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# Perturbation experiments to investigate the impact of ocean acidification: approaches and software tools

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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  $p\text{CO}_2$ . 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- $\text{CO}_2$  seawater as well as combined additions of acid and bicarbonate and/or carbonate.

## 1 Introduction

Ocean acidification refers to the on-going decrease in ocean pH as a result of the uptake of anthropogenic carbon dioxide ( $\text{CO}_2$ ) in the ocean. The partial pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ) increases in the atmosphere due to anthropogenic inputs. It has increased by more than 35% 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

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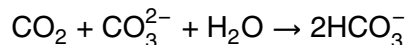
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currently absorb on average about one metric ton of  $\text{CO}_2$  produced by each person every year. It is estimated that the surface waters of the oceans have taken up over 500 Gt  $\text{CO}_2$ , about half of all that generated by human activities since 1800 (Sabine et al., 2004). The increase of  $p\text{CO}_2$  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 ( $\text{CO}_3^{2-}$ ) and the saturation state of the major shell-forming carbonate minerals:



Surface ocean pH is estimated to have decreased from approximately 8.2 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 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  $p\text{CO}_2$ . 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>).

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 version 2.0 which was recently released (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<sub>2</sub> measurements (Dickson et al., 2007), and five new functions have been added to assist the design of ocean acidification perturbation experiments. These new functions are described in the present paper.

## 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

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and atmosphere are in equilibrium with respect to  $\text{CO}_2$ . 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  $\text{CO}_2$  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  $p\text{CO}_2$  generated by bubbling gases. The syntax is (more information is available in the help files of the *seacarb* package):

`pgas(flag, var1, var2,  $p\text{CO}_2\text{g}$ , S, T, P, Sit, Pt)`

The input parameters below are required:

- **flag**: a number (1 to 24) indicating which couple of parameters of the carbonate system are known. For example, flag is 8 when  $\text{pH}_T$  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 and alkalinity in the example above). See the *seacarb* manual for more detail, especially regarding the units.
- **$p\text{CO}_2\text{g}$**  is the  $\text{CO}_2$  partial pressure (in  $\mu\text{atm}$ ) of the gas used to bubble the medium.
- **S**: salinity.
- **T**: temperature in  $^\circ\text{C}$
- **P**: hydrostatic pressure (P; 0 by default). Note that this function was only tested at  $P=0$ , that is in surface waters.

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- Pt: concentration of total phosphate ( $\text{mol kg}^{-1}$ ; 0 by default).
- Sit: concentration of total silicate ( $\text{mol kg}^{-1}$ ; 0 by default).

*Example:* Seawater with a  $p\text{CO}_2$  of  $384 \mu\text{atm}$  and TA of  $2325 \mu\text{mol kg}^{-1}$  is bubbled with a mixture of  $\text{CO}_2$  and air with a  $p\text{CO}_2$  of  $793 \mu\text{atm}$ . 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 pH goes above or below a set value. Gases are then delivered until pH reaches the set value again. Different combination of gases have been used: (1) air and pure  $\text{CO}_2$ , (2)  $\text{CO}_2$ -free air and pure  $\text{CO}_2$ , and (3) air,  $\text{CO}_2$ -free air and pure  $\text{CO}_2$ .  $\text{CO}_2$ -free air can be produced easily using  $\text{CO}_2$  scrubbers such as soda lime or NaOH and  $\text{Ca}(\text{OH})_2$  in low concentrations (Hintz, personal communication). The pH threshold is calculated using the desired  $p\text{CO}_2$  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  $\text{CaCO}_3$  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  $p\text{CO}_2$  better than  $\pm 10 \mu\text{atm}$ . The main drawback of this technique is that the pH electrode must be frequently calibrated in order to avoid drift. Hence, the other technique of bubbling with premixed gases may be attractive. Air with

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the desired  $p\text{CO}_2$  can be produced using gas mixing pumps or purchased. Another technique would be to control atmospheric  $p\text{CO}_2$  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  $p\text{CO}_2$  (Weinbauer, personal communication). Such bags are permeable to gases and small molecules in solution but impermeable to larger molecules and particles.

### 3.2 Addition of high- $\text{CO}_2$ sea water

DIC and 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:

$$[\text{DIC}]_{\text{mixed}} \times M_{\text{mixed}} = [\text{DIC}]_{\text{sw}} \times M_{\text{sw}} + [\text{DIC}]_{\text{high CO}_2} \times M_{\text{high CO}_2}$$

$$[\text{TA}]_{\text{mixed}} \times M_{\text{mixed}} = [\text{TA}]_{\text{sw}} \times M_{\text{sw}} + [\text{TA}]_{\text{high CO}_2} \times M_{\text{high CO}_2}$$

Where the subscripts “sw” and “high  $\text{CO}_2$ ”, respectively indicate concentrations in the normal and high  $\text{CO}_2$  seawater. Concentrations are in  $\mu\text{mol kg}^{-1}$  and  $M$  is the mass in kg. The *seacarb* function *pmix* estimates the carbonate chemistry after mixing of two water samples. Its syntax is:

`pmix(flag, var1, var2,  $p\text{CO}_2$ s, wf, S, T, P, Pt, Sit)`

flag, var1, var2, S, T and P where defined in Sect. 3.1. The other input parameters required are:

- $p\text{CO}_2\text{s}$ :  $p\text{CO}_2$  of the high- $\text{CO}_2$  water in  $\mu\text{atm}$
- wf: weight fraction of the high- $\text{CO}_2$  seawater vs. final weight

5 *Example:* One mixes, in a closed system, 0.99623 kg of seawater having a TA of  $2325 \mu\text{mol kg}^{-1}$ , and  $p\text{CO}_2$  of  $384 \mu\text{atm}$  with 0.00377 kg of seawater having TA of  $2325 \mu\text{mol kg}^{-1}$  and saturated with  $\text{CO}_2$  ( $p\text{CO}_2 = 1 \times 10^6 \mu\text{atm}$ ). The weight fraction, wf is  $3.77\text{e-}3$ . Salinity is 34.9, temperature is  $18.9^\circ\text{C}$  and calculations are made for surface waters. The command needed is:

10 `pmix(flag = 24, var1 = 384, var2 = 2325e-6, pCO2s = 1e6, wf = 3.77e - 3, S = 34.9, T = 18.9)`

This produces seawater with a final  $p\text{CO}_2$  of  $793 \mu\text{atm}$  and all parameters of the carbonate chemistry in 2100 are perfectly reproduced. To the best of our knowledge, this approach has only been used in the context of  $\text{CO}_2$  perturbation experiments carried out in mesocosms (Schulz et al., unpubl.).

