 (1.00)	1913.7 (1.00)	178.6 (1.00)	4.25	2.73	<b>0.0072</b> (1.00)	<b>8.145</b>	<b>8.068</b>
	2106.9	2243.9	667	25.0 (0.95)	1971.2 (0.95)	110.7 (0.95)	2.64	1.69	<b>0.0119</b> (1.00)	<b>7.924</b>	<b>7.848</b>
	2106.9	2191.4	930	34.9 (0.93)	1991.0 (0.93)	81.0 (0.93)	1.93	1.24	<b>0.0164</b> (1.00)	<b>7.784</b>	<b>7.708</b>

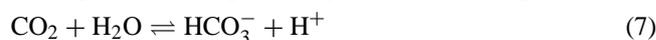
### 3 Results

#### 3.1 Changing DIC at constant TA

Invasion of anthropogenic CO<sub>2</sub> into the surface ocean increases seawater [CO<sub>2</sub>] 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  $\mu\text{mol kg}^{-1}$  (no silicate, phosphate, ammonia or bisulfide present) and a CO<sub>2</sub> fugacity ( $f\text{CO}_2$ ) in equilibrium with the atmosphere of 280  $\mu\text{atm}$ , DIC concentrations would have been about 2040  $\mu\text{mol kg}^{-1}$  at 15°C and a salinity of 35, calculated using the stoichiometric equilibrium constants for carbonic acid determined by Roy et al. (1993). It is noted that the constants of Roy et al. (1993) are recommended for artificial seawater while those of Mehrbach et al. (1973) as refitted by Lueker et al. (2000) for natural seawater (compare Dickson et al., 2007). The choice of stoichiometric equilibrium constants for carbonic acid slightly influences the absolute values of calculated carbonate system parameters, nevertheless this has no

influence on the discussion below. In the pre-industrial water mass described above, DIC has increased since then by about 67  $\mu\text{mol kg}^{-1}$  at a  $f\text{CO}_2$  of  $\sim 390$   $\mu\text{atm}$  at present day and will increase an additional  $\sim 102$   $\mu\text{mol kg}^{-1}$  when atmospheric CO<sub>2</sub> reaches 700  $\mu\text{atm}$  (Table 1 and Fig. 2a). At the same time, TA is not influenced by the oceanic CO<sub>2</sub> uptake and stays constant (Fig. 2b).

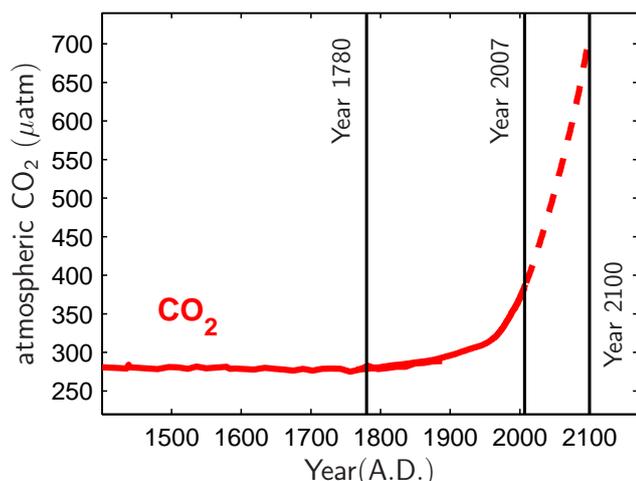
As a consequence there are changes in carbonate system speciation. Increasing [CO<sub>2</sub>] (which is equivalent to increasing DIC at constant TA) lead to an increase in [H<sup>+</sup>] (decreasing pH) and [HCO<sub>3</sub><sup>-</sup>], and decreasing [CO<sub>3</sub><sup>2-</sup>]. This re-equilibration can be thought to occur by CO<sub>2</sub> partly dissolving in seawater, producing bicarbonate and protons as



and partly by combining directly with carbonate ions forming bicarbonate as



Increasing [CO<sub>2</sub>] therefore leads to increasing [HCO<sub>3</sub><sup>-</sup>] and [H<sup>+</sup>] while [CO<sub>3</sub><sup>2-</sup>] and hence calcite and aragonite saturation states decrease (Fig. 2). It is noted that the magnitude



