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Biogeosciences

Technical Note: Approaches and software tools to investigate the impact of ocean acidification

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Abstract. Although future changes in the seawater carbonate chemistry are well constrained, their impact on marine organisms and ecosystems remains poorly known. The biological response to ocean acidification is a recent field of research as most purposeful experiments have only been carried out in the late 1990s. The potentially dire consequences of ocean acidification attract scientists and students with a limited knowledge of the carbonate chemistry and its experimental manipulation. Hence, some guidelines on carbonate chemistry manipulations may be helpful for the growing ocean acidification community to maintain comparability. Perturbation experiments are one of the key approaches used to investigate the biological response to elevated pCO_2. They are based on measurements of physiological or metabolic processes in organisms and communities exposed to seawater with normal or altered carbonate chemistry. Seawater chemistry can be manipulated in different ways depending on the facilities available and on the question being addressed. The goal of this paper is (1) to examine the benefits and drawbacks of various manipulation techniques and (2) to describe a new version of the R software package *seacarb* which includes new functions aimed at assisting the design of ocean acidification perturbation experiments. Three approaches closely mimic the on-going and future changes in the seawater carbonate chemistry: gas bubbling, addition of high-CO_2 seawater as well as combined additions of acid and bicarbonate and/or carbonate.

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1 Introduction

Ocean acidification refers to the on-going decrease in ocean pH as a result of the uptake of anthropogenic carbon dioxide (CO_2) in the ocean. The partial pressure of CO_2 (pCO_2) increases in the atmosphere due to anthropogenic inputs. It has increased by about 40% (267 to 384 µatm) since the beginning of the industrial revolution leading to changes in the Earth's climate and in the functioning of terrestrial ecosystems. The world's oceans currently absorb on average about one metric ton of CO_2 produced by each person every year. It is estimated that the surface waters of the oceans have taken up 118 Pg C, or about 25% of the carbon generated by human activities since 1800 (Sabine et al., 2004). The increase of pCO_2 profoundly affects the seawater carbonate system (Table 1). It increases the concentrations of bicarbonate and dissolved inorganic carbon, and lowers pH, the concentration of carbonate ions (CO_3^{2-}) and the saturation state of the major shell-forming carbonate minerals:

$$CO_2 + CO_3^{2-} + H_2O \rightarrow 2HCO_3^-$$

Surface ocean pH is estimated to have decreased from approximately 8.20 to 8.07 between preindustrial time and the 1990s and may reach 7.79 in 2100 (Table 1). The expression "ocean acidification" refers to the decrease in pH but does not imply that the pH of surface ocean waters will become acidic (below 7.0) any time soon.

Although the chemistry of ocean acidification is very well known, the associated impacts on marine organisms and ecosystems remain poorly known. The impact of low pH on marine organisms started to be investigated several decades ago (e.g. Bouxin, 1926), yet research into the biological response to ocean acidification is still in its infancy: the first

Table 1. Changes in the carbonate chemistry of surface seawater from the Last Glacial Maximum (LGM; 18 ky BP) to 2100. Total alkalinity, CO_2 partial pressure (pCO_2), salinity and temperature were fixed and used to derive all other parameters using the *seacarb* software (Lavigne et al., 2008) and the dissociation constant of carbonic acid of Lueker et al. (2000). It is assumed that the ocean and atmosphere are in equilibrium with respect to CO_2. Values of temperature, salinity, total alkalinity and total phosphate in 1766, 2007 and 2100 are from Plattner et al. (2001) prescribing historical CO_2 records and non-CO_2 radiative forcing from 1766 to 1990 and using the A2 IPCC SRES emissions scenario (Nakićenović and Swart, 2000) thereafter. Temperature during the LGM was set 1.9°C colder than in 2007 (MARGO Project Members, 2009). The LGM salinity was set 1.07 higher than during pre-industrial time (Paul and Schäfer-Neth, 2003). Total alkalinity in the LGM was scaled to salinity while the total phosphate concentration was assumed to be the same as in 1766. pCO_2 in 1766 and 2100 are from Plattner et al. (2001), while values in the LGM and 2007 are from Petit et al. (2001) and Keeling et al. (2008), respectively. The concentration of total silicate is assumed to have remained constant from the LGM to 2100. It was calculated using the gridded data reported by Garcia et al. (2006) between 0 and 10 m and weighing the averages using the surface area of each grid cell. pH is expressed on the total scale.

Parameter	Unit	LGM	1766	2007	2100
Temperature	°C	17.2	18.3	18.9	21.4
Salinity	–	36	34.9	34.9	34.7
Total phosphate	10^{-6} mol kg^{-1}	0.66	0.66	0.63	0.55
Total silicate	10^{-6} mol kg^{-1}	7.35	7.35	7.35	7.35
Total alkalinity	10^{-6} mol kg^{-1}	2399	2326	2325	2310
CO_2 partial pressure (seawater)	µatm	180	267	384	793
$[CO_2]$	10^{-6} mol kg^{-1}	6.26	9.05	12.8	24.7
$[HCO_3^-]$	10^{-6} mol kg^{-1}	1660	1754	1865	2020
$[CO_3^{2-}]$	10^{-6} mol kg^{-1}	299	231	186	118
Dissolved inorganic carbon	10^{-6} mol kg^{-1}	1966	1994	2064	2162
pH_T	–	8.33	8.20	8.07	7.79
$[H^+]$	10^{-9} mol kg^{-1}	4.589	6.379	8.600	16.13
Calcite saturation	–	7.1	5.5	4.5	2.8
Aragonite saturation	–	4.6	3.6	2.9	1.8

purposeful manipulative experiment was carried out only in the 1980s (Agegian, 1985) and others began only in the late 1990s. The potentially dire consequences of ocean acidification have attracted the interest of scientists and students with a limited knowledge of the carbonate chemistry and its experimental manipulation. Hence, some guidelines on carbonate chemistry and its manipulation may be helpful for the growing ocean acidification community in order to ensure that the most adequate approaches are used and that results and subsequent analyses are comparable. The need to standardize measurement of parameters of the carbonate system has been identified (Kleypas et al., 2006), a gap which was recently filled (Dickson et al., 2007). Perturbation experiments are one of the key approaches used to investigate the biological response to elevated pCO_2. They are based on measurements of physiological or metabolic processes in organisms and communities exposed to seawater with normal or altered chemistry. Seawater chemistry can be manipulated using different ways depending on the facilities available and on the question being addressed. Langdon (2000) summarized the state-of-the-art techniques used with reef builders. Here we examine the benefits and drawbacks of current methods and provide a software package to assist the design of perturbation experiments.