### 15 3.3 Addition of $\text{CO}_3^{2-}$ and/or $\text{HCO}_3^-$

Dissolved inorganic carbon (DIC) and total alkalinity (TA) can be increased by adding  $\text{CO}_3^{2-}$  in the form of  $\text{Na}_2\text{CO}_3$  and/or by adding  $\text{HCO}_3^-$  in the form of  $\text{NaHCO}_3$ . In closed systems, the change in DIC generated by these additions is proportional to the changes in concentration:  $1 \times \Delta\text{CO}_3^{2-}$  and  $1 \times \Delta\text{HCO}_3^-$ . The contribution of these anions to TA is proportional to the product of their charge and concentration. Thus: TA increases by  $2 \times \Delta\text{CO}_3^{2-}$  and  $1 \times \Delta\text{HCO}_3^-$ . The changes in the carbonate chemistry generated by manipulations of total alkalinity therefore depend on the proportion of  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  added (Fig. 3). This approach can be used to hold pH constant or combined with acid addition to maintain TA constant (see Sect. 3.5).

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The *seacarb* function *pTA* computes the changes in the carbonate chemistry due to addition of  $\text{CO}_3^{2-}$  or  $\text{HCO}_3^-$ . Its syntax is:

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

*flag, var1, var2, S, T, P, Sit* and *Pt* where defined in the previous sections. 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 its is carried out in a system open to the atmosphere (in the later case, full equilibrium between air and water is assumed).
- *pCO2a*: the value of atmospheric  $p\text{CO}_2$  in  $\mu\text{atm}$ . It is only used in systems open to the atmosphere (i.e. *sys*=1).
- *CO3*: amount of  $\text{CO}_3^{2-}$  added in  $\text{mol kg}^{-1}$ .
- *HCO*: amount of  $\text{HCO}_3^-$  added in  $\text{mol kg}^{-1}$ .

In an open system, the carbonate system re-equilibrates through air-sea  $\text{CO}_2$  gas exchange after the addition of chemicals. At equilibrium:



*Example:*  $\text{HCO}_3^-$  ( $1081 \times 10^{-6} \text{ mol kg}^{-1}$ ) is added to seawater for which  $p\text{CO}_2$  (384  $\mu\text{atm}$ ) and TA (2325  $\mu\text{mol kg}^{-1}$ ) are known. No  $\text{CO}_3^{2-}$  is added. The atmospheric  $p\text{CO}_2$  is 384  $\mu\text{atm}$ , salinity is 34.9, temperature is 18.9°C and calculations are made for surface waters. The command needed in a closed and open system are, respectively:

*pTA(flag=24, sys=0, var1=384, var2=2325e-6, pCO2a=384, co3=0e-6, HCO3=878e-6, S=34.9, T=18.9)*

*pTA(flag=24, sys=1, var1=384, var2=2325e-6, pCO2a=384, co3=0e-6, HCO3=878e-6, S=34.9, T=18.9)*

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Results are shown in Fig. 3 and Table 2. In a closed system, the target  $p\text{CO}_2$  of 793  $\mu\text{atm}$  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 (7.993 instead of 7.941) and TA, DIC,  $[\text{CO}_3^{2-}]$ ,  $\Omega_a$ , and  $\Omega_c$  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 in seawater limits coral calcification. Langdon et al. (2000) used this approach in the Biosphere 2 coral reef mesocosm. They used additions  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  to increase the  $\text{CaCO}_3$  saturation state. Since the experiment lasted several months, additions of  $\text{CaCl}_2$  were used to restore a normal  $\text{Ca}^{2+}$  concentration. The manipulations of the  $\text{CaCl}_2$  concentration is described in Sect. 3.6.

### 3.4 Addition of strong acids and bases

The carbonate chemistry can be manipulated by addition a strong acid or a strong base, such as HCl and NaOH, which dissociate fully in seawater. The addition of a strong acid or base in a system closed to the atmosphere does not alter the concentration of dissolved inorganic carbon (DIC) but modifies total alkalinity (TA). TA decreases following addition of acid and increases following 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  $\text{CO}_2$  exchange at the air-water interface in order to equilibrate the partial pressure of  $\text{CO}_2$  in the atmosphere and seawater.

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. The syntax is:

ppH(flag, sys, var1, var2, pCO<sub>2</sub>a, vol, N, S, T, P, Sit, Pt)

flag, sys, var1, var2, pCO<sub>2</sub>a, S, T, P, Sit and Pt where defined in sections 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 acid additions and a positive sign for base additions.
- N: the normality of the acid or base (mol kg<sup>-1</sup>).