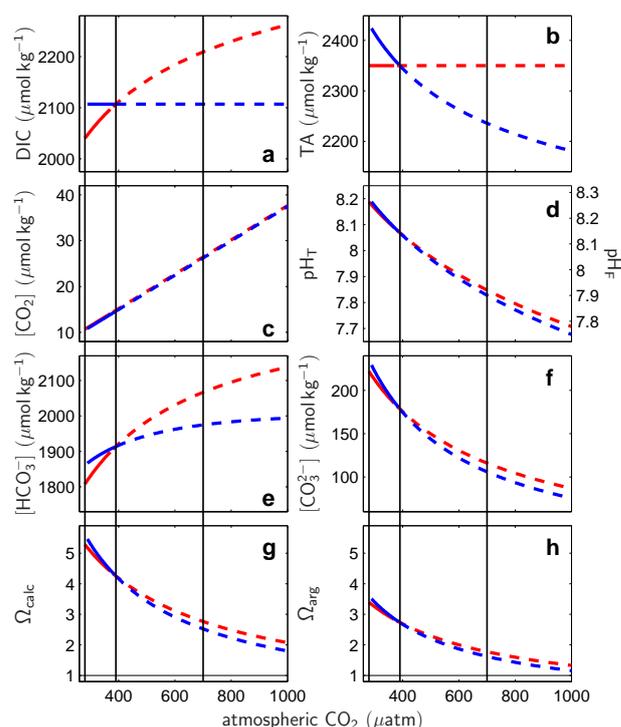
**Fig. 1.** Atmospheric CO<sub>2</sub> evolution of the last couple of hundred years together with future projections. The solid line comprises historical data from Siegenthaler et al. (2005), Enting et al. (1994) and Tans (2007), while the dashed line represents atmospheric CO<sub>2</sub> concentrations predicted for the IS92a emission scenario according to Schimel et al. (1994).

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<sub>2</sub> changes DIC but not TA, similar carbonate chemistry speciation changes could be observed at constant DIC and changing TA. By manipulating TA, it is possible to have always one of the four core parameters of the carbonate system, i.e. 1) [CO<sub>2</sub>] and *f*CO<sub>2</sub>, 2) [HCO<sub>3</sub><sup>-</sup>], 3) [CO<sub>3</sub><sup>2-</sup>], Ω<sub>calc</sub> and Ω<sub>arg</sub>, or 4) [H<sup>+</sup>] and pH, changing equally in comparison to manipulating DIC at constant TA. An exception are [HCO<sub>3</sub><sup>-</sup>] changes which cannot increase as much at variable TA because DIC becomes limiting in this respect (compare Table 1). While there is no compelling reason to favour one over the other remaining three TA manipulation approaches (they are similar in terms of carbonate speciation fractional differences between DIC and TA manipulation, ranging from 0.93 to 1.07), in the following we will adopt the case of equal CO<sub>2</sub>. This, at least, facilitates comparison between DIC and TA manipulations as future ocean carbonate chemistry changes are usually described by different CO<sub>2</sub> scenarios.

Rising TA in the parcel of seawater, described in the previous section, from 2350 by about 83 μmol kg<sup>-1</sup> at present day DIC of about 2107 μmol kg<sup>-1</sup> would reduce seawater CO<sub>2</sub> from 390 to 280 μatm, its pre-industrial value. Similarly, decreasing TA by about 114 μmol kg<sup>-1</sup> would increase oceanic CO<sub>2</sub> from 390 to 700 μatm (Table 1 and Fig. 2). Although conceptually different, the concomitant changes in carbonate chemistry speciation closely follow those occurring at con-

