

## 1 The carbon dioxide system in seawater: equilibrium chemistry and measurements

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### 1.1 Introduction

The world's oceans can be thought of as a dilute solution of sodium bicarbonate (together with other acid-base species at still lower concentrations) in a saltwater background. In the surface waters of the North Atlantic, for example, the concentration of total dissolved inorganic carbon (the sum of the concentrations of the three coexisting species: bicarbonate ion, carbonate ion, and unionised dissolved carbon dioxide) is only about 2 mmol kg<sup>-1</sup>. About 90% of this is present as bicarbonate ion, the proportion of carbonate ion is about a factor of 10 less (~10%), and that of unionised carbon dioxide yet another factor of 10 less (<1%). As a result of the equilibria between these various species (see below), seawater is buffered (weakly) with respect to changes in hydrogen ion (present at much lower concentrations: <10<sup>-8</sup> μmol kg<sup>-1</sup>).

Over the past twenty years, accurate measurement of the seawater carbon dioxide system has become a high priority for scientists who have worked to understand just how much of the carbon dioxide (CO<sub>2</sub>) created by man's activities has ended up in the ocean, where it is distributed, and how it has changed the chemistry of the oceans. The chemical changes associated with the increase of CO<sub>2</sub> in the oceans are often referred to as *ocean acidification*. As we work to design suitable experiments to understand the biological and ecological consequences of such changes, it is important that the chemistry of CO<sub>2</sub> be well characterised in the various laboratory experiments and field observations that are undertaken. Achieving this requires an understanding of the basic solution chemistry underlying ocean acidification, as well as of the relative merits of the various analytical techniques available to the investigator.

Unfortunately – from the point of view of someone desiring simplicity – in addition to carbon dioxide there are other acid-base systems in seawater that complicate things, particularly in systems that are not typical of the open surface ocean, with its low nutrient levels and relatively low amounts of dissolved organic material. The approach I shall take in this chapter is to introduce first a somewhat simplified view of acid-base chemistry in seawater involving only the primary seawater acid-base systems: carbonic acid, boric acid and water. These will be discussed in some detail, and used to introduce the classical oceanographic analytical parameters for carbon dioxide studies in seawater: total dissolved inorganic carbon, total alkalinity, pH, and p(CO<sub>2</sub>) – the partial pressure of carbon dioxide that is in equilibrium with a water sample (Box 1.1). The concept of calcium carbonate saturation state will also be introduced.

Once this basic seawater chemistry has been presented – and assimilated – it will be appropriate to revisit a number of these topics and to introduce further complexity, so as to clarify how these various concepts can be applied appropriately in the seawater systems that are of interest to investigators in ocean acidification. Finally, I shall present a brief discussion of some of the current techniques available for the measurement of the various parameters of the seawater carbon dioxide system, and will indicate their advantages and disadvantages. The advantages and disadvantages of using alternate combinations of parameters to provide a complete description of the composition of a particular seawater sample will also be discussed.

As will become clear, at this time it is not as straightforward as one might wish to characterise the state of a particular seawater sample's carbonate chemistry and to assign a well-constrained measurement uncertainty. Investigators who wish to do high quality work in ocean acidification, but who have little previous experience in seawater CO<sub>2</sub> measurements, would do well to collaborate with a scientist with experience in this area and who has access to a working laboratory that can perform the necessary measurements with the required quality.

### Box 1.1: Terminology and units for parameters relevant to the carbonate system

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Research in ocean acidification brings together various scientific disciplines such as chemistry, geology, biogeochemistry, ocean physics and various sub-disciplines of biology and ecology (biological oceanography, marine ecology and ecological physiology, biochemistry, physiological chemistry and molecular biology). Each of these disciplines generally investigates ocean acidification from its own point of view, building on its own traditions with the goal of providing the highest possible accuracy under the constraints of each field. Ideally, efficient communication should use a unified set of terms and units in scientific presentations, discussions and publications and when differences exist, they must be clearly documented and understood. A large number of terms and units are used to describe the physicochemical properties of the carbonate system in seawater and in the biological material and fluids that interact with seawater (Table). Marine chemistry uses them to quantify changes in seawater acid-base composition. Acid-base physiology uses similar terms to estimate the quantities of protons or base equivalents moving between water and organism as well as between body compartments causing changes in body fluid composition (e.g. Pörtner *et al.*, 1991). The aim of this box is to alert readers to parameters (e.g. pH, dissolved inorganic carbon) that are defined differently in marine chemistry (see chapter 1) and physiology (see chapter 9) and to describe the main terms and units used in this guide.

pH is the parameter that causes most difficulties. Marine chemistry has developed the total hydrogen ion concentration scale. It requires buffers prepared in synthetic seawater for calibration (Hansson, 1973; Dickson *et al.*, 2007). This scale includes the effect of sulfate ion in its definition. From a physiological perspective, the use of a free hydrogen ion concentration scale would be more appropriate than the total scale as it does not include sulfate protonation in its definition. It is possible to convert a pH value from the total scale to the free scale and vice versa (Zeebe & Wolf-Gladrow, 2001) in seawater of a known salinity, and software tools are available to achieve this (Lavigne & Gattuso, 2010). The free scale could also be used to express pH of the extracellular fluids of marine invertebrates. However, neither the total scale nor the free scale can be used straightforwardly for pH determinations in intracellular fluids and in extracellular fluids of vertebrates, which have ionic strengths of about one third of that of seawater. The conventional NBS pH scale is therefore commonly used in physiology for such measurements.

The sum of the concentrations of all inorganic carbon species is termed “total dissolved inorganic carbon” (DIC or  $C_T$ ) in the field of marine chemistry and “total  $\text{CO}_2$ ” ( $\text{Cco}_2$ ) in the field of physiology. These terms are not always synonymous, especially in body fluids where  $\text{Cco}_2$  may also include inorganic  $\text{CO}_2$  species bound to protein. Furthermore, different (though related) titration procedures are used to determine total alkalinity (by use of strong acid) in seawater and titratable acid(ity) (by use of strong base) in physiological fluids like urine. In tissues and blood, the  $\text{CO}_2$ /bicarbonate buffer system is distinguished from non-bicarbonate buffers, when analysing the “titration” of the latter by accumulating  $\text{CO}_2$ , by metabolic influences, or during proton-equivalent ion exchange.

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**Table:** Main parameters describing the physicochemistry of seawater and body fluids and their differences in the fields of marine chemistry and physiology. The notation and units used in this guide are also shown. Alternate notations or units are given in parentheses.

Parameter	Marine chemistry		Physiology	
	Notation	Unit	Notation	Unit
pH <sup>(1)</sup>	Total scale	-	NBS or NIST scale <sup>(2)</sup>	-
Partial pressure of CO <sub>2</sub>	p(CO <sub>2</sub> ) (pCO <sub>2</sub> , Pco <sub>2</sub> , p(CO <sub>2</sub> ))	µatm	Pco <sub>2</sub>	kPa (mm Hg, Torr, µatm)
CO <sub>2</sub> solubility	K <sub>0</sub>	mol kg <sup>-1</sup> atm <sup>-1</sup>	αco <sub>2</sub>	mmol l <sup>-1</sup> mm Hg <sup>-1</sup> (kPa <sup>-1</sup> )
Dissolved inorganic carbon or total CO <sub>2</sub>	DIC (C <sub>T</sub> , ΣCO <sub>2</sub> , Tco <sub>2</sub> )	mol kg <sup>-1</sup>	Cco <sub>2</sub>	mol l <sup>-1</sup>
Bicarbonate concentration	[HCO <sub>3</sub> <sup>-</sup> ]	mol kg <sup>-1</sup>	[HCO <sub>3</sub> <sup>-</sup> ]	mol l <sup>-1</sup>
Carbonate concentration	[CO <sub>3</sub> <sup>2-</sup> ]	mol kg <sup>-1</sup>	[CO <sub>3</sub> <sup>2-</sup> ]	mol l <sup>-1</sup>
Ammonium concentration	[NH <sub>4</sub> <sup>+</sup> ]	mol kg <sup>-1</sup>	[NH <sub>4</sub> <sup>+</sup> ]	mol l <sup>-1</sup>
Total alkalinity	A <sub>T</sub> (TA, AT, ALK)	mol kg <sup>-1</sup>	-	-

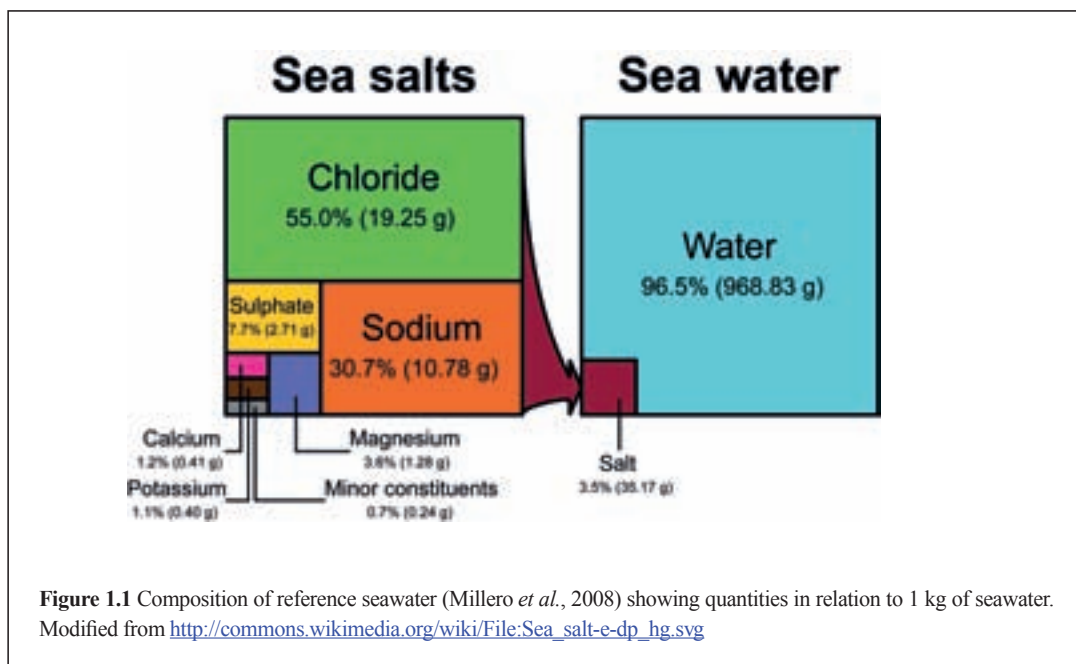
<sup>1</sup> Whenever a pH is defined, it is necessary to remember that it implicitly is based on a concentration unit, for hydrogen ion, although the pH value itself has the dimension 1.

<sup>2</sup> The free scale can be a suitable alternative.

## 1.2 Basic chemistry of carbon dioxide in seawater

### 1.2.1 Introduction

Seawater is unique among natural waters in that its relative composition is well defined (see e.g. Millero *et al.*, 2008) and dominated (>99.3% by mass) by a fairly limited number of major ions (Figure 1.1). The various acid-base species discussed in this chapter are in the remaining 0.7%, with carbonic acid and boric acid species predominating. As we shall see, this distinction between the *major* ions, that can be considered to make up a background ionic medium, and the various reacting species, that are present at relatively low concentrations, is an important convenience when discussing acid-base chemistry in seawater.

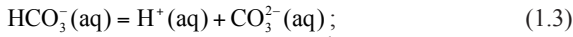
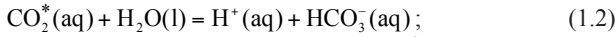


### 1.2.2 Acid-base equilibria in seawater

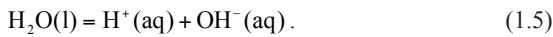
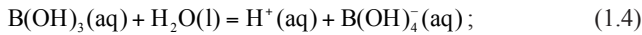
When carbon dioxide dissolves in water, it hydrates to yield carbonic acid,  $\text{H}_2\text{CO}_3$ . This reaction is slow relative to the ionisation of  $\text{H}_2\text{CO}_3$  and it is possible to distinguish between simple dissolved carbon dioxide,  $\text{CO}_2(\text{aq})$ , and the hydrated species,  $\text{H}_2\text{CO}_3$  (see e.g. Soli & Byrne, 2002). This kinetic process is relevant in some physiological systems and is catalysed by the enzyme carbonic anhydrase. At equilibrium, the concentration of carbonic acid,  $[\text{H}_2\text{CO}_3]$ , is only about 1/1000 of the concentration of dissolved carbon dioxide,  $[\text{CO}_2(\text{aq})]$  and has no special significance to the acid-base equilibria since both are uncharged (Butler, 1998). Here the total concentration of the two unionised species:  $[\text{H}_2\text{CO}_3] + [\text{CO}_2(\text{aq})]$ , will be abbreviated as the concentration of the hypothetical aqueous species  $\text{CO}_2^*$ :  $[\text{CO}_2^*]$ .<sup>1</sup> In acid solutions ( $\text{pH} < 5$ )  $\text{CO}_2^*$  is the dominant carbon dioxide species in solution, however at higher pHs it ionises to form bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ) ions.

<sup>1</sup> This corresponds to defining the standard states of  $\text{CO}_2(\text{aq})$  and of  $\text{H}_2\text{CO}_3$  using the so-called *hydrate convention* (Pitzer & Brewer, 1961).

Thus when carbon dioxide dissolves in seawater it can be considered to react with the water in accordance with the following series of chemical equilibria (Figure 1.2):



the notations (g), (l), (aq) refer to the state of the species, i.e. a gas, a liquid, or in aqueous solution respectively. Equation (1.1) refers to the solubility equilibrium of carbon dioxide between air and seawater; equations (1.2) and (1.3) are consecutive acid dissociation reactions of dissolved carbon dioxide. Two other important acid-base equilibria in seawater are the dissociation of boric acid and the self-ionisation of water:



The equilibrium relationships between the concentrations of these various species can then be written in terms of the equilibrium constants:

$$K_0 = [\text{CO}_2^*] / f(\text{CO}_2); \quad (1.6)$$

$$K_1 = [\text{H}^+][\text{HCO}_3^-] / [\text{CO}_2^*]; \quad (1.7)$$

$$K_2 = [\text{H}^+][\text{CO}_3^{2-}] / [\text{HCO}_3^-]; \quad (1.8)$$

$$K_B = [\text{H}^+][\text{B}(\text{OH})_4^-] / [\text{B}(\text{OH})_3]; \quad (1.9)$$

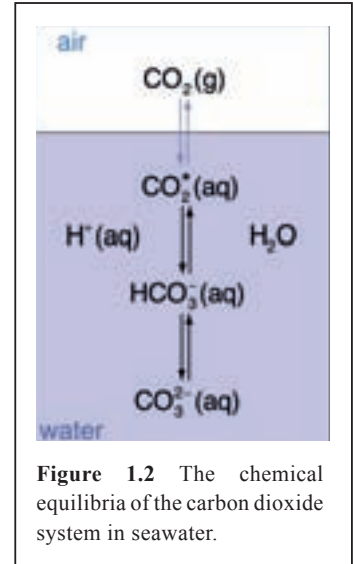
$$K_w = [\text{H}^+][\text{OH}^-]. \quad (1.10)$$

In these equations,  $f(\text{CO}_2)$  is the fugacity of carbon dioxide in the gas phase (see Standard Operating Procedure (SOP) 24 in Dickson *et al.*, 2007) and brackets represent total stoichiometric concentrations<sup>2</sup> of the particular chemical species enclosed between them, expressed as moles per kilogram of solution. The use of fugacity in equation (1.6) allows the same equilibrium constant to be used for a wide variety of gas phase compositions (Weiss, 1974). In practice, most applications in ocean acidification studies will involve air containing carbon dioxide that is in equilibrium with the seawater at a total pressure of about 1 atmosphere. This air will thus also contain water vapour that is at its equilibrium concentration (its vapour pressure, approximately). Weiss & Price (1980) defined an alternate relationship,  $F$  such that

$$F = [\text{CO}_2^*] / x'(\text{CO}_2); \quad (1.11)$$

where  $x'(\text{CO}_2)$  is the mole fraction of  $\text{CO}_2$  present in dry air (i.e. after drying to remove the water vapour), and the appropriate corrections for non-ideality have been included implicitly. There are many situations where it is more practical to use this equation directly rather than calculating the correct  $f(\text{CO}_2)$  value.

