

National Oceanography Centre, Southampton

Research & Consultancy Report No. 83

Report on a desk study to identify likely sources of error in the measurements of carbonate system parameters and related calculations, particularly with respect to coastal waters and ocean acidification experiments

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2010

Supplement to DEFRA Contract ME4133_DEFRApH monitoring project

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DOCUMENT DATA SHEET

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<i>TITLE</i> Report on a desk study to identify likely sources of error in the measurements of carbonate system parameters and related calculations, particularly with respect to coastal waters and ocean acidification experiments. Supplement to DEFRA contract ME4133 “DEFRApH monitoring project”.	
<i>REFERENCE</i> Southampton, UK: National Oceanography Centre, Southampton, 53pp. (National Oceanography Centre Southampton Research and Consultancy Report, No. 83) (Unpublished manuscript)	
<i>ABSTRACT</i> <p>Investigating the future of eco-systems in a high CO₂ world requires experiments that use seawater that has been modified beyond the limits of our knowledge of the carbonate system. The partial pressure of carbon dioxide (pCO₂) is difficult to measure directly in experimental systems and tends to be calculated from measurements of two of the other three components of the carbonate system (pH, total alkalinity - TA, total dissolved inorganic carbon - DIC). This desk study was commissioned by DEFRA following recent concerns about the accuracy with which the pCO₂ of experimental systems can be determined by calculation from measurements of TA and DIC. The evidence reported by (Hoppe et al., 2010 [1]) suggests errors as large as 25 % may occur at high concentrations of pCO₂ (~ 1000 µatm).</p> <p>This study has looked at the literature on the methodologies involved. It concludes that for work in open ocean waters the evidence is that measurements of TA and DIC can usually be used to calculate pCO₂ within recognised limits that are tighter than the errors suggested by Hoppe et al. We provide maps to illustrate how the calculated values of pCO₂ and carbonate ion vary with and are sensitive to changes in the values of the input pair of variables. These help to show graphically that accurate measurements of pH in conjunction with either TA or DIC can be used to accurately calculate both pCO₂(aq) and the concentration of carbonate ion. Conversely, the maps also show that inaccurate measurements of pH severely compromise any subsequent calculations of concentrations of pCO₂(aq) and carbonate ion. Calculation of the carbonate system is supported by three main software packages CO₂SYS, SEACARB and SWCO₂. These give closely similar results; and the scale of discrepancy, when different carbonic acid dissociation constants are used, is shown to be small relative to the errors reported by Hoppe et al. However it is not clear that the problem described by Hoppe et al., is due to inaccuracies either in the constants and equations describing the seawater chemistry or in the sampling and analytical measurements used by them. The ocean acidification community needs to be aware that seawater carbon measurements are not straightforward, and that it is as difficult to be confident in the accuracy of a measurement that is made without following best practice.</p> <p>A weakness with present procedures is in the measurement of carbonate alkalinity. Carbonate alkalinity is calculated from measurements of total alkalinity. The definition of total alkalinity is left open ended to allow for the inclusion of unknown protolytes. While the evidence from work in ocean waters is that carbonate alkalinity can generally be successfully calculated from total alkalinity, there is evidence that significant amounts of organic alkalinity may be present in enriched experimental systems and coastal waters. This is in the form of dissolved organic compounds and bacterial cells which can act as titrateable bases over the pH range of the alkalinity titration.</p> <p>We recommend that work be carried out: (i) to see if the finding of Hoppe et al. (2010) are</p>	

reproduced when measurements are done following best practice (Dickson et al., 2007 [2]); (ii) to determine in which water types and systems the presence of organic alkalinity is significant; (iii) to provide a needed improvement in the accuracy and precision of measurements of pH.

Where possible at least three of the carbonate system variables should be measured so that the system is over determined and any differences between calculated (and where possible observed) values of pCO₂ can be determined. Certified reference buffers are now available to aid improvement in the accuracy of measurements of pH. Careful use of appropriate seawater based buffer solutions may enable a range of laboratories to achieve measurements of pH to better than 0.01 units equivalent to a pCO₂ variation of 10 µatm if best practice is followed scrupulously.

[1] Hoppe, C. J. M., G. Langer, S. D. Rokitta, D. A. Wolf-Gladrow, and B. Rost, 2010. On CO₂ perturbation experiments: over-determination of carbonate chemistry reveals inconsistencies.

Biogeosciences Discuss., 7, 1707–1726.

[2] Dickson, A. G., Sabine, C. L., and Christian, J. R.: 2007. Guide to best practices for ocean CO₂

measurements, PICES Special Publication, 3, Sidney, Canada, 2007.

ACKNOWLEDGEMENTS

Preparation of this report was funded (djh and sl) by DEFRA (contract ME4133) as part of DEFRA's contribution to the UK Ocean Acidification programme of research. Toby Tyrrell's contribution was supported by the EU FP7 Integrated Project EPOCA (European Project on Ocean Acidification). The authors gratefully acknowledge the great effort that has already gone into supporting work in this field particularly documents such as Dickson et al., 2007 and Dickson, 2010 and the work of Frank Millero and co-workers. We also thank Andrew Dickson and Cynthia Dumousseaud, for useful exchanges in the early stage of preparation of this report, and Andrew Dickson, Jean Pierre Gattuso, Francois Muller, Zongpei Jiang, Clara Hoppe and Rachel Cave for comments on the final draft.

KEYWORDS

alkalinity, analysis, ocean acidification, pCO₂, pH, total dissolved inorganic carbon

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Executive summary and recommendations

Seawater carbon measurements are not straightforward, and the accuracy of a particular measurements is difficult to determine, although reproducibility can be assessed. If appropriate estimates of the overall uncertainty (bias & precision) are not available then the existence of "discrepancies" is moot. Similarly, other information that is used to compute (for example) carbonate ion concentration is based on experimental measurements with associated uncertainties. Meaningful attempts to assess the propagation of errors need to take this into account. Additionally other errors caused by an incomplete (or incorrect) model of the acid-base chemistry may be associated with particular samples, such as those caused by the presence of organic acids. Consequently, work to study the potential effects of ocean acidification (OA) on ecosystems is thrown down challenges:- (1) Marine chemists, must ascertain if their understanding of the marine carbonate system, gained largely in the deep sea, is adequate for work in coastal waters and experimental systems with high levels of organic matter and CO₂ and (2) Experimentalists, must carry out their experiments and analyses with sufficient rigor that the carbonate chemistry of their systems can be replicated in future validation experiments.

Our findings are:-

1. Three of the four components of the carbonate system - DIC (total dissolved inorganic carbon), pCO₂ and pH - can be measured by direct techniques, so that uncertainty in the quantity measured is limited only by experimental error.
2. Total alkalinity is only measured by titration with strong acid. Carbonate alkalinity is then arrived at indirectly by calculation based on the measurement of total alkalinity. This requires that all the components of the sample, which will be titrated in the pH range 3.6 to 3.0 are known and accounted for in the equations used to calculate carbonate alkalinity. Three independent studies show titrateable organic matter can be present in samples from coastal waters and mesocosms at levels equivalent to a few 10s of $\mu\text{M kg}^{-1}$ of alkalinity. Because of non-linearity in the equations, when such quantities of organic alkalinity are present then equivalent errors of 10s of % will occur in the values of pCO₂ calculated from measurements of total alkalinity and total dissolved inorganic carbon.
3. However the discrepancy was reported in a notionally low organic system. So that there are three possibilities: (i) the discrepancy is real and none of the available sets of

equations and constants are really appropriate for work at high levels of CO₂, (ii) the discrepancy is an artefact of the way the measurements were made or (iii) the notionally inorganic system in which the discrepancy was recognised contained high levels of bacteria the surfaces of which react during the titration.

4. The three main different software packages (CO2SYS, CO2SW and SEACARB) available for the calculation of CO₂ system variables give identical results when calculations are done with the same input parameters. At a pCO₂ of 1000 µatm, a maximum sensitivity of only 5 % was found to the choice of constants.
5. Calculations of pCO₂ and [CO₃²⁻] from a measurement paired with pH were found to be more sensitive to errors in pH than errors in the other measurement. Accurate pH measurements will thus yield accurate determinations of parameters of interest to OA, whereas poor pH measurements can only yield inaccurate and misleading determinations of OA-relevant parameters. With TRIS buffers an accuracy of 0.005 pH units may be achievable by experienced analysts using the best techniques, equivalent to an error in pCO₂ of 10 µatm at a pCO₂ level of 1000 µatm, when pCO₂ is calculated from measurements of pH and DIC.

Our recommendations are that:-

1. The measurements of Hoppe et al. (2010) should be repeated by other laboratories. Best practice methods should be used to check if the discrepancies reported by Hoppe et al. are reproducible.
2. Further work should be carried out using state of the art techniques in a range of environments - coastal water to laboratory experiments - to determine the locations and scale of the contribution of organic material to measured total alkalinity.
3. Where possible the carbonate system should be over-determined to provide evidence of problems. The simplest way to do this is by measuring three or all four carbonate variables. Measurements of TA and DIC are commonly made in most laboratories carrying out high quality carbonate system measurements. Now that reference materials are available, measurement of pH can potentially provide the needed third determinand.
4. To assist with the development of high quality pH measurements outside the core community of carbonate chemists a more closely specified standard operating procedure (SOP) than the current CO₂-SOP-6a (Dickson et al., 2007) for the electrode

based measurements of pH is recommended – this would be based on experience already available in the community on the most appropriate pH electrodes and temperature sensors to use and the appropriate design of a measurement cell.

5. Development of instruments for the automated colorimetric determination of pH should be encouraged as these offer the possibility of making high precision measurements (down to < 0.001 pH unit) with small (< 5 ml) volumes of sample).
6. A SOP appropriate to sampling experimental systems is needed; this would include recommendations on the collection of small volume samples (< 100 ml) and filtering of samples. Research is needed into reliable and easy to use (by non expert) storage containers particularly for the storage of small samples (10 to 100 ml).
7. Research is needed to find a “safe” efficient biocide to replace the use of mercuric chloride. Note: The testing of biocides and containers must be done in ways that the results are statistically valid.
8. Appropriate meta-data for all measurements of carbonate data in natural waters and experimental systems should be recorded. This is to enable carbonate system errors to be assessed and experiments replicated within known limits of reproducibility. A model template for such reporting is provided in this document (Appendix 1).

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1 Background

1.1 How well is the system known?

Investigating the future of ecosystems in a high CO₂ world requires experiments that use seawater, which has been modified beyond the limits of our present knowledge of the carbonate system. This raises the question - how far can the known characteristics of the carbonate system be extrapolated? It consequently requires that new data be reported in a way that enables the reproducibility of reported anomalies to be verified.

The dissolved inorganic carbonate system is generally well characterised when measurements are made in open ocean waters (e.g. Lamb et al., 2001). When the values of any two of the four components (pH, pCO₂, total alkalinity-TA and total dissolved inorganic carbon-DIC) are measured, as well as the temperature and salinity of the water, then the rest of the carbonate system can be calculated within known limits (Zeebe and Wolf-Gladrow, 2001; Millero et al., 1993a; Lee et al., 2000). This ability has been developed through fitting of experimental data (e.g Roy et al., 1993; Meherbach et al., 1973; Dickson and Millero, 1987) and in tests of the match up of in-situ data (Lueker et al., 2000; Millero et al., 1993a; Lamb et al., 2001; Millero et al., 2002). However the measurement of total alkalinity (TA) and total CO₂ by titration (Dickson et al., 2007; Edmond, 1970) has been thought for some time (Bradshaw & Brewer 1988a; 1988b) to produce results which suggest that the titration may be biased by the presence of protolytes which are not accounted for in the equations used to calculate the concentration of carbonate components. The use of measurements of **total alkalinity** to calculate the other components of the carbonate system requires that **carbonate alkalinity** can be reliably calculate from measurements of TA. While this has generally appeared to be the case; the definition of total alkalinity has been left open to admit unknown contributions to TA (Dickson, 1981; 2010a).

Research into ocean acidification (OA) is leading to an increase in the number of laboratory and mesocosm experiments that are being carried out at high CO₂ partial pressures: OA experiments are thus conducted under conditions which are beyond the bounds for which existing best fits of carbonate system data have been developed (Gattuso et al., 2010). This is in terms of both the ranges of pH and pCO₂ developed in the systems and the concentrations of added media (e.g. buffers such as HEPES (Wiki, 2010)) and nutrients and organic matter produced in the systems. The recent discussion paper by Hoppe et al. (2010) has raised

concern over the degree to which problems may be arising in the determination of true $p\text{CO}_2$ levels in such artificially manipulated systems. Similar concerns have been raised for work in coastal environments where the results may be distorted by contamination by suspended sediment and organic matter. The comment on Hoppe et al. (2010) by Tyrrell et al. (2010) shows that the anomalies found by Hoppe et al. could be the result of the presence of organic protolytes. This suggestion was based on the results of Kim and Lee (2009), which appear to confirm the likely presence of such uncharacterised protolytes in experimental systems. Other papers (Hernandez-Ayon et al., 1999; 2007 and Muller and Bleie, 2008) show that sufficient titrate-able organic matter may be present in incubations, coastal waters and mesocosms to produce the scale of discrepancy reported by Hoppe et al. (2010).

1.2 How well are the data reported?

The published referee reports of the Hoppe et al. (2010) paper suggest that there were considerable flaws in how the data were presented and consequently the degree to which it could be reliably interpreted (Dickson, 2010b, Wanninkhof, 2010). This in turn highlights causes for concern over the degree to which best practice (Dickson et al., 2007; Dickson, 2010a; Gattuso et al., 2010), is generally being followed and achieved.

The concern that Hoppe et al. (2010) expressed was that although marine chemists have recognised discrepancies in calculated values of $p\text{CO}_2$, the significance of this phenomenon for CO_2 perturbation experiments has so far not been determined. They reported on the application of two common perturbation methods for seawater carbonate chemistry (changing either DIC or TA). The carbonate system was over-constrained by measuring DIC, TA, pH, and $p\text{CO}_2$. They found that calculated $p\text{CO}_2$ matched directly measured $p\text{CO}_2$ if pH and TA or pH and DIC were chosen as input parameters, whereas $p\text{CO}_2$ calculated from measurements of TA and DIC was considerably ($\sim 25\%$) lower than measured values. This has important implications for CO_2 perturbation experiments. Firstly, calculated $p\text{CO}_2$ values may not be comparable between experiments if different input parameters are used; and secondly, responses of organisms to acidification may be wrongly estimated when using the TA/DIC pair for calculations. For instance, an experiment thought to have been conducted at $750\ \mu\text{atm}$, may have actually been run at $1000\ \mu\text{atm}$. This is may be particularly troublesome for interpretation of results from experiments with calcifiers, as carbonate ion concentrations and calcite/aragonite saturation states will be wrongly calculated.