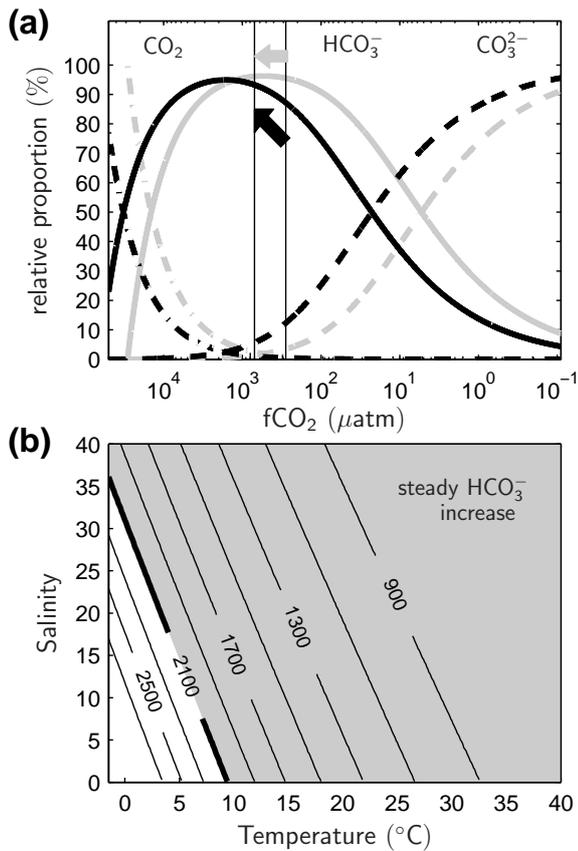


**Fig. 2.** Redistributions in the seawater carbonate system in response to DIC changes at constant TA (red) and TA changes at constant DIC (blue). Shown are changes in surface seawater DIC (a), TA (b), [CO<sub>2</sub>] (c), pH on the total and free scale (d), [HCO<sub>3</sub><sup>-</sup>] (e), [CO<sub>3</sub><sup>2-</sup>] (f), and calcite and aragonite saturation states (g and h, respectively). The black vertical lines denote atmospheric CO<sub>2</sub> values representative for pre-industrial (~280 μatm) and present day (~390 μatm), and projected values for the year 2100 (~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 and Table 1.

stant TA and variable DIC (compare Fig. 2). The drop in pH, [CO<sub>3</sub><sup>2-</sup>], and calcite and aragonite saturation states, and the increase in [HCO<sub>3</sub><sup>-</sup>] in response to increasing [CO<sub>2</sub>] are very similar. At 700 μatm the fractional difference between both manipulation approaches is 0.91 for [CO<sub>3</sub><sup>2-</sup>] and hence CaCO<sub>3</sub> saturation states, 1.05 for pH and [H<sup>+</sup>], and 0.96 for [HCO<sub>3</sub><sup>-</sup>]. These differences become more pronounced towards higher CO<sub>2</sub> levels (compare Table 1).

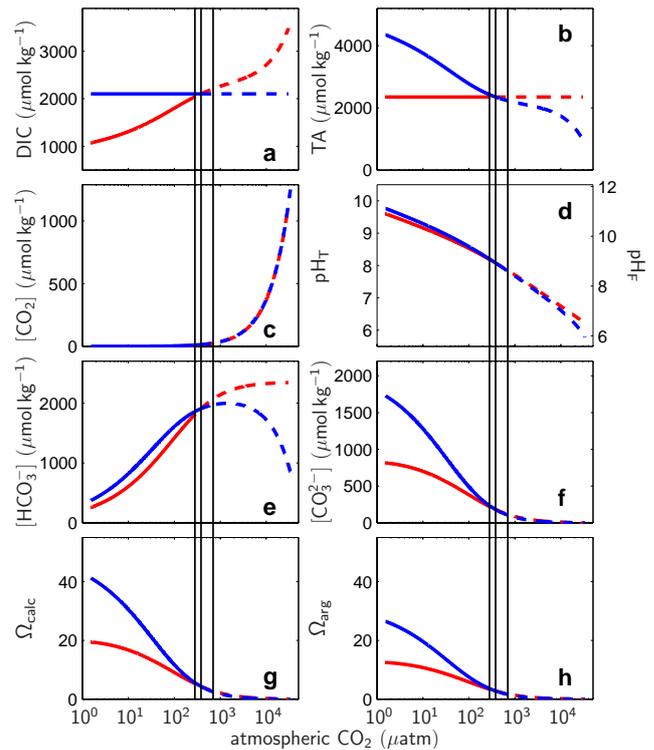
### 3.3 Temperature, salinity and CO<sub>2</sub> range considerations

While in the variable DIC at constant TA scenario [HCO<sub>3</sub><sup>-</sup>] is progressively increasing in the 280 to 700 μatm CO<sub>2</sub> range, the variations in [HCO<sub>3</sub><sup>-</sup>] in response to changes in TA at constant DIC depend on temperature and salinity. This is because DIC remains constant and cannot compensate for



**Fig. 3.** The influence of salinity and temperature on dissolved inorganic carbon partitioning, in particular on HCO<sub>3</sub><sup>-</sup> concentration changes. **(a)** relative proportions of CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> in response to changing fugacity of carbon dioxide ( $f\text{CO}_2$ ) 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  $f\text{CO}_2$  levels of 280 and 700 μatm, the black and grey arrows the change in [HCO<sub>3</sub><sup>-</sup>] in this range. **(b)** DIC isopleths (concentrations given in μmol kg<sup>-1</sup>) in response to salinity and temperature changes. The right hand side of an isopleth denotes the temperature-salinity space in which [HCO<sub>3</sub><sup>-</sup>] are steadily increasing in the 280 to 700 μatm range in response to changing TA. This is exemplified for the 2100 μmol kg<sup>-1</sup> DIC isopleth (thick black line) by the grey shaded area. See text for details.