2 Method

Detailed information on the carbonate chemistry, including the merit of units and scales, as well as on the measurement methods can be found in Dickson et al. (2007), Zeebe and Wolf-Gladrow (2001) and in the *Guide for Best Practices in Ocean Acidification Research and Data Reporting* in preparation (see http://www.epoca-project.eu/index.php/Best-Practices-Guide/).

The R package *seacarb* was used to calculate the carbonate chemistry parameters. *R* is a free cross-platform language and environment for statistical computing and graphics (R Development Core Team, 2008). The previous version, *seacarb* 1.2.3 (Proye and Gattuso, 2003) is superseded by the recently released version 2.0 (Lavigne et al., 2008). The new version has been updated in order to be fully consistent with the most recent guidelines to best practices for ocean CO_2 measurements (Dickson et al., 2007). All equations are solved analytically except when total alkalinity is one of the two input parameters of the carbonate system that are required. In that case, the concentration of H^+ or dissolved inorganic carbon are solved numerically using the R function *uniroot*. Five new functions have been added to *seacarb* 2.0 to assist the design of ocean acidification perturbation experiments. The syntax of these commands is briefly

described in Appendices A1 to A5. Additional information is available in the help files of the *seacarb* package.

3 Main approaches

The seawater chemistry can be manipulated in various ways in order to alter parameters of the carbonate system. The following sections examine the five techniques that are most useful in the context of ocean acidification. Calculations were carried out using the first and second dissociation constants of carbonic acid given by Lueker et al. (2000). In systems open to the atmosphere, it is assumed that the seawater and atmosphere are in equilibrium with respect to CO_2. It must be pointed out that the methods described below enable to set the carbonate chemistry at the beginning of a perturbation experiment. However, initial values can be altered by biological (e.g. photosynthesis, respiration and calcification) and physical processes (e.g. air-seawater exchange of CO_2 and temperature changes), which must also be taken into account in the experimental design.

3.1 Gas bubbling

Bubbling seawater with gases is an efficient way to manipulate its carbonate chemistry. The *seacarb* function pgas estimates the changes in the carbonate chemistry resulting from changes in pCO_2 generated by bubbling gases.

Example: seawater with a pCO_2 of 384 µatm and total alkalinity (TA) of 2325 µmol kg^{-1} is bubbled with a mixture of CO_2 and air with a pCO_2 of 793 µatm. Salinity is 34.9, temperature is 18.9°C and calculations are made for surface waters. The command needed is:

```
pgas(flag=24, var1=384, var2=2325e-6, pCO2g=793,
S=34.9, T=18.9)
```

This approach exactly reproduces the changes of all parameters of the carbonate system expected in the year 2100 (Fig. 1 and Table 2). Smith and Roth (1979) is perhaps the first publication which manipulated the carbonate chemistry via gas bubbling within a range compatible with that of the on-going ocean acidification.

Two techniques have been used in the context of ocean acidification: pH-stat and bubbling with premixed gases. In pH-stat systems, pH is monitored continuously and a controller opens or closes valves when the pH goes above or below a set value. Gases are then delivered until the pH reaches the set value again. Different combination of gases have been used: (1) air and pure CO_2, (2) CO_2-free air and pure CO_2, and (3) air, CO_2-free air and pure CO_2. CO_2-free air can be produced easily using CO_2 scrubbers such as soda lime or NaOH and $Ca(OH)_2$ (C. J. Hintz, personal communication, 2008). The pH threshold is calculated using the desired pCO_2 and total alkalinity which must either be constant or frequently measured. This method allows one to compensate for any change in the carbonate chemistry due to photosynthesis and respiration or, in the case of open culture systems, to changes in the source water. However, it does not compensate for changes in total alkalinity that would result from precipitation and dissolution of $CaCO_3$ which could occur between measurements of total alkalinity. Hence, the carbonate chemistry can be maintained with good efficiency in the culture vessel, e.g. with variability of pCO_2 better than ±10 µatm. The main drawback of this technique is that the pH electrode must be frequently calibrated in order to account for drift. Hence, the second technique of bubbling with premixed gases may be attractive. Air with the desired pCO_2 can be produced using gas mixing pumps or purchased.

Another approach would be to control atmospheric pCO_2 to the desired level in the laboratory or growth cabinet in which the experiments are carried out. A simple air pump can then be used to bubble the experimental seawater. To the best of our knowledge, this technique has yet to be used in the context of ocean acidification.

Seawater aeration by bubbling may lead to difficulties in phytoplankton cultures (Shi et al., 2009). It may also enhance the coagulation of organic matter (Engel et al., 2004). This coagulation may be critical for studies investigating the response of microbial communities because their metabolism depends on the respective abundance of dissolved and particulate organic matter. This drawback may be avoided by enclosing the community in a dialysis bag, which is itself enclosed in a container bubbled with a gas having the desired pCO_2 (M. Weinbauer, personal communication, 2008). Such bags are permeable to gases and small molecules in solution but impermeable to larger molecules and particles.

3.2 Addition of high-CO_2 sea water

Dissolved inorganic carbon (DIC) and total alkalinity (TA) are conservative quantities with respect to mixing (Wolf-Gladrow et al., 2007). Hence, when two water parcels are mixed, the amount of a solute in the mixture equals the sum of the amounts of this solute in the two initial water parcels. Hence:

$$[\mathrm{DIC}]_{\mathrm{mixed}} \times M_{\mathrm{mixed}} = [\mathrm{DIC}]_{sw} \times M_{sw} + [\mathrm{DIC}]_{\mathrm{high\ CO_2}} \times M_{\mathrm{high\ CO_2}}$$
$$[\mathrm{TA}]_{\mathrm{mixed}} \times M_{\mathrm{mixed}} = [\mathrm{TA}]_{sw} \times M_{sw} + [\mathrm{TA}]_{\mathrm{high\ CO_2}} \times M_{\mathrm{high\ CO_2}}$$

Where the subscripts "sw" and "high CO_2", respectively indicate concentrations in the normal and high CO_2 seawater. Concentrations are in µmol kg^{-1} and M is the mass in kg. The *seacarb* function pmix estimates the carbonate chemistry after mixing of two water samples.