A discrepancy is real when it is greater than the possible error in its value. Although Hoppe et al. looked at the propagation of errors they did not clearly enunciate the limitations of calculation of $p\text{CO}_2$ with different pairs of other components relative to their own errors and possible biases. None of the methods they used was a standard best practice procedure and it is likely that they under-reported the actual error in their methods. Dickson (2010b) and Wanninkhof (2010), in their reviews published in Biogeosciences Discussions, highlighted a number of problems with the approaches taken by Hoppe et al. They considered that overall the large discrepancy of 25% in the calculation of $p\text{CO}_2$ from TA and DIC and direct measurements of $p\text{CO}_2$ were not comparable with previous work. Although Lueker et al., (2000) and Millero et al., (2002) did find discrepancies they were of smaller magnitude ($1/5^{\text{th}}$) and only at high $p\text{CO}_2$ levels. In response to the form in which the paper was originally submitted Dickson pointed out there were a number of ways in which the data are reported that make it difficult to evaluate. The critical point is that the data in Hoppe et al., (2010) are presented with inadequate information to support an assessment of the precisions of the individual measurements. When possible errors in the Hoppe et al. paper are taken into account, Dickson considered that these might be sufficient to explain the reported offset.

1.3 Purpose of this document

(1) It is clear from the reviews of Dickson, (2010b) and Wanninkhof, (2010) that workers must strive to assess the likely scale of errors in the data that they report and that they detail their assessment in the metadata accompanying their measurements. Then any identified anomalies can be assessed in the context of the precision and accuracy of the measurements. It will assist any subsequent work done to test the existence of a reported anomaly. To this end, in Appendix 1 we set out a formal list of requirements for meta-data – which builds on the best practice recommendations of Dickson et al. (2007) and Gattuso et al. (2010).

(2) Of the different carbonate system measurement methods, it is those which use titration that are the least reliable in terms of certainty about what is actually being measured. And only in the case of the determination of carbonate alkalinity via the measurement of total alkalinity does another independent method not exist to check the determination (e.g. total carbonate can be determined both by titration and by measurement of evolved CO_2 following the addition of excess acid). The determination of carbonate alkalinity assumes that all the components contributing to total alkalinity are known (see section 3). The definition of total alkalinity is left open ended (Dickson, 2010) and there is now independent evidence to suggest that organic protolytes can be present in cultures, mesocosms and coastal waters at

sufficiently high concentrations to produce a mismatch between values of $p\text{CO}_2$ and values calculated from the TA/ DIC pair and from pH/TA or DIC (Hernández-Ayón et al., 1999; 2007; Muller and Bleie, 2008; Kim et al., 2006; Kim and Lee, 2009). This was demonstrated by Tyrrell et al. (2010).

Consequently we review in detail the measurement and calculation procedures relating to the determination of total and carbonate alkalinity.

(3) For completeness we provide an updated overview of the methods available for the determination of carbonate system parameters – this is based on the tables in Dickson (2010).

(4) Although Hoppe et al. (2010) began a discussion of the propagation of errors this was not followed through nor did they recognise the established idea that certain pairs of the carbonate system provide a more independent assessment of the concentration of the other components. That is, when $p\text{CO}_2$ or $[\text{CO}_3^{2-}]$ is calculated using pH the concentration of $p\text{CO}_2$ determined is not sensitive to changes in the concentration of the other component used – TA or DIC. To illustrate this, we provide a series of maps of the relative relationships between calculations based on different choices of the pair of carbonate components. We hope these are instructive and reinforce in peoples' minds what has been recognised for some time, that different pairs have different sensitivities to errors (Zeebe and Wolf-Gladrow, 2001).

This document will:-

- Identify known and potential sources of error in measurements of individual carbonate system variables (TA, DIC, $p\text{CO}_2$ and pH)
- Consider in detail the measurement of total alkalinity and calculation of carbonate alkalinity
- Compare differences in the output of different software packages and different sets on constants available for the calculation of CO_2 system variables.
- Provide a graphical interpretation of the sensitivity of calculated values of $p\text{CO}_2$ and carbonate ion to variations in concentrations of the pair of system variables chosen for the calculation.
- Provide a template for the reporting of meta-data in association with all measurements of carbonate data in marine waters.
- Where possible, make recommendations on required research and improvements or modifications to best practice.

2 Sampling and measurement of carbonate system variables

2.1 Notes relevant to the development of SOPs appropriate for the sampling and storage of waters from ocean acidification experiments

There are few references in the literature providing advice on the collection and storage of samples to be used for the measurement of components of the carbonate system. The growth of experimental studies requires that best practice is developed which is appropriate to these systems and that the limitations of this best practice are known including the collection and preservation of samples. Best practice essentially for work on deep seas cruises for the collection of samples is outlined in SOP 1 (Dickson et al., 2007). For the determination of TA and DIC a relatively large sample 250-500 ml is collected in hard glass bottle, poisoned with mercuric chloride and then well sealed. Hard glass is used to limit the dissolution of silicate from the bottle walls.

Extra guidelines are needed for ocean acidification (OA) sampling because the samples are different both in their nature and the volumes of solution that are available for sampling. Compared to deep sea water these samples are likely to contain higher levels of suspended solids, organic matter, nutrients and other modifying additions etc. This requires that recommendations be made with respect both to sampling and storage procedures. Three points to consider in drawing up appropriate SOPs are filtering, preservation and containers.

(a) Filtering – Suspended material that will react with strong acid needs to be removed from TA and DIC samples. This may be especially true in coastal waters, incubations and mesocosm. Kim et al. (2006) have provided one of the few reports on the need for filtration of samples for the determination of TA. They clearly showed that TA can be influenced in the presence of high plankton bio-mass and that at levels of bacteria found in natural samples reactions with cell surfaces may measurably effect the value of TA determined. Chanson & Millero (2007) followed up the work of Kim et al. (2006) and found that the measured TA of open ocean waters from the Pacific was not changed by filtration. Similar tests in coastal waters and experimental systems are required.

Studies on the affects of filtering on DIC and pH samples are required and suitable procedures need to be developed and evaluated. The problem is that during filtration exposing a free surface of the solution will permit uptake or loss of CO₂ depending on the relative pCO₂ in the surrounding atmosphere and the degree of exchange that is possible. This is not a significant problem for alkalinity, as CO₂ exchange has no effect on alkalinity (see Figure 1.1.3 in Zeebe

and Wolf-Gladrow 2001). At this stage anyone who filters samples for DIC or pH analysis should test the procedure and report the results of those tests in the accompanying meta-data and more publicly. Suitable filtering systems, perhaps using a peristaltic pump could ensure that sample modification would be kept to a minimum. It needs to be established in which types of water CaCO_3 and/or other particles that will react with strong acid are present at levels that create a significant error and what those levels are.

(b) Poisoning – Mercuric chloride, is the chemical of choice within the carbonate chemistry community (See SOP 1) for the preservation of samples and references to other methods have not been found. It seems to work well and samples appear to be well preserved over many months of storage. Again it should be said that much of this experience is for deep sea samples which are relatively sterile. Preservation of experimental samples containing higher levels of organic matter needs to be checked, as greater quantities of poison may be required. The draw back of mercuric chloride is the perceived hazard of using mercury. Research into an equally efficient but “safe” biocide should be encouraged.

(c) Container type - SOP 1 recommends the use of hard glass bottles of pyrex-type composition. These have been found to be suitable for the storage of alkalinity samples for periods of years (Dickson, pers comm.). The key requirement is that the sample does not react with the material of the bottle and that the bottle retains water vapour and is also impervious to the transport of carbon dioxide. (Zhang et al. (1999), reported on short term dissolution rates of glass flasks used for preparing nutrient standard solution). Bottles made from PET (Polyethylene terephthalate - used widely for carbonated drinks) have been used for short periods - up to days (Dickson pers comm.). More information is needed on the performance of these bottles. For all containers the security of the closure is the other key thing. The SOP 1 procedure recommends use of a greased ground glass stopper with a retaining band. This procedure works well with experienced users. Research is needed into the reliability of suitable screw tops with compression seals or serum caps, as these might be suitable for small (< 10 ml) volume samples.

Any new developments must be tested in a statistically valid way.

2.2 Overview of measurement methods

The following section extends the tables “Table 1.3” and “Table 1.4” in Dickson 2010. It provides a brief description of the methods available to measure carbonate system components and links to their description in the CO_2 -SOPs and/or literature references. A

summary is also provided of the recognized likely scale of the errors associated with the different methods. In Section 3 of this report we focus on the measurement of alkalinity by titration in particular. There are unique grounds for concern about this parameter and method. We describe the methods used to calculate total alkalinity and carbonate from the data obtained from the titration procedure. Each of the other carbonate parameters is most often measured directly (except for total carbonate when that is also measured by titration and $p\text{CO}_2$ when it is measured via a measurement of pH change).

2.2.1 Total dissolved inorganic carbon (DIC)

For total dissolved inorganic carbon there are five methods in use (A) Acidification / vacuum extraction / manometric determination; (B) Acidification / gas stripping / coulometric determination; (C) Acidification / gas stripping / infrared detection; (D) Closed-cell acidimetric titration; (E) Auto-analyser-colorimetric.

(A) This method is used in Dickson's lab at UC (University of California San Diego - Scripps Institution of Oceanography) for the certification of reference materials. It does not lend itself to use for large numbers of samples. Total dissolved inorganic carbon is assayed following C. D. Keeling's procedures using vacuum extraction and manometry. The weighed sample is acidified with phosphoric acid; the CO_2 evolved is extracted under vacuum and condensed in a trap cooled by liquid nitrogen. The water and CO_2 are separated from one another by sublimation and the CO_2 is transferred into an electronic constant-volume manometer. There its pressure, volume and temperature are measured and the amount of CO_2 separated is computed from the virial equation of state for gasses.

(B) This is the method described in SOP 2 of Dickson *et al.* (2007). A system for implementing this, the (VINDTA 3C), is commercially available from Marianda (<http://www.marianda.com>) and this instrument has superseded the successful earlier SOMA system (Johnson *et al.*, 1987).

(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://apollosciotech.com>), and from Marianda (AIRICA: <http://www.marianda.com>). For practical reasons smaller sample volumes are used than in method B, this reduces the precision of individual measurements relative to a coulometer based system.

(D) Closed cell titrations have been used frequently for the measurement of DIC. The method is prone to a number of recognized errors, for example it relies on the electrode used

having a Nernstian response and a significant error is introduced in the estimation of DIC if the response is non-Nernstian (Millero 1993) and good cell design is important to ensure rapid mixing. The calculated DIC value is affected by the presence of unidentified protolytes and is biased by errors in the calculation of TA.

(E) This method is not recommended for high precision work. As with all auto-analyser based methods it requires skill to achieve precision and accuracy better than 1 % on a routine basis. This method could be considered where large numbers of samples need to be processed and only limited volumes (< 10 ml) are available. Stoll et al., (2001) suggest that a typical analysis speed of 45 samples hour⁻¹ can be reached with an accuracy of 2-3 μM and a precision of 2.5 μM . It should be born in mind that this work was carried out in one of the world's more experienced groups working with auto-analyser based methods.

2.2.2 Total alkalinity

There are two methods that are described in the CO₂-SOPs (Dickson et al 2007) and a number of other procedures which are in common use but which do not give the same precision as the SOP methods. **(F)** Closed-cell acidimetric titration; **(G)** Open-cell acidimetric titration; **(H)** Other titration systems

(F) This method is described as SOP 3a of Dickson *et al.* (2007). A system for implementing this is the (VINDTA 3S) available from Marianda (<http://www.marianda.com>). The cell is actually an open cell but the sample is titrated as if it were in closed cell system hence DIC data are generated but are not reliable.

(G) This method is used in Dickson's lab at UC for the certification of reference materials (Dickson *et al.*, 2003). It is described in 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." A number of titration systems are now available for this: from the Kimoto Electric Co. (<http://www.kimotoelectric.co.jp/english/product/ocean/alkali.html>), from Apollo SciTech, Inc. (<http://apolloscitech.com>), and from Langdon Enterprises (clangdon920@yahoo.com). Although all are described as capable of good repeatability, their reproducibility and uncertainty are not yet established in the literature.

(H) 1. A number of other variations of the titration procedure are in use and/or have been reported (e.g. Van den Berg and Rogers, 1987; Haraldson et al., 1997) which calculate TA based only on a few points (< 5). The ability of such systems to deliver data of sufficient precision for marine carbonate system work is therefore questionable. See section 3.3.3 for

the discussion of the improvements in the precision of TA determination that are achieved by having sufficient data to apply a curve fitting method. **2.** The difference derivative readout method of Hernández-Ayón et al. (1999) does not produce as high a precision as a titration method based on an assumed model of the carbonate system and a least squares fit, but is important in that the procedure allows the effects of organic protolytes on the titration to be identified and quantified. **3.** Martz et al. (2006) suggested a colorimetric titration method that would be a suitable basis for an automated method. Dilution of a pulse of titrant in the titration vessel is tracked spectrophotometrically following the change in total indicator concentration. The concentrations of reacted and un-reacted indicator species, derived from Beer's law, are used to calculate the relative proportions of titrant and sample in addition to the equilibrium position (pH) of the titration mixture.

2.2.3 pH

Essentially two methods are in use for pH, which are described in SOPs 6a and 6b. (I) Electrometric determination with standards based on TRIS and AMP buffers; (J) Spectrophotometric determination using *m*-cresol purple. Several laboratories are working on the automation of the spectrophotometric method. (It is important to remember to compare pH data on the same scale. For work on the carbonate system the “total scale” should be used for reporting pH data. The numerical values output by the different scales are significantly different. For example, at T=25 °C and salinity=35, pH (free scale) can be ~0.11 higher than pH(total), while the difference between pH(total scale) and pH(seawater scale) is smaller ~0.01.)

(I) This requires a high-quality volt-meter (readable to > 0.05 mV equivalent to 0.001 in pH) and access to certified TRIS and AMP buffers. (The method is described in SOP 6a “Determination of the pH of sea water using a glass/reference electrode cell” of Dickson et al., 2007). The availability of certified TRIS buffers from Dickson's laboratory means that the routine use of electrode measurements should be re-evaluated. If an enhanced and more detailed version of SOP 6a were to be followed scrupulously, pH measurements with a precision of 0.005 may be obtainable in the future.