These equilibrium constants<sup>3</sup> are functions of the temperature, pressure and salinity of the seawater and have been measured at one atmosphere pressure in a variety of studies (see Millero, 2007). Recommended values are given in Table 1.1 as a function of salinity and temperature.



**Figure 1.2** The chemical equilibria of the carbon dioxide system in seawater.

<sup>2</sup> The *total* stoichiometric concentration of a species is the sum of the concentrations of the *free* species itself, together with the concentrations of all complexes that are formed between that species and the components of the ionic medium (for seawater:  $\text{H}_2\text{O}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , &  $\text{SO}_4^{2-}$ ).

<sup>3</sup> Strictly, equilibrium expressions such as equations (1.6) to (1.10) should be expressed in terms of activities rather than total stoichiometric concentrations so as to be *equilibrium constants*. However, as activity coefficients remain approximately constant for small amounts of reacting species in a background ionic medium, these expressions are valid and correspond to *ionic medium* equilibrium constants where the corresponding standard states are based on a seawater medium of a specified composition (Dickson *et al.*, 1981). Note that the activity of water is assumed to be unity.

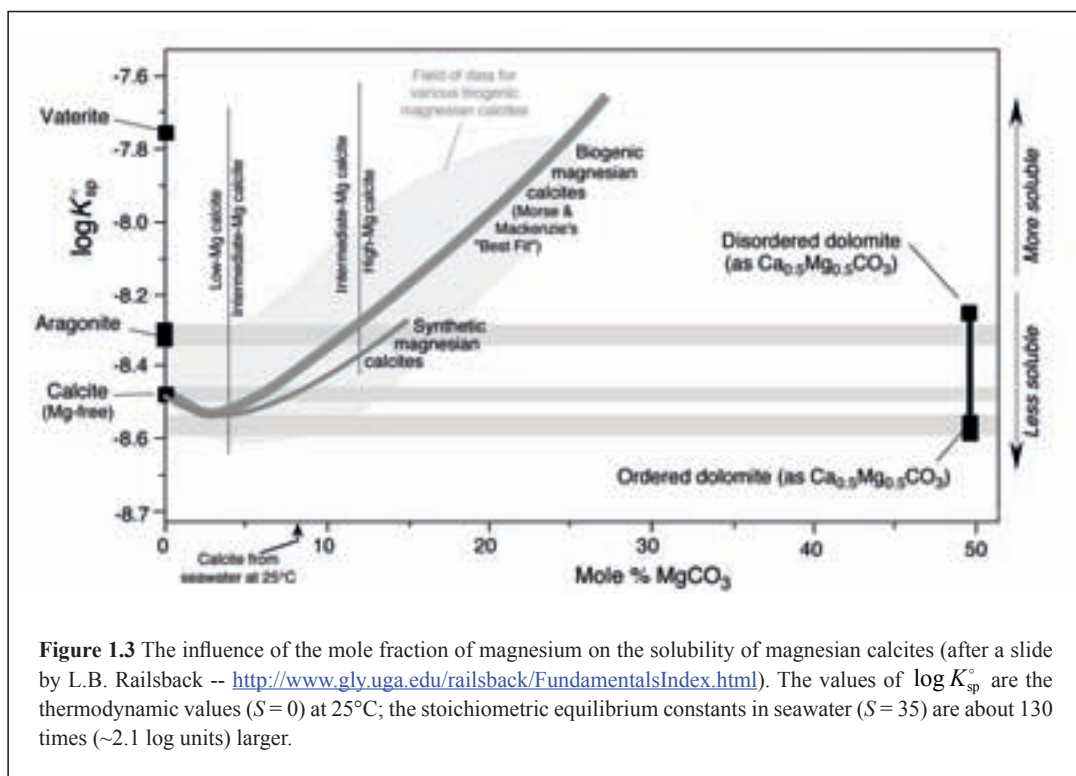
**Table 1.1** Expressions for calculating equilibrium constants (on the total hydrogen ion scale) as a function of salinity ( $S$ ) and temperature ( $T$ , in Kelvin) (Weiss & Price, 1980; Millero, 1995; Dickson *et al.*, 2007).

Note:  $I / m^\circ = \frac{19.924S}{1000 - 1.005S} \approx 0.02S$ ;  $k^\circ = 1 \text{ mol kg}^{-1}$ .

Equilibrium constant expression	Equation in text
$\ln(K_a / k^\circ) = 93.4517 \left( \frac{100}{T} \right) - 60.2409 + 23.3585 \ln \left( \frac{T}{100} \right) + S \left( 0.023517 - 0.023656 \left( \frac{T}{100} \right) + 0.0047036 \left( \frac{T}{100} \right)^2 \right)$	(1.6)
$\log(K_1 / k^\circ) = \frac{-3633.86}{T} + 61.2172 - 9.67770 \ln(T) + 0.011555S - 0.0001152S^2$	(1.7)
$\log(K_2 / k^\circ) = \frac{-471.78}{T} - 25.9290 + 3.16967 \ln(T) + 0.01781S - 0.0001122S^2$	(1.8)
$\ln \left( \frac{K_{a1}}{k^\circ} \right) = \frac{-8966.90 - 2890.53S^{1/2} - 77.942S + 1.728S^{3/2} - 0.0996S^2}{T} + (148.0248 + 137.1942S^{1/2} + 1.62142S) + (-24.4344 - 25.0855S^{1/2} - 0.2474S) \ln(T) + 0.053105S^{1/2}T$	(1.9)
$\ln(K_w / (k^\circ)^2) = \frac{-13847.26}{T} + 148.9652 - 23.6521 \ln(T) + \left( \frac{118.67}{T} - 5.977 + 1.0495 \ln(T) \right) S^{1/2} - 0.01615S;$	(1.10)
$\ln \left( \frac{F}{\text{atm } k^\circ} \right) = 218.2968 \left( \frac{100}{T} \right) - 162.8301 + 90.9241 \ln \left( \frac{T}{100} \right) - 1.47696 \left( \frac{T}{100} \right)^2 + S \left( 0.025695 - 0.025225 \left( \frac{T}{100} \right) + 0.0049867 \left( \frac{T}{100} \right)^2 \right)$	(1.11)
$\lg K_{\text{ap}}(\text{aragonite}) = -171.945 - 0.077993T + 2903.293/T + 71.595 \lg(T) + (-0.068393 + 0.0017276T + 88.135/T)S^{0.5} - 0.10018S + 0.0059415S^{1.5}$	(1.14)
$\lg K_{\text{ap}}(\text{calcite}) = -171.9065 - 0.077993T + 2839.319/T + 71.595 \lg(T) + (-0.77712 + 0.0028426T + 178.34/T)S^{0.5} - 0.07711S + 0.0041249S^{1.5}$	(1.15)
$\ln(K'_s / k^\circ) = \frac{-4276.1}{T} + 141.328 - 23.093 \ln(T) + \left( \frac{-13856}{T} + 324.57 - 47.986 \ln(T) \right) \times \left( \frac{I}{m^\circ} \right)^{1/2} + \left( \frac{35474}{T} - 771.54 + 114.723 \ln(T) \right) \times \left( \frac{I}{m^\circ} \right) - \frac{2698}{T} \left( \frac{I}{m^\circ} \right)^{3/2} + \frac{1776}{T} \left( \frac{I}{m^\circ} \right)^2 + \ln(1 - 0.001005S),$	(1.30)
$\ln(K_f / k^\circ) = \frac{874}{T} - 9.68 + 0.1115^{1/2}$	(see 1.41)
$\ln(K_{\text{si}} / k^\circ) = \frac{-8904.2}{T} + 117.385 - 19.334 \ln(T) + \left( \frac{-458.79}{T} + 3.5913 \right) (I / m^\circ)^{1/2} + \left( \frac{188.74}{T} - 1.5998 \right) (I / m^\circ) + \left( \frac{-12.1652}{T} + 0.07871 \right) (I / m^\circ)^2 + \ln(1 - 0.001005S);$	(1.45)
$\ln(K_{11} / k^\circ) = \frac{-4576.752}{T} + 115.525 - 18.453 \ln(T) + \left( \frac{-106.736}{T} + 0.69171 \right) S^{1/2} + \left( \frac{0.65643}{T} - 0.01844 \right) S;$	(1.46)
$\ln(K_{2p} / k^\circ) = \frac{-8814.715}{T} + 172.0883 - 27.927 \ln(T) + \left( \frac{-160.340}{T} + 1.3566 \right) S^{1/2} + \left( \frac{0.37335}{T} - 0.05778 \right) S,$	(1.47)
$\ln(K_{3p} / k^\circ) = \frac{-3070.75}{T} - 18.141 + \left( \frac{17.27039}{T} + 2.81197 \right) S^{1/2} + \left( \frac{-44.99486}{T} + 0.09984 \right) S;$	(1.48)
$\ln K_{\text{NH3}} = -6285.33/T + 0.0001635T - 0.25444 + (0.46532 - 123.7184/T)S^{0.5} + (-0.01992 + 3.17556/T)S$	(1.49)

### 1.2.3 The saturation state of calcium carbonate minerals in seawater

There are three primary biogenic carbonate-containing mineral phases that occur in seawater: aragonite, calcite, and magnesian calcite. Aragonite and calcite are naturally occurring polymorphs of calcium carbonate with differing crystal lattice structures and hence solubilities, aragonite being about 1.5 times more soluble than calcite at 25°C. Magnesian calcite is a variety of calcite with magnesium ions randomly substituted for the calcium ions in a disordered calcite lattice. At low mole fractions of magnesium (<4%) the solubility of this phase is lower than that of calcite, whereas at high mole fractions (>12%) the solubility is greater than that of aragonite (see Figure 1.3).



The dissolution equilibria for calcite and aragonite can be written as



where (s) indicates the solid phase. The corresponding equilibrium constant is the solubility product

$$K_{sp} = [\text{Ca}^{2+}][\text{CO}_3^{2-}]; \quad (1.13)$$

where the effect of the different crystal structure of the particular solid phase is now implicit in the solubility product itself. Strictly, aragonite and calcite have different standard Gibbs free energies, thus even when ionic medium standard states are used; the solubility products for the two minerals will have different values. It is necessary to indicate the mineral of interest explicitly, e.g.

$$K_{sp}(\text{aragonite}) = [\text{Ca}^{2+}][\text{CO}_3^{2-}]; \quad (1.14)$$

$$K_{sp}(\text{calcite}) = [\text{Ca}^{2+}][\text{CO}_3^{2-}]. \quad (1.15)$$

Clearly these equations cannot both hold true simultaneously. Aragonite is often referred to as a metastable form of calcium carbonate as it is not the form that would be expected at complete thermodynamic equilibrium.

Nevertheless it is often convenient to treat the solubility of aragonite in seawater as though it were a stable phase and to apply equation (1.14) to investigate its saturation state – equation (1.17) below.

Magnesian calcites can be problematic. Their solubility is not unique, nor do they necessarily form or dissolve congruently (i.e., maintaining the same molar ratio throughout the formation or dissolution process). As a result, they do not have unique solubility products (see Figure 1.3). Nevertheless, it is sometimes useful to define *apparent* solubility products for these minerals in seawater (i.e., with essentially fixed proportions of magnesium and calcium ions) as

$$K'_{sp}(\text{mag. calcite}) = [\text{Ca}^{2+}][\text{CO}_3^{2-}]; \quad (1.16)$$

the exact value of  $K'_{sp}(\text{mag. calcite})$  at any particular salinity and temperature will then depend on the mole fraction of magnesium in the solid (see e.g. Busenberg & Plummer, 1989).

The most common use of such solubility products – particularly in ocean acidification research – is to calculate the saturation state of seawater with respect to a particular calcium carbonate mineral X. The saturation state,  $\Omega(X)$ , is defined by the expression:

$$\Omega(X) = [\text{Ca}^{2+}][\text{CO}_3^{2-}] / K'_{sp}(X). \quad (1.17)$$

This expresses the ratio between the observed ion product,  $[\text{Ca}^{2+}][\text{CO}_3^{2-}]$ , and its expected value were the solution to be in equilibrium with the particular calcium carbonate mineral. If  $\Omega(X) = 1$ , the solution is in equilibrium with that mineral phase, if  $\Omega(X) > 1$  the solution is supersaturated with respect to that particular mineral phase, and if  $\Omega(X) < 1$  it is undersaturated. Insofar as the kinetics of dissolution (and formation) of such minerals have been shown to be functions of saturation state (see e.g. Morse & Arvidson, 2002; Morse *et al.*, 2007) this is a useful parameter for studies of calcification and dissolution.

## 1.2.4 Analytical parameters for the carbon dioxide system in seawater

It is usually not practical to measure the individual concentrations of each of these acid-base species in seawater directly so as to get a complete description of the composition of a particular seawater sample. Typically, the concentrations are inferred from a combination of analytical measurements made on the particular sample, together with published values for the various equilibrium constants (Table 1.1) as well as published information about the boron to salinity ratio of seawater (Table 1.2).

**Table 1.2** Reference composition of seawater (Millero *et al.*, 2008) at a practical salinity of 35.\*The DIC is 0.0019663 mol kg<sup>-1</sup>; the total concentration of boron is 0.0004151 mol kg<sup>-1</sup>. To calculate the composition at another salinity,  $[Y]_S = [Y]_{35} \times (S/35)$ , where Y refers to species that are dependent on salinity such as calcium ion concentration or total boron.

Constituent	Concentration mol kg <sup>-1</sup>
Sodium	0.4689674
Magnesium	0.0528171
Calcium	0.0102821
Potassium	0.0102077
Strontium	0.0000907
Chloride	0.5458696
Sulphate	0.0282352
Bicarbonate	0.0017177

Constituent	Concentration mol kg <sup>-1</sup>
Bromide	0.0008421
Carbonate	0.0002390
Borate	0.0001008
Fluoride	0.0000683
Hydroxide	0.0000080
Boric acid	0.0003143
Dissolved carbon dioxide	0.0000096

\*The concentrations of the various acid-base species were estimated assuming that the pH = 8.1 (on the seawater scale), and that the  $A_T = 2300 \mu\text{mol kg}^{-1}$ . The atmospheric CO<sub>2</sub> fugacity was chosen as 33.74 Pa = 333  $\mu\text{atm}$ , i.e. appropriate for the time period the original salinity/conductivity relationship was characterised (see Millero *et al.*, 2008 – p. 59).