Example: one mixes, in a closed system, 0.99623 kg of seawater having a TA of 2325 µmol kg^{-1}, and pCO_2 of 384 µatm with 0.00377 kg of seawater having a TA of 2325 µmol kg^{-1} and saturated with CO_2 ($pCO_2 = 1 \times 10^6$ µatm). The weight fraction, wf, is 3.76×10^{-3}. Salinity is 34.9, temperature is 18.9°C

Table 2. Seawater carbonate chemistry in 2007, 2100, and after perturbation. The total alkalinity (TA), partial pressure of CO_2 in seawater (pCO_{2sw}), salinity and temperature were used to derive all other parameters using the *seacarb* package (Lavigne et al., 2008) except for manipulations of the calcium concentration for which DIC was used rather than pCO_{2sw}. For simplicity, it is assumed that temperature, salinity and total alkalinity in 2100 are identical to their 2007 values, respectively 18.9°C, 34.9 and 2325×10^{-6} mol kg^{-1}, and the concentrations of total phosphate and silicate were set to 0. The seawater pCO_2 was set to 384 µatm in 2007 (Keeling et al., 2008) and 793 µatm in 2100 (according to the A2 SRES emission scenario; Plattner et al., 2001). The main body of the text provides further information. (a): $\times10^{-9}$ mol kg^{-1}, (b): $\times10^{-6}$ mol kg^{-1}.

	pCO_{2sw} (µatm)	pH_T (–)	$[H^+]$ (a)	TA (b)	DIC (b)	$[CO_2]$ (b)	$[HCO_3^-]$ (b)	$[CO_3^{2-}]$ (b)	Ω_c (–)	Ω_a (–)
Year 2007	384	8.065	8.6	2325	2065	12.8	1865	187	4.5	2.9
Year 2100	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Gas bubbling	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Addition of high-CO_2 seawater	792	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Addition of CO_3^{2-} and HCO_3^-; closed sys.	793	7.942	11.4	3406	3146	26.4	2901	218	5.2	3.4
Addition of CO_3^{2-} and HCO_3^-; open sys.	384	8.207	6.2	3406	2950	12.8	2580	357	8.5	5.5
Acid addition; closed sys.	793	7.768	17.1	2184	2065	26.4	1940	98	2.3	1.5
Acid addition; open sys.	384	8.042	9.1	2184	194	12.8	1767	167	4	2.6
Addition of:										
CO_3^{2-} and HCO_3^-; closed sys.	400	8.073	8.4	2467	2191	13.3	1977	201	4.8	3.1
followed by acid addition; closed sys.	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Manipulation of $[Ca^{2+}]$	384	8.065	8.6	2325	2065	12.8	1866	187	2.6	1.7

and calculations are made for surface waters. The command needed is:

pmix(flag=24, var1=384, var2=2325e-6, pCO2s=1e6, wf=3.76e-3, S=34.9, T=18.9)

This produces seawater with a final pCO_2 of 792 µatm (the 1 µatm difference with the target value is due to rounding errors) and all parameters of the carbonate chemistry in 2100 are perfectly reproduced. To the best of our knowledge, this approach has only been used twice: in mesocosms by Schulz et al. (unpublished data) and in the laboratory by C. McGraw (personal communication, 2009). It is therefore not possible to evaluate its pros and cons at this stage.

3.3 Addition of CO_3^{2-} and/or HCO_3^-

Dissolved inorganic carbon and total alkalinity can be increased by adding CO_3^{2-} in the form of Na_2CO_3 and/or by adding HCO_3^- in the form of $NaHCO_3$. In closed systems, the change in DIC generated by these additions is proportional to the changes in concentration: $1\times\Delta CO_3^{2-}$ and $1\times\Delta HCO_3^-$. The contribution of these anions to TA is proportional to the product of their charge and concentration. Thus: TA increases by $2\times\Delta CO_3^{2-}$ and $1\times\Delta HCO_3^-$. The changes in the carbonate chemistry generated by manipulations of total alkalinity therefore depend on the proportion of CO_3^{2-} and HCO_3^- added (Fig. 3). This approach can be used to keep the pH constant or combined with addition of acid to keep TA constant (see Sect. 3.5).

In an open system, the carbonate system re-equilibrates through air-sea CO_2 gas exchange after adding chemicals. At equilibrium:

$$CO_3^{2-} + H^+ \rightarrow HCO_3^- \quad \text{(R1)}$$

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \quad \text{(R2)}$$

The *seacarb* function pTA computes the changes in the carbonate chemistry due to addition of CO_3^{2-} and/or HCO_3^-.

Example: HCO_3^- (1081×10^{-6} mol kg^{-1}) is added to seawater for which pCO_2 (384 µatm) and TA (2325 µmol kg^{-1}) are known. No CO_3^{2-} is added. The atmospheric pCO_2 is 384 µatm, salinity is 34.9, temperature is 18.9°C and calculations are made for surface waters. The respective commands in a closed and open system are:

pTA(flag=24, sys=0, var1=384, var2=2325e-6, pCO2a=384, co3=0e-6, hco3=1081e-6, S=34.9, T=18.9)

pTA(flag=24, sys=1, var1=384, var2=2325e-6, pCO2a=384, co3=0e-6, hco3=1081e-6, S=34.9, T=18.9)

Results are shown in Fig. 3 and Table 2. In a closed system, the target pCO_2 of 793 µatm is reached but all other parameters of the carbonate system are very different from their values expected in 2100. pH is higher than it should be (7.942 instead of 7.793) and TA, DIC, $[CO_3^{2-}]$, and the