(J) The colorimetric method is described in SOP 6b “Determination of the pH of sea water using the indicator dye *m*-cresol purple” of Dickson *et al.* (2007). The manual method has been used successfully over a number of years by some laboratories (Perez and Fraga, 1987; Perez et al., 2010). To obtain accurate data, biases due to contamination of the dye need to be

taken into account (Yao *et al.*, 2007). The method has been automated (e.g. Friis *et al.*, 2004) but work is on-going in a number of laboratories to produce reliable systems that can be run autonomously. A system is available from Sunburst Sensors (<http://www.sunburstsensors.com/>, Seidel *et al.*, 2008). Wang *et al.* (2007) reported on an autonomous multi-parameter flow-through CO₂ system measuring surface seawater pH, carbon dioxide fugacity ($f\text{CO}_2$), and total dissolved inorganic carbon (DIC). All three measurements are based on spectrophotometric determinations of solution pH at multiple wavelengths using sulfonephthalein indicators. The field precisions were reported to be 0.0008 units for pH, 0.9 μatm for $f\text{CO}_2$, and 2.4 mol kg⁻¹ for DIC.

Getting pH measurements of high accuracy will continue to be difficult even with the availability of reference solutions. For example, the dyes used must be calibrated on an individual batch basis and calibration data extrapolated to true ionic medium composition (Dickson pers comm.: Yao *et al.*, 2007), and uncertainties still exist with regard to what is achievable with electrometric measurements. Currently in the absence of gross error an accuracy of 0.01 might be achievable by both approaches (Dickson pers comm.).

2.2.4 xCO₂ / pCO₂

A range of methods exist for the measurement of pCO₂ – (K) Direct - equilibrator infrared determination of xCO₂; (L) Indirect - membrane colorimetric determination of xCO₂; (M) Direct - membrane infrared determination of xCO₂; (N) MIMS. pCO₂ methods have been successfully adapted to provide continuous measurements in autonomous systems on buoys and ships of opportunity.

(K) This method is described in SOP 5 “Determination of pCO₂ in air that is in equilibrium with a continuous stream of sea water” of Dickson *et al.* (2007). Systems for autonomous use on ships of opportunity have evolved over the past 20 years (Schuster *et al.*, 2009) and have been improved through community effort and testing (e.g. Koertzing *et al.*, 2000). Systems are marketed by General Oceanics: <http://www.genocean.com/genocean/8050/8050.htm>. and Dartcomm-PML (contact nhm@pml.ac.uk). Detailed correction procedures for this method are described in Pierrot *et al.* (2009).

Discrete samples of seawater can be measured in a similar way; the uncertainty is increased to between 0.5 and 1.0%. One such method is described in SOP 4 “Determination of pCO₂ in air that is in equilibrium with a discrete sample of sea water” of Dickson *et al.* (2007); another in a paper by Neill *et al.* (1997).

(L) Two systems have been successfully developed based on the indirect determination of

pCO₂ by reaction with dye c.f method J. They have been used on moored systems and floating buoy. On the CARIOCA (CARbon Interface OCEan Atmosphere) buoys (Merlivat et al., 1995), the method uses thymol blue as the indicator dye (Lefevre et al., 1993). A similar indirect system, the SAMI, is produced by Sunburst Sensors (<http://www.sunburstsensors.com/>, DeGrandpre et al., 1995).

(M) Newer systems designed for use on moorings measure pCO₂ by direct equilibrium across a silicon membrane into a miniature gas stream pumped through an infra-red detector. Values for xCO₂ are output after corrections for measurement of temperature, gas stream pressure and humidity. These systems are available from ProOceanus (www.pro-oceanus.com) and Contros (<http://www.contros.eu>). These two systems and the NOAA surface float based system have recently been the subject of ACT (Alliance for Coastal Technologies) field evaluations.

(N) MIMS Membrane inlet mass spectrometry has been reported to provide a detector system that can be used for underway observations (Tortell, 2005). Additionally it can be used in the laboratory to measure pCO₂ levels in incubations (Hoppe et al., 2010). Rost et al. (2007) reported results from incubations in 8 ml thermostatted cuvettes, which were attached to a sector-field multi-collector-mass-spectrometer via a gas-permeable membrane (PTFE, 0.01 mm) inlet system.

Summary tables of total errors and biases for individual methods

Table 2.1 Generally accepted levels of error associated with each method				
		Ref Method	State of art	Other
	Total dissolved inorganic carbon			
(A)	Acidification / vacuum extraction / manometric determination	1.0		
(B)	Acidification / gas stripping / coulometric determination		2-3	
(C)	Acidification / gas stripping / infrared detection			4
(D)	Closed-cell acidimetric titration			10+
(E)	Auto-analyser colorimetric			5+
	Total alkalinity			
(F)	Closed-cell acidimetric titration		2-3	
(G)	Open-cell acidimetric titration	1.2		
(H)	Other titration systems			2-10
	pH			

(I)	Electrometric determination with standard TRIS buffer.		0.005	0.01-0.03
(J)	Spectrophotometric determination using I. <i>m</i> -cresol purple	0.003		
	xCO₂ / pCO₂			
(K)	Direct - equilibrator infrared determination of xCO ₂		2	
(M)	Indirect - membrane colorimetric determination of xCO ₂			2-10
(L)	Direct - membrane infrared determination of xCO ₂			1-10

Table 2.2 Present status (2010) of certified reference materials for the quality control of oceanic carbon dioxide measurements based on Dickson (2010).

Analytical Measurement n	Desired Accuracy ¹	Uncertainty ²	Certification
total dissolved inorganic carbon	$\pm 1 \mu\text{M kg}^{-1}$	$\pm 1 \mu\text{M kg}^{-1}$	since 1991 ³
total alkalinity	$\pm 1 \mu\text{M kg}^{-1}$	$\pm 1 \mu\text{M kg}^{-1}$	since 1996 ⁴
pH	± 0.002	± 0.003	since 2009 ⁵
Mole fraction of CO ₂ in dry air	$0.5 \mu\text{mol mole}^{-1}$	$\pm 0.1 \mu\text{mol mole}^{-1}$	since 1995 ⁶

¹ Based on considerations outlined in the report of SCOR Working Group 75 (SCOR, 1992). They reflect the desire to measure changes in the CO₂ content of sea water that allow the increases due to the burning of fossil fuels to be observed.

² Estimated standard uncertainties for the reference materials described here..

³ Sterilised natural seawater, certified using a definitive method based on acidification, vacuum extraction, and manometric determination of the CO₂ released. Available from UC San Diego (<http://andrew.ucsd.edu/co2qc/>).

⁴ 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/>).

⁵ Standard buffer solutions based on TRIS and AMP in synthetic seawater (Nemzer & Dickson, 2005; DeVallis & Dickson, 1998). Available from UC San Diego (<http://andrew.ucsd.edu/co2qc/>). These now available, in at present limited quantities, from Dickson's laboratory for the validation of locally prepared buffers. SOP 6a describes the preparation of buffers using 2-amino-2-hydroxy-1,3-propanediol (TRIS) and 2-aminopyridine (AMP) in synthetic sea water.

⁶ For calibration of continuous pCO₂ measurement systems, cylinders of air certified by non-dispersive infrared spectrometry are available from NOAA/ESRL, (<http://www.esrl.noaa.gov/gmd/ccgg/refgases/stdgases.html>). For a discrete $f(\text{CO}_2)$ measurements however, it is desirable to use a sterilized sea water sample as a reference material. The thermodynamics of the sea water system suggest that, since the CRMs are certified stable for CT, AT, and pH, they should be stable for $f(\text{CO}_2)$, however a reliable technique for independently determining $f(\text{CO}_2)$ to allow proper certification has not yet been developed.

3 Measurement of total alkalinity and the determination of carbonate alkalinity

Attention is given here to methods for measuring alkalinity because of known difficulties in deriving carbonate alkalinity from total alkalinity, and because Tyrrell et al. (2010) showed that errors in the determination of alkalinity could explain the discrepancies in the Hoppe et al (2010) data.

3.1. Available methods and procedures

For the last few decades, the measurement of carbonate alkalinity in seawater has been done indirectly, by measuring the total alkalinity of samples of seawater by titration with a strong acid (Dickson, 1992; Wolf-Gladrow et al., 2007). Briefly, a strong acid (usually HCl) is added in a stepwise manner to a known volume of seawater, while the electromotive force (e.m.f.) generated is measured using a pH electrode/reference electrode cell. TA is calculated from the volume of acid added and the e.m.f. data, usually using a modified Gran approach or a least-squares non-linear curve fitting method. Good precision and accuracy (~0.1%) can be achieved, when the titration procedure is automated and special care is taken to follow standard operating procedures (Dickson et al., 2007).

Total alkalinity may be measured using either a closed-cell or an open-cell titration. ***Closed cell TA titrations*** allow the concentration of total dissolved inorganic carbon (DIC) to be derived from the titration data (Edmond, 1970; Bradshaw and Brewer, 1988a; 1988b; Muller and Bleie, 2008). Since the coulometric technique for measuring DIC has proven more accurate and reliable (Dickson et al., 2007), true closed cell titrations are now less used than ***open cell titrations***. Open cell titrations are used to measure TA. Compared to closed cell titrations, the open cell procedure offers two main advantages: a) the volume of the seawater sample can be measured more easily and accurately, and b) the carbon dioxide is easily removed from the sample during the titration, this allows for a more reliable determination of the equivalence point. Dickson et al. (2003) describe the application of a true open cell method. It should be noted that the method employed by the Marianda VINDTA 3C a system, which is now commonly used for high precision measurements, uses a closed cell procedure with an open cell. This means that the values of DIC returned by the instrument are unreliable mainly due to the loss of CO₂ from the titration cell.

3.2 Errors associated with the TA titration procedures.

The accuracy and reproducibility of the TA determination depend on the quality of individual measurements performed during the analytical process. In what follows, we present possible errors associated with each step of the TA titration procedure.

Acid preparation The intrinsic accuracy of the TA determination (before correction relative to CRM measurements) is directly related to the accuracy with which the titrant acid is prepared and maintained. Dickson et al. (2003) discuss problems inherent in the standardisation procedures. The most common technique for preparing titrant HCl is by using commercially prepared ampoules of standardised concentrated HCl (e.g.. TITRISOL[®] by MERCK chemicals). The concentration accuracy of such ampoules is typically in the range of 0.2%. The acid concentrate from the ampoules is diluted with pure water to the desired concentration gravimetrically or volumetrically (this should be at a known temperature). The ionic strength of the titrant is adjusted to about that of average seawater (0.7 M) using analytical grade NaCl before the volumetric flask is filled to its calibration mark. The dilution step and addition of NaCl can introduce additional error to the final acid concentration due to impurities of the NaCl salt and volumetric or gravimetric uncertainties (~3 % has been suggested as possible by Dickson et al., 2003; Millero et al., 1993), therefore, a standardisation procedure is recommended. The HCl titrant can be standardised by titrating weighed amounts of Na₂CO₃ or TRIS dissolved in 0.7 M NaCl solutions (Millero et al., 1993). Alternatively, the concentration of the titrant can be determined precisely by coulometry where this possible (< 0.02 %)

Piston burette calibration/accuracy

Accurate and precise additions of the titration acid are key to accurate determination of the titration endpoints. Modern automated titration burettes are capable of high precision (~ 0.001 ml), although their accuracy may not be as good. Therefore, it is recommended that the accuracy of the titration-burette be tested periodically. The precision of piston burettes is a function of their volume. A smaller burette is usually capable of higher precision. It is recommended, however, that the burette is large enough to contain enough acid for the complete titration of a seawater sample.

A detailed procedure for calibrating the accuracy of automated titration-burettes can be found in Annex 2 of SOP 3a (Dickson et al., 2007).

Sample volume determination

Similarly the volume of seawater titrated must be accurately and precisely known. For closed-cell titrations, the volume of the seawater sample is essentially the volume of the cell. Therefore, the volume of the titration cell must be carefully calibrated, by weighing the titration cell with and without pure water. An analytical balance with a minimum sensitivity of 1 part in 10^5 should be used to weigh the titration cell (including electrodes, temperature sensors and expansion plunger). Ideally the calibration must be at the same temperature as the titrations to avoid errors associated with the expansion or contraction of the titration cell or electrodes. It must be noted that when closed-cell titration cells are used, the calibration of the cell must be checked every time the electrodes or temperature sensors are replaced or moved in any way.

For *open-cell titrations* the volume of the seawater sample is measured by a pipette before it is delivered to the titration cell. This pipette must be calibrated gravimetrically. Following the procedure described by Dickson et al. (2007) allows this to be done with an accuracy greater than 0.02 %.

Electrode accuracy/sensitivity

Models used to calculate TA from titration data (e.m.f. versus acid volume) assume that the pH electrodes used have a “Nernstian behaviour” (i.e. a slope = 59.16 mV/pH unit at 25°C). The slope of the output from pH electrodes can and does deviate from this value, leading to errors in the calculation of TA. Millero et al. (1993) investigated the sensitivity of TA calculations to electrode deviation from Nernstian response. They found that a 1 mV deviation in slope can lead to an error of $10 \mu\text{M kg}^{-1}$ in TA when pK_1 is fixed or $2.1 \mu\text{M kg}^{-1}$ when pK_1 is treated as a variable in the least-square calculations. To avoid electrode related errors in calculating TA, both the slope (Millero et al., 1993) and precision (Dickson et al., 2003) of the pH electrodes must be evaluated prior to titrations.

Electrode change in slope

Failure to maintain constant temperature during titrations, will lead to errors in TA values due to the change in the slope and standard potential of the electrode. The slope of the electrode (mV/pH) is a function of temperature:

$$E = E_0 - k \cdot T \cdot \text{pH}$$

Where E is the electrode's potential reading in mV, and E_0 is the standard potential of the electrode. The slope of the electrode is equal to kT and at 25°C its theoretical value is equal to 59.2 mV per pH unit. A 5°C fluctuation in temperature or error in temperature measurement can cause a 1 mV change in slope. As mentioned above, a 1 mV deviation in slope, can lead to 10 or $2.1 \mu\text{M kg}^{-1}$ error in TA depending on how pK_1 is treated in the least-square calculations (Millero et al., 1993).

Temperature control of titration cell and measuring pipettes.