**Salinity and temperature:** It is always important to measure salinity and temperature. The various equilibrium constants are all functions of salinity and temperature (see Table 1.1), and the composition of the solution that is inferred from the various other analytical measurements will depend on these values.

**Total dissolved inorganic carbon:** The total dissolved inorganic carbon of a seawater sample:

$$\text{DIC} = [\text{CO}_2^*] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]; \quad (1.18)$$

can be measured directly by acidifying the sample, extracting the resulting unionised carbon dioxide, and measuring its amount. The result is expressed in moles per kilogram of solution, and is independent of the temperature (and pressure) of the sample.

**Total alkalinity:** The total alkalinity of a sample of seawater is a type of mass-conservation expression for hydrogen ion relative to a chosen zero value. For simple, open-ocean surface seawater it can be approximated by the expression:

$$A_T \approx [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] - [\text{H}^+]. \quad (1.19)$$

The total alkalinity of a seawater sample is estimated using some form of acidimetric titration. Again, the result is expressed in moles per kilogram of solution and is independent of the temperature (and pressure) of the sample. Thus although the concentration of each of the individual species making up alkalinity changes when the temperature or pressure changes, the particular linear combination of these concentrations given in equation (1.19) remains constant.

**Hydrogen ion concentration:** The hydrogen ion concentration in seawater is reported as a pH:

$$\text{pH} = -\lg[\text{H}^+]. \quad (1.20)$$

where  $\lg x = \log_{10} x$  (Thompson & Taylor, 2008)<sup>4</sup>. Here hydrogen ion concentration is also expressed on a total scale (footnote 2) in moles per kilogram of solution. The pH of a seawater sample can be measured by one of two techniques: a potentiometric technique using an electrode that is sensitive to hydrogen ion together with a suitable reference electrode, and a spectrophotometric technique in which an indicator dye – a dye for which the acid and base forms have different colours – is added to the solution and the pH inferred from the resulting absorbance spectrum. Note that the pH of a particular sample of seawater depends upon its temperature (and pressure). If either of these is changed, the pH will change.

**$p(\text{CO}_2)$ :** The partial pressure of carbon dioxide in air in equilibrium with a seawater sample (at a specified temperature) is a measure of the degree of saturation of the sample with  $\text{CO}_2$  gas. The  $p(\text{CO}_2)$  of a particular seawater sample is a strong function of temperature, changing about 4.2% per Kelvin.

The partial pressure of a gas in a mixture is given by the expression:

$$p(\text{CO}_2) = x(\text{CO}_2)p, \quad (1.21)$$

where  $x(\text{CO}_2)$  is the mole fraction of the  $\text{CO}_2$  in the gas phase (air), and  $p$  is the total pressure. If these are known – usually from direct measurements on the gas phase – it is possible to estimate the corresponding fugacity of  $\text{CO}_2$  (see SOP 24 in Dickson *et al.*, 2007). This can then be used with the solubility constant,  $K_0$ , in equation (1.6) to calculate the concentration of dissolved, unionised carbon dioxide,  $[\text{CO}_2^*]$ . The units for fugacity are the same as for pressure, and must correspond to those used to define  $K_0$ .

Commonly it is not the  $p(\text{CO}_2)$  that is measured directly, but rather the mole fraction of  $\text{CO}_2$  in air that was in equilibrium with a water sample and which was subsequently dried before measurement. In that case, the function  $F$  presented in equation (1.11) often provides a more convenient way to calculate  $[\text{CO}_2^*]$  provided that the total pressure is approximately 1 atm. In the inverse case, where seawater is equilibrated with dry air containing a known mole fraction of  $\text{CO}_2$  at a total pressure of 1 atm, the same expression may prove useful.

<sup>4</sup> Strictly  $\text{pH} = -\log_{10} \{[\text{H}^+]/(\text{mol kg}^{-1})\}$  so as to allow the taking of the logarithm. This nicety will not be adhered to in this chapter.

There are a variety of approaches to measuring each of these parameters. At this time, the oceanographic community studying the carbon cycle in the open ocean environment has made available a *Guide to Best Practices for Ocean CO<sub>2</sub> Measurements* (Dickson *et al.*, 2007) that describes the present state-of-the-art techniques for each of these CO<sub>2</sub> parameters.

## 1.2.5 Calculation of carbon species concentrations in seawater

It is conventional to provide thermodynamic information about acid-base reactions that are written as acid dissociations – e.g. equations (1.2) to (1.5); however, these are not the only possible ways to write the various reactions. Although it is possible to write many balanced chemical reactions relating the seven individual acid-base species mentioned above (CO<sub>2</sub><sup>\*</sup>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, B(OH)<sub>3</sub>, B(OH)<sub>4</sub><sup>-</sup>, H<sup>+</sup> and OH<sup>-</sup>), the equilibrium constant for every one of these possible reactions can be calculated from a knowledge of the four simple acid dissociation constants, (1.7) to (1.10). For example, a particularly convenient formulation that encapsulates the equilibrium relationship between the concentrations of the various carbon dioxide species is

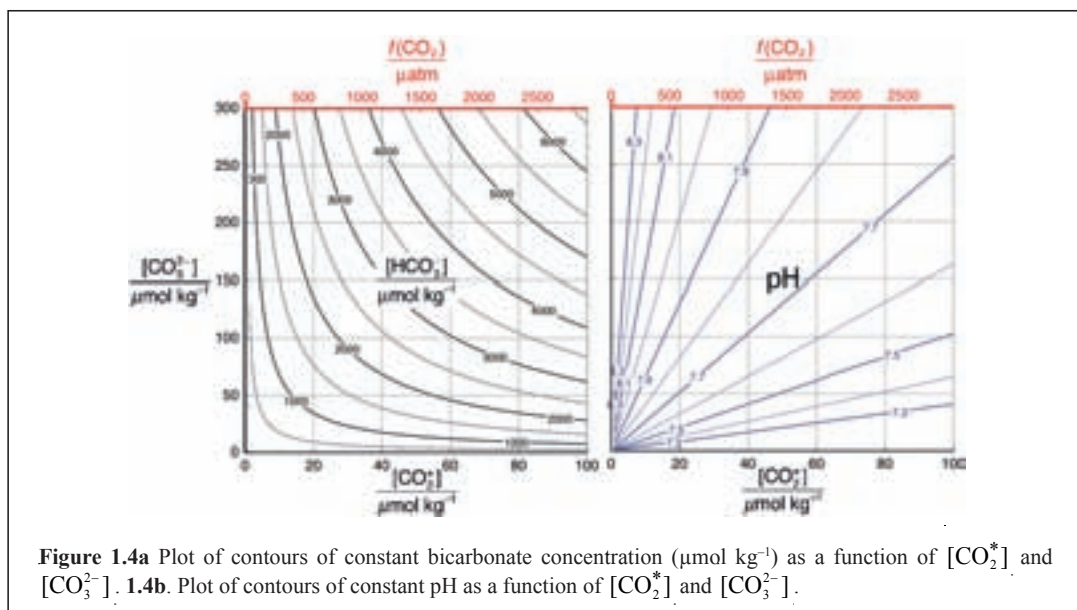


notations in parentheses indicating the state of the various species (g, l, aq, s) are omitted from now on to simplify the various expressions.

An examination of this equation tells us essentially what happens as the dissolved carbon dioxide concentration increases (for example by dissolution of CO<sub>2</sub> from the atmosphere). The additional carbon dioxide reacts with carbonate ion to form bicarbonate ion. The net effect is to increase the concentrations of dissolved carbon dioxide and bicarbonate ion, while decreasing the concentration of carbonate ion. The extent to which this occurs (at any particular salinity and temperature) can be inferred from the equilibrium constant corresponding to reaction (1.22), which in turn can be derived from equations (1.7) and (1.8):

$$K = K_1 / K_2 = [\text{HCO}_3^-]^2 / ([\text{CO}_2^*][\text{CO}_3^{2-}]) \quad (1.23)$$

In Figure 1.4a, I have used equation (1.23) to construct a contour plot indicating how the concentrations of bicarbonate ion ([HCO<sub>3</sub><sup>-</sup>]) in seawater media can be viewed as a function of the concentration of dissolved carbon dioxide ([CO<sub>2</sub><sup>\*</sup>]) and of carbonate ion ([CO<sub>3</sub><sup>2-</sup>]) at *S* = 35 and *t* = 25°C (*T* = 298.15 K). The *x*-axis is also marked in terms of *f*(CO<sub>2</sub>), which is directly proportional to [CO<sub>2</sub><sup>\*</sup>]— equation (1.6).



**Figure 1.4a** Plot of contours of constant bicarbonate concentration (μmol kg<sup>-1</sup>) as a function of [CO<sub>2</sub><sup>\*</sup>] and [CO<sub>3</sub><sup>2-</sup>]. **1.4b.** Plot of contours of constant pH as a function of [CO<sub>2</sub><sup>\*</sup>] and [CO<sub>3</sub><sup>2-</sup>].

Another convenient reaction relates carbonate ion directly to the dissolved carbon dioxide:



The equilibrium constant for this reaction is then the product of  $K_1$  and  $K_2$ :

$$K_1 K_2 = [\text{H}^+]^2 [\text{CO}_3^{2-}] / [\text{CO}_2^*] \quad (1.25)$$

Examination of this expression shows that the concentration ratio  $[\text{CO}_3^{2-}] / [\text{CO}_2^*]$  is a function of the hydrogen ion concentration and thus of the pH – equation (1.20). This is shown in Figure 1.4b.

If one picks a particular point on these graphs, i.e. specifying  $[\text{CO}_2^*]$  and  $[\text{CO}_3^{2-}]$ , all the other information about the concentrations of the other acid-base species is necessarily defined in terms of the various equilibrium constants (which in turn depend on the salinity and temperature). For example,  $[\text{HCO}_3^-]$  can be calculated from equation (1.23) and  $[\text{H}^+]$  from equation (1.25). Then once  $[\text{H}^+]$  is known,  $[\text{OH}^-]$  can be calculated from equation (1.10) and the ratio  $[\text{B}(\text{OH})_4^-] / [\text{B}(\text{OH})_3]$  from equation (1.9). We also know the total boron concentration in the seawater:  $[\text{B}(\text{OH})_4^-] + [\text{B}(\text{OH})_3]$ , which varies in direct proportion to the salinity (Table 1.2), so the individual concentrations of  $[\text{B}(\text{OH})_4^-]$  and  $[\text{B}(\text{OH})_3]$  can be estimated. Note too that each of the analytical parameters mentioned above: DIC,  $A_T$ , pH and  $p(\text{CO}_2)$ , can in turn be estimated once these various concentrations are known.

In general therefore, the composition of the carbon dioxide system in any seawater sample is specified completely once one knows the salinity and temperature (and hence the values for all the various equilibrium constants), together with two other concentration-related parameters (in addition to the total boron/salinity ratio). These other concentration-related parameters are typically chosen from those mentioned above: DIC,  $A_T$ , pH and  $p(\text{CO}_2)$ . The advantages and disadvantages of choosing a particular pair are discussed later in this chapter.

If one also wishes to estimate the saturation state – equation (1.17) – with regard to a particular calcium carbonate mineral, in addition to the appropriate solubility product, one also needs the concentration of calcium ion. For unmodified seawaters this too can be estimated from the salinity (Table 1.2).

There are a variety of programs available to do these calculations. Perhaps the most widely known is CO2SYS which was originally made available as a DOS executable (Lewis & Wallace, 1998), but which is now also available as Excel macros or as MATLAB code at <http://cdiac.ornl.gov/oceans/co2rprt.html>. Other similar programs are available, for example csys, a series of MATLAB files based on the book by Zeebe & Wolf-Gladrow (2001), is available at [http://www.soest.hawaii.edu/oceanography/faculty/zebe\\_files/CO2\\_System\\_in\\_Seawater/csys.html](http://www.soest.hawaii.edu/oceanography/faculty/zebe_files/CO2_System_in_Seawater/csys.html); seacarb, a series of functions written in R (Lavigne & Gattuso, 2010), is available at <http://cran.at.r-project.org/web/packages/seacarb/index.html>; and SWCO2, a package available from Keith Hunter at [http://neon.otago.ac.nz/research/mfc/people/keith\\_hunter/software/swco2/](http://neon.otago.ac.nz/research/mfc/people/keith_hunter/software/swco2/).

When doing such calculations, with or without a standard package, there are two important considerations. First, it is desirable to use the best available values for the equilibrium constants. However, it may not always be clear from a particular program, just which constants have been selected for use. The expressions given in Table 1.1 are those recommended in the recently published *Guide to best practices for ocean CO<sub>2</sub> measurements* (Dickson *et al.*, 2007) and are on the *total* hydrogen ion concentration scale. Second, if pH measurements are made, it is essential that the pH be defined in the same way it was for the equilibrium constants. This is discussed in more detail below.

## 1.3 The definition and measurement of pH in seawater

### 1.3.1 Introduction

Unfortunately, as noted by Dickson (1984), the field of pH scales and the study of acid-base reactions in seawater is one of the more confused areas of marine chemistry. The primary intent of measuring pH is to use it together with appropriated acid-dissociation constants (and other information – see section 1.2.5 above) to calculate the speciation of the various acid-base systems in seawater. For a particular acid-dissociation, e.g.



the corresponding equilibrium expression can be written in the form

$$\text{pH} + \lg K(\text{HB}) = \lg \{[\text{B}^-]/[\text{HB}]\} \quad (1.27)$$

If the pH scale is changed, changing the numerical value of pH, the corresponding value of the equilibrium constant must also change, and by the same amount, to ensure that the right hand side of this equation remains constant. It is thus essential that the pH is defined on the same pH scale as that of all acid-dissociation constants that are used with it.

The pH of seawater is best defined in terms of the concentration of hydrogen ion on the total hydrogen ion concentration scale (strictly the activity of hydrogen ion referenced to an *ionic medium* standard state, see footnote 3), and that is the approach recommended here. The equilibrium constants recommended in Dickson *et al.* (2007) and provided in this chapter (Table 1.1) are also defined using this pH scale.

### 1.3.2 The total hydrogen ion concentration scale

A key feature underlying the study of acid-base chemistry in seawater is the (often implicit) use of ionic medium standard states making it practical to define equilibrium constants that are based on concentration products, e.g., equations (1.7) to (1.10). The pH is defined as

$$\text{pH} = -\lg[\text{H}^+]; \quad (1.28)$$

where the square brackets again imply *total* concentration, that is the sum of the concentration of the *free* species itself, together with the concentrations of all complexes that are formed between that species and the components of the ionic medium (for seawater:  $\text{H}_2\text{O}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$ ).