*Example:* A volume of 14.18 ml of 0.01 N HCl is added to 1 kg of seawater having a known pCO<sub>2</sub> (384 μatm) and TA (2325×10<sup>-6</sup> mol kg<sup>-1</sup>). The atmospheric pCO<sub>2</sub> is 384 μatm, salinity is 34.9 and temperature is 18.9°C. Calculations are made for surface waters. The command needed in a closed and open system are:

```
ppH(flag=24, sys=0, var1=384, var2=2325e-6, pCO2a=384, vol=-0.1418e-3, N=0.01, S=34.9, T=18.9)
```

```
ppH(flag=24, sys=1, var1=384, var2=2325e-6, pCO2a=384, vol=-0.1418e-3, N=0.01, S=34.9, T=18.9)
```

Results are shown in Fig. 4 and Table 2. The target pCO<sub>2</sub> of 793 μatm is reached in a closed system but pH is lower than the value expected in 2100 (7.768 instead of 7.993, corresponding to a 5.6% increase in [H<sup>+</sup>] that results from the decrease in total alkalinity generated by acid addition). 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<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and the CaCO<sub>3</sub> saturation states are lower than their target values. However, it is possible to restore TA to its initial level by adding of CO<sub>3</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup>, an approach that is described in Sect. 3.5. Obviously, due to gas exchange at the air-water interface, pCO<sub>2</sub> and other parameters of the carbonate chemistry cannot be successfully adjusted by a one-time acid addition in an open system.

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Acid addition 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 $\text{CO}_3^{2-}$ and/or $\text{HCO}_3^-$

As mentioned in Sect. 3.4, the addition of acid does not enable to fully mimic the changes in carbonate chemistry expected during the present century but the addition of  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$  followed by acid circumvents this problem. The first addition elevates DIC to the desired level and the acid addition (at constant DIC) precisely cancels the increase in TA resulting from the addition of  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$

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

*Example:*  $\text{HCO}_3^-$  ( $111.3 \times 10^{-6} \text{ mol kg}^{-1}$ ) and  $\text{CO}_3^{2-}$  ( $15.3 \times 10^{-6} \text{ mol kg}^{-1}$ ) are added to seawater for which  $p\text{CO}_2$  ( $384 \mu\text{atm}$ ) and TA ( $2325 \mu\text{mol kg}^{-1}$ ) are known. The atmospheric  $p\text{CO}_2$  is  $384 \mu\text{atm}$ , salinity is 34.9, temperature is  $18.9^\circ\text{C}$  and calculations are made for surface waters. Then, 14.18 ml of 0.01 N HCl is added. The commands needed are:

```
tmp = pTA(flag=24, sys=0, var1=384, var2=2325e-6, pCO2a=384,  
CO3=15.3e-6, HCO3=111.3e-6, S=34.9, T=18.9, P=0, Pt=0, Sit=0)
```

```
ppH(flag=24, sys=0, var1=tmp pCO2[2], var2=tmp ALK[2], pCO2a=384,  
vol=-0.1418e-3, N=1, S=34.9, T=18.9, P=0, Pt=0, Sit=0))
```

Results are shown in Table 2. The first addition raises DIC to the desired level of  $2190 \times 10^{-6} \text{ mol kg}^{-1}$  but increases TA to a value higher than target ( $2467$  vs.  $2325 \times 10^{-6} \text{ mol kg}^{-1}$ ). The subsequent addition of HCl restores TA to the desired value without affecting DIC. All carbonate parameters after both additions are on the target values (Table 2).

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To our knowledge, the first paper which used addition of acid, base and  $\text{NaHCO}_3$  to manipulate the carbonate chemistry within a range compatible with ocean acidification is that of Borowitzka (1981) who investigated, in the laboratory, the response of photosynthesis and calcification of an articulated coralline red algae.

### 5 3.6 Manipulation of the $\text{Ca}^{2+}$ concentration

Although manipulating the calcium concentration is not technically altering 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 ( $\Omega$ ) which is expressed as:

$$10 \quad \Omega = \frac{[\text{Ca}^{2+}]_{\text{SW}} \times [\text{CO}_3^{2-}]_{\text{SW}}}{K_{\text{sp}}^*}$$

where  $[\text{Ca}^{2+}]_{\text{SW}}$  and  $[\text{CO}_3^{2-}]_{\text{SW}}$  are the concentrations of calcium and carbonate in seawater, respectively, and  $K_{\text{sp}}^*$  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  $\Omega$  resulting from decrease in  $[\text{CO}_3^{2-}]$  driven by ocean acidification can be mimicked by altering  $[\text{Ca}^{2+}]_{\text{SW}}$ . This approach involves the use of artificial seawater (Gattuso et al., 1998). It is recommended to use the simplified synthetic sea water recipe described by Gattuso et al. (1998) and based on DOE (1994) because it is the basis of the synthetic seawater that has been used to determine a variety of equilibrium constants for use in seawater.

20 The *seacarb* function  $p\text{Ca}$  estimates the changes in  $\Omega_c$  and  $\Omega_a$  resulting from the manipulation of the  $\text{Ca}_{\text{SW}}^{2+}$  concentration. Note that this function does not account for the effect of the changes in the calcium concentration of the dissociation constants of carbonic acid and on the solubility product of  $\text{CaCO}_3$  (Ben-Yaakov and Goldhaber, 1973). The syntax is:

25  $p\text{Ca}(\text{flag}, \text{sys}, \text{var1}, \text{var2}, \text{Ca}, \text{S}, \text{T}, \text{P}, \text{Sit}, \text{Pt})$

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flag, sys, var1, var2, S, T, P, Sit and Pt where defined in the previous sections. Ca is the calcium concentration in  $\text{mol kg}^{-1}$ .

*Example:* Artificial seawater is made with a known TA ( $2325 \mu\text{mol kg}^{-1}$ ) and DIC ( $2064 \mu\text{mol kg}^{-1}$ ). The calcium concentration is set to  $5.98 \times 10^{-6} \text{mol kg}^{-1}$ . The command needed is:

$p\text{Ca}(\text{flag} = 15, \text{var1} = 2325\text{e}-6, \text{var2} = 2063\text{e} - 6, \text{Ca} = 5.98\text{e}-3, \text{S} = 34.9, \text{T} = 18.9)$

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).