Significant errors in the determination of TA can result from unaccounted fluctuations in temperature during the potentiometric titration. When the sample and acid titrant are measured volumetrically, special care must be taken so that temperature is kept as close as possible to the calibration temperature of the measuring apparatus (i.e. glass pipettes, burettes). For example, a 5°C change in temperature can lead to a 0.0045-0.009 % error in the volume of the glass pipette or burette. More significant however are changes in the volume of the titrating acid or seawater sample due to changes in temperature. A change in temperature of about 5°C could lead to a change in volume and consequently the concentration of the titrant (0.6 M kg^{-1} NaCl and 0.1 M kg^{-1} HCl) in the order of 0.1% (Dickson et al., 2007).

3. 3 Determining TA from raw analytical data

3.3.1 Gran function

For the calculation of the TA of a sample of seawater, the second inflection point of the titration curve must be accurately determined (See Figure 3.1). Dyrssen, (1965) first applied the ***Gran function*** method to calculate the TA and DIC of seawater samples from potentiometric titration data. Early applications of this method used simple Gran functions which ignored the contribution of non-carbonate species (Dyrssen, 1965; Dyrssen and Sillen, 1967). A major disadvantage of the Gran function method is that not all points of the titration curve are used for the calculation of TA and DIC. In particular, only ranges of acid additions, where the Gran function is linear, can be used while points near the buffer minima (near the inflection points) are omitted. Because the TA value calculated with simple Gran plots depends on the range of acid additions chosen for the linear fit, systematic errors can be as high as 1 % (Barren et al., 1983; Hansson and Jagner, 1973). Gran functions are used to estimate V_1 and V_2 ; the volumes of acid added to reach the carbonate/bicarbonate and bicarbonate/carbonic acid equivalence points. The TA can be derived directly from V_2 while

DIC is the difference between V_2 and V_1 . The simple Gran function method assumes that for $V > V_2$, all H^+ added forms free hydrogen ions. The mass balance condition for H^+ is then:

$$(V_0 + V) [H^+] = c(V - V_2) \quad \text{for} \quad (V > V_2)$$

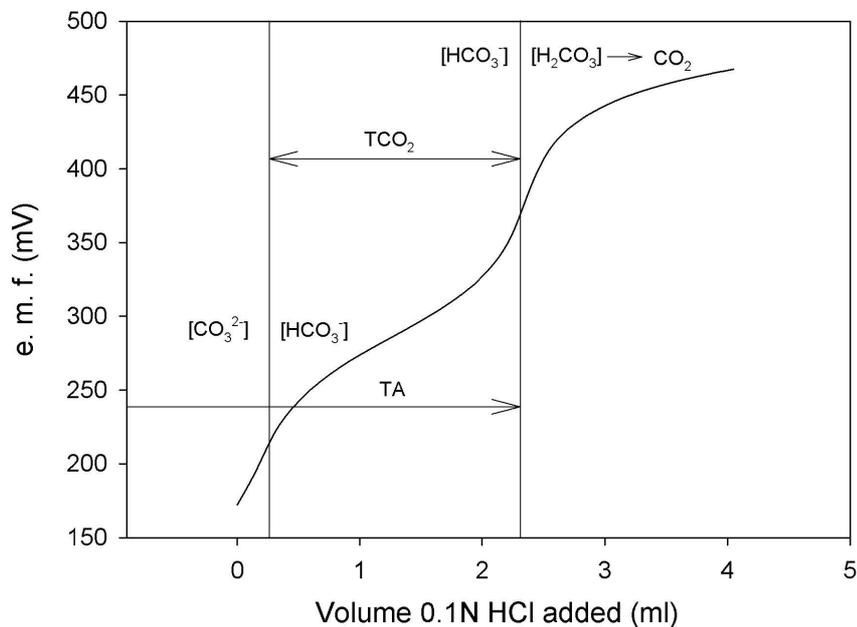
where V_0 is the volume of the seawater sample and c the concentration of the titrant.

At 25 °C $[H^+] \propto 10 \exp(E/59.16)$, therefore:

$$F_2 = (V_0 + V) 10 \exp(E/59.16) \propto (V - V_2)$$

If the above assumptions are true, F_2 is linear and intersects the x-axis at $V = V_2$.

Figure 3.1 Shows a typical titration curve for a sample of surface seawater - volume of 25 ml and salinity 35.



3.3.2 Modified Gran functions, were developed when more complex computational techniques became available. They enable more accurate determinations of TA by taking into account the effect of other ionic species including HSO_4^- , HF , $B(OH)_4^-$, and OH^- (Hansson and Jagner, 1973). The inclusion of these additional species decreased systematic error to about 0.1 % (Hansson and Jagner, 1973). Where the simple Gran function assumes that for $V > V_2$, all H^+ added forms only free hydrogen ions, the reality is that some bicarbonate still remains un-titrated while a significant amount of hydrogen sulphate is being formed.

Furthermore, some of the acid added above V_2 is consumed by the formation of hydrofluoric acid. Therefore, the modified Gran function can be more accurately described by:

$$F_2 = (V_0 + V)([H^+] + [HSO_4^-] + [HF] - [HCO_3^-]) \propto (V - V_2)$$

(Note where $V > V_2$ the concentrations of borate, hydroxide and carbonate ions are low and can be neglected.)

The contribution of nutrients (i.e. phosphate and silicate) are included in more sophisticated Gran functions which improve the estimation of TA especially in nutrient rich waters (Bradshaw et al., 1981). Such models offer lower errors in estimating TA (< 0.1) although the biggest improvement was in the estimation of DIC (Barron et al., 1983).

3.3.3 Non-linear least squares method

To avoid calculating TA based only on a small number of titration points, a non-linear least square estimates method can be employed to fit a model curve directly to the titration data (Dickson, 1981; Johansson and Wedborg, 1982). The quality of the fit and the TA value calculated by the model is dependent on the number of parameters treated as variables. For calculations of TA and DIC from titration data, higher TA accuracy is achieved by using a 4-parameter fit (TA, DIC, E_0 , and pK_1 are used as variables) (Barron et al., 1983; Millero et al., 1993b). Millero et al. (1993b) found that the precision with which the model fitted the data was less than $0.4 \mu\text{M kg}^{-1}$ (0.02 %) when pK_1 was allowed to vary (along with DIC, TA and E_0) and $1.5 \mu\text{M kg}^{-1}$ (0.07%) when it was not. This was also reported elsewhere (Barron et al., 1983; Buler, 1992). It must be noted that when only TA is determined from potentiometric titrations, only data between pH 3 and 3.5 are needed (Dickson et al., 2003). In this case only E_0 and TA are treated as fitting parameters.

For the least squares procedure it is necessary to start with reasonable estimates for TA and E_0 . This can be done using Gran plots (see previous section). The TA and E_0 values are then refined using a non-linear least squares calculation. At each point of the titration curve, TA can be calculated from:

$$\frac{(V_0 \cdot TA - V \cdot c)}{(V_0 + V)} = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [HS^-] + [NH_3] - [H^+] - [HSO_4^-] - [HF] - [H_3PO_4]$$

where V_0 is the initial volume of the sample, c is the normality of the titrant, and V is the volume of the acid added. In the calculations all the volumes are converted to mass using the known densities of the solutions. Computer software can be used to fit the above expression to the experimental data by minimizing the sum of squares of residuals through the fine adjustment of E_0 , TA, pK_1 , and DIC. E_0 is used for the calculation of $[H^+]$ from:

$$[H^+] = \exp\left(\frac{E - E_0}{RT/F}\right)$$

More details on the non-linear least squares method can be found in Dickson et al. (2007).

Simpler methods have been used to calculate TA from potentiometric titration data including the derivative method (Hernandez-Ayon et al., 1999; 2007; Muller and Bleie, 2008; Rigobello-Masinis and Masinis, 2001). This method involves plotting the slope of the titration curve dpH/dV_{HCl} against V_{HCl} . The TA is calculated as with the Gran function from the second inflection point, which in this case is represented by a sharp slope maximum. Simple computational techniques can be used to calculate V_{HCl} at the slope maximum, which is then used to calculate TA. The biggest disadvantage of this method is again that only a few points of the titration curve are used for the calculation of TA while the estimation of dissociation constants and the electrode's standard potential is not possible. The precision and accuracy of this method is around 0.2-0.4% (Hernandez-Ayon et al., 1999; Muller and Bleie, 2008).

3.4 Other sources of error in alkalinity titrations, including organic acids

3.4.1 Influence of suspended particulate matter

Suspended particles found in seawater including phytoplankton, bacteria, biogenic and lithogenic minerals generally carry a negative charge which originates from the protonation and de-protonation of ionizable surface species, i.e. carboxyl, hydroxide, phosphate and amino groups (Kim et al., 2006; Loucaides et al., 2010). Because of their net negative surface charge, suspended particles can significantly contribute to TA if present in high concentrations. Kim et al. (2006) found that high concentrations of phytoplankton in cultures ($POC = 200 \mu M l^{-1}$) contributed between 3 and $5 \mu M kg^{-1}$ to TA. Such concentrations, however, are unlikely to be encountered in the ocean where POC usually ranges between 1 and $30 \mu M kg^{-1}$ (Poulton et al., 2007). Bacteria are abundant in seawater. Kim et al. (2006) found that the contribution of bacteria (> 0.45 and $< 0.7 \mu m$ mesh) at the eastern coastal waters of the Korean peninsula ranged between 1 and $6 \mu M kg^{-1}$ to TA. Chanson and Millero

(2007) followed up the work of Kim et al. (2006) (c.f. section 2.1 above) and found that samples of open ocean water from the Pacific did not contain significant enough amounts of bacteria for the measured TA to change when filtered through 0.45 micron filters. More studies are needed to determine the contribution of bacteria to measured TA in a wider range of marine waters.

The contribution of charged biogenic silica particles to TA is less significant. If we assume a maximum charge density of about $4 \mu\text{M g}^{-1}$ (Loucaides et al., 2010), a biogenic silica concentration in the sample would need to be greater than 4 mM kg^{-1} to cause a change in TA of about $1 \mu\text{M kg}^{-1}$. Biogenic silica concentrations even during diatom blooms are generally much lower (Kristiansen et al., 2000).

Dissolution of CaCO_3 particles (PIC) during acidimetric titrations can cause significant errors in the determination of TA as dissolving one mole of CaCO_3 increases TA by two moles. The concentration of PIC during coccolithophore blooms in high latitudes can reach concentrations up to $300\text{-}500 \text{ mg m}^{-3}$ (Fernández et al., 1993; Holligan et al., 1993). Such high PIC concentrations in unfiltered seawater samples could lead to errors in TA of between 6 and $10 \mu\text{M kg}^{-1}$.

3.4.2 Influence of dissolved organic matter

Discrepancies between measured and calculated TA (from DIC, pH, pCO_2) have been reported since precise measurements of TA and DIC became available (Bradshaw and Brewer, 1988a). The over-estimation of carbonate alkalinity from TA lead to speculation that unidentified organic bases contributed significantly to TA (Bradshaw and Brewer, 1988a; 1988b; Rigobello-Masini and Masini, 2001). Without direct evidence of the presence of such organic species, workers have attempted to quantify their contribution to TA by comparing measured and calculated TA in over determined systems where pH has been measured along with TA and DIC (Hernandez-Ayon et al., 2007; Kim and Lee, 2009; Muller and Bleie, 2008). Hernandez-Ayon et al. (2007) estimated the concentration of organic bases in cultures to be as high as $800 \mu\text{M kg}^{-1}$ and in the northern Gulf of California as high as $109 \mu\text{M kg}^{-1}$. Higher values were estimated in San Diego Bay ($190 \mu\text{M kg}^{-1}$) while in San Quintin Bay concentrations of organic bases were lower ($\sim 75 \mu\text{M kg}^{-1}$). Kim and Lee (2009) found that the difference between measured and calculated TA increased in phytoplankton cultures with increasing DOC in a near 1:1 ratio. The highest difference between measured and calculated

TA in their cultures was about $80 \mu\text{M kg}^{-1}$. Muller and Bleie (2008), found a smaller but significant differences ($\sim 20 \mu\text{M kg}^{-1}$) in mesocosms in a Norwegian fjord.

Table 3.1 Provides a summary of the errors due to factors discussed above. Errors are expressed as % of the final value, and the change in the scale of the error as the sophistication of the calculation is increased from a simple Gran plot to least squares fit with 4 parameters is applied is shown.

Source of error		Error (%)				
		Gran	Derivative	Mod. Gran	N.L.L.S. 3 par.	N.L.L.S. 4 par.
Temperature 5 °C error	electrode	0.4	0.4	0.4	0.4	0.1
	acid dosage ¹	0.1	0.1	0.1	0.1	0.1
	sample measurement ²	0.1	0.1	0.1	0.1	0.1
Electrode slope ³ 1 mV/pH error		0.4	0.4	0.4	0.4	0.1
Acid concentration ⁴		3	3	3	3	3
	Standardized	0.02	0.02	0.02	0.02	0.02
Piston burette precision ⁵		0.02	0.02	0.02	0.02	0.02
Sample volume precision ⁶		0.02	0.02	0.02	0.02	0.02
Data fitting model ⁷		1	0.2	0.1	<0.1	<0.05

¹. Based on the change in concentration of the acid and expansion or contraction of the glass burette

². Taking into account the expansion of seawater and the glass pipette

³. From Millero et al. (1993b)

⁴. From Millero et al. (1993b) and Dickson et al. (2003)

⁵. Based on the precision of automated titrator burettes (~ 0.001 ml) from Millero et al. (1993b).

⁶. The precision of measuring a sample of seawater gravimetrically or by a calibrated pipette for an open cell titration.

⁷. From Barron et al. (1983), Millero et al. (1993b), Dickson et al. (2003), and Hernandez-Ayon et al. (2007).

3.5 Recommendations on the determination of TA in seawater

Above we have identified a number of problems that can potentially reduce the accuracy and precision of measurements TA and therefore the determination of carbonate alkalinity. Below we summarise the evidence and make recommendations for further work. This in terms of best practice and investigation of the identified problems with measurement of TA. (In the following Section 4, we demonstrate how errors in the determination of a carbonate system component effect the calculation of the other components.)

- High concentrations of suspended material in seawater can be a significant source of TA. Bacteria can lead to TA errors in the order of $5 \mu\text{M kg}^{-1}$. Calcite particle concentrations during coccolithophore blooms can lead to an overestimation of TA of the order of $6\text{-}10 \mu\text{M kg}^{-1}$ for PIC values between $300\text{-}500 \text{ mg m}^{-3}$. Therefore, in high productivity waters,

filtration ($\sim 0.45 \mu\text{m}$ filter) is recommended. In particular further work to identify which waters contain sufficient bacteria to effect the measurements of TA is needed.