In the case of hydrogen ion, such complexes occur with water (there are no unhydrated protons present in aqueous solution), and with sulphate ion to form the hydrogen sulphate anion:  $\text{HSO}_4^-$ . This interaction is usually written as the dissociation:



with the associated equilibrium constant,

$$K'_S = [\text{H}^+]_F [\text{SO}_4^{2-}] / [\text{HSO}_4^-]. \quad (1.30)$$

The term  $[\text{H}^+]_F$  indicates that here the hydrogen ion concentration is the *free* concentration (i.e., including only the hydrated forms of the ion), and the prime indicates that the equilibrium constant is defined accordingly. Thus one might expect the total hydrogen ion concentration to be expressed as:

$$[\text{H}^+] = [\text{H}^+]_F + [\text{HSO}_4^-]. \quad (1.31)$$

Substituting equation (1.30) in this, gives

$$[\text{H}^+] = [\text{H}^+]_F (1 + [\text{SO}_4^{2-}] / K'_S). \quad (1.32)$$

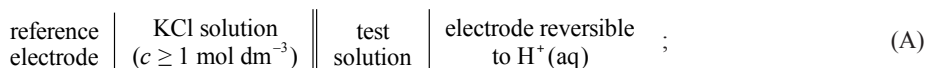
The term in parentheses is essentially constant at  $\text{pH} > 5$ . To ensure that  $[\text{H}^+] \propto [\text{H}^+]_F$  even at lower pH's such as are found in an alkalinity titration or other solutions that have high acid levels, Dickson (1990) proposed that the *total* hydrogen ion scale for seawater be defined as

$$[\text{H}^+] = [\text{H}^+]_F (1 + S_T / K'_S); \quad (1.33)$$

where  $S_T$  is the total amount of sulphate ion present in the seawater and this is the current definition. At  $\text{pH} > 5$ , equations (1.32) and (1.33) are essentially equivalent.

### 1.3.3 Measuring total hydrogen ion concentration using a pH cell

The standard potentiometric technique (Dickson, 1993; Dickson *et al.*, 2007) uses the pH cell:



where typically the electrode reversible to hydrogen ion is a glass electrode, often in a combination format with the associated reference electrode. The pH of a sample is then defined in terms of electromotive force measurements on the sample itself (X) and on a standard buffer solution (S) of assigned pH:

$$\text{pH}(X) = \text{pH}(S) - \frac{E_x - E_s}{RT \ln 10 / F} \quad (1.34)$$

In this equation pH(X) and pH(S) are the pHs of the sample and standard buffer, respectively;  $E_x$  and  $E_s$  are the corresponding e.m.f.s obtained with cell (A) on these solutions;  $T$  is the measurement temperature (note that both sample solution and standard buffer *must* be at the same temperature); and  $R$  and  $F$  are the gas and Faraday constants, respectively. The primary standard buffer for the measurement of total hydrogen ion concentrations in seawater media is based on 2-amino-2-methyl-1,3-propanediol (Tris) in synthetic seawater, and its pH values are assigned using Harned cells – cells with hydrogen and silver/silver chloride electrodes (DelValls & Dickson, 1998; Nemzer & Dickson, 2005).

Although it is practical to make up one's own Tris buffers in accordance with the recipe given by Nemzer & Dickson (2005), it is not particularly straightforward and requires some care to ensure values that are in good agreement (0.005) with those published by DelValls & Dickson (1998). It is recommended that such “home-made” buffers be calibrated against a primary standard buffer wherever practical.

A further complication with using such buffers is that, ideally, the salinity of the buffer matches the salinity of the sample being tested. This is rarely the case, however it has been shown that if the salinity is relatively close (within 5) of the buffer (usually prepared with a nominal salinity of 35), then the likely error is less than 0.01 in pH (Whitfield *et al.*, 1985).

If this electrode-based technique is used to measure pH, the overall uncertainty for the pH measurement is probably less than 0.02 for seawater measurements in the pH range 7.5-8.5, provided that the electrode slope is Nernstian or nearly so (>99%). If the quality of the electrode has not been assessed independently this uncertainty can be larger, but as the pH(S) of Tris buffer is about 8.1, the errors will not be very large within the usual seawater pH range (7.5-8.5).

### 1.3.4 Measuring total hydrogen ion concentration using an indicator dye

The spectrophotometric approach to pH measurement involves adding a small amount of a solution of a pH indicator dye to the seawater sample (e.g. Clayton & Byrne, 1993; Dickson *et al.*, 2007). The dye is an acid-base compound such as *m*-cresol purple whose second dissociation:



occurs at around seawater pH thus ensuring that both species are present in measurable amounts. The expression for the acid-dissociation constant for this dye

$$K(\text{HI}^-) = [\text{H}^+][\text{I}^{2-}] / [\text{HI}^-]; \quad (1.36)$$

can be rewritten as

$$\text{pH} = -\lg K(\text{HI}^-) + \lg \{[\text{I}^{2-}] / [\text{HI}^-]\}. \quad (1.37)$$

The spectrophotometric approach uses the fact that the acid and base forms of the indicator have substantially different absorbance spectra. Thus the information contained in the spectrum for the indicator dye in the seawater solution is sufficient to estimate the second term on the right hand side of equation (1.37). The total absorbance at a particular wavelength  $\lambda$ ,

$$A_\lambda = \varepsilon_\lambda(\text{HI}^-)[\text{HI}^-] + \varepsilon_\lambda(\text{I}^{2-})[\text{I}^{2-}]; \quad (1.38)$$

where  $\varepsilon_\lambda(\text{HI}^-)$  and  $\varepsilon_\lambda(\text{I}^{2-})$  are the extinction coefficients at that wavelength of the acid and base forms of the dye, respectively.

Typically absorbance information from two wavelengths (1) and (2) suffices to compute the pH:

$$\text{pH} = -\lg K(\text{HI}^-) + \lg \left( \frac{A_1 / A_2 - \varepsilon_1(\text{HI}^-) / \varepsilon_2(\text{HI}^-)}{\varepsilon_1(\text{I}^{2-}) / \varepsilon_1(\text{HI}^-) - (A_1 / A_2) \varepsilon_2(\text{I}^{2-}) / \varepsilon_2(\text{HI}^-)} \right). \quad (1.39)$$

These two wavelengths are usually chosen to correspond to the absorbance maxima for the base (1) and acid (2) forms of the dye. This choice of wavelengths is at once the most sensitive to pH changes as well as forgiving of minor deviations in wavelength reproducibility. The properties of the indicator dye *m*-cresol purple: equilibrium constant<sup>5</sup> and extinction coefficient ratios, have been described by Clayton & Byrne (1993). This method is also calibrated by assigning the value of  $K(\text{HI}^-)$ , in solutions of known  $[\text{H}^+]$ , ideally using primary standard buffers certified using a Harned Cell.

For the most accurate measurements, it is important to allow for the pH change resulting from the dye addition (Clayton & Byrne, 1993; Dickson *et al.*, 2007). This is usually minimised by adjusting the pH of the dye stock solution to be similar to that of the samples being measured. Unfortunately, it has also been suggested recently that these dyes, when obtained commercially, may have small amounts of coloured impurities that can vary from lot to lot. This will affect the apparent extinction coefficient ratios and can contribute as much as 0.01 to the overall uncertainty of the resulting pH data (Yao *et al.*, 2007). Thus the appropriate overall uncertainty estimate for spectrophotometric pH measurements is probably about 0.01 in pH.

### 1.3.5 Other pH scales (that are not recommended for use)

Two other pH scales have been used for seawater measurements in the past:

1. The so-called NBS scale (more correctly now referred to as the IUPAC scale) was based originally on recommendations and primary buffer standards from the US National Bureau of Standards (NBS), renamed the National Institute of Standards and Technology in 1988.
2. The seawater pH scale (SWS) which includes fluoride ion in the ionic medium (in addition to sulphate) and thus includes the species HF in the definition of the SWS hydrogen ion concentration:

$$[\text{H}^+]_{\text{SWS}} = [\text{H}^+]_{\text{F}} + [\text{HSO}_4^-] + [\text{HF}]; \quad (1.40)$$

or, more strictly,

$$[\text{H}^+]_{\text{SWS}} = [\text{H}^+]_{\text{F}} (1 + S_{\text{T}} / K'_{\text{S}} + F_{\text{T}} / K'_{\text{F}}); \quad (1.41)$$

where  $F_{\text{T}}$  is the total concentration of fluoride ion in the seawater, and  $K'_{\text{F}}$  is the dissociation constant for HF with hydrogen ion concentration expressed as the *free* concentration.

The uncertainty inherent in using the IUPAC scale for seawater measurements may be as large as 0.05 in pH, even for careful measurements. For the seawater scale, the errors will be approximately the same as for the total scale, provided that measurements are made in a similar fashion. It will however be important to assure oneself that indeed the standard buffer or the indicator dye's *pK* have been assigned values on this scale. Note that if it is necessary to calculate the amount of hydrogen fluoride in a particular sample, it can be estimated from knowledge of the total hydrogen ion concentration, the total fluoride concentration (proportional to salinity) and the corresponding equilibrium constant.

Whatever pH scale is employed, it is essential that it be used with equilibrium constants defined on the same scale. If one were to use pH measurements on the IUPAC scale with the constants of Table 1.1 (on the total hydrogen ion scale) an additional systematic error of about 0.15 pH units would be incurred at 25°C ( $\text{pH} \approx \text{pH}(\text{NBS}) - 0.15$ ). For seawater scale pH measurements, the error is about 0.01 units ( $\text{pH} \approx \text{pH}(\text{SWS}) + 0.01$ ).

<sup>5</sup> The paper of DelValls & Dickson (1998) suggests that the buffer used by Clayton & Byrne (1993) to estimate  $K(\text{HI}^-)$  may have been assigned an inappropriate pH. This has not yet been confirmed. Recent work in my laboratory, however, suggests an additional systematic error may largely counteract the proposed original error.

## 1.4 Implications of other acid-base equilibria in seawater on seawater alkalinity

### 1.4.1 Natural seawater

In addition to the various species detailed above, i.e. those from carbon dioxide, boric acid or water, natural seawater can contain a number of other acid-base species in significant amounts. The most common are a variety of minor nutrient species that also have acid-base behaviour (e.g. silicate, phosphate, and ammonia):



The dissociation constants for these various equilibria are thus

$$K_{\text{Si}} = [\text{H}^+][\text{SiO(OH)}_3^-]/[\text{Si(OH)}_4]; \quad (1.45)$$

$$K_{1\text{P}} = [\text{H}^+][\text{H}_2\text{PO}_4^-]/[\text{H}_3\text{PO}_4]; \quad (1.46)$$

$$K_{2\text{P}} = [\text{H}^+][\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]; \quad (1.47)$$

$$K_{3\text{P}} = [\text{H}^+][\text{PO}_4^{3-}]/[\text{HPO}_4^{2-}]; \quad (1.48)$$

$$K_{\text{NH}_3} = [\text{H}^+][\text{NH}_3]/[\text{NH}_4^+]. \quad (1.49)$$

Although ammonia is typically present at very low amounts ( $< 1 \mu\text{mol kg}^{-1}$ ) in oxygenated seawater and can usually be ignored, the other species are present at significant concentrations in deep water, and can be upwelled to the surface in various regions. In addition, there is the potential for additional organic acid-base species to be present, especially in enclosed systems with significant biological activity (Hernández-Ayon *et al.*, 2007; Kim & Lee, 2009).

The net effect is to add additional species into the expression for the total alkalinity of seawater which is rigorously defined (Dickson, 1981) as “. . . the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant  $K \leq 10^{-4.5}$  at 25°C and zero ionic strength) over proton donors (acids with  $K > 10^{-4.5}$ ) in 1 kilogram of sample.” Thus

$$\begin{aligned} A_T = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] \\ & + 2[\text{PO}_4^{3-}] + [\text{SiO(OH)}_3^-] + [\text{NH}_3] + [\text{HS}^-] + \dots \\ & - [\text{H}^+]_F - [\text{HSO}_4^-] - [\text{HF}] - [\text{H}_3\text{PO}_4] - \dots \end{aligned} \quad (1.50)$$

where the ellipses stand for additional minor acid or base species that are either unidentified or present in such small amounts that they can be safely neglected.  $[\text{H}^+]_F$  is the *free* concentration of hydrogen ion. Wolf-Gladrow *et al.* (2007) provide a detailed discussion of the origins of this expression and its application to biogeochemical processes.

For natural seawater these additional components do not usually complicate the *measurement* of total alkalinity, the value of which can be determined fairly accurately even if the existence of such species is ignored. However, it will affect significantly the *use* of this measured total alkalinity in inferring the composition of the seawater solution (section 1.2.5). One way to think about this is to consider how much each acid-base system contributes to the total alkalinity of a particular sample. Thus the “phosphate alkalinity” ( $[\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] - [\text{H}_3\text{PO}_4]$ ) in most samples (pH range 7–8) is approximately equal to the total concentration of phosphate in the sample, whereas for silicate the “silicate alkalinity” ( $[\text{SiO(OH)}_3^-]$ ) depends strongly on pH, and at pH 8 will be about 3% of the total silicate concentration (less at lower pH’s). Ignoring such contributions from these minor acid-base systems is thus equivalent to the alkalinity value being in error by the corresponding amount.

Essentially any computation involving total alkalinity requires (as with borate) that the total concentrations and the various equilibrium constants of all these other acid base systems be known so that they can be accounted for (see Table 1.2). If they are not well known there will be an inherent uncertainty in the computed speciation.

In the case of substantial organic contributions to the alkalinity such information may well not be available, and total alkalinity, though measurable, may not be fully interpretable.

### 1.4.2 Modified seawater media – implications for alkalinity

Often when culturing organisms in the laboratory, it will be common to have high concentrations of these nutrient species (and possibly of ammonia and of various organic acid-base species). If this is indeed the case, it is unlikely that measurements of total alkalinity will provide the information desired about the sample's carbon dioxide speciation without significant (and possibly a prohibitive amount of) extra work. This is particularly true if other acid-base species are deliberately added to the culture medium (for example the use of HEPES to control pH). It will then almost certainly be impossible to infer anything useful from a total alkalinity measurement and other techniques must be used.

## 1.5 Choosing the appropriate measurement techniques

### 1.5.1 Introduction

As was discussed in section 1.2.5, there are no analytical methods that measure directly the individual concentrations of all the various acid-base species that are present in seawater. These concentrations are usually inferred from measurements of salinity, temperature, and at least two of the analytical parameters introduced in section 1.2.4, in addition to the various other equilibrium constants, etc. The question for an investigator then becomes: which two parameters should I choose to measure? Furthermore, what measurement techniques should I use to estimate them?

A key aspect of making appropriate choices is that the measurements chosen *benefit for purpose*, that is, able to achieve the goals: uncertainty, convenience, speed, cost, etc., of the ocean acidification study being undertaken. An important first step is to define clearly the purpose for which the measurements are being made and to specify the associated constraints on the uncertainty required of the analytical measurements as well as other necessary considerations.

### 1.5.2 Available measurement techniques

In the 1990s a group of US investigators decided to document the techniques that they were using for open ocean studies of the carbon dioxide system in seawater. The resulting handbook (DOE, 1994) was made available through CDIAC as a printed book, as well as electronically. Recently an effort was made to update this information. This resulting document was published as the *Guide to best practices for ocean CO<sub>2</sub> measurements* (Dickson *et al.*, 2007) by PICES; it is available on the web at: [http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html).

This *Guide* provides detailed standard operating procedures for each of the current state-of-the-art techniques for measuring the various parameters of the seawater carbon dioxide system. Unfortunately, none of these techniques can be described as routine. Each requires trained analytical staff to perform the technique described, and much of the instrumentation described in the *Guide* is not commercially available. In fact most such instrumentation in use is, to some degree or another, “home-built” and it involves a significant cost to acquire (or build) a working instrument (including the necessary training of personnel). Furthermore, these instruments have not usually been optimised for ease of use or even ease of maintenance.