## 4 Discussion and conclusion

Three approaches closely mimic the on-going and future changes in the seawater carbonate chemistry: gas bubbling, addition of high- $\text{CO}_2$  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, which 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  $\text{CaCO}_3$ , nutrient uptake and release) can alter the carbonate chemistry by changing total alkalinity even though

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the concentration of dissolved inorganic carbon remains constant. Also, in all three approaches, calcification can deplete the concentration of  $\text{Ca}^{2+}$  when the organism to volume ratio is high or the incubation time long.

The approach that adds  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$  appears of limited practical because most carbonate chemistry variables reach values that far from target values. Several studies have used acid addition to manipulate the carbonate chemistry. Although this technique offers precise control of  $p\text{CO}_2$  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 under natural ocean acidification), and the  $\text{CaCO}_3$  saturation state) as well as the concentrations of bicarbonate and carbonate. 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  $p\text{CO}_2$ . 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 acid addition (changing TA at constant DIC) (Iglesias-Rodriguez et al., 2008a,b). The argument is that acid addition does not reproduce the increase in the  $\text{HCO}_3^-$  concentration generated by natural ocean acidification whereas gas bubbling does reproduce it. The authors claim that  $\text{HCO}_3^-$  may stimulate photosynthesis and, in turn, calcification. They conclude that experiments which used acid addition and demonstrated 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. They also reported lower rates of calcification of coccolithophores at lower pH and higher  $p\text{CO}_2$  (Sciandra et al., 2003; Delille et al., 2005; Feng et al., 2008). Second, the concentrations of  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,

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$\text{CO}_3^{2-}$ , and  $\text{H}^+$  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 in the same direction. Differences in the two approaches cannot explain the opposite directions of the biological responses. 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 is clearer in zooxanthellate scleractinian corals. Schneider and Erez (2006) measured the rate of calcification under constant DIC, constant pH and constant  $p\text{CO}_2$  and showed that calcification is controlled by the concentration of  $\text{CO}_3^{2-}$ , the future value of which is relatively well mimicked by acid addition.

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://epoca-project.eu>).

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## References

- Agegian, C. R.: The biogeochemical ecology of *Porolithon gardineri* (Foslie), Ph.D. thesis, University of Hawaii, 1985. 4415, 4424
- 10 Ben-Yaakov, S. and Goldhaber, M.: The influence of sea water composition on the apparent constants of the carbonate system, *Deep-Sea Res.*, 20, 87–99, 1973. 4425
- Borowitzka, M. A.: Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps* and *A. foliacea*, *Mar. Biol.*, 62(1), 17–23, 1981. 4425
- Bouxin, H.: Action des acides sur le squelette des larves de l'oursin *Paracentrotus lividus*. Influence du pH, *C. R. Soc. Biol.*, 94, 453–455, 1926. 4415
- 15 Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Borges, A. V., Riebesell, U., and Gattuso, J.-P.: Response of primary production and calcification to changes of  $p\text{CO}_2$  during experimental blooms of the coccolithophorid *Emiliania huxleyi*, *Global Biogeochem. Cy.*, GB2023, doi:10.1029/2004GB002318, 2005. 4427
- 20 Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean  $\text{CO}_2$  measurements, *PICES Special Publication*, 3, 1–191, 2007. 4415, 4416
- DOE: Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water, version 2, ORNL/CDIAC; location is Oak Ridge, Tennessee, 1994. 4425

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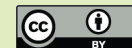
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- Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbruggen, A., and Zondervan, I.: Transparent exopolymer particles and dissolved organic carbon production by *Emiliana huxleyi* exposed to different CO<sub>2</sub> concentrations: a mesocosm experiment, *Aquat. Microb. Ecol.*, 34(1), 93–104, 2004. 4419
- 5 Feng, Y., Warner, M., Zhang, Y., Sun, J., Fu, F., Rose, J., and Hutchins, D.: Interactive effects of increased pCO<sub>2</sub>, temperature and irradiance on the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae), *Eur. J. Phycol.*, 43, 87–98, 2008. 4427
- Garcia, H., Locarnini, R., Boyer, T., and Antonov, J.: World Ocean Atlas 2005. Volume 4: Nutrients (phosphate, nitrate, silicate), in: NOAA Atlas NESDIS 64, edited by: Levitus, S.,  
10 1–396, US Government Printing Office, Washington, DC, 2006. 4433
- Gattuso, J.-P., Frankignoulle, M., Bourge, I., Romaine, S., and Buddemeier, R. W.: Effect of calcium carbonate saturation of seawater on coral calcification, *Glob. Planet Change*, 18, 37–46, 1998. 4425, 4426
- Iglesias-Rodriguez, M., Halloran, P., Rickaby, R., Hall, I., Colmenero-Hidalgo, E., Gittins, J.,  
15 Green, D., Tyrrell, T., Gibbs, S., von Dassow, P., Rehm, E., Armbrust, E., and Boessenkool, K.: Phytoplankton calcification in a high-CO<sub>2</sub> world, *Science*, 320, 336–340, 2008a. 4427
- Iglesias-Rodriguez, M. D., Buitenhuis, E. T., Raven, J. A., Schofield, O., Poulton, A. J., Gibbs, S., Halloran, Paul, R., and de Baar, H. J. W.: Response to Comment on Phytoplankton calcification in a high-CO<sub>2</sub> world, *Science*, 322, 1466c–1466c, 2008b. 4427
- 20 Keeling, R., Piper, S., Bollenbacher, A., and Walker, J.: Atmospheric CO<sub>2</sub> records from sites in the SIO air sampling network, in: Trends: a compendium of data on global change, Oak Ridge National Laboratory, US Department of Energy, 2008. 4433, 4434
- Kleypas, J. A., Feely, R. A., Fabry, V. J., Langdon, C., Sabine, C. L., and Robbins, L. L.: Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research, Institute for the Study of Society and Environment (ISSE) of the University Corporation for Atmospheric Research (UCAR), Boulder, Colorado, 2006. 4415
- 25 Langdon, C.: Review of experimental evidence for effects of CO<sub>2</sub> on calcification of reef builders, in: Proceedings of the 9th International Coral Reef Symposium, 1091–1098, Bali, 2000. 4416
- 30 Langdon, C., Takahashi, T., Marubini, F., Atkinson, M., Sweeney, C., Aceves, H., Barnett, H., Chipman, D., and Goddard, J.: Effect of calcium carbonate saturation state on the rate of calcification of an experimental coral reef, *Global Biogeochem. Cy.*, 14, 639–654, 2000. 4422, 4426