- Interference from suspended including bacteria can be removed by filtering. Interference from dissolved organic matter cannot be removed. Further investigation of contribution of dissolved organic alkalinity is needed to establish the extent of the problem identified by Hernandez-Ayon et al. (2007), Kim and Lee (2009) and Muller and Bleie (2008). This is a critically needed piece of work, and as Tyrrell et al. (2010) have demonstrated this problem may be the source of the discrepancies identified by Hoppe et al. (2010).
- The open cell titration is the recommended method for the determination of TA. When only TA is determined, the pH range of the titration should be between ~ 3.6 and ~ 3 . An initial acid addition step to reduce the pH to 3.6, followed by no less than 6 minutes during which the sample is stirred and allowed to degas, should allow for a more accurate determination of the equivalence point, as CO_2 species do not have to be taken into account. A detailed methodology can be found in CO2-SOP 3b (Dickson et al., 2007).

The existence of certified reference materials is a great boon to those making carbonate system measurements and has greatly enhanced the inter-comparability of data. However they have also lead to a degree of laziness. Effort should be made to achieve results as close as possible to the CRM value. To achieve this, the following points need to be taken into account.

- The acid titrant should be standardised to avoid concentration errors as high as 3%.
- Titrations should be performed at a controlled temperature ($\Delta T < 0.5 \text{ }^\circ\text{C}$). Reagents must be prepared at a know temperature when the preparation is done volumetrically. The titration cell, and ideally the titrant solution bottle and burette should be thermostatted.
- The precision and response (slope) of the pH electrodes must be monitored. Electrodes with non-Nernstian behaviour (slope $> 1.0 \text{ mV}$ different from the Nernst value) should not be used.
- The accuracy of the titration burette must be determined and monitored periodically following CO2-SOP 3a-Annex 2 (Dickson et al., 2007).
- The seawater samples must be measured gravimetrically or volumetrically with a calibrated thermostated glass pipette. A detailed method for calibrating a glass pipette can

be found in SOP 13 (Dickson et al., 2007). Note: If precision of the pipette is not better than 0.1 % the precision of TA and DIC analyses can never be better than $2 \mu\text{M kg}^{-1}$.

- For open cell titrations between $\sim \text{pH } 3$ and $\sim \text{pH } 3.6$, the Gran method can be used to estimate E_0 and TA. Then, a non-linear least square estimates method can be used to refine the E_0 and TA values.
- Nutrient concentrations must be measured in conjunction with TA measurements. Failure to do so will lead to an over-estimation of carbonate alkalinity. When pCO_2 is calculated from TA, an error of $4.5 \mu\text{atm}$ for $1 \mu\text{M kg}^{-1}$ phosphate and $0.1 \mu\text{atm}$ for $1 \mu\text{M kg}^{-1}$ of silicate should be expected if concentrations of nutrients are not taken into account.

4 Variation in magnitude of a calculated carbonate variable relative to changes in magnitude of the two in-put variables, illustrated in contour plots.

Much of the new work on ocean acidification is concerned with the impact of the future degree of acidification on ecosystems. This requires the manipulation of experimental systems (e.g. Hurd et al., 2009) beyond present conditions in ocean waters. It also requires an awareness of the modification of carbonate system parameters needed to produce the required change in $p\text{CO}_2$ in the system (Hurd et al., 2009). Ocean acidification experiments are designed to run at target atmospheric CO_2 (and hence $p\text{CO}_2(\text{aq})$) values, between about 200 and 1000 μatm (Barry et al., 2010). It is important to be able to ascertain from measurements whether or not the experiments have actually been run at those target values. It is not straightforward to measure $p\text{CO}_2(\text{aq})$ in experimental cultures. Therefore $p\text{CO}_2(\text{aq})$ has often to be calculated from other measurements. Here we provide a set of maps that illustrate the relationship between the values of a measurement pair and the calculated third carbonate variable. This has been done so that the sensitivity of the calculated value to errors in the input variables can be judged. Consistent ranges of axis values are used across the diagrams based on field observations (Table 4.1; Key et al., 2004). It can be seen in Table 4.1, that while the range of values of DIC is only about 20%, values of $p\text{CO}_2$ change by 3 fold. The maps in Figures 4.1 to 4.3 provide a graphical illustration of how the components interact (leaving aside the uncertainty in the calculation of carbonate alkalinity in coastal water and experimental system containing significant concentrations of organic matter).

Table 4.1. Range of observed values of measurable carbonate system parameters in open ocean surface waters. The ranges of DIC and alkalinity were obtained from the GLODAP database (Key et al., 2004) and represent surface water (0-10 m) of the open ocean excluding coastal or shelf environments. The ranges of pH and $p\text{CO}_2(\text{aq})$ were calculated from DIC and alkalinity using the CSYS software (Zeebe, 2010), with temperature and salinity taken from the World Ocean Atlas database.

Parameter	Observed Range (present-day)	Units
[DIC]	1840 to 2200	$\mu\text{M kg}^{-1}$
[Alk]	2170 to 2460	$\mu\text{M kg}^{-1}$
pH	7.9 to 8.4	(total scale)
$p\text{CO}_2(\text{aq})$	127 to 524	μatm

The maps have to be viewed in the context of likely errors in the measurements, these have been summarised by Dickson (2010). These are repeated below in Table 4.2. In Table 4.2 these errors are compared to the shift in the value of each variable required to change the pH

of a sample by 0.01 with the other variable kept constant at a temperature of 15 °C and a salinity of 35.

Table 4.2 Change in carbonate system variable required to change pH by 0.01 units all other components kept constant. Calculated using CO2SYS with the Mehrbach constants refitted by Dickson and Millero, pH on total scale.

	Change	Uncertainty state of the art (Dickson, 2010)
DIC	5.00 $\mu\text{M kg}^{-1}$	3 $\mu\text{M kg}^{-1}$
TA	-5.00 $\mu\text{M kg}^{-1}$	3 $\mu\text{M kg}^{-1}$
pCO ₂	10.00 μatm	2 μatm
pH		0.005
Temp	0.60 °C	0.01 ^a
Salt	1.00	0.01 ^a
PO ₄	5.00 $\mu\text{M kg}^{-1}$	0.01
Si	170.00 $\mu\text{M kg}^{-1}$	0.1

^a Likely quality of determination

For completeness in Table 4.3 we give an indication of the likely error in the calculation of pCO₂, pH and Ω from other pairs of variables for a typical surface ocean water.

Table 4.3 Paired variable calculation errors based on likely state of the art analyses and associated uncertainties (Dickson, 2010; Table 4.2 above) for samples at salinity 35, temperature 15 °C, and concentrations of DIC and TA around DIC 2070 $\mu\text{M kg}^{-1}$, TA 2300 $\mu\text{M kg}^{-1}$

	pCO ₂	pH	Ω_{cal}
DIC/TA	12	0.012	0.10
DIC/pH	6		0.03
DIC/pCO ₂		0.005	0.06
TA/pH	6		0.03
TA/pCO ₂		0.002	0.04
pH/pCO ₂			0.18

4.1 Calculation of pCO₂

Accurate estimates of pCO₂ are essential for the design of reproducible ocean acidification experiments, and for being able to inter-compare different sets of experimental results. Figures 4.1 a, b and c illustrate the variation in pCO₂ when calculated from other variable pairs. The contour plots show pCO₂ (μatm) as calculated from (a) DIC and pH, (b) DIC and TA (c) pH and TA. These plots are calculated for a surface water temperature = 15°C and salinity = 35. In Fig 4.1b it can be seen from the near 1:1 gradient of the contour lines that calculated values of pCO₂ from DIC and TA are similarly sensitive to the values of both

parameters. Similar sized errors in either DIC or TA have similar effects on the calculated $p\text{CO}_2$. The error in $p\text{CO}_2$ increases rapidly at higher DIC and lower values of TA. At higher values of $p\text{CO}_2$ the contour lines are more closely packed in Figure 4.1b. Figures 4.1a and 4.1c show the calculation using pH with DIC and TA respectively. In these figures the gradient of change of $p\text{CO}_2$ with respect to either DIC or TA is small. This behaviour is consistent with the discrepancies in calculated values of $p\text{CO}_2$ reported by Hoppe et al. (2010). That is to say, when $p\text{CO}_2$ is calculated from DIC and TA, the derived value will be sensitive to an error in the value of TA. If, as we suspect, TA is over estimated due to the presence of organic alkalinity, the apparent higher value of TA will depress the calculated value of $p\text{CO}_2$. However, when either DIC or TA is paired with pH the calculation is sufficiently insensitive to variations in DIC or TA that the error in DIC or TA does not lead to a significant discrepancy between measured and calculated $p\text{CO}_2$ (again c.f. Hoppe et al., 2010). In Figure 4.1a the reason for the lack of sensitivity of calculated $p\text{CO}_2$ to DIC is that for any particular value of pH we know the relative proportions of $[\text{CO}_2(\text{aq})]$, $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$ in [DIC]. This can be illustrated in Bjerrum plots (see for instance Zeebe and Wolf-Gladrow, 2001; Figure 1.1.4). At a fixed pH, $[\text{CO}_2(\text{aq})]$ is linearly proportional to [DIC]. The same is true for $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$. Therefore, $p\text{CO}_2$ is also linearly proportional to [DIC] at constant pH, when T, S and pressure are also fixed (because solubility is then fixed). At a given value of pH, therefore:

$$p\text{CO}_2(\text{aq}) = k * [\text{DIC}]$$

where k is a constant. When [DIC] varies by 20% (1840 to 2200; the range on the x-axis of Fig 4.1a and 4.1b), then the calculated $p\text{CO}_2$ necessarily varies by exactly 20% as well. This is a relatively small range compared to the overall range of change in $p\text{CO}_2$ that is possible. The same reasoning holds for the insensitivity of calculated $p\text{CO}_2$ values to TA, when the measurement pair is TA and pH - the range in TA values on the y-axis of Fig 4.1c is 13% and the range in $p\text{CO}_2$ values along any vertical line through the plot is also 13%.

4.2 Calculation of $[\text{CO}_3^{2-}]$ and Ω_{calcite} and $\Omega_{\text{aragonite}}$

The calculation of $[\text{CO}_3^{2-}]$ is especially important in OA experiments for two reasons: (1) unlike $p\text{CO}_2(\text{aq})$ it cannot be measured and (2) it determines the degree of saturation of seawater for CaCO_3 shells (Ω_{calcite} and $\Omega_{\text{aragonite}}$). Accurate determination of the degree of saturation is key in OA research, because evidence shows vulnerability of many calcifiers to OA-related changes in the corrosiveness of seawater with respect to CaCO_3 .

Similar results are obtained when $[\text{CO}_3^{2-}]$ is calculated from the different measurement pairs out of (DIC & TA), (DIC & pH) and (pH & TA) (Figures 4.2 a, b & c). The determination of $[\text{CO}_3^{2-}]$ is less vulnerable to errors in TA or DIC measurements, when these are combined with accurate measurements of pH.

Figures 4.3 a, b & c show results of calculating $[\text{CO}_3^{2-}]$ from different parameter pairs when pCO_2 is one of the pair. It can be seen that pCO_2 and pH are a poor measurement pair for this purpose. Closely-spaced contour lines indicate a high degree of sensitivity to any errors in the measurements of either pCO_2 or pH. This is because pCO_2 and pH are not fully independent measurements of the carbonate system.

4.3 Findings

Interpretation of the above figures leads to the following implication in terms of how best to determine pCO_2 from pairs of the other carbonate variables. If the actual value of TA is thought to be unreliable, because of an organic-alkalinity contribution, it is better to combine TA with pH than with DIC, because in the former case the error in TA leads to a much smaller associated error in pCO_2 . This conclusion depends critically on the assumption that pH can be measured with appropriate accuracy. High priority should be accorded to improving methods for measuring pH. When pH can be measured accurately, then, when combined with even moderately accurate measurements of DIC or TA, it enables accurate and robust determinations of $\text{pCO}_2(\text{aq})$, $[\text{CO}_3^{2-}]$, Ω_{calcite} and $\Omega_{\text{aragonite}}$. All of these determinations will be relatively little affected by the presence of organic-alkalinity if pH and TA are used, and not at all affected if pH and DIC are used. The diagrams also suggest that accurate measurements of pH can be the key that opens the door to accurate determinations of all of the most important parameters for OA research. Conversely, however, inaccurate pH measurements will impair the determinations of all the other parameters, when pH is one of the measured variables used in the calculation. Hence the quality of the carbonate system calculations stands or falls depending on the accuracy of the measurements of pH.

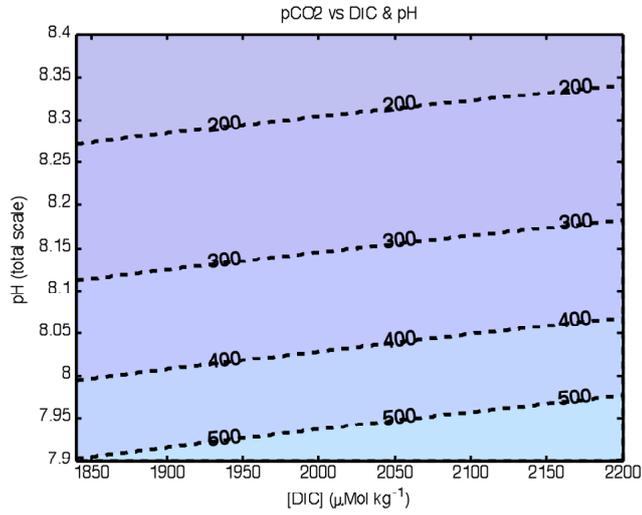
The introduction of certified TRIS buffers from Dickson's laboratory is a major advance towards achieving reliable measurements of pH. Using certified TRIS buffers it should be possible to make acceptable pH measurements using both the spectrophotometric and potentiometric methods. Laboratory measurements should then be possible with precision approaching 0.005.

To help a wide range of laboratories achieve this precision an updated and more detailed version of CO₂-SOP 6a (Dickson et al., 2007) is needed. This should include specific advice on the design and configuration of the measurement cell, specification and recommendation of electrode types and volt-meters, and advice on temperature measurement with probes internal or external to the electrode, the need for temperature stabilisation and stirring. Additional recommendation should be provided on the maintenance of electrodes.