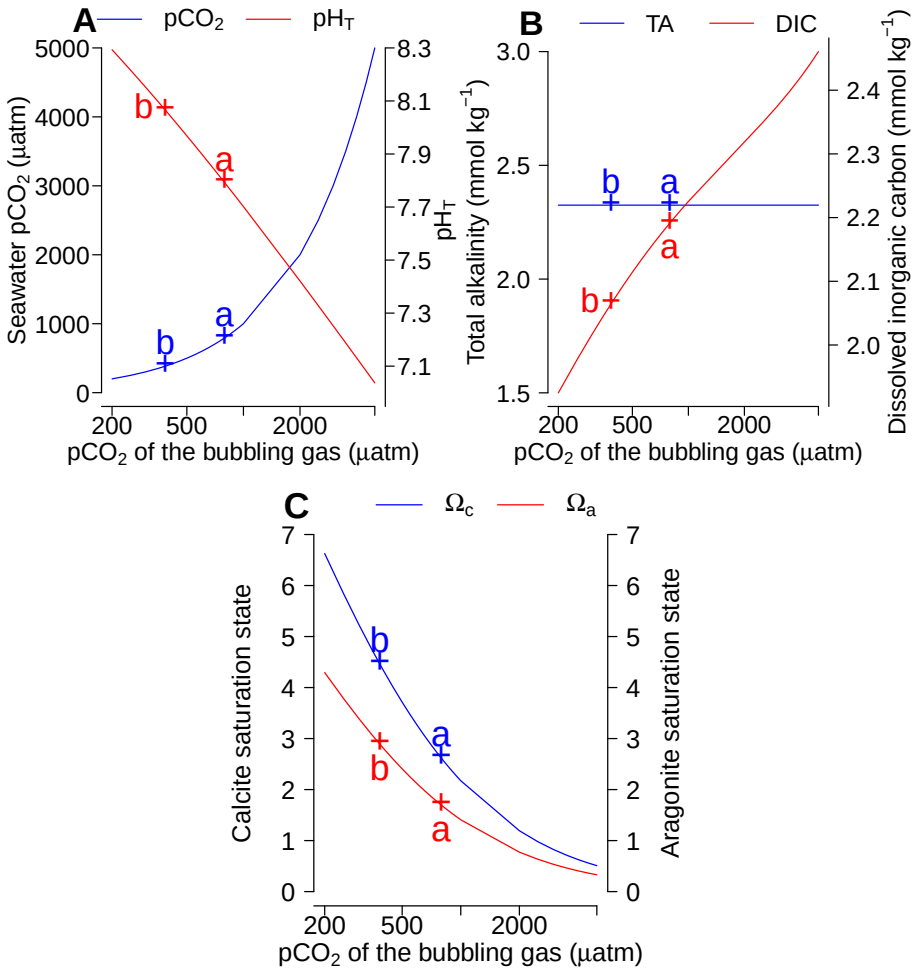

Fig. 1. Effect of bubbling seawater with gases of varying pCO_2 on the carbonate chemistry of seawater as computed with the *seacarb* function pgas. The letters "b" and "a" indicate, respectively, the value of a parameter before and after perturbation for the example described in the text.

saturation states of aragonite and calcite (Ω_a and Ω_c) are higher than the target values. They are even higher than the values of the initial seawater. Those differences are magnified in open systems.

Manipulation of total alkalinity has often been used to investigate the physiological response to changes in the carbonate chemistry (e.g. Swift and Taylor, 1966). Marubini and Thake (1999) used bicarbonate additions to test the hypothesis that the supply of DIC limits coral calcification. Langdon et al. (2000) used this approach in the Biosphere 2 coral reef mesocosm. They added $NaHCO_3$ and Na_2CO_3 to increase the $CaCO_3$ saturation state. Since the experiment lasted several months, additions of $CaCl_2$ were used to restore a normal Ca^{2+} concentration. The manipulations of the Ca^{2+} concentration is described in Sect. 3.6.

3.4 Addition of strong acids and bases

The carbonate chemistry can be manipulated by addition of a strong acid or a strong base, such as HCl and NaOH, which completely dissociate in seawater. Adding of a strong acid or base to a system closed to the atmosphere does not alter the concentration of dissolved inorganic carbon but modifies total alkalinity. TA decreases when adding an acid and increases after addition of a base.

The change of total alkalinity after addition of a strong acid or base in a system open to the atmosphere is identical to that described above for a closed system. However, the concentration of DIC is modified through CO_2 exchange at the air-water interface because the partial pressures of CO_2 in the atmosphere and seawater equilibrate.

The *seacarb* function ppH calculates the changes in the carbonate chemistry during pH manipulations. The change in salinity due to the addition of acid or base is negligible.

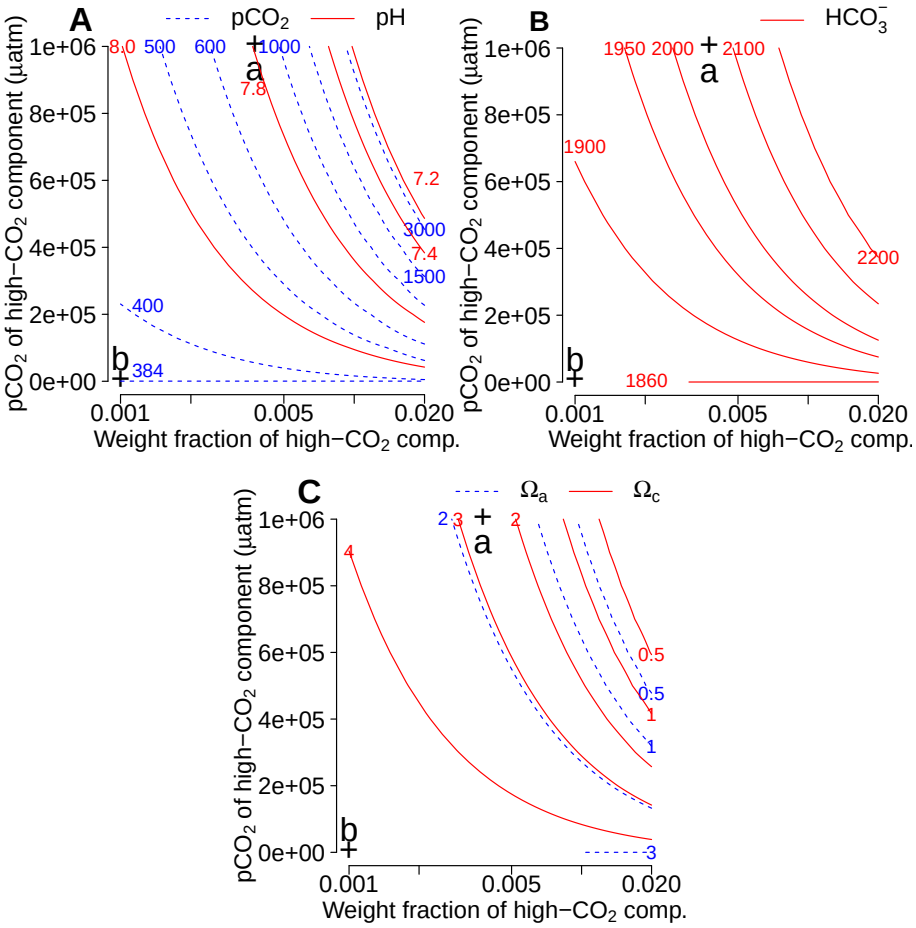

Fig. 2. Effect of mixing with various weight fractions of high-pCO_2 seawater on the carbonate chemistry of seawater in a closed system as computed with the *seacarb* function pmix. The letters "b" and "a" indicate, respectively, the value of a parameter before and after perturbation for the example described in the text.