the salinity and temperature dependent redistributions in the carbonate system. Those redistributions are such that colder and less saline seawater has a higher [CO<sub>2</sub>] and [HCO<sub>3</sub><sup>-</sup>] and lower [CO<sub>3</sub><sup>2-</sup>] at a given DIC and  $f\text{CO}_2$  (compare Fig. 3a). Hence, for certain combinations of low salinity and low temperature [HCO<sub>3</sub><sup>-</sup>] is not progressively increasing with decreasing TA at constant DIC, in the 280 to 700 μatm CO<sub>2</sub> range. For instance, in seawater at typical DIC concentrations of 2100 μmol kg<sup>-1</sup> and a salinity of 35 the temperature must be about 0°C or warmer in order to allow [HCO<sub>3</sub><sup>-</sup>] to steadily increase between 280 and 700 μatm in response to changes in TA (Fig. 3b).



**Fig. 4.** Redistributions in the seawater carbonate system spanning a broad range of atmospheric CO<sub>2</sub> 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<sub>2</sub>] **(c)**, pH on the total and free scale **(d)**, [HCO<sub>3</sub><sup>-</sup>] **(e)**, [CO<sub>3</sub><sup>2-</sup>] **(f)**, and calcite and aragonite saturation states **(g)** and **(h)**, respectively). Line style follows that of Fig. 2 where black vertical lines mark CO<sub>2</sub> levels of 280, 390 and 700 μatm.

Apart from temperature and salinity, another factor to be considered when choosing between TA and DIC manipulations is the range of experimental CO<sub>2</sub> levels. Both extending the CO<sub>2</sub> range towards relatively high levels, considerably exceeding those expected for the end of this century ( $\gg 700$  μatm), and towards relatively low levels considerably beyond, for instance, Pleistocene glacial minima ( $\ll 180$  μatm) will result in considerable differences between the two fundamental approaches. At high CO<sub>2</sub> levels, [HCO<sub>3</sub><sup>-</sup>] eventually starts to decrease in the changing TA at constant DIC scenario while at low levels the increase in [CO<sub>3</sub><sup>2-</sup>] and hence calcium carbonate saturation states is larger in comparison to the change at constant TA and variable DIC. For the other two carbonate chemistry parameters ([CO<sub>2</sub>], and pH), however, the two approaches give basically the same results both in terms of trend and magnitude (Fig. 4). Extreme  $f\text{CO}_2$  levels beyond the range of seawater carbonate chemistry changes of the recent past in the Pleistocene and projected ocean acidification in the next centuries ( $\ll 180$  μatm and  $\gg 700$  μatm) therefore need special

consideration regarding carbonate chemistry manipulation, especially because on the natural time scales involved neither TA nor DIC can be considered constant.

### 3.4 Experimental manipulation methods

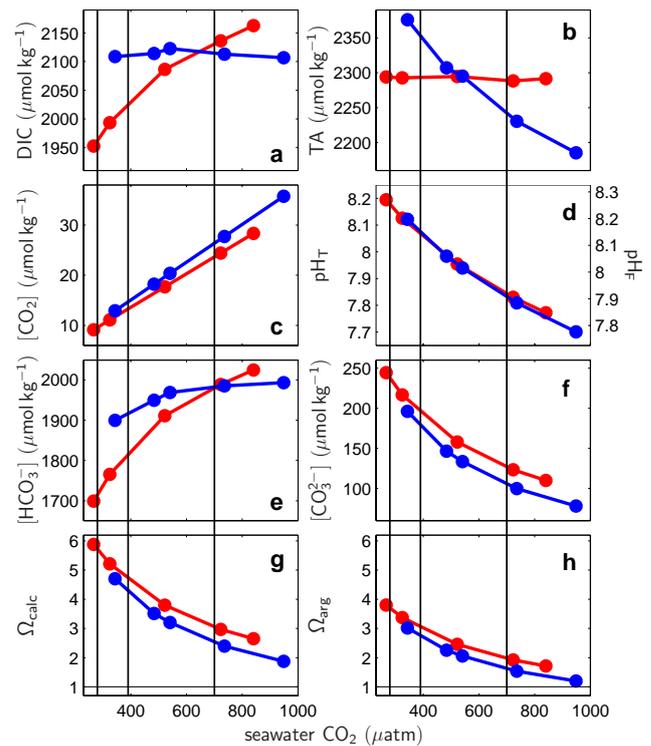
As we have shown both fundamental carbonate chemistry manipulation approaches (changing DIC at constant TA and changing TA at constant DIC) generally give similar results in terms of variations in [CO<sub>2</sub>], [HCO<sub>3</sub><sup>-</sup>], [CO<sub>3</sub><sup>2-</sup>], [H<sup>+</sup>] (pH), Ω<sub>calc</sub> and Ω<sub>arg</sub>. 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 Riebesell et al., 2009).