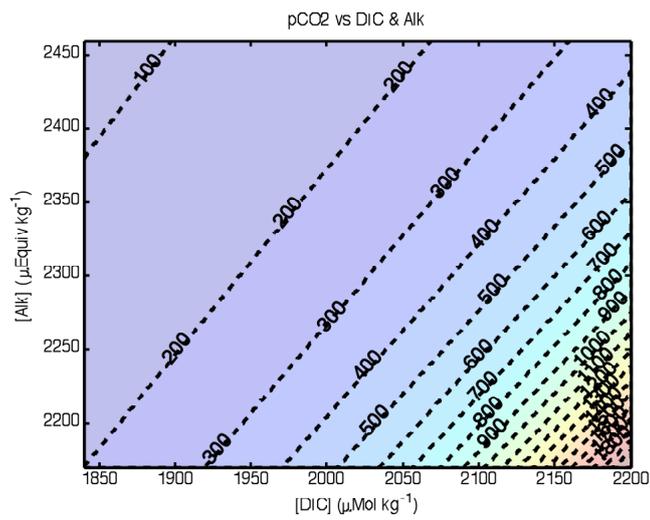
It is pertinent to remember previous recommendations (Orr et al., 2009) that “*the uninformed use of cheap, ‘off-the-shelf’ pH sensors should be discouraged for ocean acidification-related purposes, because high-quality results are unlikely to be obtained.*” For the same reason continued use of measurements on the NBS scale is discouraged. We would say that such pH sensors should never be used and that for consistency all data should be reported on the “total hydrogen ion” pH scale.

Figure 4.1: Contour plots of partial pressure of carbon dioxide in seawater ($p\text{CO}_2$ μatm) as calculated from (a) DIC and pH, (b) DIC and TA (c) pH and TA. These plots are calculated for surface waters temperature = 15°C and salinity = 35.

4.1a



4.1b



4.1c

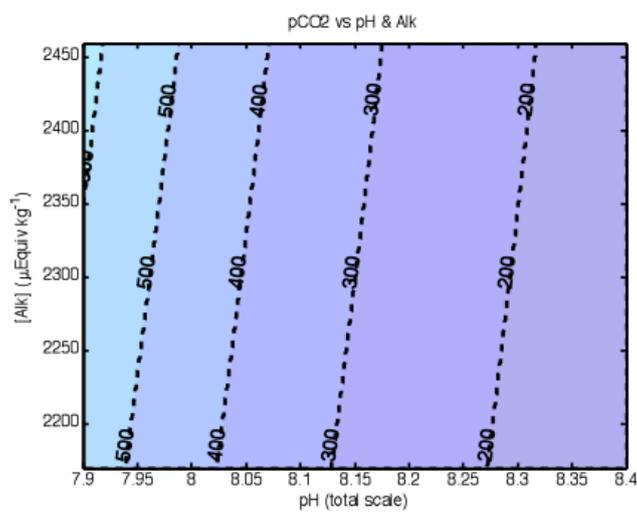
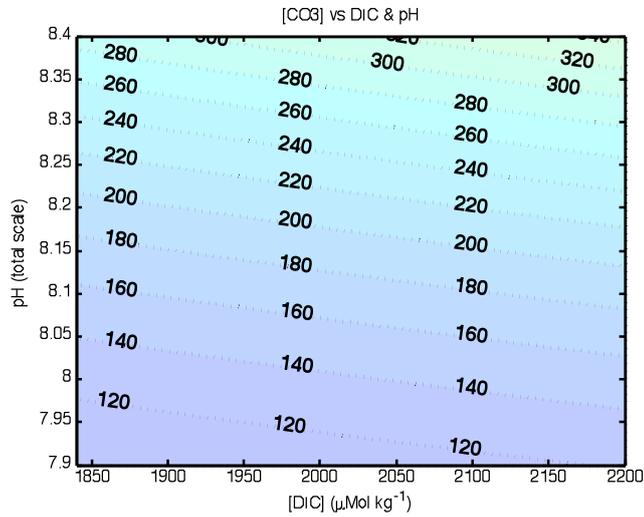
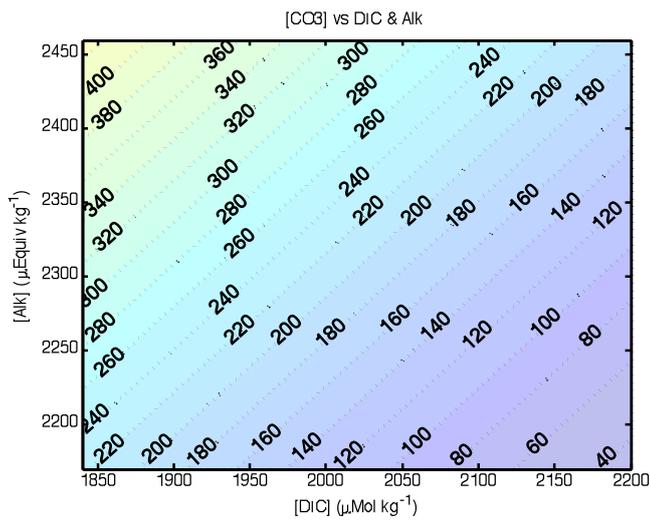


Figure 4.2: Contour plots of carbonate ion concentration in seawater ($[\text{CO}_3^{2-}]$ $\mu\text{M kg}^{-1}$) as calculated from (a) DIC and pH, (b) DIC and TA, and (c) pH and TA. These plots are calculated for surface waters, temperature = 15°C and salinity = 35.

4.2a



4.2b



4.2c

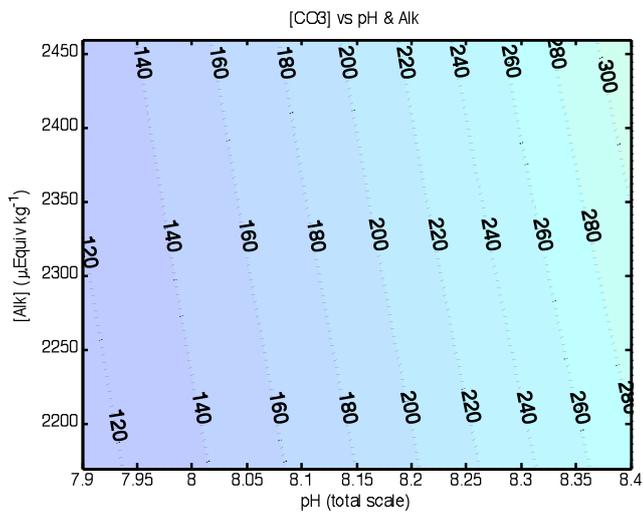
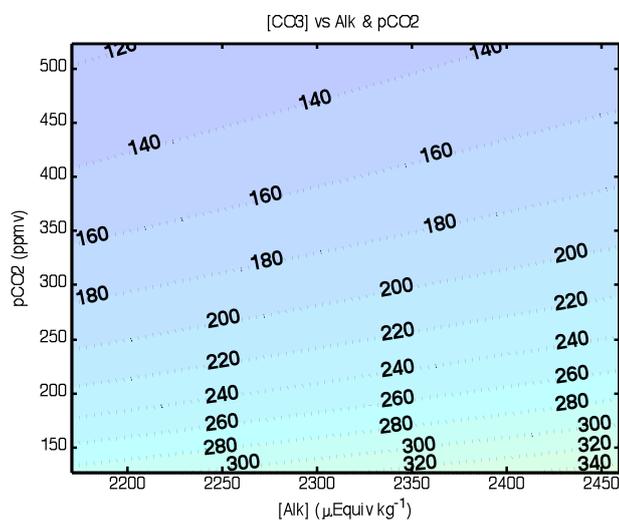
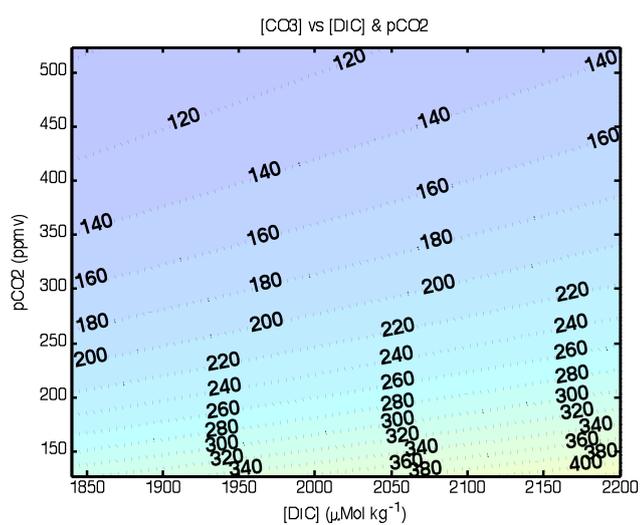


Figure 4.3: Contour plots of carbonate ion concentration in seawater ($[CO_3^{2-}] \mu M kg^{-1}$), calculated from (a) DIC and pCO_2 , (b) TA and pCO_2 , and (c) pH and pCO_2 . These plots are calculated for surface waters, temperature = $15^\circ C$ and salinity = 35.

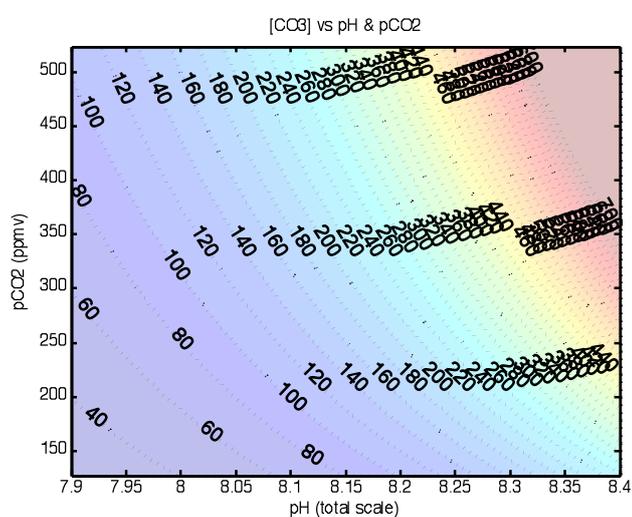
4.3a



4.3b



4.3c



5 Calculation of carbonate speciation in seawater using computer software

The speciation of the carbonate system in seawater can be determined knowing two of the four concentration-related parameters (DIC, TA, pH, pCO₂) along with the salinity and temperature of the sample. The calculations are elaborate and time consuming if done from first principles, therefore, one of several freely distributed computer programs tends to be used. The most commonly used are CO2SYS¹, SEACARB², and SWCO2³. Because no standard software package has been recommended, the choice of software is made strictly based on personal preference and familiarity with a particular program. Therefore, we believed it is appropriate in this work to compare these computer programs in order to establish if the choice of software presents a bias to the calculation of the carbonate system speciation.

The three computer packages all follow the same principle in which the carbonate system speciation is calculated based on two input parameters (DIC, TA, pH, pCO₂), temperature, salinity, pressure and concentrations of phosphate and silicate. In return, the software calculates the other two parameters, and provides a detailed break down of the speciation of DIC and the calcite and aragonite saturation states and Revelle factor. The user has a range of choices for the first and second dissociation constants of carbonic acid and pH scales. CO2SYS and SWCO2 are available as Windows Excel macros while, SEACARB is written in the R language. Unlike the other packages, which only calculate the carbonate speciation of seawater, SEACARB also includes a number of other functions designed to assist with the design and analysis of ocean acidification perturbation experiments (Gattuso and Lavigne, 2009). This advantage has made SEACARB increasingly popular throughout the ocean acidification community. SEACARB is updated frequently with new features and applications. The latest version at the time this publication was composed was 2.3.2. (March, 2010).

To test how closely the three packages agree in terms of calculating the carbonate speciation of seawater, we used a range of TA and DIC values and the carbonic acid dissociation constants from Roy et al. (1993) to calculate pCO₂ and pH_T. The results produced by SWCO2 were identical with those calculated by CO2SYS. SEACARB, however, produced slightly different values, although the difference from the output of the other two packages was insignificant ($\Delta\text{pH} < 0.0001$, $\Delta\text{pCO}_2 < 0.5$).

The choice of dissociation constants for carbonic acid can be significant in determining the calculated speciation of the carbonate system. To provide a quantitative comparison of these differences, in a consistent way, we used CO2SYS to calculate pH and pCO₂ at a fixed alkalinity (2300 μM kg⁻¹), a temperature of 15.0 °C and salinity of 35.0. This was done over a range of values of DIC that generate values of pCO₂ between 200 and 1000 μatm using the constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). These data were taken as the reference as they are generally taken to be the most appropriate constants for use in real seawater (Wanninkhof et al., 1999; Lueker et al., 2000). This was then repeated using the same values of DIC, stepping through the 6 different sets of published constants used in CO2SYS. The results of these calculations are shown in Table 5.1. At the highest levels of pCO₂ the other sets of constants predict pCO₂ levels some 10s of μatm higher. Roy et al are highest at 1053 μatm. The constants of Mojica Prieto and Millero give the closest fit to the refitted Mehrbach constants.

The deviation between refitted Mehrbach and Roy is 5 %. This much smaller than the 25 % deviation reported by Hoppe et al. (2010). Using the Roy constants actually reduced the deviation reported by Hoppe et al., relative to what they would have reported using the refitted Mehrbach constants. Choice of constants cannot be the explanation for the Hoppe et al. discrepancies.