Example: a volume of 14.08 ml of 0.01 N HCl is added to 1 kg of seawater having a known pCO_2 (384 µatm) and TA (2325 µmol kg^{-1}). The atmospheric pCO_2 is 384 µatm, salinity is 34.9 and temperature is 18.9°C. Calculations are made for surface waters. The commands needed in a closed and open system are:

ppH(flag=24, sys=0, var1=384, var2=2325e-6, pCO2a=384, vol=−14.08e-3, N=0.01, S=34.9, T=18.9)

ppH(flag=24, sys=1, var1=384, var2=2325e-6, pCO2a=384, vol=−14.08e-3, N=0.01, S=34.9, T=18.9)

Results are shown in Fig. 4 and Table 2. The target pCO_2 of 793 µatm is reached in a closed system but the pH is lower than the value expected in 2100 (7.768 instead of 7.793, corresponding to an increase of 2.9% in $[H^+]$ that resulting from the decrease in total alkalinity generated by the addition of acid). This is an undesirable effect of the direct manipulation of pH as TA will not change significantly during the course of this century (Table 1). As a result, DIC, HCO_3^-, CO_3^{2-}, Ω_a and Ω_c are lower than their target values. However, it is possible to restore TA to its initial level by adding CO_3^{2-} and/or HCO_3^-, an approach that is described in Sect. 3.5. Obviously, due to gas exchange at the air-water interface, pCO_2 and other parameters of the carbonate chemistry cannot be successfully adjusted by a one-time addition of acid in an open system.

Addition of acid has often been used in studies of the impact of ocean acidification on organisms. The first purposeful experiment is that of Agegian (1985) who looked at the response of coralline algae.

3.5 Addition of strong acid as well as CO_3^{2-} and/or HCO_3^-

As mentioned in Sect. 3.4, the addition of acid does not allow to fully mimic the changes in carbonate chemistry expected during the present century but the addition of CO_3^{2-} and/or HCO_3^- followed by addition of acid circumvents this

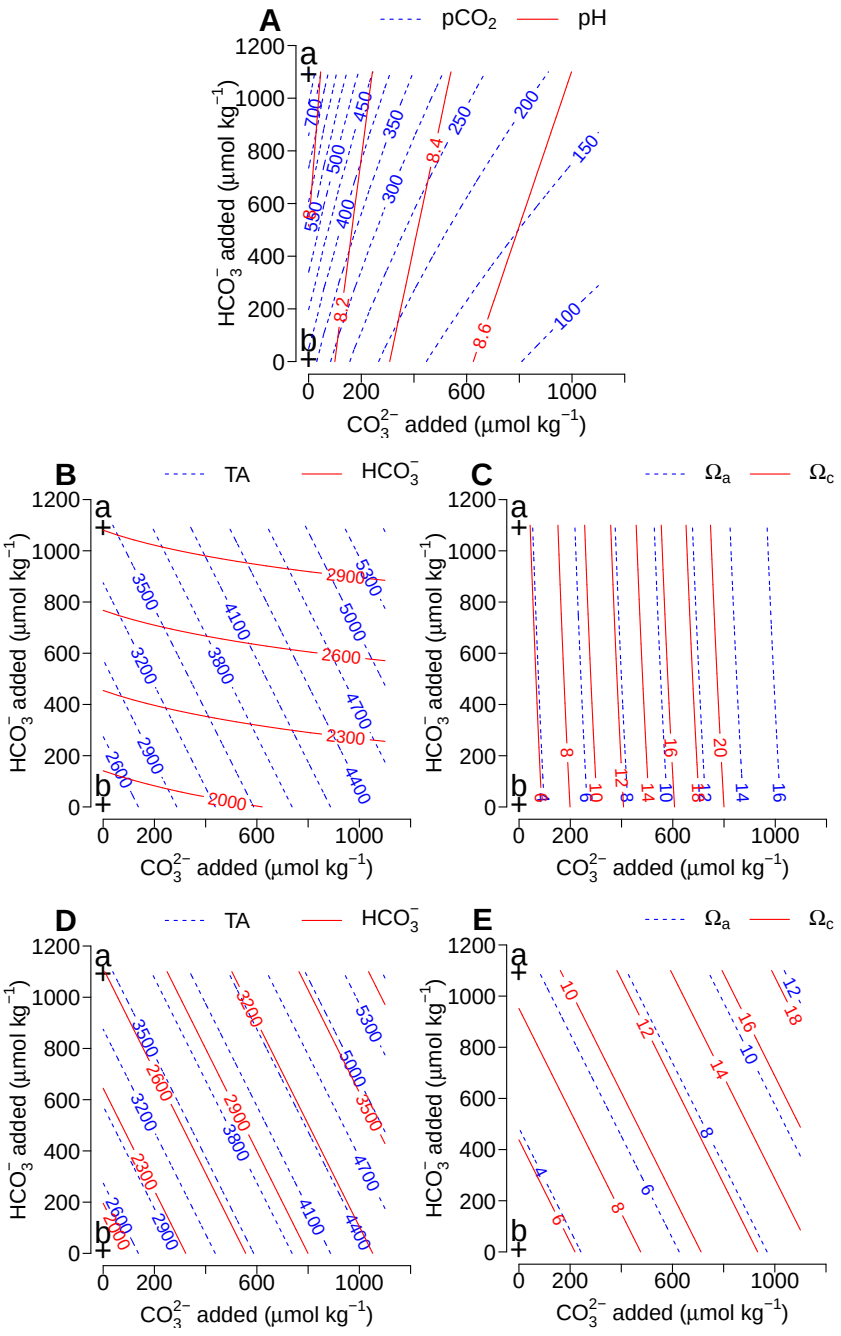

Fig. 3. Effect of adding CO_3^{2-} and/or HCO_3^- on the carbonate chemistry of seawater in closed (**A**, **B** and **C**) and open systems (**D** and **E**) as computed with the *seacarb* function pTA. The letters "b" and "a" indicate, respectively, the value of a parameter before and after perturbation for the example described in the text.

problem. The first addition elevates DIC to the desired level while adding acid (at constant DIC) precisely cancels out the increase in TA resulting from the addition of CO_3^{2-} and/or HCO_3^-.