The combined standard uncertainty of these various techniques has – as yet – not been evaluated fully, but it is fair to say that on the whole the techniques detailed in the *Guide* are aimed at getting the best possible quality of measurement data for the carbon dioxide system in seawater. Furthermore, many of them have been used extensively in multiple laboratories and there is – within the user community – a reasonable understanding of their uncertainty, as well as of their advantages and disadvantages.

In addition to the techniques outlined in the *Guide*, a few other approaches (see Table 1.3) are worthy of consideration as being of appropriate quality for ocean acidification studies while being – perhaps – more cost-effective. Instrumentation for some of these techniques is now available commercially (typically from individual scientists who have established



companies to build and sell such instrumentation), but – as a result of the limited market for such instrumentation – none of them is available as a “turnkey” system, nor is there a well-developed support infrastructure providing the necessary training or instrument servicing. Furthermore, at this time such techniques have rarely been described with the level of detail outlined in the *Guide* nor have they been independently and rigorously tested.

**Table 1.3** Methods for the measurement of parameters of the carbon dioxide system in seawater (also see notes below).

<b>Total dissolved inorganic carbon</b>
A. Acidification / vacuum extraction / manometric determination
B. Acidification / gas stripping / coulometric determination
C. Acidification / gas stripping / infrared detection
D. Closed-cell acidimetric titration
<b>Total alkalinity</b>
E. Closed-cell acidimetric titration
F. Open-cell acidimetric titration
G. Other titration systems
<b>pH</b>
H. Electrometric determination with standard Tris buffer
I. Spectrophotometric determination using <i>m</i> -cresol purple
<b><math>x'(\text{CO}_2) / p(\text{CO}_2)</math></b>
J. Direct infrared determination of $x'(\text{CO}_2)$

- A. This method is used in my laboratory for the certification of reference materials.
- B. This is the method described in SOP 2 of Dickson *et al.* (2007). A system for implementing this (VINDTA 3C) is available from Marianda (<http://www.marianda.com>)
- C. This approach has been described in various publications (e.g. Goyet & Snover, 1993). Systems for implementing it are available from Apollo SciTech, Inc. (<http://apolloscitech.com>), and from Marianda (AIRICA: <http://www.marianda.com>).
- D. This method is not recommended. If the electrode used is non-Nernstian, a significant error is introduced in the estimation of DIC.
- E. This method is described as SOP 3a of Dickson *et al.* (2007). A system for implementing this (VINDTA 3S) is available from Marianda (<http://www.marianda.com>).
- F. This method is used in my laboratory for the certification of reference materials (Dickson *et al.*, 2003). It is described as SOP 3b of Dickson *et al.* (2007), and also as ISO 22719:2008 “Water quality – Determination of total alkalinity in seawater using high precision potentiometric titration.”
- G. A number of titration systems are now available for this: from the Kimoto Electric Co. (<http://www.kimoto-electric.co.jp/english/product/ocean/alkali.html>), from Apollo SciTech, Inc. (<http://apolloscitech.com>), and from Langdon Enterprises ([clangdon920@yahoo.com](mailto:clangdon920@yahoo.com)). Although all are described as capable of good repeatability, their reproducibility and uncertainty are unknown.
- H. This requires a high-quality pH meter (readable to 0.1 mV, 0.001 in pH) and access to certified Tris buffers. (The method is described in SOP 6a of Dickson *et al.*, 2007).
- I. This method is described in SOP 6b of Dickson *et al.* (2007), however see Yao *et al.* (2007).
- J. This method is described in SOP 5 of Dickson *et al.* (2007), and requires a significant amount of seawater such as a flowing stream of seawater: e.g., the system marketed by General Oceanics: <http://www.generaloceanics.com/genocean/8050/8050.htm>. If however, it is desired to make the measurement on a discrete sample of seawater, the uncertainty is increased to between 0.5 and 1.0%. One such method is described in SOP 4 of Dickson *et al.* (2007); another in a paper by Neill *et al.* (1997).

In Table 1.4, I provide estimates of the measurement uncertainty for the various primary analytical parameters. These are only for guidance, the magnitudes of these uncertainties depend not only on the measurement technique employed, but also on the metrological traceability of the measured results, as well as on the implementation of the technique in a particular laboratory (skill of analysts, quality assurance program, etc.); thus the measurement uncertainty value should be estimated separately by each individual laboratory.

**Table 1.4** Estimated measurement uncertainties for the measurement of parameters of the carbon dioxide system in seawater (for a single measurement on a sample of surface seawater). RM: Reference materials.

Parameter	Reference method	State-of-the-art (using RMs)*	Other techniques (using RMs)	Without using RMs†
Total alkalinity	1.2 $\mu\text{mol kg}^{-1}$	2-3 $\mu\text{mol kg}^{-1}$	4-10 $\mu\text{mol kg}^{-1}$	?
Total dissolved inorganic carbon	1.0 $\mu\text{mol kg}^{-1}$	2-3 $\mu\text{mol kg}^{-1}$	4-10 $\mu\text{mol kg}^{-1}$	?
pH	0.003‡	~0.005‡	0.01-0.03	?
$x'(\text{CO}_2)/p(\text{CO}_2)$	1.0 $\mu\text{atm}$	~2 $\mu\text{atm}$	5-10 $\mu\text{atm}$	?

\*The methods described in Dickson *et al.* (2007), performed by an experienced laboratory with well-trained analysts, and with a good quality assurance program in place.

†If appropriate reference materials are not used, it is usually not practical to assign a measurement uncertainty.

‡These levels of uncertainty in pH require that the apparent dye extinction coefficient ratios be appropriate to the particular lot of dye being used (see discussion in Yao *et al.* (2007)).

### 1.5.3 Quality assurance of measurements

Quality assurance constitutes the system by which an analytical laboratory can assure outside users that the analytical results they produce are of proven and known quality (Dux, 1990). A formal quality assurance program will be required for the carbon dioxide measurements performed in association with ocean acidification studies. A quality assurance program consist of two separate related activities (Taylor, 1987):

**Quality control:** The overall system of activities whose purpose is to control the quality of a measurement so that it meets the needs of users. The aim is to ensure that data generated are of known accuracy to some stated, quantitative, degree of probability, and thus provides quality that is satisfactory, dependable, and economic.

**Quality assessment:** The overall system of activities whose purpose is to provide assurance that quality control is being done effectively. It provides a continuing evaluation of the quality of the analyses and of the performance of the associated analytical systems.

These are discussed in detail in the books of Taylor (1987) and of Dux (1990), and a brief description appropriate to ocean carbon dioxide measurements is given in Chapter 3 of Dickson *et al.* (2007). In particular, effective quality control requires at a minimum the following:

- Suitable and properly maintained equipment and facilities,
- Well documented measurement procedures (SOPs),
- Regular and appropriate use of reference materials to evaluate measurement performance,
- Appropriate documentation of measurements and associated quality control information.

As noted above, regular use of reference materials is the preferred approach to evaluating measurement quality. Reference materials are stable substances for which one or more properties are established sufficiently well to calibrate a chemical analyser or to validate a measurement process (Taylor, 1987). Ideally, such materials are based on a matrix similar to that of the samples of interest, in this case seawater. The most useful reference

materials are those for which one or more of the properties have been *certified* as accurate, preferably by the use of a definitive method in the hands of two or more analysts.

The US National Science Foundation has, since 1988, supported my laboratory at the Scripps Institution of Oceanography to produce and distribute such reference materials for the quality control of ocean CO<sub>2</sub> measurements (see Table 1.5). They should be used regularly to ensure the quality of measurements performed in support of ocean acidification studies.

**Table 1.5** Availability of reference materials for the quality control of carbon dioxide measurements in seawater. RM: Reference materials.

Analytical measurement	Desired accuracy <sup>†</sup>	Uncertainty <sup>††</sup>	Availability
Total dissolved inorganic carbon	± 1 μmol kg <sup>-1</sup>	± 1 μmol kg <sup>-1</sup>	Since 1991 <sup>(a)</sup>
Total alkalinity	± 1 μmol kg <sup>-1</sup>	± 1 μmol kg <sup>-1</sup>	Since 1996 <sup>(b)</sup>
pH	± 0.002	± 0.003	Since 2009 <sup>(c)</sup>
Mole fraction of CO <sub>2</sub> in dry air	± 0.5 μmol/mol	± 0.1 μmol/mol	Since 1995 <sup>(d)</sup>

<sup>†</sup>These values are based on considerations outlined in the report of SCOR Working Group 75 (SCOR, 1985). They reflect the desire to measure changes in the CO<sub>2</sub> content of seawater that allow the increases due to the burning of fossil fuels to be observed.

<sup>††</sup>Estimated standard uncertainties for the reference materials described here.

<sup>(a)</sup>Sterilised natural seawater, certified using a definitive method based on acidification, vacuum extraction, and manometric determination of the CO<sub>2</sub> released. Available from UC San Diego (<http://andrew.ucsd.edu/co2qc/>).

<sup>(b)</sup>Certified using a definitive method based on an open-cell acidimetric titration technique (Dickson *et al.*, 2003). Available from UC San Diego (<http://andrew.ucsd.edu/co2qc/>).

<sup>(c)</sup>Standard buffer solutions based on Tris in synthetic seawater (Nemzer & Dickson, 2005). Available from UC San Diego (<http://andrew.ucsd.edu/co2qc/>).

<sup>(d)</sup>Cylinders of air certified by non-dispersive infrared spectrometry. Available from NOAA/ESRL, Boulder, CO (<http://www.esrl.noaa.gov/gmd/ccgg/refgases/stdgases.html>). However, gas mixtures certified to a lesser accuracy can be obtained from a variety of manufacturers.

### 1.5.4 Error propagation and its implications

Although, mathematically (in a system without any uncertainties) the use of different combinations of analytical parameters should provide equivalent information, in practice that is not the case. The inherent measurement uncertainties propagate through all further computations aimed at estimating other aspects of the carbon dioxide system in a particular seawater sample. Furthermore, such results will include additional measurement uncertainties associated with the various equilibrium constants, and with other information such as the boron/salinity ratio, the total concentration of phosphate, etc.

The general relationship between the combined standard uncertainty  $u_c(y(x_1, x_2, \dots, x_n))$  of a value  $y$  and the uncertainties of the independent parameters  $x_1, x_2, \dots, x_n$  on which it depends is:

$$u_c(y(x_1, x_2, \dots, x_n)) = \sqrt{\sum_{i=1, n} \left( \frac{\partial y}{\partial x_i} \right)^2 u(x_i)^2} \quad (1.51)$$

where  $y(x_1, x_2, \dots, x_n)$  is a function of several parameters (Ellison *et al.*, 2000), and  $u(x_i)$  is the uncertainty in  $x_i$ . The various partial derivatives ( $\partial y / \partial x_i$ ) can be estimated either algebraically (where convenient) or

numerically. This is the approach that should be used to estimate the combined measurement uncertainty of an analytical measurement (see Ellison *et al.*, 2000).

In the case of the calculation of some aspect of the carbonate system (e.g.  $[\text{CO}_3^{2-}]$ ) from various combinations of the possible analytical parameters, alternate approaches are *not* necessarily equivalent. Dickson and Riley (1978) first pointed out the implications of uncertainties in the various analytical parameters (and in the equilibrium constants) in detail. They chose to rewrite equation (1.51) in terms of the *relative uncertainties*:

$$\frac{u_c(y(x_1, x_2, \dots, x_n))}{y} = \sqrt{\sum_{i=1, n} \left( \frac{\partial y}{\partial x_i} \frac{1}{y} \right)^2 \left( \frac{u(x_i)}{x_i} \right)^2}, \quad (1.52)$$

and their Table II provides values of the sensitivity coefficients  $\{(\partial y / y) / (\partial x_i / x_i)\}$  for a surface seawater. It is important to note that the values of these sensitivity coefficients, and similarly of the partial differentials  $(\partial y / \partial x_i)$ , are not constant but depend on the approximate composition of the seawater itself. For particular ocean acidification experiments (whose  $\text{CO}_2$  levels are likely to be significantly different from that of the seawaters used in Dickson and Riley) one should plan to estimate them numerically using a program such as CO2SYS.

The uncertainties in the values of the equilibrium constants and other ancillary data such as the boron to salinity ratio are often forgotten. Furthermore, it is usually not straightforward to use CO2SYS (or other easily available software) to estimate the likely contribution of these additional uncertainties. In that case, values provided by Dickson and Riley (1978) provide reasonable estimates of the sensitivity coefficients with respect to  $K_1$  and  $K_2$  (except perhaps when using the measurement pair  $A_T$  and DIC).

**Table 1.6** Estimated relative uncertainties\* in calculating  $[\text{CO}_2^*]$  and  $[\text{CO}_3^{2-}]$  (or saturation state) resulting from the measurement uncertainties in Table 1.4, and based on the sensitivity parameters calculated by Dickson & Riley (1978) for surface seawater. The uncertainties for the various equilibrium constants are assumed to be 0.01 in  $\log_{10}(K_1)$ ; 0.02 in  $\log_{10}(K_2)$ ; and 0.002 in  $\log_{10}(K_0)$ . RM: Reference materials.

Pair of parameters	Relative uncertainty	Reference methods	State-of-the-art (using RMs)*	Other techniques (using RMs)
pH, $A_T$	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	2.6% 3.6%	2.9% 3.7%	6.1-8.7% 5.1-6.5%
pH, DIC	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	2.4% 4.1%	2.6% 4.2%	5.6-8.0% 5.7-7.3%
$A_T$ , DIC	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	4.9% 0.6%	5.4% 1.7%	5.8-9.3% 2.2-5.5%
pH, $p(\text{CO}_2)$	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	0.6% 5.3%	0.8% 5.7%	1.5-2.9% 10.6-15.0%
$A_T$ , $p(\text{CO}_2)$	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	0.6% 3.3%	0.8% 3.3%	1.5-2.9% 3.4-3.8%
DIC, $p(\text{CO}_2)$	$\frac{u_c([\text{CO}_2^*])}{[\text{CO}_2^*]}$ $\frac{u_c([\text{CO}_3^{2-}])}{[\text{CO}_3^{2-}]}$	0.6% 4.0%	0.8% 4.1%	1.5-2.9% 4.2-4.9%

\*These values are certainly not accurate to two significant figures. However, one can easily see the implications of the estimated measurement uncertainties, and can also infer the importance of the uncertainties ascribed to the various equilibrium constants (which dominate the relative uncertainty when using methods with the lowest possible uncertainty).

The principal difficulty in performing a rigorous error propagation to estimate the overall uncertainty of, for example, saturation state is that it is often not straightforward to obtain the necessary information about the uncertainties  $u(x_i)$  of the various input data. The marine chemistry community has rarely attempted to estimate the combined standard uncertainty for the various measurement techniques discussed here, instead usually providing only precision information, and then often only data obtained under repeatability conditions, i.e. the variability within a single laboratory, over a short time, using a single operator, item of equipment, etc. This is necessarily a *lot* smaller than the combined standard uncertainty for a particular measurement technique (Ellison *et al.*, 2000). Table 1.4 provides (my personal) estimates of the measurement uncertainties associated with the various parameters. Table 1.6 uses these values, together with the sensitivity coefficients estimated by Dickson and Riley (1978) to calculate the relative uncertainties of  $[\text{CO}_2^*]$  and  $[\text{CO}_3^{2-}]$  resulting from the various possible pairs of parameters.