Links

¹CO2SYS

<http://cdiac.ornl.gov/oceans/co2rprt.html#aboutco2sys>

²SEACARB

Latest version now: Lavigne, H. and Gattuso, J.-P. (2010) seacarb: seawater carbonate chemistry with R. R package version 2.3.4. <http://cran-project.org/package=seacarb>

³SWCO2

http://neon.otago.ac.nz/research/mfc/people/keith_hunter/software/swco2/

Table 5.1. Calculations of $p\text{CO}_2$ and pH_T using COSYS and constants for the dissociation of carbonic acid from several sources. TA and DIC were used as input parameters. TA and DIC in $\mu\text{M kg}^{-1}$ and $p\text{CO}_2$ in μatm .

k_1 k_2 from \rightarrow		Mehrbach et al. (1973) ¹		Roy et al. (1993)		Goyet and Poisson, (1989)		Hansson (1973)		Hansson and Mehrbach ²		Mojica Prieto (2002)		Mojica Prieto (2002) ³	
TA	DIC	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T	$p\text{CO}_2$	pH_T
2300	1942	200	8.292	215	8.273	214	8.280	212	8.286	211	8.284	201	8.291	201	8.291
2300	2081	400	8.045	427	8.028	426	8.034	422	8.041	419	8.038	402	8.045	405	8.041
2300	2120	501	7.961	534	7.945	532	7.951	527	7.957	524	7.955	503	7.961	511	7.953
2300	2183	750	7.805	795	7.791	793	7.797	786	7.803	781	7.800	753	7.804	773	7.792
2300	2223	1000	7.691	1053	7.680	1051	7.686	1043	7.691	1037	7.688	1003	7.691	1035	7.676
Difference from values derived using the refitted Mehrbach constants															
2300	1942	200	8.292	-15	0.019	-14	0.012	-12	0.006	-11	0.008	-1	0.001	-1	0.001
2300	2081	400	8.045	-27	0.017	-26	0.011	-22	0.004	-19	0.007	-2	0.000	-5	0.004
2300	2120	501	7.961	-33	0.016	-31	0.010	-26	0.004	-23	0.006	-2	0.000	-10	0.008
2300	2183	750	7.805	-45	0.014	-43	0.008	-36	0.002	-31	0.005	-3	0.001	-23	0.013
2300	2223	1000	7.691	-53	0.011	-51	0.005	-43	0.000	-37	0.003	-3	0.000	-35	0.015

¹Constants refitted by Dickson and Millero (1987) ²Constants refitted by Dickson and Millero (1987) ³Corrected for DIC

6 References

- Barron, J. L., Dyrssen, D., Jones, E. P., and Wedborg, M., 1983. A comparison of computer methods for seawater alkalinity titrations. *Deep Sea Research Part A. Oceanographic Research Papers* 30, 441-448.
- Barry, J. P., Tyrrell, T., Hansson, L., Plattner, G.-K., and Gattuso, J.-P., 2010. Atmospheric CO₂ targets for ocean acidification perturbation experiments, in: *Guide to best practices in ocean acidification research and data reporting*, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P., Luxembourg: Publications Office of the European Union, 53–66.
- Bradshaw, A. L. and Brewer, P. G., 1988a. High precision measurements of alkalinity and total carbon dioxide in seawater by potentiometric titration -- 1. Presence of unknown protolyte(s)? *Marine Chemistry* 23, 69-86.
- Bradshaw, A. L. and Brewer, P. G., 1988b. High precision measurements of alkalinity and total carbon dioxide in seawater by potentiometric titration. 2. Measurements on standard solutions. *Marine Chemistry* 24, 155-162.
- Bradshaw, A. L., Brewer, P. G., Shafer, D. K., and Williams, R. T., 1981. Measurements of total carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program. *Earth and Planetary Science Letters* 55, 99-115.
- Butler, J. N., 1992. Alkalinity titration in seawater: How accurately can the data be fitted by an equilibrium model? *Marine Chemistry* 38, 251-282.
- Byrne, R.H., DeGrandpre, M.D., Short, R.T., Martz, T.R., Merlivat, L., McNeil, C., Sayles, F.L., Bell, R. and P. Fietzek. 2009. Sensors and systems for observation of marine CO₂ system variables, *Proceedings for Ocean Obs '09*, Venice.
- Chanson, M. and F.J. Millero, 2007. Effect of filtration on the total alkalinity of open-ocean seawater, *Limnol. Oceanogr. Methods*, 5, 293-295.
- Clayton, T., Byrne, R. H., Breland, J. A., Feely, R. A., Millero, F. J., Campbell, D. M., Murphy, P. P., and Lamb, M. F., 1995. The role of pH measurements in modern oceanic CO₂-system characterizations: Precision and thermodynamic consistency, *Deep-Sea Res.*, 42, 411–429.
- DelValls T. A. & Dickson A. G., 1998. The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. *Deep-Sea Research I* 45:1541-1554.
- DeGrandpre, M.D., T. R. Hammar, S. P. Smith, and F. L. Sayles, 1995. In situ measurements of seawater pCO₂ *Limnol. Oceanogr.*, 40, 969-975
- Dickson, A. G., 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep Sea Research Part A. Oceanographic Research Papers* 28, 609-623.
- Dickson, A. G., 1992. The development of the alkalinity concept in marine chemistry. *Marine Chemistry* 40, 49-63.
- Dickson, A. G.:2010a. The carbon dioxide system in sea water: equilibrium chemistry and measurements, *Guide for Best Practices in Ocean Acidification Research and Data Reporting*, Office for Official Publications of the European Union, Luxembourg.
- Dickson, A. 2010b. (Referee): Interactive comment on “On CO₂ perturbation experiments: over-determination of carbonate chemistry reveals inconsistencies” by C. J. M. Hoppe et al. *Biogeosciences Discuss.*, 7, C1286–C1293, 2010 www.biogeosciences-discuss.net/7/C1286/2010/
- Dickson, A. G. and Millero, F. J.:1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, *Deep-Sea Res.*, 34, 1733–1743.
- Dickson, A. G. and Riley, J. P., 1978. The effect of analytical error on the evaluation of the components of the aquatic carbon-dioxide system, *Mar. Chem.*, 6, 77–85.
- Dickson, A. G., Afghan, J. D., and Anderson, G. C., 2003. Reference materials for oceanic CO₂ analysis: a method for the certification of total alkalinity. *Marine Chemistry* 80, 185-197.
- Dickson, A. G., Sabine, C. L., and Christian, J. R.: 2007. *Guide to best practices for ocean CO₂ measurements*, PICES Special Publication, 3, Sidney, Canada, 2007.
- Dyrssen, D. and Sillén, L. G., 1967. Alkalinity and total carbonate in sea water. A plea for independent data. *Tellus* 19, 113-121.

- Dyrssen, D., 1965. A Gran titration of sea water onboard Sagitta. *Acta Chemica Scandinavica* 19, 1265.
- Edmond, J.M., 1970. High precision determination of titration alkalinity and total carbon dioxide content of seawater by potentiometric titration, *Deep-Sea Res.*, 17, 737-750.
- Fernández, E., Boyd, P., Holligan, P. M., and Harbour, D. S., 1993. Production of organic and inorganic carbon within a large-scale coccolithophore bloom in the northeast Atlantic Ocean. *Marine Ecology Progress Series* 97, 271-285.
- Friis, K., Körtzinger, A., and Wallace, D., 2004. Spectrophotometric pH measurement in the ocean: Requirements, design, and testing of an autonomous charge-coupled device detector system, *Limnol. Oceanogr. Methods*, 2, 126–136.
- Gattuso, J.-P., Lavigne, H., 2009. Technical note: Approaches and software tools to investigate the impact of ocean acidification. *Biogeosciences*, 6, 2121-33.
- Gattuso, J.-P., Lee, K., Rost, B., and Schulz, K.: 2010. Approaches and tools to manipulate the carbonate chemistry, *Guide for Best Practices in Ocean Acidification Research and Data 10 Reporting*, Office for Official Publications of the European Union, Luxembourg, in press..
- Goyet, C. and Poisson, A., 1989. New determination of carbonic acid dissociation constants in seawater as a function of temperature and salinity, *Deep-Sea Res.*, 36, 1635–1654.
- Gran, G.:1952. Determination of the equivalence point in potentiometric titrations of seawater with 15 hydrochloric acid, *Oceanol. Acta*, 5, 209–218, 1952.
- Hansson, I. 1973a. A new set of acidity constants for carbonic acid and boric acid in seawater. *Deep-Sea Research* 20:461-478.
- Hansson, I. and Jagner, D., 1973. Evaluation of the accuracy of Gran plots by means of computer calculations : Application to the potentiometric titration of the total alkalinity and carbonate content in sea water. *Analytica Chimica Acta* 65, 363-373.
- Haraldsson, C. L.G. Anderson, M. Hassellöv, S. Hulth, K. Olsson 1997. Rapid, high-precision potentiometric titration of alkalinity in ocean and sediment pore waters *Deep Sea Research Part I*: 44, 2031-2044
- Hernández-Ayón, J. M., Belli, S. L., and Zirino, A., 1999. pH, alkalinity and total CO₂ in coastal seawater by potentiometric titration with a difference derivative readout. *Analytica Chimica Acta* 394, 101-108.
- Hernandez-Ayon, J. M., Zirino, A., Dickson, A. G., Camiro-Vargas, T., and Valenzuela-Espinoza, E., 2007. Estimating the contribution of organic bases from microalgae to the titration alkalinity in coastal seawaters. *Limnology and Oceanography: Methods* 5, 225-232
- Holligan, P. M., Fernandez, E., Aiken, J., Balch, W. M., Boyd, P., Burkill, P. H., Finch, M., Groom, S. B., Malin, G., Muller, K., Purdie, D. A., Robinson, C., Trees, C. C., Turner, S. M., and van der Wal, P., 1993. A Biogeochemical Study of the Coccolithophore, *Emiliania Huxleyi*, in the North Atlantic. *Global Biogeochemical Cycles* 7.
- Hoppe, C. J. M., G. Langer, S. D. Rokitta, D. A. Wolf-Gladrow, and B. Rost, 2010. On CO₂ perturbation experiments: over-determination of carbonate chemistry reveals inconsistencies. *Biogeosciences Discuss.*, 7, 1707–1726.
- Hurd, C.L., C.D. Hepburn, K.I. Currie, J.A. Raven, K.A. Hunter, 2009. Testing the Effects of Ocean Acidification on Algal Metabolism: Considerations for Experimental Designs, *Journal of Phycology* , 45, 1236-1251
- IPPC: Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Core Writing Team, edited by: Pachauri, R. K. and Reisinger, A., IPCC, Geneva, Switzerland, 2007.
- Johansson, O. and Wedborg, M., 1982. On the evaluation of potentiometric titrations of seawater with hydrochloric acid. *Oceanologica Acta* 5, 209-218.
- Johnson, K. M. , J. M. Sieburth, P. J. L. Williams, L. Brandstrom, 1987. Coulometric total carbon dioxide analysis for marine studies: Automation and calibration, *Marine Chemistry*, 21, , 117-133
- Key, R. M., A. Kozyr, C. L. Sabine, K. Lee, R. Wanninkhof, J. L. Bullister, R. A. Feely, F. J. Millero, C. Mordy and T.-H. Peng, 2004. A global ocean carbon climatology: Results from Global Data Analysis Project (GLODAP) *Global Biogeochemical Cycles*, 18, GB4031, doi:10.1029/2004GB002247.

- Kim, H.-C., and K. Lee, 2009. Significant contribution of dissolved organic matter to seawater alkalinity, *Geophys. Res. Lett.*, 36, L20603, doi:10.1029/2009GL040271.
- Kim, H. C., K. Lee, and W. Y. Choi (2006), Contribution of phytoplankton and bacterial cells to the measured alkalinity of seawater, *Limnol. Oceanogr.*, 51, 331–338.
- Koertzinger, A., Mintrop, L., Wallace, D. W. R., Johnson, K. M., Neill, C., Tilbrook, B., Towler, P., Inoue, H. Y., Ishii, M., Shaffer, G., Saavedra, R. F. T., Ohtaki, E., Yamashita, E., Poisson, A., Brunet, C., Schauer, B., Goyet, C., and Eiseid, G., 2000. The international at-sea intercomparison of $f\text{CO}_2$ systems during the R/V Meteor Cruise 36/1 in the North Atlantic Ocean, *Mar. Chem.*, 72, 171–192.
- Kristiansen, S., Farbrot, T., and Naustvoll, L.-J., 2000. Production of Biogenic Silica by Spring Diatoms. *Limnology and Oceanography* 45, 472-478.
- Lamb, M. F., C. L Sabine, R. A Feely, R Wanninkhof, R. M Key, G. C Johnson, F. J Millero, K Lee, T. -H Peng, A Kozyr, J. L Bullister, D Greeley, R. H Byrne, D. W Chipman, A. G Dickson, C Goyet, P. R Guenther, M Ishii, K. M Johnson, C. D Keeling, T Ono, K Shitashima, B Tilbrook, T Takahashi, D. W. R Wallace, Y. W Watanabe, C Winn, C. S Wong, 2001. Consistency and synthesis of Pacific Ocean CO_2 survey data, *Deep Sea Research II*: 49, 21-54
- Lee, K., Millero, F. J., Byrne, R. H., Feely, R. A., and Wanninkhof, R., 2000. The recommended dissociation constants for carbonic acid in seawater, *Geophys. Res. Lett.*, 27, 229–232, 2000.
- Lefèvre, N., J.P. Ciabrini, G. Michard, B. Briant, M. Duchaffaut, and L. Merlivat, 1993. A new optical sensor for pCO_2 measurement, *Marine Chemistry* 42, 189-198.
- Lewis, E. and Wallace, D. W. R.:1998. Program Developed for CO_2 System Calculations, ORNL/CDIAC-105, Carbon Dioxide Information Analysis Centre, Oak Ridge National Laboratory, US Department of Energy, USA.
- Loucaides, S., Behrends, T., and Van Cappellen, P., 2010. Reactivity of biogenic silica: Surface versus bulk charge density. *Geochimica et Cosmochimica Acta* 74, 517-530.
- Lueker, T., A.G. Dickson, C.D. Keeling, 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2 : validation based on laboratory measurements of CO_2 in gas and *Marine Chemistry*, 70, 105-119.
- Martz, T. R., A. G. Dickson, and M. D. DeGrandpre, (2006) Tracer Monitored Titrations: Measurement of Total Alkalinity *Anal. Chem.* 78,1817-1826.
- Merlivat, L. and P. Brault, 1995. CARIOCA Buoy: Carbon Dioxide Monitor, *Sea Technology*, 10, 23-30.
- Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M.:1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, *Limnol. Oceanogr.*, 18, 897–907, 1973.
- Millero, F. J.: 1995. Thermodynamics of the carbon dioxide system in the oceans, *Geochim. Cosmochim. Acta*, 59, 661–667.
- Millero, F. J. R. H Byrne, R. Wanninkhof, R. Feely, T. Clayton, P. Murphy, M. F Lamb 1993a The internal consistency of CO_2 measurements in the equatorial Pacific *Marine Chemistry*, 44, 269-280
- Millero, F. J., Zhang, J.-Z., Lee, K., and Campbell, D. M., 1993b. Titration alkalinity of seawater. *Marine Chemistry* 44, 153-165.
- Millero, F. J., Pierrot, D., Lee, K., Wanninkhof, R., Feely, R., Sabine, C. L., Key, R. M., and Takahashi, T., 2002. Dissociation constants for carbonic acid determined from field measurements, *Deep-Sea Res.*, 49, 1705–1723.
- Millero, F. J., Graham, T. B., Huang, F., Bustos-Serrano, H., and Pierrot, D., 2006. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature, *Mar. Chem.*, 100, 80–94, 2006.
- Mojica Prieto, F.J. and F.J. Millero, 2002. The values of $\text{p}K_1 + \text{p}K_2$ for the dissociation of carbonic acid in seawater, *Geochim. Cosmochim. Acta*, 66, 2529-2540.
- Muller, F. L. L. and Bleie, B., 2008. Estimating the organic acid contribution to coastal seawater alkalinity by potentiometric titrations in a closed cell. *Analytica Chimica Acta* 619, 183-191.
- Neill C., Johnson K. M., Lewis E. & Wallace D. W. R., 1997. Accurate headspace analysis of $f\text{CO}_2$ in discrete water samples using batch equilibration. *Limnology and Oceanography* 42:1774-1783.