There is no specific *seacarb* function to handle this approach. The changes in the carbonate chemistry can be calculated using successively the functions pTA and ppH.

Example: HCO_3^- (111.2×10^{-6} mol kg^{-1}) and CO_3^{2-} (15.3×10^{-6} mol kg^{-1}) are added to seawater for which pCO_2 (384 µatm) and TA (2325 µmol kg^{-1}) are known. The atmospheric pCO_2 is 384 µatm, salinity is 34.9, temperature is 18.9°C and calculations are made for surface waters. Then, 14.18 ml of 0.01 N HCl is added. The commands needed are:

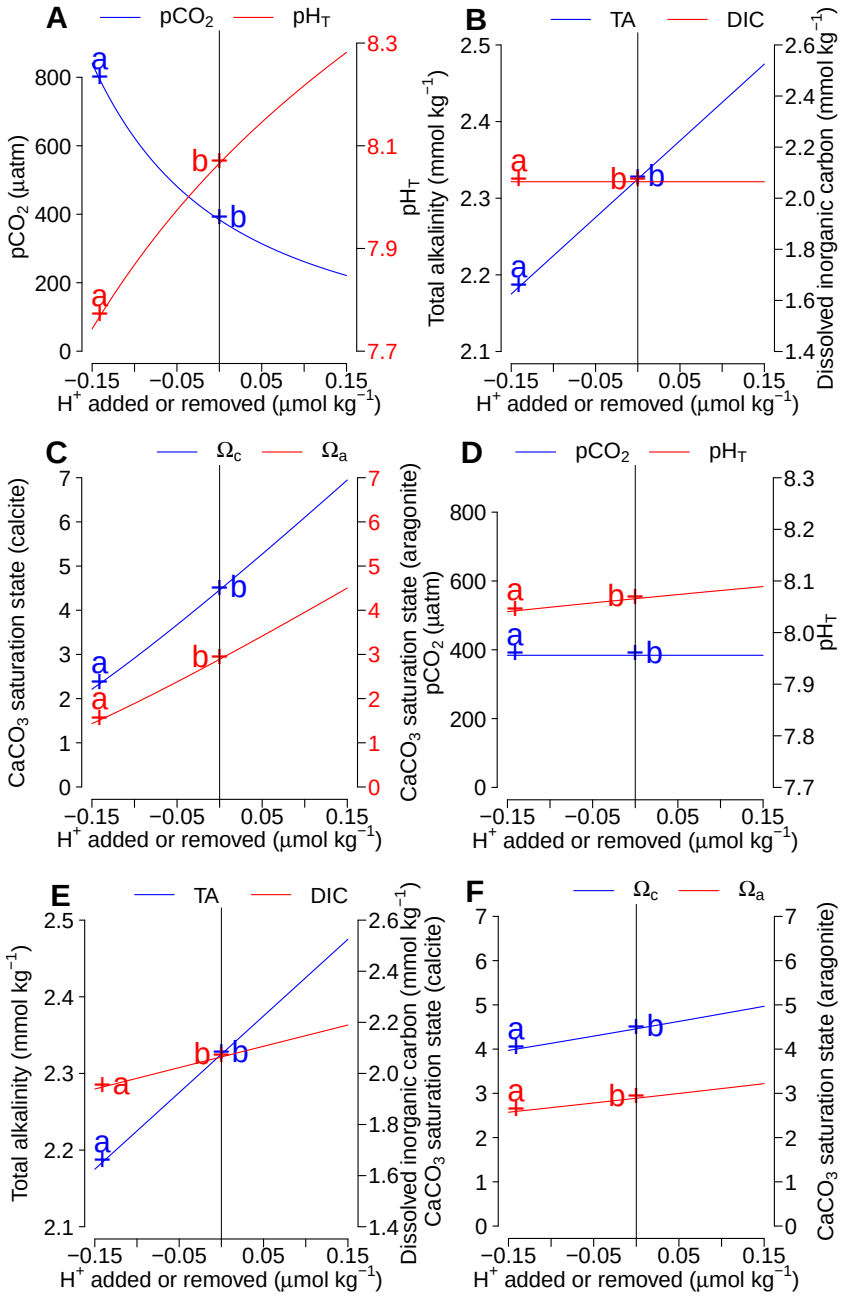

Fig. 4. Effect of adding a strong acid or a strong base on the carbonate chemistry of seawater in closed (**A**, **B** and **C**) and open systems (**D**, **E** and **F**) as computed with the *seacarb* function pgas. By convention, addition of acid is negative whereas addition of base is positive. The letters "b" and "a" indicate, respectively, the value of a parameter before and after perturbation for the example described in the text.

```
tmp=pTA(flag=24, sys=0, var1=384, var2=2325e-6,
pCO2a=384, co3=15.3e-6, hco3=111.2e-6, S=34.9, T=18.9)

ppH(flag=24, sys=0, var1=tmp$pCO2[2],
var2=tmp$ALK[2], pCO2a=384, vol=−14.18e-3, N=0.01,
S=34.9, T=18.9)
```

Results are shown in Table 2. The first addition raises DIC to the desired level of 2191×10^{-6} mol kg^{-1} but increases TA to a value higher than the target (2467 vs. 2325×10^{-6} mol kg^{-1}). The subsequent addition of HCl restores TA to the desired value without affecting DIC. After both additions, all carbonate parameters are at the target values (Table 2).

To our knowledge, the first paper which used addition of acid, base and $NaHCO_3$ to manipulate the carbonate chemistry within a range comparable to ocean acidification is that of Borowitzka (1981) who investigated, in the laboratory, the response of photosynthesis and calcification of an articulated coralline red algae.