As can be seen, the likely relative uncertainty in estimating the concentration of unionised  $\text{CO}_2$ :  $[\text{CO}_2^*]$ , is always smallest if  $\text{p}(\text{CO}_2)$  is measured directly, and is otherwise of an approximately similar magnitude whichever parameter pair is chosen. Also, the relative uncertainty in  $[\text{CO}_3^{2-}]$  (or saturation state) is similar for different combinations, with the exception of  $\text{pH}$  and  $\text{p}(\text{CO}_2)$  where it is twice as large.

### 1.5.5 Advantages (and disadvantages) of different parameters

There are a variety of possible metrics for choosing suitable parameter combinations to characterise the seawater composition in an ocean acidification experiment. At present, I feel it is fair to say that there is not really an *optimal* choice of parameters. Here I briefly summarise the advantages and disadvantages of each parameter (prices are for 2009 and expressed in US dollars).

**Total alkalinity:** Equipment for this measurement can be purchased for \$10,000-20,000. It is relatively straightforward to use, though troubleshooting can be problematic. It typically has a stable calibration, and reference materials are available. Samples are easy to handle, as gas exchange is not typically a problem. The lowest uncertainty is obtained with sample sizes of about 100 ml, although it is practical to titrate samples that are as small as 15 ml without much difficulty. An analysis takes about 10–15 min in all. The most obvious disadvantage is that it is more difficult to interpret alkalinity accurately in samples with high concentrations of nutrients or of dissolved organic material. Reference materials are available.

**Total dissolved inorganic carbon:** Equipment for this measurement (using the infrared technique) can be purchased for \$40,000-50,000. It is relatively straightforward to use and quite quick (~10 min per sample), however the calibration is, in many cases, achieved using reference materials and is not highly stable. The sample size needed for analysis is small (<10 ml), however samples must be protected from gas exchange, particularly at higher  $\text{p}(\text{CO}_2)$ . Reference materials are available.

**pH:** Equipment for spectrophotometric pH measurement can be obtained for less than \$20,000. The procedure is relatively straightforward, and capable of some automation. It is necessary to minimise gas exchange when handling samples. The most obvious disadvantage at this time is the need for concern about the dye purity (Yao & Byrne, 2007), which causes the measurement uncertainty to be about 10 times its reproducibility. At this time, there are only limited amounts of pH reference materials available, though I hope my laboratory will be able to supply them in larger quantities in the future.

**$\text{p}(\text{CO}_2)$ :** Equipment for this measurement is typically quite expensive (about \$50,000). It usually requires a flowing stream of seawater and is calibrated using cylinders of air with known  $\text{CO}_2$  levels. It is cumbersome to set up, but can be relatively straightforward to use once running. One advantage is that such systems are usually designed to run autonomously.

At this time only  $\text{pH}$  and  $\text{p}(\text{CO}_2)$  can be used for continuous measurement allowing relatively straightforward monitoring of an experiment over time. However, if the experiment is arranged such that the alkalinity of the seawater remains constant (or nearly so), one need only monitor one of these continually, though it will be desirable to measure two parameters explicitly on any discrete samples taken to characterise the experiment.

At this time, I believe that the best combination of parameters for studying the  $\text{CO}_2$  system in open ocean water is probably total alkalinity and total carbon. It is straightforward to collect and preserve samples for later analysis, the equipment is reasonably readily available, and reference materials are also available to ensure metrological traceability. Also, there will be a close link to the extensive set of open-ocean studies that have been, and will be performed in the future.

Nevertheless, there are occasions when an alkalinity measurement will be difficult to interpret. In that case, I believe that the optimal combination of parameters is pH (measured spectrophotometrically) and total dissolved inorganic carbon (measured using infrared spectroscopy). These two parameters allow a description of the  $\text{CO}_2$  system alone (without concern as to other co-existing acid-base systems), equipment for making the measurements is available, and reference materials are also available (though pH reference materials are in shorter supply). This pair can also be applied to study normal seawaters and may well be the best all-round choice.

### 1.6 Conclusions and recommendations

As can be seen from the extensive discussion above, seawater acid base chemistry is necessarily complicated. It involves a variety of different acid-base species in addition to the three forms of carbon dioxide: dissolved carbon dioxide, bicarbonate ion, and carbonate ion. Although care has gone into defining and measuring the various equilibrium constants, the uncertainty of these is still discussed extensively (see for example Millero, 1995; Millero *et al.*, 2006).

At present there are four parameters that can be reliably measured for the seawater carbon dioxide system ( $A_T$ , DIC, pH,  $p(\text{CO}_2)$ ), and one of these, pH, has multiple possible definitions which in turn can result in multiple values for acid-dissociation constants (Dickson, 1984). This chapter follows the recommendation of the original *Handbook of methods for the analysis of the various parameters of the carbon dioxide system in seawater* (DOE, 1994) and of the more recent *Guide to best practices for ocean  $\text{CO}_2$  measurements* (Dickson *et al.*, 2007) and recommends use of the so-called *total hydrogen ion concentration scale* to define pH in seawater media. Values of equilibrium constants that correspond to this pH scale are given in Table 1.1.

The various equilibrium and mass-balance equations that describe the acid-base chemistry of seawater comprise a set of equations with a limited number of linearly independent variables (the rank of the system of equations). It is possible to obtain a complete description of the acid-base composition of a seawater sample at a particular temperature and pressure provided the following is known:

- the salinity and temperature, and hence the solubility constant of carbon dioxide in the seawater as well as the equilibrium constant for each of the acid dissociation reactions that is assumed to exist in the solution;
- the total concentrations for each of these non- $\text{CO}_2$  acid-base systems;
- the values for at least two of the  $\text{CO}_2$ -related parameters:  $A_T$ , DIC, pH,  $p(\text{CO}_2)$ .

At this time, the analytical methods described in the *Guide to best practices for ocean  $\text{CO}_2$  measurements* (Dickson *et al.*, 2007) are presently the best understood and have the lowest uncertainty. For studies on natural seawater, my recommendation would be to measure  $A_T$  and DIC (as samples for these can be preserved easily and the measurements made with low uncertainty). However, as was noted above, there may be samples from ocean acidification experiments where it is not possible to fully interpret an alkalinity measurement. In such cases, it is probably best to measure pH and DIC, and this combination is also acceptable for the study of ocean acidification in natural seawaters. However, in that case the uncertainty of the calculated parameters is typically dominated by the uncertainty in the (spectrophotometric) pH measurement, and a total carbon value obtained using a simpler system (such as one based on infrared measurement) is ideal.

Nevertheless, it is not – as yet – straightforward to make accurate measurements of seawater  $\text{CO}_2$  parameters. Most of the methods require trained analysts, and in many cases equipment is not easily available. At this time, it is probably desirable for individuals studying ocean acidification to plan to work closely with a scientist with a good understanding of seawater acid-base chemistry and with access

to a working laboratory that can perform the necessary measurements. Alternately, it may be practical to send samples to a central laboratory for analysis provided that such a laboratory has an appropriate quality assurance program in place, and can provide the results in a timely fashion.

As we move into the future, we need to develop robust analytical techniques that can be used conveniently for ocean acidification studies (involving in many cases smaller samples than are typical for open ocean studies). Although some such techniques already exist (Table 1.3), they still require additional efforts to document them effectively and to establish a community-wide quality assurance scheme for each technique. Such a scheme will involve:

1. writing appropriate Standard Operating Procedures for the techniques in use;
2. interlaboratory comparison exercises to assess the various figures of merit for each technique (trueness and precision);
3. regular use of certified reference materials to assist in the quality control;
4. regular laboratory performance testing using blind samples.

To date it is fair to state that few ocean acidification experiments have been conducted where scrupulous care has been directed at the – apparently straightforward – task of measuring the associated carbon dioxide chemistry. Although this may well not be the largest source of uncertainty in such experiments, it is appropriate to plan to control it effectively.

## 1.7 References

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## 2 Approaches and tools to manipulate the carbonate chemistry

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### 2.1 Introduction

Although the chemistry of ocean acidification is very well understood (see chapter 1), its impact on marine organisms and ecosystems remains poorly known. The biological response to ocean acidification is a recent field of research, the first purposeful experiments have only been carried out as late as the 1980s (Agegian, 1985) and most were not performed until 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. Perturbation experiments are one of the key approaches used to investigate the biological response to elevated  $p(\text{CO}_2)$ . Such experiments are based on measurements of physiological or metabolic processes in organisms and communities exposed to seawater with normal and altered carbonate chemistry. The basics of the carbonate chemistry must be understood to perform meaningful  $\text{CO}_2$  perturbation experiments (see chapter 1).

Briefly, the marine carbonate system considers  $\text{CO}_2^*(\text{aq})$  [the sum of  $\text{CO}_2$  and  $\text{H}_2\text{CO}_3$ ],  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{H}^+$ ,  $\text{OH}^-$ , and several weak acid-base systems of which borate-boric acid ( $\text{B}(\text{OH})_4^-$ ,  $\text{B}(\text{OH})_3$ ) is the most important. As discussed by Dickson (chapter 1), if two components of the carbonate chemistry are known, all the other components can be calculated for seawater with typical nutrient concentrations at given temperature, salinity, and pressure. One of the possible pairs is of particular interest because both components can be measured with precision, accuracy, and are conservative in the sense that their concentrations do not change with temperature or pressure. Dissolved inorganic carbon (DIC) is the sum of all dissolved inorganic carbon species while total alkalinity ( $A_T$ ) equals  $[\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] - [\text{H}^+] + \text{minor components}$ , and reflects the excess of proton acceptors over proton donors with respect to a zero level of protons (see chapter 1 for a detailed definition).  $A_T$  is determined by the titration of seawater with a strong acid and thus can also be regarded as a measure of the buffering capacity. Any changes in any single component of the carbonate system will lead to changes in several, if not all, other components. In other words, it is not possible to vary a single component of the carbonate system while keeping all other components constant. This interdependency in the carbonate system is important to consider when performing  $\text{CO}_2$  perturbation experiments.

To adjust seawater to different  $p(\text{CO}_2)$  levels, the carbonate system can be manipulated in various ways that usually involve changes in  $A_T$  or DIC. The goal of this chapter is (1) to examine the benefits and drawbacks of various manipulation methods used to date and (2) to provide a simple software package to assist the design of perturbation experiments.

### 2.2 Approaches and methodologies

Seawater chemistry can be manipulated in various ways that alter the carbonate system differently. The following sections examine the five techniques that are most useful in the context of ocean acidification. To illustrate the discussions, each section below is followed by a numerical example. The R package seacarb was used to calculate the carbonate chemistry parameters (Lavigne & Gattuso, 2010) and the syntax used for

each example is available in Gattuso & Lavigne (2009). Note that other packages are available (see chapter 1). 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  $\text{CO}_2$  and the target  $p(\text{CO}_2)$  is the projected value for the year 2100 (Gattuso & Lavigne, 2009; Table 2.1).

**Table 2.1** Seawater carbonate chemistry in 2007, 2100, and after perturbations intended to simulate year 2100 carbonate chemistry (modified from Gattuso & Lavigne, 2009). Total alkalinity ( $A_T$ ), partial pressure of  $\text{CO}_2$  in seawater ( $p(\text{CO}_2)$ ), salinity and temperature were used to derive all other parameters using the seacarb package v.2.1.5 (Lavigne & Gattuso, 2010) except for manipulations of the calcium concentration for which DIC was used rather than  $p(\text{CO}_2)$ . Temperature (18.9°C) and salinity (34.9) were assumed to remain constant, the concentrations of total phosphate and silicate were set to 0 and the seawater  $p(\text{CO}_2)$  was set at 384  $\mu\text{atm}$  in 2007 and 793  $\mu\text{atm}$  in 2100. (a):  $\times 10^{-9}$  mol  $\text{kg}^{-1}$ , (b):  $\times 10^{-6}$  mol  $\text{kg}^{-1}$ .

	$p(\text{CO}_2)_{\text{sw}}$ ( $\mu\text{atm}$ )	$\text{pH}_T$ (-)	$[\text{H}^+]$ (a)	$A_T$ (b)	DIC (b)	$[\text{CO}_2]$ (b)	$[\text{HCO}_3^-]$ (b)	$[\text{CO}_3^{2-}]$ (b)	$\Omega_c$ (-)	$\Omega_a$ (-)
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- $\text{CO}_2$ seawater	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Addition of $\text{HCO}_3^-$ ; closed sys.	793	7.942	11.4	3406	3146	26.4	2901	218	5.2	3.4
Addition of $\text{HCO}_3^-$ ; 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	1947	12.8	1767	167	4	2.6
Addition of:										
$\text{CO}_3^{2-}$ and $\text{HCO}_3^-$ ; 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 $[\text{Ca}^{2+}]$	384	8.065	8.6	2325	2065	12.8	1866	187	2.6	1.7

It must be pointed out that the methods described below enable one to set the carbonate chemistry at the beginning of a perturbation experiment. The impact of biological (e.g. photosynthesis, respiration and calcification) and physical processes (e.g. air-seawater exchange of  $\text{CO}_2$  and temperature changes) on the carbonate chemistry can distort the initial values and must be taken into account in the experimental design (see section 2.4.2).

There are several experimental approaches to adjust seawater  $\text{CO}_2$  by either changing DIC at constant  $A_T$  (e.g. aeration with air at target  $p(\text{CO}_2)$ , injections of  $\text{CO}_2$  saturated seawater and combined additions of  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$  and HCl) or changing  $A_T$  at constant DIC (NaOH and/or HCl additions). Each experiment has different requirements depending on organisms, experimental duration, incubation volumes or sampling intervals and hence one of the possible carbonate chemistry manipulations will probably be favoured.

## 2.2.1 Changing DIC at constant $A_T$

### Aeration at target $p(\text{CO}_2)$

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

**Example:** seawater with  $p(\text{CO}_2)$  of 384  $\mu\text{atm}$  and an  $A_T$  of 2325  $\mu\text{mol kg}^{-1}$  can be 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 done for surface waters. This approach exactly reproduces the values of all parameters of the carbonate system expected in the year 2100 (Table 2.1).

Two aeration techniques have been used in ocean acidification studies: pH-stat and bubbling with premixed gases. In pH-stat systems, pH is monitored continuously and a controller opens or closes the valves that deliver the gases when pH goes above or below a set value. Gases are then delivered until pH reaches the target value again. Different combinations of gases have been used: (1) air and pure CO<sub>2</sub>, (2) CO<sub>2</sub>-free air and pure CO<sub>2</sub> and (3) air, CO<sub>2</sub>-free air and pure CO<sub>2</sub>. CO<sub>2</sub>-free air can be produced easily using either molecular sieves or CO<sub>2</sub> scrubbers such as soda lime or NaOH and Ca(OH)<sub>2</sub> (C. Hintz, pers. comm.). The pH threshold is calculated using the desired p(CO<sub>2</sub>) and total alkalinity which is either assumed to be constant or frequently measured. This method has the potential to compensate for changes in the carbonate chemistry due to photosynthesis and respiration or, in the case of open culture systems, to changes in the chemistry of the source water. However, the air-water gas exchange and CO<sub>2</sub> hydration is relatively slow and the system may not reach equilibrium when there is high biological activity (high biomass to volume ratio). Like with other approaches, it does not compensate for changes in total alkalinity resulting from the precipitation and dissolution of CaCO<sub>3</sub> that occur between measurements of total alkalinity.