#### 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<sub>2</sub> levels (aka bubbling). By equilibration of the gas bubbles with the water phase, CO<sub>2</sub> and hence DIC is adjusted to desired values. Seawater CO<sub>2</sub> 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<sub>2</sub> enriched seawater. Such seawater can easily be prepared by aeration with pure CO<sub>2</sub> 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<sub>3</sub> or Na<sub>2</sub>CO<sub>3</sub>, will also increase DIC. However, both salts NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> also introduce alkalinity in form of HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, 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<sub>2</sub>CO<sub>3</sub> increases TA twice as much as NaHCO<sub>3</sub> 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<sub>2</sub> levels, for instance when initial seawater values are higher than intended. This can be achieved again by aeration with air at target CO<sub>2</sub> (aka bubbling) which would decrease DIC when CO<sub>2</sub> levels prior to aeration were higher. Another possibility is the injection of CO<sub>2</sub> free seawater which, in analogy to the CO<sub>2</sub> enriched seawater, can be prepared by aeration with CO<sub>2</sub> free air. A third option could be a combination of TA and DIC manipulation where



**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<sup>-2</sup> s<sup>-1</sup> and a 24/0 light/dark cycle), respectively. Resulting redistributions of the seawater carbonate system are shown in (c) ([CO<sub>2</sub>]), (d) (pH on the total and free scale), (e) ([HCO<sub>3</sub><sup>-</sup>]), (f) ([CO<sub>3</sub><sup>2-</sup>]), 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<sub>2</sub> slopes (c) are due to a 4°C difference in incubation temperature.

in a first step TA is increased (see below) to values corresponding to the lowest of the desired CO<sub>2</sub> levels and then, in a second step, combined additions of NaHCO<sub>3</sub> or Na<sub>2</sub>CO<sub>3</sub> and HCl are used to adjust DIC and CO<sub>2</sub> 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<sub>2</sub> treatments.

#### 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<sup>+</sup> reduce TA while bases and hence OH<sup>-</sup> increase TA. (compare Eq. 3).

**Table 2.** Comparison of experimental carbonate system manipulation approaches in terms of practical aspects and feasibility.

Method	Feasibility	Practical aspects
<i>TA manipulation at constant DIC</i>		
– 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 <sub>2</sub> ranges considerably exceeding 700 $\mu\text{atm}$
<i>DIC manipulation at constant TA</i>		
– Aeration at target CO <sub>2</sub> (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 <sub>2</sub> and pH change gradually during aeration
– Injection of CO <sub>2</sub> enriched/free seawater	– Ideal for large volumes – Difficult to precisely adjust carbonate chemistry	– About one liter of CO <sub>2</sub> enriched seawater per cubic meter reduces pH by about 0.2 pH units, while about fifty liters of CO <sub>2</sub> free seawater increase pH by about 0.1
– Combined NaHCO <sub>3</sub> /Na <sub>2</sub> CO <sub>3</sub> and HCl additions	– Ideal for small scale bottle experiments – Manipulation can be extremely precise	