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Lavigne, H., Proye, A., and Gattuso, J.-P.: seacarb 2.0, an R package to calculate parameters of the seawater carbonate system, <http://cran.r-project.org/web/packages/seacarb/index.html>, 2008. 4416, 4433, 4434

Lueker, T. J., Dickson, A., and Keeling, C. D.: Ocean  $p\text{CO}_2$  calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of  $\text{CO}_2$  in gas and seawater at equilibrium, *Mar. Chem.*, 70(1–3), 105–119, 2000. 4416, 4433

MARGO Project Members: Constraints on the magnitude and patterns of ocean cooling at the Last Glacial Maximum, *Nat. Geosci.*, 2, 127–132, 2009. 4433

Marubini, F. and Thake, B.: Bicarbonate addition promotes coral growth, *Limnol. Oceanogr.*, 44(3), 716–720, 1999. 4422

Nakićenović, N. and Swart, R. (eds.): Special report on emissions scenarios : a special report of Working Group III of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, 2000. 4433

Paul, A. and Schäfer-Neth, C.: Modeling the water masses of the Atlantic Ocean at the Last Glacial Maximum, *Paleoceanography*, 18, 1058, doi:10.1029/2002PA000783, 2003. 4433

Petit, J. R., Jouzel, J., Raynaud, D., Barkov, N., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, J., Delaygue, G., Delmotte, M., Kotlyakov, V., Legrand, M., Lipenkov, V., Lorius, C., Ppin, L., Ritz, C., Saltzman, E., and Stievenard, M.: Vostok ice core data for 420,000 years, vol. Contribution Series #2001-076, NOAA/NGDC Paleoclimatology Program, 2001. 4433

Plattner, G., Joos, F., Stocker, T., and Marchal, O.: Feedback mechanisms and sensitivities of ocean carbon uptake under global warming, *Tellus B*, 53, 564–592, 2001. 4433, 4434

Proye, A. and Gattuso, J.-P.: seacarb, an R package to calculate parameters of the seawater carbonate system, <http://cran.r-project.org/web/packages/seacarb/index.html>, 2003. 4416

R Development Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>, ISBN 3-900051-07-0, 2008. 4416

Riebesell, U., Bellerby, R. G. J., Engel, A., Fabry, V. J., Hutchins, D. A., Reusch, T. B. H., Schulz, K. G., and Morel, F. M. M.: Comment on “Phytoplankton calcification in a high- $\text{CO}_2$  world”, *Science*, 322, 1466b–1466b, 2008. 4428

Sabine, C., Feely, R., Gruber, N., Key, R., Lee, K., Bullister, J., Wanninkhof, R., Wong, C., Wallace, D., Tilbrook, B., Millero, F., Peng, T., Kozyr, A., Ono, T., and Rios, A.: The oceanic

- sink for anthropogenic CO<sub>2</sub>, *Science*, 305(5682), 367–371, 2004. 4415
- Schneider, K. and Erez, J.: The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*, *Limnol. Oceanogr.*, 51, 1284–1293, 2006. 4428
- 5 Sciandra, A., Harlay, J., Lefvre, D., Leme, R., Rimmelin, P., Denis, M., and Gattuso, J.-P.: Response of the coccolithophorid *Emiliana huxleyi* to elevated partial pressure of CO<sub>2</sub> under nitrate limitation, *Mar. Ecol.-Prog. Ser.*, 261, 111–122, 2003. 4427
- Shi, D., Xu, Y., and Morel, F.: Effects of the pH/pCO<sub>2</sub> control method in the growth medium of phytoplankton, *Biogeosciences Discuss.*, 6, 2415–2439, 2009,  
10 <http://www.biogeosciences-discuss.net/6/2415/2009/>. 4419, 4428
- Smith, A. D. and Roth, A. A.: Effect of carbon dioxide concentration on calcification in the red coralline alga *Bossiella orbigniana*, *Mar. Biol.*, 52, 217–225, 1979. 4418
- Swift, E. and Taylor, W. R.: The effect of pH on the division rate of the coccolithophorid *Cricosphaera elongata*, *J. Phycol.*, 2, 121–125, 1966. 4422
- 15 Trimborn, S., Langer, G., and Rost, B.: Effect of varying calcium concentrations and light intensities on calcification and photosynthesis in *Emiliana huxleyi*, *Limnol. Oceanogr.*, 52, 2285–2293, 2007. 4426
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Krtzinger, A., and Dickson, A. G.: Total alkalinity: The explicit conservative expression and its application to biogeochemical processes, *Mar. Chem.*, 106, 287–300, 2007. 4419
- 20 Zeebe, R. E. and Wolf-Gladrow, D. A.: CO<sub>2</sub> in seawater: equilibrium, kinetics, isotopes, Elsevier, Amsterdam, 2001. 4416, 4425