Overall, the carbonate chemistry can be maintained with good efficiency in the culture vessel, for example p(CO<sub>2</sub>) can be controlled usually better than ±10 μatm. The main drawback of this technique is that the pH electrode must be frequently calibrated in order to correct for drift. Hence, the technique that involves bubbling with premixed gases may be attractive. Air with the desired p(CO<sub>2</sub>) can be produced using gas mixing pumps or purchased. Another technique would be to maintain atmospheric p(CO<sub>2</sub>) to the desired level in the laboratory or in the growth cabinets in which the experiments are carried out (such cabinets for maintaining terrestrial plant are commercially available). A simple air pump can then be used to bubble the experimental seawater. To the best of our knowledge, this technique has not yet been used in the context of ocean acidification.

Aeration of seawater should be used with care for two reasons. First, bubbling may enhance the surface coagulation of organic matter (Engel *et al.*, 2004). This may be critical for studies investigating the response of microbial communities since 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 maintained in a container bubbled with a gas of the desired p(CO<sub>2</sub>) (M. G. Weinbauer, pers. comm.). Such bags are permeable to gases and small molecules but impermeable to larger molecules and particles. It is highly recommended to check that the membranes are chemically neutral as some materials leak unwarranted chemical compounds. Second, some species of phytoplankton, for instance dinoflagellates, are known to be negatively affected by turbulence and especially by continuous bubbling (Shi *et al.*, 2009). For those cases, it is useful to equilibrate the media to the desired p(CO<sub>2</sub>) level prior to the inoculation or use other means to achieve a carbonate system close to reality, i.e. where DIC varies and A<sub>T</sub> remains constant.

#### ***Addition of high-CO<sub>2</sub> seawater***

DIC and A<sub>T</sub> 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. The seacarb function *pmix* estimates the carbonate chemistry after mixing of two water samples.

**Example:** one can mix, in a closed system, 0.99623 kg of seawater having an A<sub>T</sub> of 2325 μmol kg<sup>-1</sup>, and p(CO<sub>2</sub>) of 384 μatm with 0.00377 kg of seawater having an A<sub>T</sub> of 2325 μmol kg<sup>-1</sup> and saturated with CO<sub>2</sub> (p(CO<sub>2</sub>) = 1 × 10<sup>6</sup> μatm). The weight fraction of the high-CO<sub>2</sub> seawater relative to the final weight is 3.76 × 10<sup>-3</sup>. Salinity is 34.9, temperature is 18.9°C and calculations are made for surface waters. This produces seawater with a final p(CO<sub>2</sub>) of 793 μatm and all parameters of the projected carbonate chemistry in 2100 are perfectly reproduced.

To the best of our knowledge, this approach has been used only twice. To create a p(CO<sub>2</sub>) range from 200 to 1300 μatm, Schulz *et al.* (unpubl.) added about 20 to 200 dm<sup>3</sup> of seawater enriched in CO<sub>2</sub> to 60 m<sup>3</sup> mesocosms (Figure 2.1). C. McGraw (pers. comm., 2009) used this technique in laboratory experiments. As this approach uses water with very high p(CO<sub>2</sub>), caution has to be taken to avoid gas exchange during mixing and handling.



**Figure 2.1** Aeration system for seawater carbon dioxide ( $\text{CO}_2$ ) enrichment consisting of a bottle of pure  $\text{CO}_2$  gas and two 250 l seawater containers (photo credit: K. Schulz).

### *Addition of strong acid as well as $\text{CO}_3^{2-}$ and/or $\text{HCO}_3^-$*

As will be outlined below, the addition of acid alone does not fully mimic the changes in carbonate chemistry expected during the present century. 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  $A_T$ , resulting from the addition of  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$ .

**Example:**  $\text{HCO}_3^-$  ( $111.2 \mu\text{mol kg}^{-1}$  of  $\text{NaHCO}_3$ ) and  $\text{CO}_3^{2-}$  ( $15.3 \mu\text{mol kg}^{-1}$  of  $\text{Na}_2\text{CO}_3$ ) can be added to seawater for which  $p(\text{CO}_2)$  ( $384 \mu\text{atm}$ ) and  $A_T$  ( $2325 \mu\text{mol kg}^{-1}$ ) are known, salinity is 34.9, temperature is  $18.9^\circ\text{C}$  and calculations are done for surface waters. Then,  $14.18 \text{ ml kg}^{-1}$  of 0.01 N HCl is added. The first addition raises DIC to the desired level of  $2191 \mu\text{mol kg}^{-1}$  but increases  $A_T$  to a value higher than target (2467 vs.  $2325 \mu\text{mol kg}^{-1}$ ; Table 2.1). The subsequent addition of HCl, in a closed system to prevent gas exchange, restores  $A_T$  to the desired value without affecting DIC. All carbonate parameters after both additions reach the target values.

### **2.2.2 Addition of strong acids and bases**

The addition of a strong acid, such as HCl, or base, such as NaOH, in a system closed to the atmosphere does not alter the concentration of dissolved inorganic carbon but modifies total alkalinity.  $A_T$  decreases following addition of an acid whereas it increases following addition of a base. The change in 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. The seacarb function *ppH* estimates the changes in the carbonate chemistry during pH manipulations. The change in salinity due to the addition of acid or base is minor and can therefore be neglected.

**Example:** a volume of  $14.08 \text{ ml}$  of 0.01 N HCl is added to 1 kg of seawater with known  $p(\text{CO}_2)$  ( $384 \mu\text{atm}$ ) and  $A_T$  ( $2325 \mu\text{mol kg}^{-1}$ ); 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 done for surface waters. The target  $p(\text{CO}_2)$  of  $793 \mu\text{atm}$  is reached in a closed system (Table 2.1) but the pH is lower than the value expected in 2100 ( $7.768$  vs  $7.793$ , corresponding to a 2.9 % increase in  $[\text{H}^+]$  that results from the decrease in total alkalinity generated by acid addition). This is an undesirable effect of the direct manipulation of pH, as  $A_T$  will not change significantly during the course of this century. As a result, DIC,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$  and the  $\text{CaCO}_3$  saturation states are lower than their target values. However, it is possible to restore  $A_T$  to its initial level by adding  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$ , an approach that is described above.

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

DIC and  $A_T$  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  $A_T$  is proportional to the product of their charge and concentration. Thus,  $A_T$  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. This approach can be used to hold pH constant or be combined with acid addition to maintain  $A_T$  constant (see section 2.2.1).

The seacarb function  $pTA$  estimates the changes in the carbonate chemistry following addition of  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$ . In an open system, the carbonate system re-equilibrates through air-sea  $\text{CO}_2$  gas exchange after the addition of chemicals but  $A_T$  remains at a level higher than the target value.

**Example:**  $\text{HCO}_3^-$  ( $1081 \mu\text{mol kg}^{-1}$  of  $\text{NaHCO}_3$ ) is added to seawater for which  $p(\text{CO}_2)$  ( $384 \mu\text{atm}$ ) and  $A_T$  ( $2325 \mu\text{mol kg}^{-1}$ ) 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  $18.9^\circ\text{C}$  and calculations are done for surface waters. Results are shown in Table 2.1. 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 ( $7.942$  vs.  $7.793$ ) and  $A_T$ , DIC,  $[\text{CO}_3^{2-}]$  as well as the saturation states of aragonite ( $\Omega_a$ ) and calcite ( $\Omega_c$ ) are higher than the target values and are even higher than the values of the initial seawater. Differences are magnified in open systems.

### 2.2.4 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:

$$\Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{\text{sp}}^*} \quad (2.1)$$

where  $[\text{Ca}^{2+}]$  and  $[\text{CO}_3^{2-}]$  are the concentrations of calcium and carbonate ions in seawater, respectively, and  $K_{\text{sp}}^*$  is the solubility product at the *in situ* conditions of temperature, salinity and pressure (Zeebe & Wolf-Gladrow, 2001). It can readily be appreciated that the changes in  $\Omega$  resulting from a decrease in  $[\text{CO}_3^{2-}]$  (driven by ocean acidification) can be mimicked by altering  $[\text{Ca}^{2+}]$ . Uncoupling  $\Omega$  from the carbonate chemistry can also be useful, for instance, to examine a possible dependence of photosynthesis on calcification (Gattuso *et al.*, 2000; Trimborn *et al.*, 2007). It is also useful to replenish calcium when its concentration decreases below its natural levels during long-term experiments with calcifiers (Langdon *et al.*, 2000).

The seacarb function  $pCa$  estimates the changes in  $\Omega_c$  and  $\Omega_a$  resulting from the manipulation of the concentration of  $\text{Ca}^{2+}_{\text{sw}}$ . It is recommended to use the simplified recipe for synthetic seawater based on DOE (1994) described by Gattuso *et al.* (1998) because it is the basis of the synthetic seawater that has been used to determine a variety of equilibrium constants for use in seawater. Note that the effect of the changes in the calcium concentration on the dissociation constants of carbonic acid and on the solubility product of  $\text{CaCO}_3$  may have to be considered (Ben-Yaakov & Goldhaber, 1973).

**Example:** artificial seawater with a known  $A_T$  ( $2325 \mu\text{mol kg}^{-1}$ ) and ( $2064 \mu\text{mol kg}^{-1}$ ) and with a calcium concentration set to  $6.03 \mu\text{mol kg}^{-1}$  reproduces well the saturation states of aragonite and calcite expected in 2100 without affecting any of the other parameters of the carbonate system which remain at their 2007 values (Table 2.1).

## 2.3 Strengths and weaknesses

The seacarb function  $oa$  (Lavigne & Gattuso, 2010) describes the various approaches that can be used to alter the seawater carbonate system. It provides precise guidelines on how the target carbonate chemistry can be reached as well as a plot showing, in the  $A_T$  vs. DIC space, the changes generated by the five main perturbation techniques.

It can readily be seen from Figure 2.2 and Table 2.1 that 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 additions 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 occurred during the past 200 years and are expected to continue in the next few hundreds years. All three approaches therefore allow precise control of all carbonate parameters to 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. Note that, in all approaches, biological processes (e.g. photosynthesis, respiration, calcification, dissolution of  $\text{CaCO}_3$ , nutrient uptake and release) can significantly distort the target carbonate chemistry by changing DIC and  $A_T$  when experiments are run with high biomass (Rost *et al.*, 2008). Gas bubbling can maintain the concentration of DIC constant, provided that the dissolution of  $\text{CO}_2$  is faster than its biological uptake, but does not compensate the drift in  $A_T$ . Also, in all three methods, in addition to  $A_T$ , calcification can also deplete the concentration of  $\text{Ca}^{2+}$  when the organism to volume ratio is high or the incubation time is long.

The approach that adds  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$  appears of limited practical value because most carbonate chemistry variables deviate from target values. Obviously, due to gas exchange at the air-water interface, only gas bubbling can successfully be used to adjust  $p(\text{CO}_2)$  and other parameters of the carbonate chemistry in an open system.

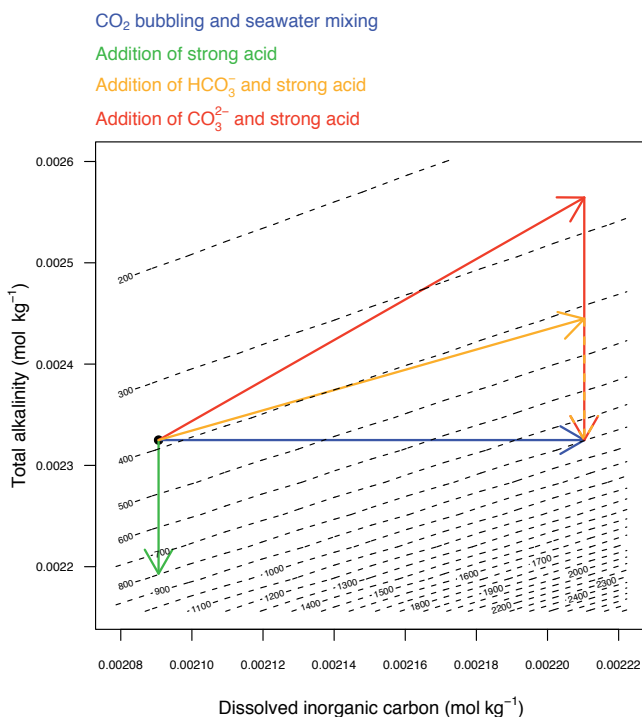
Several studies have used acid addition to manipulate the carbonate chemistry. Although this technique enables to precisely control  $p(\text{CO}_2)$  in systems closed to the atmosphere, it also alters  $A_T$  which results in carbonate parameters which are different from those expected in the future: pH is lower than its target value, DIC remains unchanged (whereas it increases under natural ocean acidification), and the concentrations of bicarbonate and carbonate as well as  $\text{CaCO}_3$  saturation states are lower than expected. Gattuso & Lavigne (2009) and Schulz *et al.* (2009) provide a detailed analysis of the similarities and differences between acid addition and DIC manipulations. It is recommended to combine the addition of bicarbonate and/or carbonate, to increase DIC, with acid addition to avoid this drawback.

Iglesias-Rodriguez *et al.* (2008a) recently reported that, in contrast to all previous reports, calcification of coccolithophorids increases at elevated  $p(\text{CO}_2)$ . They argued that this is due to the approaches used to manipulate the carbonate chemistry and that the approach of gas bubbling is superior to that of acid addition (Iglesias-Rodriguez *et al.*, 2008a and 2008b). The argument is that acid addition does not reproduce the increase of the  $\text{HCO}_3^-$  concentration generated by natural ocean acidification whereas gas bubbling does. The authors claim that since  $\text{HCO}_3^-$  may stimulate photosynthesis and, in turn, calcification, experiments that 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 (Table 2.1). Several previous perturbation experiments were carried out with gas bubbling and also reported lower rates of calcification of coccolithophores at lower pH or higher  $p(\text{CO}_2)$  (e.g. Sciandra *et al.*, 2003; Delille *et al.*, 2005; Feng *et al.*, 2008). It should also be pointed out that perturbation experiments carried out using gas bubbling can, like all other approaches, also lead to poor control of the carbonate chemistry, for example when the duration of the experiments is too long, when experiments are run at high biomass, or when the DIC uptake is larger than the dissolution of  $\text{CO}_2$  (see above). It therefore seems that the different responses of coccolithophores reported in the literature do not originate from the approach used to manipulate the carbonate chemistry. The situation is clearer in reef-building corals as Schneider & 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.

## 2.4 Potential pitfalls and suggestions for improvements

### 2.4.1 Seawater filtration and autoclaving

As filtration can significantly shift the carbonate chemistry of seawater, the carbonate system should be manipulated after filtration. If this cannot be done, samples for carbonate chemistry determination must be



**Figure 2.2** Changes in the carbonate chemistry generated by various perturbation techniques, in the  $A_T$  vs. DIC space. The plot was generated using the seacarb function *oa*.

taken after filtration to account for any gas exchange. Gentle pressure filtration through a cartridge should be performed because vacuum filtration or strong pressure filtration alter  $p(\text{CO}_2)$  and DIC (see Grasshoff *et al.*, 1999) and could lead to cell rupture, which in turn increases total alkalinity.