- Nemzer B. V. & Dickson A. G., 2005. The stability and reproducibility of Tris buffers in synthetic seawater. *Marine Chemistry* 96:237-242.
- Pérez, F. and Fraga, F., 1987. The pH measurements in seawater on NBS scale, *Mar. Chem.*, 21, 315–327.
- Perez, F. F., M. Vazquez-Rodriguez, H. Mercier, A. Velo, P. Lherminier and A. F. Rios, 2010. Trends of anthropogenic CO₂ storage in North Atlantic water masses, *Biogeosciences*, 7, 1789–1807.
- Pierrot, D., C. Neill, K. Sullivan, R. Castle, R. Wanninkhof, H. Lueger, T. Johannessen, A. Olsen, R.A. Feely, C.E. Cosca, 2009. Recommendations for autonomous underway pCO₂ measuring systems and data-reduction routines. *Deep-Sea Research II* 56, 512–522
- Poulton, A.J. T. R. Adey, W. M. Balch, P. M. Holligan, 2007. Relating coccolithophore calcification rates to phytoplankton community dynamics: Regional differences and implications for carbon export, *Deep Sea Research Part II*: 54, 538-557
- Rigobello-Masini, M. and Masini, J. C., 2001. Application of modified Gran functions and derivative methods to potentiometric acid titration studies of the distribution of inorganic carbon species in cultivation medium of marine microalgae. *Analytica Chimica Acta* 448, 239-250.
- Rost, B., S. A. Kranz, K.U. Richter and P.D. Tortell, 2007. Isotope disequilibrium and mass spectrometric studies of inorganic carbon acquisition by phytoplankton *Limnol. Oceanogr. Methods* 5, 328–337.
- Roy, R. N., Roy, L. N., Lawson, M., Vogel, K. M., Porter-Moore, C., Davis, W., Millero, F. J., and Campbell, D. M.: 1993. The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 C, *Mar. Chem.*, 44, 249–259.
- Schuster, U., A. Hannides, L. Mintrop, and A. Koertzing, 2009. Sensors and instruments for oceanic dissolved carbon measurements. *Ocean Sci. Discuss.*, 6, 491–524.
- Seidel, M.P., DeGrandpre, M.D. and A.G. Dickson, 2008. A sensor for in situ indicator-based measurements of seawater pH, *Mar. Chem.*, 109, 18–28.
- Schneider, K. and Erez, J.: 2006. The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurytoma*, *Limnol. Oceanogr.*, 51, 1284–1293.
- Schwartz, L.M, 1989. Statistical uncertainties of end-points in Gran plots *Analytica Chimica Acta*, 225, 1989, 205-215.
- SCOR Working Group 75, 1992. Methodology for oceanic CO₂ measurements. UNESCO technical papers in marine science 75: 1-37.
- Stoll, M. H. C. K. Bakker, K. Nobbe, G. H., Haese R. R. 2001 Continuous-Flow Analysis of Dissolved Inorganic Carbon Content in Seawater *Anal. Chem.* 73, 4111-4116
- Suratman, S., K. Weston. T. Jickells and L. Fernand (2009) Spatial and seasonal changes of dissolved and particulate organic C in the North Sea. *Hydrobiologia*, 628: 13-25.
- Tortell, P. D.: 2005. Dissolved gas measurements in oceanic waters made by membrane inlet mass spectrometry, *Limnol. Oceanogr. Methods*, 3, 24–37, 2005.
- Tyrrell, T., S. Loucaides, B. Kelly-Gerreyn, E. Achterberg, (2010) Interactive comment on “On CO₂ perturbation experiments: over-determination of carbonate chemistry reveals inconsistencies” by C. J. M. Hoppe et al. *Biogeosciences Discuss.*, 7, C257–C260.
- Van Den Berg, C.M.G., H. Rogers, 1987. Determination of alkalinities of estuarine waters by a two-point potentiometric titration *Marine Chemistry*, 20, 219-226.
- Wang, Z. A., X. Liu, R. H. Byrne, R. Wanninkhof, R. E. Bernstein, E.A. Kaltenbacher, J. Patten (2007) Simultaneous spectrophotometric flow-through measurements of pH, carbon dioxide fugacity, and total inorganic carbon in seawater. *Anal. Chim. Acta*, 596, 23–36.
- Wanninkhof, R., Lewis, E., Feely, R.A., Millero, F.J., 1999. The optimal carbonate dissociation constants for determining surface water pCO₂ from alkalinity and total inorganic carbon. *Mar. Chem.* 65, 291-301.
- Wanninkhof, R. 2010. (Referee): Interactive comment on “On CO₂ perturbation experiments: over-determination of carbonate chemistry reveals inconsistencies” by C. J. M. Hoppe et al. *Biogeosciences Discuss.*, 7, C1342–C1344, 2010 www.biogeosciences-discuss.net/7/C1342/2010/. 2010
- Weiss, R. F.: 1974 Carbon dioxide in water and seawater. The solubility of a non-ideal gas, *Mar. Chem.*, 2, 203–215.
- Wiki 2010 <http://en.wikipedia.org/wiki/HEPES> accessed 30/7/10

- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Koertzing, A. and Dickson, A. G., 2007. Total alkalinity: The explicit conservative expression and its application to biogeochemical processes, *Mar. Chem.*, 106, 287–300.
- Yao W., Liu X. & Byrne R. H., 2007. Impurities in indicators used for spectrophotometric seawater pH measurements: assessment and remedies. *Marine Chemistry* 107:167–172.
- Zhang, J.-Z., C. Fischer and P. B. Ortner, 1999. Dissolution of silicate from glassware as a contaminant in silicate analysis of natural water samples. *Water Research*, 33:2879-2883.
- Zeebe, R. E. and Wolf-Gladrow, D. A.:2001 *CO₂ in Seawater: Equilibrium, Kinetics, Isotopes*, Elsevier Science, Amsterdam, Netherlands.
- Zeebe, R.E. 2010.
http://www.soest.hawaii.edu/oceanography/faculty/zeebe_files/CO2_System_in_Seawater/csyste.html) accessed 02 August 2010.

Appendix 1

Recommendations for Meta data reporting

A significant part of the information required below is specific for the carbonate system measurements. Several of these fields will be generic for a particular lab, i.e. will only have to be filled out once by each lab; variations to the standard procedures can then easily be edited in for each cruise, mesocosm and/or process study.

General Information: (this information is generic to all metadata collected during a scientific cruise but will be more unique in mesocosm and laboratory studies):

1. Cruise information:
 - Vessel (name; country; vessel ID)
 - Principal Scientist for cruise
 - Date and Port of departure
 - Date and Port of arrival
 - Cruise ID (EXPOCODE)
 - Name of experiment (e.g. P16 or M60/5)
 - Leg
 - Geographical coverage (e.g. North Atlantic; 30°N to 50°N and 60°W to 10°W)
 - Number of CTD stations
2. Mesocosm or laboratory information
 - Name of supporting programme (e.g. EPOCA)
 - Principal Scientist for study
 - Scientist reporting data
 - Name of individual process study
 - Mesocosm/laboratory location
 - Start and end dates of study
 - Name of experiment (e.g. P16 or M60/5)
 - Number of systems and samples measured
 - Brief description of environmental modifications to carbonate saturation state

Carbonate measurements:

1. PI and analysts carrying out analyses
 - Name project PI reporting data (e.g. Toby Tyrrell)
 - Name analytical service PI (e.g. Eric Achterberg)
 - Name senior analyst (e.g. Cynthia Dumousseaud)
 - Organization: (e.g. UKOA Carbonate Analytical Service)

- Address:
 - Phone:
 - Email:
2. Variables description:
 - Variable names (e.g. xCO₂ or pCO₂ or fCO₂ at say 25 °C or in-situ temperature)
 - Reporting units
 3. Date of collection; (date of reception by service); date of measurement

Sampling procedures and storage

1. Date of collection
2. Sampling containers (e.g. 500 ml Schott Glass Duran clear glass). Grease for the stopper.
3. Processing of sampling during collection (e.g. Filtering of alkalinity samples – method e.g. vacuum filter size e.g. GF/F).
4. Poisoning of samples (e.g. 100 micro-litre saturated HgCl₂)
5. Storage (e.g. in dark refrigerated 4 °C)

Method description (separate details for each method DIC/TA/pCO₂/pH):

1. Instrument: - instrumentation used for the measurements. (For instance: VINDTA 3C.)
2. Method for each measured parameter, and appropriate reference. (For instance: Dickson et al., (2007) SOP 6)
3. Note deviations in your set-up from the reference method or any modification from the standard instrument.
4. Note temperature control and variability during analysis for sample, acid, volumetric burettes.
5. Note electrode slope and stability if titrations are used

Reagents:

1. Brands and stock information of the reagents/salts used.
2. Where the solutions prepared on the ship, or pre-made in the lab prior to cruise
3. Which medium was used for the reagents (e.g. MilliQ, NaCl solution)

Standardisation:

1. How were your stock solutions prepared (initial salts, medium), + method (volumetric; mass,.)

2. How were the stock solutions diluted to working concentrations (medium) + method (volumetric; mass)
3. How were stock solutions standardised (i.e. coulometric standardization of acid titrant)
4. Blank measurements (medium)
5. Which pipettes were used? State calibration information of the pipettes.(or balance(calibration, precision,...))

Reference material:

1. Certified reference materials used (state batch numbers, producer etc.).
2. pCO₂ calibration gasses and reference gasses used for calibration of working standard gasses.
3. State calibration method used for coulometer (gas loops) and how often calibration is performed.
4. State when and how often reference materials are used for QC during a run.
5. State how precision and stability is tested.

Quantification procedures:

1. Mathematical formula used for the calculation of concentration (e.g. Alkalinity – Gran plot/Curve fitting; pCO₂ – Pierrot et al (2009))
2. Calibration curves/ranges (e.g pCO₂ no of standard gasses and concentration)
3. Blank corrections
4. Recalculation of run

Data quality:

1. Best estimate of accuracy and precision
2. State how these numbers were obtained (e.g. by measurements of n duplicates and by running n number of CRM samples).
3. Number of samples/duplicates measured
4. Provide relevant control charts (see Dickson et al., (2007) SOP 22)

Flagging used

Data should be flagged following the WOCE codes as first step by the analyst and then by the project scientist before reporting the data to e.g. CDIAC; following the SOCAT procedure: 2 (good), 3 (questionable) or 4 (bad).

Notes

1. Environmental information during analysis (e.g lab temperature (e.g. 20-24°C variable)).

Appendix 2

Definition of Alkalinity

The most widely accepted definition of Total Alkalinity (TA) is that given by Dickson (1981). Based on his definition, the TA of a seawater sample is equal to the number of M of hydrogen ion equivalent to the excess of proton acceptors. Since weak acids can act as both proton donors and proton acceptors (as conjugate bases), Dickson defined proton donors as bases formed from weak acids with $pK_a \leq 4.5$ at 25 °C and at zero ionic strength. The choice of 4.5 as a pK_a threshold value is purely for convenience as it roughly corresponds to the pH endpoint of a conventional alkalinity titration.

Based on Dickson's definition, the TA of a seawater sample can be expressed in terms of proton balance as follows:

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

where brackets represent total concentrations in solution and the ellipses signify additional acids or bases which are either unidentified or present in concentrations too low to be considered. TA is considered to be a close to conservative quantity in seawater – directly predictable from the salinity of the sample and independent of temperature and pressure (Lee et al. 2006). However the concentration of each individual species in the above expression is not. In the ocean changes occur due to various physicochemical and biogeochemical processes including $CaCO_3$ dissolution, calcification, nutrient up-take and organic matter production etc. Calculating the changes in TA with the above expression, however, is not at all straightforward because a change in PO_4 or CO_3 , for example will also change the pH, and therefore the speciation of the other chemical species. This, however, can be overcome if we consider TA in terms of charge balance.

Seawater must obey the law of electro-neutrality which means that the sum of all charges in solution is zero. This can be expressed as follows:

$$\sum_i q_i [c_i] = 0$$

where q_i is the electric charge in elementary units and $[c_i]$ is the concentration in $M \text{ kg}^{-1}$. If the most important seawater ions are considered the above expression reads

$$\begin{aligned} & [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}] + [K^+] + 2[Sr^{2+}] + [NH_4^+] + [H^+] + \dots - [Cl^-] - 2[SO_4^{2-}] - [Br^-] \\ & - [NO_3^-] - [NO_2^-] - [HCO_3^-] - 2[CO_3^{2-}] - [B(OH)_4^-] - [OH^-] - [HS^-] - [H_3SiO_4^-] - [HSO_4^-] \\ & - [F^-] - [H_2PO_4^-] - 2[HPO_4^{2-}] - 3[PO_4^{3-}] - \dots = 0 \end{aligned}$$

where the eclipses signify ions with negligible concentrations. If the TA proton balance equation is added to both sides of the charge balance expression then the following can be derived:

$$\begin{aligned} & [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}] + [K^+] + 2[Sr^{2+}] + [NH_4^+] + [NH_3] + \dots - [Cl^-] - [Br^-] - [NO_3^-] - [NO_2^-] \\ & - 2[HSO_4^-] - 2[SO_4^{2-}] - [F^-] - [HF] - [H_3PO_4] - [H_2PO_4^-] - [HPO_4^{2-}] - [PO_4^{3-}] - \dots = TA \end{aligned}$$

If $TN = [NO_3^-] + [NO_2^-]$, $TP = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]$, $TS = [HSO_4^-] + [SO_4^{2-}]$, $TF = [F^-] + [HF]$, and $TNH_3 = [NH_3] + [NH_4^+]$, then

$$TA = [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}] + [K^+] + 2[Sr^{2+}] + TNH_3 + \dots - [Cl^-] - [Br^-] - TN - 2TS - TF - TP - \dots$$

The advantage of this last expression is that all constituents are conservative. Please note that even though the acid-base speciation of NH_3 , H_3PO_4 , HNO_2 , HF , and H_2SO_4 is a function of temperature and pressure, total concentrations (i.e. TP, TN, TNH_3 , TS, TF) are conservative. Therefore, this expression permits a direct examination of the role of different aquatic processes in modifying the alkalinity of sewerage. For example, it can readily be seen that calcite precipitation leads to an alkalinity decrease of 2 equivalents per mole of calcite precipitated.