3.6 Manipulation of the Ca^{2+} concentration

Although manipulating the calcium concentration does not technically alter the carbonate chemistry *per se*, this approach has been used in the context of ocean acidification. The reason is that some calcifying organisms, such as corals, respond to the calcium carbonate saturation state of seawater (Ω) which is expressed as:

$$\Omega = \frac{[Ca^{2+}]_{sw} \times [CO_3^{2-}]_{sw}}{K^*_{sp}}$$

where $[Ca^{2+}]_{sw}$ and $[CO_3^{2-}]_{sw}$ are the concentrations of calcium and carbonate in seawater, respectively, and K^*_{sp} is the solubility product at the *in situ* conditions of temperature, salinity and pressure (Zeebe and Wolf-Gladrow, 2001). It can readily be appreciated that the changes in Ω resulting from the decrease in $[CO_3^{2-}]_{sw}$ driven by ocean acidification can be mimicked by altering $[CO_3^{2-}]_{sw}$. This approach involves the use of artificial seawater (Gattuso et al., 1998). It is recommended to use the simplied synthetic seawater recipe based on DOE (1994) as described by Gattuso et al. (1998) and because it is the basis of the synthetic seawater that has been used to determine a variety of equilibrium constants for use in seawater.

The *seacarb* function pCa estimates the changes in Ω_c and Ω_a resulting from the manipulation of the $[Ca^{2+}]_{sw}$ concentration. Note that this function does not account for the effect of the changes on the calcium concentration of the dissociation constants of carbonic acid and on the solubility product of $CaCO_3$ (Ben-Yaakov and Goldhaber, 1973).

Example: artificial seawater is made with a known TA (2325 µmol kg^{-1}) and DIC (2064 µmol kg^{-1}). The calcium concentration is set to 6.03×10^{-6} mol kg^{-1}. The command needed is:

```
pCa(flag=15, var1=2325e-6, var2=2064e-6,
Ca=6.03e-3, S=34.9, T=18.9)
```

Results are shown in Fig. 5 and Table 2. The saturation states of aragonite and calcite expected in 2100 are well reproduced without affecting any of the other parameters of the carbonate system which remain at their 2008 values.

This approach has been used to investigate the response of corals (Gattuso et al., 1998) and coccolithophores (Trimborn et al., 2007) to changes in the saturation state of calcium carbonate. It has also been used to replenish calcium when its concentration decreases below its natural levels during long-term experiments with calcifiers (e.g. Langdon et al., 2000).

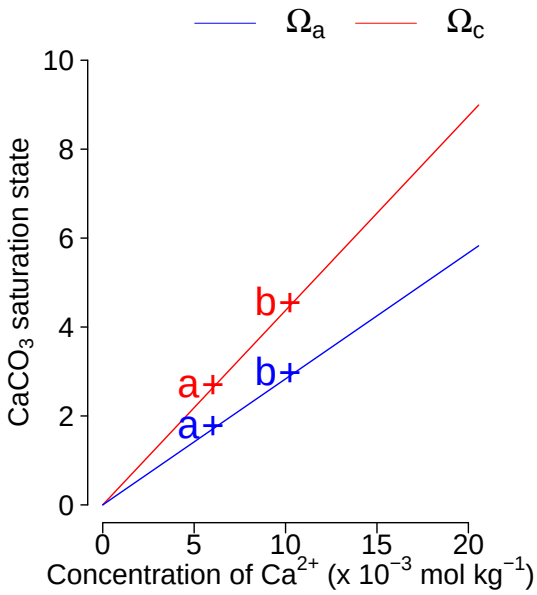

Fig. 5. Calcium carbonate saturation state as a function of the calcium concentration as computed with the *seacarb* function pCa. The letters "b" and "a" indicate, respectively, the value of the calcium carbonate saturation state before and after perturbation for the example described in the text.

4 Discussion and conclusion

Three approaches closely mimic the on-going and future changes in the seawater carbonate chemistry: gas bubbling, addition of high-CO_2 seawater, and combined addition of acid and bicarbonate and/or carbonate. All three methods increase dissolved inorganic carbon at constant total alkalinity, a situation that closely resembles the changes in the carbonate chemistry that have occurred during the last 200 years and are expected to continue during the next few hundreds years. All three approaches therefore offer precise control of carbonate chemistry variables, that reach target values at the beginning of a perturbation experiment. Gas bubbling is the easiest to implement and can be used to maintain constant conditions over long periods of time. However, during bubbling, biological processes (e.g., calcification, dissolution of $CaCO_3$, nutrient uptake and release) can alter the carbonate chemistry by changing total alkalinity even though the concentration of pCO_2 remains constant. Also, in all three approaches, calcification can deplete the concentration of Ca^{2+} when the organism to volume ratio is high or the incubation time long.

The approach of adding CO_3^{2-} and/or HCO_3^- appears to be of limited practical use because most carbonate variables reach values that are far from target values. Several studies have added acid to manipulate the carbonate chemistry. Although this technique offers precise control of pCO_2 in systems closed to the atmosphere, it also alters total alkalinity

which drives other carbonate system variables to levels far from those expected in the future. That is, pH is lower than its target value, DIC remains unchanged (whereas it increases with natural ocean acidification), and the $CaCO_3$ saturation states as well as the concentrations of bicarbonate and carbonate are higher than the target values. To avoid this drawback, it is recommended to first add bicarbonate or carbonate, or both, to increase DIC, before adding acid.

Iglesias-Rodriguez et al. (2008a) have recently reported that, in contrast to previous reports, calcification of the coccolithophore *Emiliania huxleyi* increases at elevated pCO_2. They argued that this is due to the different approaches used to manipulate the carbonate chemistry and that the approach of gas bubbling (changing DIC at constant TA) is superior to the approach of adding acid (changing TA at constant DIC) (Iglesias-Rodriguez et al., 2008a,b). The argument is that the addition of acid does not reproduce the increase in the HCO_3^- concentration occurring from natural ocean acidification whereas gas bubbling does reproduce it. The authors claim that HCO_3^- may stimulate photosynthesis and, in turn, calcification. They conclude that experiments using addition of acid demonstrating that calcification declines at lower pH, confounded the issue. This statement is misleading, although there is no doubt that gas bubbling better mimics the future carbonate chemistry (see above as well as Table 2). In contrast to the claims by Iglesias-Rodriguez et al. (2008a,b), several previous perturbation experiments were also carried out with gas bubbling and reported lower rates of calcification of coccolithophores at lower pH and higher pCO_2 (Sciandra et al., 2003; Delille et al., 2005; Feng et al., 2008). Additionally, the concentrations of CO_2, HCO_3^-, CO_3^{2-}, and H^+ all change in the same direction with both approaches: gas bubbling and acid addition (compare Fig. 1a–c with Fig. 4a–c; Table 2). Hence, the magnitude of biological responses may be different, but should at least tend to go in the same direction. Differences in the two approaches cannot explain biological responses going in opposite directions. Finally, perturbation experiments carried out using gas bubbling can also lead to poor control of the carbonate chemistry, for example, when the experiments last too long or when the biomass-to-volume ratio is too large (see above). Although the cause of the discrepancy in the response of calcification by the coccolithophore *E. huxleyi* to ocean acidification remains a matter of debate (e.g., Riebesell et al., 2008; Shi et al., 2009), it does not appear to be related to the approach used to perform the manipulation of the carbonate chemistry in perturbation experiments.