Seawater autoclaving should also be performed prior to manipulating the carbonate chemistry. It can severely change the carbonate chemistry as boiling seawater strips off gases and most of the DIC is lost.  $A_T$  has been reported to change as well: it increases, together with salinity, due to water evaporation but can also decrease due to precipitation of carbonate. Overall, the  $p(\text{CO}_2)$  of autoclaved water is initially relatively low and pH quite high. When the seawater cools, some of the DIC is likely to re-dissolve from the headspace into the water phase. When autoclaving natural seawater, it is recommended to sample for DIC and  $A_T$  determinations before and after autoclaving in order to ascertain the impact of the operating procedure on the carbonate chemistry. In case of artificial seawater, autoclaving will not change the carbonate chemistry if it is carried out before the addition of  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$ .

#### 2.4.2 Reaching and maintaining target values

When seawater is manipulated via bubbling with gases of different  $p(\text{CO}_2)$ , one must ascertain that equilibrium has been reached before starting an experiment. The required time to reach equilibrium depends on several factors such as the biomass to volume ratio,  $p(\text{CO}_2)$ , gas flow rate, bubble size, volume and shape of the flask and temperature, and may require several days. Since both bottled gas mixtures and  $\text{CO}_2$ -free air from generators do not contain any water vapour, it is important to humidify the dry air

before bubbling in order to avoid evaporation that would increase salinity, itself leading to changes in the parameters of the carbonate system.

Once the carbonate chemistry has reached a target level, it is critical to avoid any process leading to gas exchange between seawater and the atmosphere such as autoclaving, vacuum filtration or temperature changes. Unless the experimental set-up is open and bubbling is running continuously, gas-tight bottles filled without headspace should be used.

When working with high cell densities, processes such as photosynthesis and calcification can severely shift the carbonate chemistry. This problem is most pronounced in closed systems but must also be considered in open systems subject to continuous bubbling because biologically-driven changes can exceed the capability of the regulation system and thus cause a departure from the desired carbonate chemistry. This problem is more pronounced when investigating calcifying systems because calcification decreases  $A_T$ . The drawdown of  $\text{Ca}^{2+}$  associated to the decline of  $A_T$  might also need to be considered as it could also impact on the  $\text{CaCO}_3$  saturation state.

### 2.4.3 Contributions of dissolved organic matter, dissolved inorganic nutrients and pH buffers to total alkalinity

During photosynthesis, phytoplankton release dissolved organic compounds containing basic functional groups that readily react with protons during seawater titration, and thereby contribute to total alkalinity. The magnitude of the contribution of dissolved organic compounds to  $A_T$  depends on the species and on the age of the culture, suggesting that individual phytoplankton species exude dissolved organic compounds with unique proton accepting capacities (Kim & Lee, 2009). This contribution could be significant in perturbation experiments carried out at relatively high biomass:volume ratios. In that case, which must be avoided in perturbation experiments, one might consider the use of calculated carbonate parameters (e.g. total alkalinity derived from pH and DIC or from  $\text{p}(\text{CO}_2)$  and DIC) rather than the measured total alkalinity.

Some salts, such as inorganic nutrients, must be considered in experiments manipulating the carbonate chemistry as they contribute to  $A_T$ . The use of pH buffers causes large deviations from the natural carbonate chemistry as they increase  $A_T$  to values too high for accurate measurements and therefore precludes the calculation of the carbonate system using  $A_T$ . DIC and pH or  $\text{p}(\text{CO}_2)$  then have to be used instead. Depending on chemical form and concentration, inorganic nutrient addition can change  $A_T$  and should be included in carbonate chemistry calculations. Furthermore, possible changes of experimental inorganic nutrient concentrations and speciation, and their impact on  $A_T$  (for details see Brewer & Goldman (1976); Wolf-Gladrow *et al.* (2007)) highlight the importance of concomitant sampling for nutrients such as phosphate, ammonium and silicate, together with those for carbonate chemistry determination.

Phosphate ( $\text{PO}_4^{3-}$ ) is usually added to seawater as the sodium salt  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (see Guillard & Ryther, 1962). Its addition does not alter  $A_T$  as the immediate dissociation products  $\text{Na}^+$  and  $\text{H}_2\text{PO}_4^-$  do not contribute to  $A_T$  (see equation 1.50). In fact, since the other three phosphate species ( $\text{H}_3\text{PO}_4$ ,  $\text{HPO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ; equation 1.50) are included in  $A_T$ , the addition of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  do increase the alkalinity contribution by phosphate but concomitantly reduces the contribution of other  $A_T$  components by the same amount (equation 1.43). However, if phosphate is added as phosphoric acid ( $\text{H}_3\text{PO}_4$ ), total alkalinity is reduced by one mole per mole of phosphoric acid added. Hence, when calculating carbonate system speciation from measured  $A_T$ , the phosphate contribution should be taken into account. However, the error made by ignoring the contribution of phosphate to  $A_T$  is negligible at concentrations below  $1 \mu\text{mol kg}^{-1}$ .

Nitrate ( $\text{NO}_3^-$ ) is usually added to seawater as the sodium salt  $\text{NaNO}_3$  which does not alter  $A_T$ . Furthermore, as nitrate has no  $A_T$  component, it does not need to be considered when calculating the carbonate system from  $A_T$  measurements. However, the addition of nitric acid ( $\text{HNO}_3$ ) decreases  $A_T$ . Ammonia is usually added as ammonium chloride ( $\text{NH}_4$ ), which does not change  $A_T$ . Nevertheless, since  $\text{NH}_3$  contributes to  $A_T$ , it must be considered in carbonate chemistry calculations. In practice it can probably be ignored in most cases because of its relatively low concentration.



Silica in the form of  $\text{H}_4\text{SiO}_4$  is generally added as the sodium salt  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ . This changes  $A_T$  as  $\text{SiO}_3^{2-}$  combines with  $\text{H}_2\text{O}$  to form  $\text{H}_2\text{SiO}_4^{2-}$ , which quickly converts to  $\text{H}_3\text{SiO}_4^-$  by consuming a proton. At seawater pH, most of the  $\text{H}_3\text{SiO}_4^-$  further converts to  $\text{H}_4\text{SiO}_4$ , consuming another proton. Therefore,  $A_T$  increases by two moles for each mole of  $\text{Na}_2\text{SiO}_3$  added. Additions of silica to either natural seawater or artificial seawater can be relatively high, in the  $100 \mu\text{mol kg}^{-1}$  range. In these cases it might be advisable to counterbalance the otherwise relatively large increase of  $A_T$  by additions of HCl. As  $\text{H}_3\text{SiO}_4^-$  contributes to  $A_T$ , it should be included in the carbonate system calculations, although in many cases it can safely be ignored due to its relatively low concentrations at typical seawater pH (see Zeebe & Wolf-Gladrow, 2001).

#### 2.4.4 Isotopic labelling of dissolved inorganic carbon

Labelling of the dissolved inorganic carbon (DIC) pool with  $^{13}\text{C}$  or  $^{14}\text{C}$  requires the same precautions during preparation and handling as described above for the carbonate chemistry. In fact, it is a carbonate chemistry manipulation in itself and hence should be the last step in the preparation of an experiment. Any headspace should be avoided as seawater-atmosphere  $\text{CO}_2$  gas exchange reduces the concentration of the label.

Even if seawater is close to ambient  $p(\text{CO}_2)$ , any  $^{13}\text{C}$  or  $^{14}\text{C}$  added would outgas with time, driven by the difference in seawater and atmosphere  $^{13}\text{CO}_2$  or  $^{14}\text{CO}_2$  as their respective atmospheric partial pressure are close to 0: about  $4 \mu\text{atm}$  for  $^{13}\text{CO}_2$  and  $1 \times 10^{-13} \mu\text{atm}$  for  $^{14}\text{CO}_2$  (calculated according to Coplen *et al.* (2002) and Nydal & Lövseth (1996)). Therefore, aeration of seawater with air at target  $\text{CO}_2$  would increase  $^{13}\text{C}$  or  $^{14}\text{C}$  outgassing even though  $p(\text{CO}_2)$  would be kept constant. Furthermore, depending on the amount of  $^{13}\text{C}$  or  $^{14}\text{C}$  sodium salts added, DIC and  $A_T$  and hence  $p(\text{CO}_2)$  can change significantly.

#### 2.4.5 Sampling of carbonate chemistry parameters

Measuring and reporting of at least two quantities of the carbonate system prior to, after and ideally during experiments will ensure constant conditions or reveal possible shifts. Discrete samples for determination of DIC or pH should be taken with care because  $\text{CO}_2$  gas exchange between sample water and atmosphere could otherwise compromise the measurements. Sampling for  $A_T$  measurements, however, is not critically influenced by changes in DIC or pH related to gas exchange. Even if the water were stripped of any dissolved inorganic carbon, for example by warming the sample,  $A_T$  would stay constant provided that there is no evaporation and that salinity remains the same.

Samples should be poisoned for storage (see Grasshoff *et al.* (1999) and Dickson *et al.* (2007) for details) and kept at low temperatures before analysis. Headspace within DIC or pH sample vials must be avoided. Depending on organism and experimental setup, DIC and  $A_T$  measurements should be performed on filtered seawater. For instance, phytoplankton cells grown to relatively high densities in comparison to oceanic waters, can disintegrate during  $A_T$  or DIC measurements because of necessary acid additions. This could release  $A_T$  or DIC components from the particulate to the dissolved phase and compromise analysis. Furthermore, negatively charged groups in cellular plasma membranes can absorb protons added during  $A_T$  titration, thereby compromising the measurement (Kim *et al.*, 2006). Similarly, the study of calcifying organisms can also make DIC and  $A_T$  filtration necessary because  $\text{CaCO}_3$  dissolves during measurements due to acid additions, which artificially increases both DIC and  $A_T$ . Filtration of DIC samples must be carried out with care to avoid water-atmosphere  $\text{CO}_2$  gas exchange.

#### 2.4.6 Headspace and storage

Seawater in which the carbonate system has been manipulated and that is to be used in experiments should be handled very carefully. Wherever possible, headspace should be avoided as concentrations and speciation will otherwise change through water-atmosphere  $\text{CO}_2$  gas exchange if seawater  $p(\text{CO}_2)$  differs from atmospheric  $p(\text{CO}_2)$  (current atmospheric values are about  $390 \mu\text{atm}$  while those inside closed rooms are usually higher). An exception is seawater that is constantly aerated at target  $p(\text{CO}_2)$  throughout the experiment. Nevertheless, frequent sampling for at least two carbonate chemistry parameters is necessary for quality control.

Likewise, when storing manipulated seawater prior to the experiment, there should be no headspace. In this respect, it is important to keep in mind that temperature variations will change the carbonate chemistry. For instance, cooling seawater will initially decrease  $p(\text{CO}_2)$ , while increasing pH. If there is no headspace and the seawater is afterwards adjusted to intended temperatures, the carbonate system will shift back to pre-set conditions. This would not be the case if the water were stored with a headspace. The same applies for seawater that was aerated at target  $\text{CO}_2$ . If aeration is carried out at different temperatures than experimental incubation,  $p(\text{CO}_2)$  and hence carbonate chemistry speciation will change.

### 2.5 Data reporting

It is essential to report not only on the results obtained, but also on the methods used. The metadata should be included in databases (see chapter 15) in order to enable comparisons of different studies and meta-analysis. Table 2.2 provides a checklist of the information on the manipulation of the carbonate chemistry that should be reported when describing a perturbation experiment

**Table 2.2** Checklist of the information on the manipulation of the carbonate chemistry that should be reported when describing a perturbation experiment.

Method used to manipulate the carbonate chemistry	<p>Which of the methods below was used:</p> <ul style="list-style-type: none"> <li>– Aeration with air at target <math>p(\text{CO}_2)</math> (indicate <math>p(\text{CO}_2)</math> level and flow rate).</li> <li>– Addition of high-<math>\text{CO}_2</math> seawater (indicate <math>p(\text{CO}_2)</math> and mixing ratio).</li> <li>– Addition of strong acid as well as <math>\text{CO}_3^{2-}</math> and/or <math>\text{HCO}_3^-</math> (indicate volume and normality of acid added as well as the quantity of inorganic carbon added).</li> <li>– Addition of strong acids and bases (indicate volume and normality).</li> <li>– Manipulation of the <math>\text{Ca}^{2+}</math> concentration (indicate the recipe of artificial seawater used).</li> </ul>
Type of manipulation	<ul style="list-style-type: none"> <li>– Once before the experiment.</li> <li>– Continuous control during the experiment.</li> </ul>
Parameters of the carbonate chemistry	<ul style="list-style-type: none"> <li>– Values at the beginning and end of the experiment. If available, values during the experiment should also be provided.</li> <li>– At least two parameters of the carbonate system should be reported together with temperature and salinity.</li> <li>– The pH scale must be indicated.</li> </ul>
History of organisms investigated	<ul style="list-style-type: none"> <li>– Describe the conditions under which the organisms were maintained prior to the experiment. Were they pre-acclimated? If so, indicate the environmental conditions and the duration of the pre-acclimation phase.</li> </ul>

## 2.6 Recommendations for standards and guidelines

Several factors can collectively affect the success of CO<sub>2</sub> manipulation experiments. Here we make recommendations regarding the method of seawater CO<sub>2</sub> manipulation; the choice of which can be critical in obtaining significant and reproducible results in CO<sub>2</sub> perturbation experiments.

The method of manipulating carbonate chemistry in seawater is of utmost importance. Bubbling seawater with CO<sub>2</sub> enriched air may be the first choice because it is a very efficient way to manipulate seawater carbonate chemistry and, more importantly, it exactly mimics carbonate chemistry changes occurring in the years to come; the future scenario for ocean carbon chemistry being an increase in p(CO<sub>2</sub>) and DIC (decrease in pH) without alteration of A<sub>T</sub>. However, seawater aeration by bubbling may lead to difficulties in phytoplankton cultures (Shi *et al.*, 2009). Bubbling must therefore be sufficiently gentle to cause minimal impact on the phytoplankton assemblage or the cells should be separated from the bubbles by a gas-permeable membrane. Alternative but equally effective methods, when species are highly sensitive to bubbling, is to equilibrate the culture media with air at target p(CO<sub>2</sub>) or to mix it with high-CO<sub>2</sub> seawater prior to cell inoculation. Although the later method has yet to be widely tested, it also exactly mimics changes in carbonate chemistry in the future ocean just as the method of bubbling with high-CO<sub>2</sub> gases. The third method, equally reliable, is the combined addition of acid and bicarbonate and/or carbonate (increase in p(CO<sub>2</sub>) and DIC, but decrease in pH and A<sub>T</sub>) and then addition of Na<sub>2</sub>CO<sub>3</sub> and/or NaHCO<sub>3</sub> (to restore A<sub>T</sub>). This method also yields the conditions predicted to occur in the future ocean. Other methods (manipulation of A<sub>T</sub> and Ca<sup>2+</sup>) can be useful in the context of specific process studies (such as calcification).

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