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**Table 1.** Changes in the carbonate chemistry of surface seawater from the Last Glacial Maximum (LGM; 18 ky B.P.) to 2100. Total alkalinity, CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ), 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<sub>2</sub>. Values of temperature, salinity, total alkalinity and total phosphate in 1766, 2007 and 2100 are from Plattner et al. (2001) prescribing historical CO<sub>2</sub> records and non-CO<sub>2</sub> 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.  $p\text{CO}_2$  in 1766 and 2100 are from Plattner et al. (2001), while values in the LGM and 2007 are, respectively from Petit et al. (2001) and Keeling et al. (2008). 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 <sup>-6</sup> mol kg <sup>-1</sup>	0.66	0.66	0.63	0.55
Total silicate	10 <sup>-6</sup> mol kg <sup>-1</sup>	7.35	7.35	7.35	7.35
Total alkalinity	10 <sup>-6</sup> mol kg <sup>-1</sup>	2399	2326	2325	2310
CO <sub>2</sub> partial pressure (seawater)	µatm	180	267	384	793
[CO <sub>2</sub> ]	10 <sup>-6</sup> mol kg <sup>-1</sup>	6.26	9.05	12.8	24.7
[HCO <sub>3</sub> <sup>-</sup> ]	10 <sup>-6</sup> mol kg <sup>-1</sup>	1660	1754	1865	2020
[CO <sub>3</sub> <sup>2-</sup> ]	10 <sup>-6</sup> mol kg <sup>-1</sup>	299	231	186	118
Dissolved inorganic carbon	10 <sup>-6</sup> mol kg <sup>-1</sup>	1966	1994	2064	2162
pH <sub>T</sub>	–	8.33	8.2	8.07	7.79
[H <sup>+</sup> ]	10 <sup>-9</sup> mol kg <sup>-1</sup>	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

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**Table 2.** Seawater carbonate chemistry in 2007, in 2100, and after perturbation. Total alkalinity (TA), partial pressure of CO<sub>2</sub> in seawater ( $p\text{CO}_{2\text{sw}}$ ), 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  $p\text{CO}_{2\text{sw}}$ . For the sake of 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  $2325 \times 10^{-6} \text{ mol kg}^{-1}$ . The concentrations of total phosphate and silicate were set to  $0.63 \times 10^{-6}$ – $0.55 \times 10^{-6}$ , respectively. The seawater  $p\text{CO}_2$  was set at 384 in 2007 (Keeling et al., 2008) and 793  $\mu\text{atm}$  in 2100 (according to the A2 SRES emission scenario; Plattner et al., 2001). The main body of the text provides further information. (a):  $\times 10^{-9} \text{ mol kg}^{-1}$ , (b):  $\times 10^{-6} \text{ mol kg}^{-1}$ .

	$p\text{CO}_{2\text{sw}}$ ( $\mu\text{atm}$ )	$p\text{H}_T$ (–)	$[\text{H}^+]$ (a)	TA (b)	DIC (a)	$[\text{CO}_2]$ (a)	$[\text{HCO}_3^-]$ (a)	$[\text{CO}_3^{2-}]$ (a)	$\Omega_c$ (–)	$\Omega_a$ (–)
Year 2007	384	8.065	8.6	2325	2064	12.8	1865	186	4.5	2.9
Year 2100	793	7.793	16.1	2325	2190	26.4	2054	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 <sub>2</sub> seawater	793	7.793	16.1	2325	2190	26.5	2054	110	2.6	1.7
Addition of CO <sub>3</sub> <sup>2-</sup> and HCO <sub>3</sub> <sup>-</sup> ; closed	793	7.942	11.4	3406	3145	26.4	2900	218	5.2	3.4
Addition of CO <sub>3</sub> <sup>2-</sup> and HCO <sub>3</sub> <sup>-</sup> ; open	384	8.206	6.2	3406	2949	12.8	2579	357	8.5	5.5
Acid addition; closed	793	7.768	17.1	2183	2064	26.4	1940	98	2.3	1.5
Acid addition; open	384	8.042	9.1	2183	1946	12.8	1766	167	4	2.6
Addition of:										
CO <sub>3</sub> <sup>2-</sup> and HCO <sub>3</sub> <sup>-</sup> ; closed	400	8.073	8.4	2467	2190	13.3	1976	201	4.8	3.1
followed by acid addition; closed	793	7.795	16.0	2325	2190	26.3	2054	110	2.6	1.7
Manipulation of $[\text{Ca}^{2+}]$	384	8.065	8.6	2325	2063	12.8	1865	186	2.6	1.7

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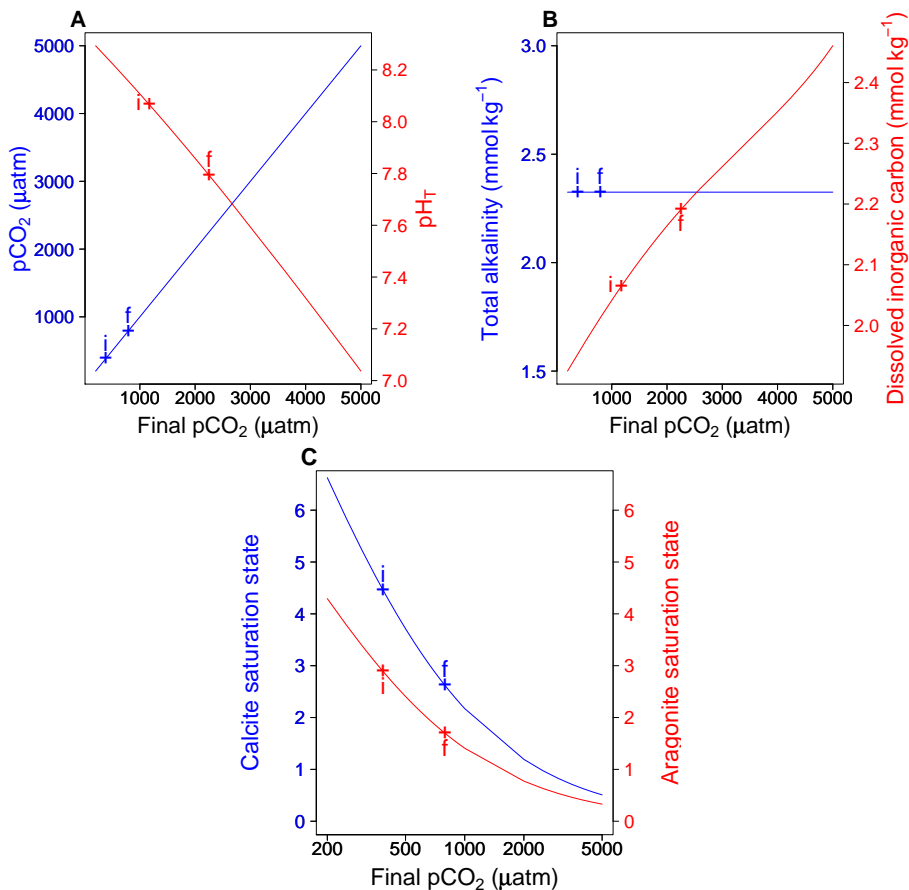
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**Fig. 1.** Effect of bubbling seawater with gases of varying  $p\text{CO}_2$  on the carbonate chemistry of seawater as computed with the *seacarb* function *pgas*. The letters “i” and “f” are, respectively, the value of a parameter before and after perturbation for the example described in the text.