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

Experiment	Strain	culture type	PAR	L/D cycle	Temp., Sal.	Nutrients	CO <sub>2</sub> range ( $\mu\text{atm}$ )	division rate	POC prod. rate	PIC prod. rate
<i>TA manipulation at constant DIC</i>										
Riebesell et al. (2000)	PML B92/11	dilute batch	150	16/8	15, 31	replete	180–750	const.	~10% $\uparrow$	~10% $\downarrow$
Zondervan et al. (2001)	PML B92/11	dilute batch	150	24/0	15, –	replete	280–800	const.	~20% $\uparrow$	~25% $\downarrow$
Zondervan et al. (2002)	PML B92/11	dilute batch	15–80	24/0, 16/8	15, –	replete	150–850	0.5–1.1 <sup>a</sup>	up to 50% $\uparrow$	const. $\leftrightarrow$
<i>DIC manipulation at constant TA</i>										
Riebesell et al. (2000)	nat. assembl.	North Pacific	–	–	13, –	–	250, 800	–	const. $\leftrightarrow$	~50–80% $\downarrow$
Sciandra et al. (2003)	TW1	chemostat	170	14/10	17, –	$\text{NO}_3^-$ lim.	400, 700	0.5	~15% $\downarrow$	~10–25% $\downarrow$
Delille et al. (2005)	nat. assembl.	mesocosm	150–650	16/8	~10, 30	final lim.	190–710	–	const. $\leftrightarrow$	~40% $\downarrow$
Leonardos & Geider (2005)	PML 92A, (non calcifying)	chemostat	80	14/10	18, –	$\text{PO}_4^{3-}$ lim.	380, 2000	0.3	const. $\leftrightarrow$	–
		chemostat	80	14/10	18, –	$\text{NO}_3^-$ lim.	380, 2000	0.3	~30% $\downarrow$	–
		chemostat	500	14/10	18, –	P/N lim.	380, 2000	0.3	~30% $\uparrow$	–
Feng et al. (2008)	CCMP 371	semi-cont.	50	12/12	20/24, –	replete	375,700	0.28–0.7 <sup>b</sup>	$\downarrow$ , $\uparrow$ <sup>b</sup>	$\uparrow$ , $\downarrow$ <sup>b</sup>
		semi-cont.	400	12/12	20/24, –	replete	375,700	0.58–0.73 <sup>b</sup>	$\uparrow$ <sup>b</sup>	$\downarrow$ <sup>b</sup>
Igl.-Rodriguez et al. (2008b)	CAWPO6	batch <sup>c</sup>	150	12/12	19,34	replete <sup>c</sup>	280–780	0.8–0.5 <sup>d</sup>	~100% $\uparrow$	~100% $\uparrow$

<sup>a</sup> Differences in growth rate are primarily due to variations in light intensities.

<sup>b</sup> Relative change was estimated from reported division rates and cellular quotas. Differences in growth rate are primarily due to differences in temperature.

<sup>c</sup> Compare Riebesell et al. (2008); Iglesias-Rodriguez et al. (2008a).

<sup>d</sup> The variability in growth rate at a certain CO<sub>2</sub> level is higher than the overall reduction trend observed, for instance growth rates at 300  $\mu\text{atm}$  range from 0.5 to 0.9.

#### 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 2). For instance, seawater manipulation by acid/base addition is very quick, precise and simple in comparison to aeration at target CO<sub>2</sub> (aka bubbling) or injection

of CO<sub>2</sub> 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.

Changes in DIC and/or TA should be less than 3% of initial concentrations in order to avoid pronounced shifts in carbonate chemistry. 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.

With respect to ocean acidification 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<sup>+</sup>, CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> or possibly CO<sub>3</sub><sup>2-</sup> 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 should be the same independent of whether the carbonate system manipulation was achieved by changing TA or DIC. This is because under most experimental conditions (see Fig. 3) the changes in individual carbonate chemistry species are of similar magnitude, but most importantly, have the same directional trend. Extreme CO<sub>2</sub> levels, however, result in considerable differences in terms of [HCO<sub>3</sub><sup>-</sup>] (>>700 μatm), and [CO<sub>3</sub><sup>2-</sup>] and hence calcium carbonate saturation state (<<180 μatm) changes between both manipulation approaches. Under such extreme conditions, biological responses in TA and DIC manipulated experiments may deviate from each other if the underlying processes are sensitive to changes in [HCO<sub>3</sub><sup>-</sup>] or [CO<sub>3</sub><sup>2-</sup>] and CaCO<sub>3</sub> saturation states.

A comparison of experimental results on sensitivities of the coccolithophore *Emiliana huxleyi* to ocean acidification indeed indicates no systematic differences in observed responses between the two approaches (Table 3). With the exception of cultures grown under low light levels, calcification of *Emiliana huxleyi* decreases with increasing CO<sub>2</sub> 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<sub>2</sub> aeration (DIC), finding no carbonate chemistry related difference in the responses of *Emiliana huxleyi* (Shi et al., 2009).

In summary, for most experimental conditions in the context of ocean acidification both CO<sub>2</sub> 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<sub>3</sub><sup>-</sup>] changes. 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<sub>3</sub><sup>-</sup>]. 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 2). With the exceptions outlined above, no systematic differences in the biological responses are expected between the different approaches.

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