Fortunately, the situation seems clearer in zooxanthellate scleractinian corals. Schneider and Erez (2006) measured the rate of calcification under constant DIC, constant pH and constant pCO_2 and showed that calcification is controlled by the concentration of CO_3^{2-}, the future value of which is relatively well mimicked by adding acid.

Our goal here has been to provide the appropriate computational tools and to highlight the positive and negative aspects of the different approaches that are used to investigate ocean acidification by perturbing the carbonate chemistry. We have not addressed practical issues that must be taken into account to design and conduct perturbation experiments. To mention just a few: response time, bubbling rate, impact of autoclaving, evaporation, heat flux, impact of nutrients and temperature. These issues, as well as others, will be covered in a *Guide for Best Practices in Ocean Acidification Research and Data Reporting* in preparation (see http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/).

Appendix A

A1 Gas bubbling: syntax of pgas

The function pgas calculates the carbonate chemistry after perturbation using the CO_2 partial pressure of the gas used to bubble the medium and the initial total alkalinity which is not altered by gas bubbling. The syntax is:

pgas(flag, var1, var2, pCO2g, S, T, P, Pt, Sit, k1k2, Kf, pHscale)

The input parameters below are required:

- flag: a number (1 to 24) indicating which couple of parameters of the carbonate system is known. For example, flag is 8 when pH_T and total alkalinity are known.
- var1 and var2: the values of the two parameters of the carbonate system prior to the perturbation which are known (pH_T and alkalinity in the example above). See the *seacarb* manual for more details, especially regarding the units.
- pCO2g is the CO_2 partial pressure (in µatm) of the gas used to bubble the medium.
- S: salinity.
- T: temperature in °C
- P: hydrostatic pressure (P; 0 by default). Note that this function was only tested at P=0, that is for surface waters.
- Pt: concentration of total phosphate (mol kg^{-1}; 0 by default).
- Sit: concentration of total silicate (mol kg^{-1}; 0 by default).
- k1k2: "l" for using K_1 and K_2 from Lueker et al. (2000) and "r" for using K_1 and K_2 from Roy et al. (1993), default is "l"

– Kf: "pf" for using K_f from Perez and Fraga (1987) and "dg" for using K_f from Dickson and Riley (1979), default is "pf"

– pHscale: "T" for using the total scale, "F" for using the free scale and "SWS" for using the seawater scale, default is "T"

A2 Addition of high-CO_2 seawater: syntax of pmix

It is important to note that pmix assumes that the initial and high-CO_2 seawater have the same total alkalinity. pmix calculates the carbonate chemistry after perturbation using the final DIC concentration calculated as described in Sect. 3.2 and the initial total alkalinity which is not altered by the mixing. The syntax is:

pmix(flag, var1, var2, pCO2s, wf, S, T, P, Pt, Sit, k1k2, Kf, pHscale)

flag, var1, var2, S, T, P, Pt, Sit, k1k2, Kf and pHscale were defined in Appendix A1. The other input parameters required are:

– pCO2s: pCO_2 of the high-CO_2 water in µatm

– wf: weight fraction of the high-CO_2 seawater vs. final weight

A3 Addition of CO_3^{2-} and/or HCO_3^-: syntax of pTA

For closed systems, the function pTA calculates the carbonate chemistry after perturbation using the final DIC concentration and total alkalinity calculated as described in Sect. 3.3. For open systems, the carbonate chemistry is calculated using atmospheric pCO_2, therefore assuming equilibrium of pCO_2 between air and seawater, and total alkalinity calculated as described in Sect. 3.3 (as is not further altered by air-sea CO_2 exchange). The syntax is:

pTA(flag, sys, var1, var2, pCO2a, co3, hco3, S, T, P, Pt, Sit, k1k2, Kf, pHscale)

flag, var1, var2, S, T, P, Pt, Sit, k1k2, Kf and pHscale where defined above. The other input parameters below are required:

– sys: 0 if the manipulation is carried out in a system closed to the atmosphere or 1 if it is carried out in a system open to the atmosphere (in the latter case, full equilibrium between air and water is assumed).

– pCO2a: the value of atmospheric pCO_2 in μatm. It is only used in systems open to the atmosphere (i.e. sys=1).

– co3: amount of CO_3^{2-} added in mol kg^{-1}.

– hco3: amount of HCO_3^- added in mol kg^{-1}.

A4 Addition of strong acids and bases: syntax of ppH

For closed systems, the function ppH calculates the carbonate chemistry after perturbation using the DIC concentration (which is unchanged by the addition of acid or base) and the total alkalinity which can easily be estimated as strong acids and bases completely dissociate in seawater. For open systems, the carbonate chemistry is calculated using atmospheric pCO_2, therefore assuming equilibrium of pCO_2 between air and seawater, and the total alkalinity after addition of acid or base as it is not further altered by air-sea CO_2 exchange. The syntax is:

ppH(flag, sys, var1, var2, pCO2a, vol, N, S, T, P, Pt, Sit, k1k2, Kf, pHscale)

flag, sys, var1, var2, pCO_2a, S, T, P, Pt, Sit, k1k2, Kf and pHscale were defined above.

The other input parameters required are:

– vol: the volume of acid or base added in liter. By convention, it is given a negative sign for additions of acid and a positive sign for additions of base.

– N: the normality of the acid or base (mol kg^{-1}).

A5 Manipulation of the Ca^{2+} concentration: syntax of pCa

The function pCa calculates the carbonate chemistry using the standard equations of Dickson et al. (2007) except that the actual calcium concentration is used rather than the standard concentration of $0.01028\times(S/35)$ mol kg^{-1}. The syntax is:

pCa(flag, sys, var1, var2, Ca, S, T, P, Pt, Sit, k1k2, Kf, pHscale)

flag, var1, var2, S, T, P, Pt, Sit, k1k2, Kf and pHscale were defined above. Ca is the calcium concentration in mol kg^{-1}.

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