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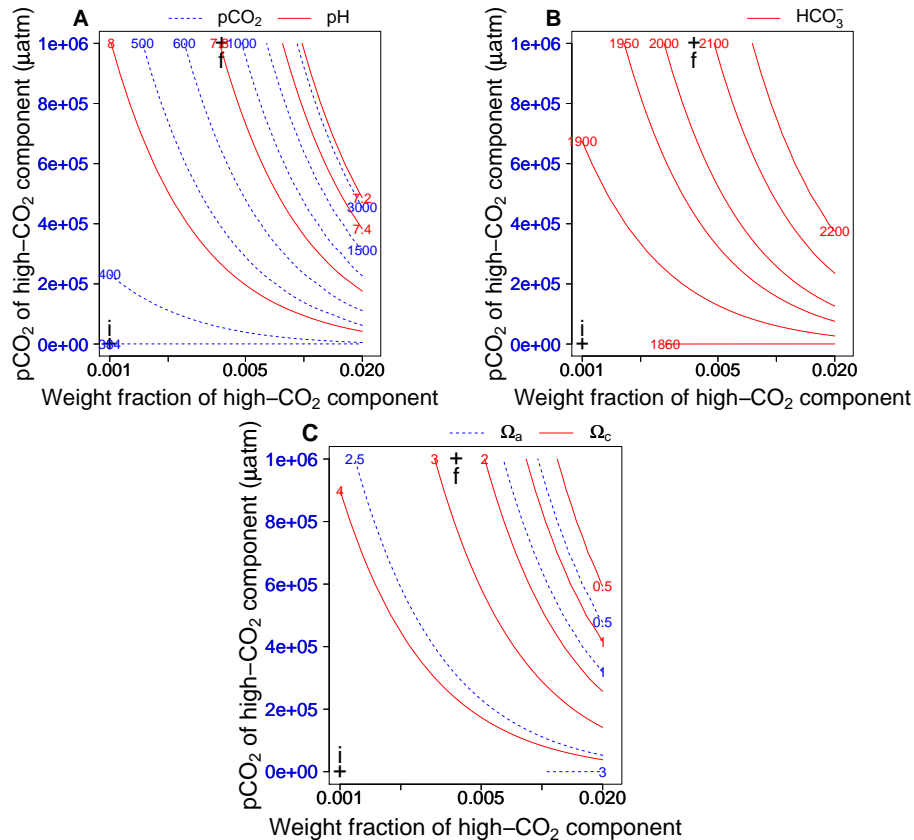
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**Fig. 2.** Effect of mixing with high- $p\text{CO}_2$  seawater in a closed system as computed with the *seacarb* function *pmix*. The letters “i” and “f” are, respectively, the value of a parameter before and after perturbation for the example described in the text.

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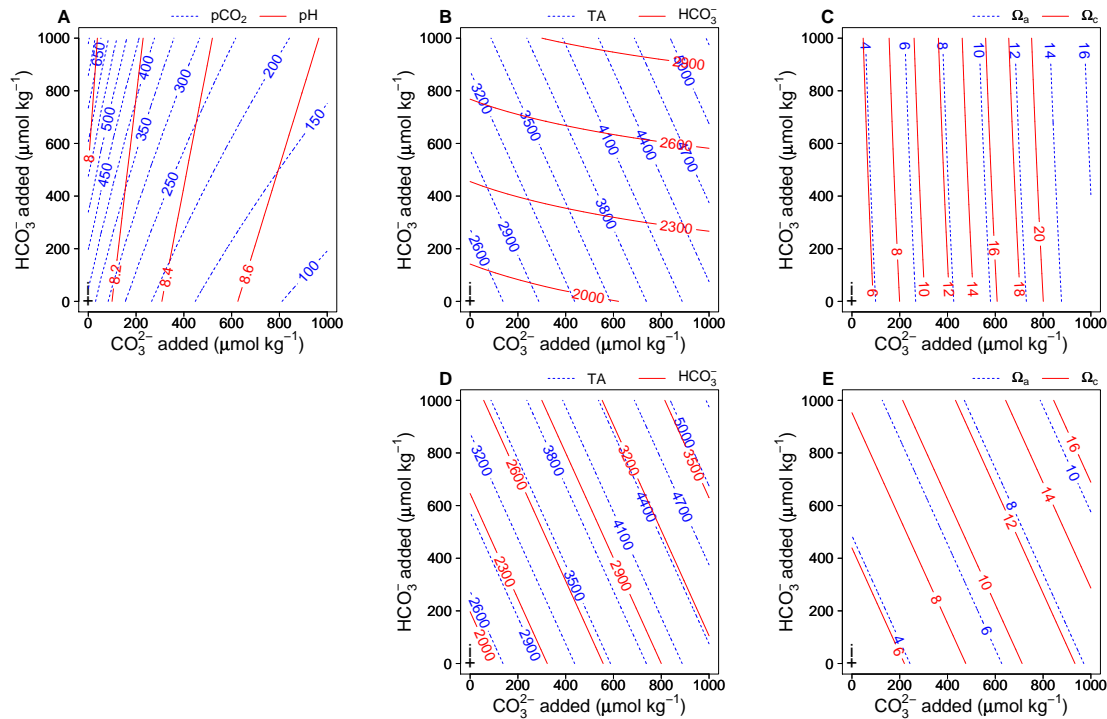
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**Fig. 3.** Effect of  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  addition in a closed (**A**, **B** and **C**) and open system (**D** and **E**) as computed with the *seacarb* function pTA. The letters “i” and “f” are, respectively, the value of a parameter before and after perturbation for the example described in the text.

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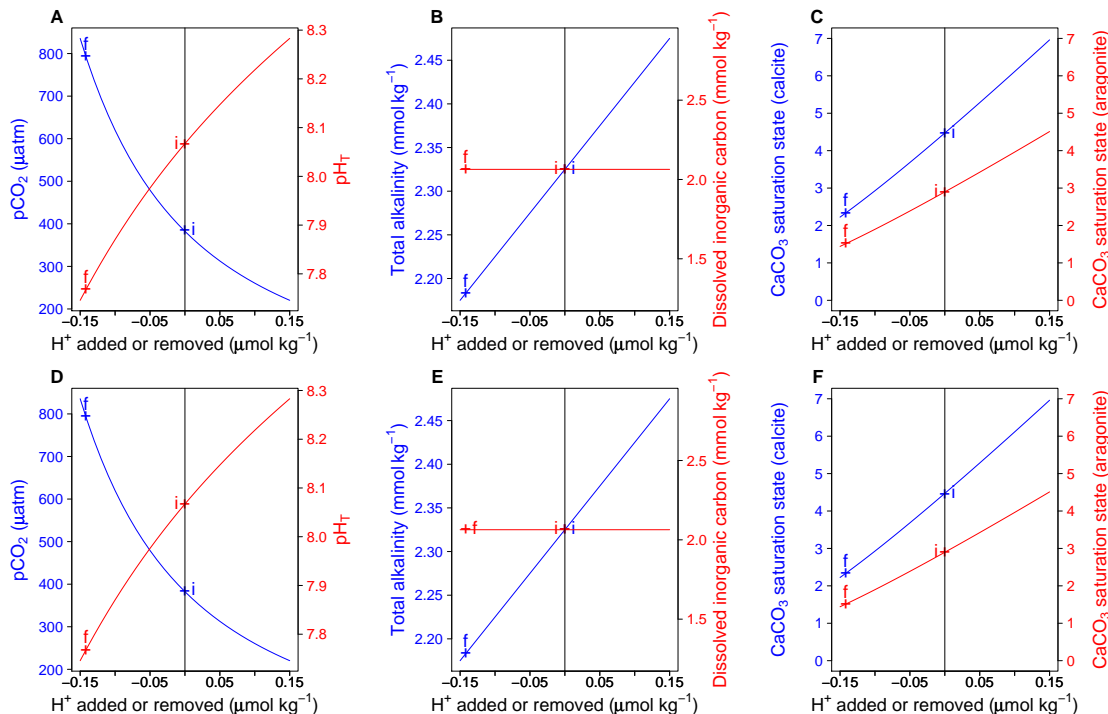
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**Fig. 4.** Effect of addition of a strong acid or a strong base in closed (A, B and C) and open systems (D, E and F) as computed with the *seacarb* function pgas. By convention, addition of acid is negative whereas addition of base is positive. The letters “i” and “f” are, respectively, the value of a parameter before and after perturbation for the example described in the text.

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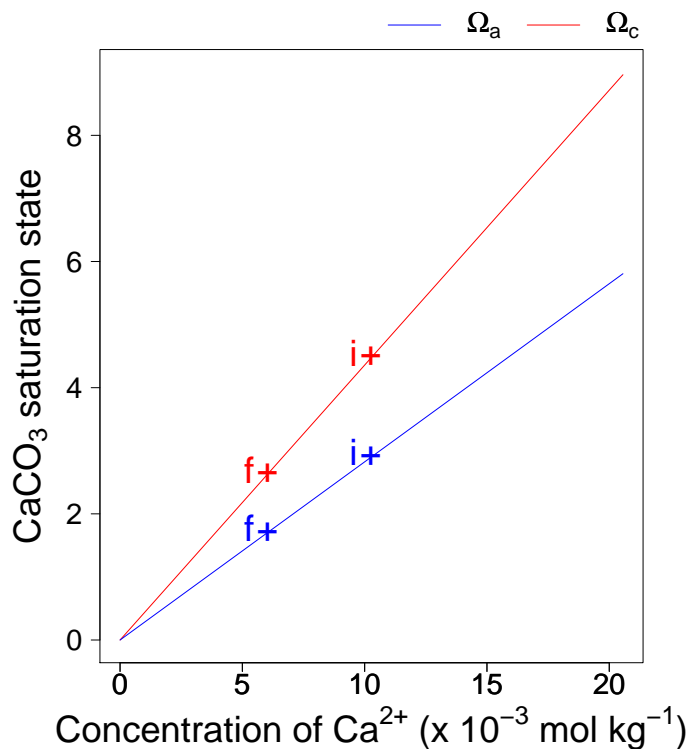
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**Fig. 5.** Calcium carbonate saturation state as a function of the calcium concentration as computed with the *seacarb* function  $pCa$ . The letters “i” and “f” are, respectively, the value of Calcium carbonate saturation state before and after perturbation for the example described in the text.

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