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# Guidelines for Measuring Changes in Seawater pH and Associated Carbonate Chemistry in Coastal Environments of the Eastern United States

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National Health and Environmental Effects Research Laboratory

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# Guidelines for Measuring Changes in Seawater pH and Associated Carbonate Chemistry in Coastal Environments of the Eastern United States

by

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## NOTICE

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## PURPOSE AND SCOPE

These guidelines are written for a variety of audiences ranging from shellfish growers interested in monitoring pH with inexpensive equipment to citizen monitoring groups to advanced chemistry laboratories interested in expanding existing capabilities. The purpose is to give an overview of available sampling, analytical and data reporting approaches that will contribute to the usefulness of coastal acidification measurements for both the needs of those intending to monitor as well as those of other interested stakeholders along the Atlantic seaboard of the US. The state of the science, including recommended best practices, is rapidly evolving, so certain sections may be either too sparse or too detailed. Thus, we encourage users of the guidelines to begin with a careful review of the detailed contents listing and to take note of references to other guidelines available in the open literature.

## ACKNOWLEDGEMENTS

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# 1. INTRODUCTION

Coastal and estuarine systems hold significant economic and recreational importance for communities along the Atlantic seaboard. These systems support finfish, bivalve, crustacean and seabird populations and play vital roles in water quality and the cycling of nitrogen and carbon. However, seawater pH and other characteristics of coastal carbonate chemistry are changing through a process known as coastal acidification, which is a fundamentally similar but more complex version of ocean acidification. Coastal acidification has the potential to disrupt the species composition and ecological functioning of coastal biological communities and threaten commercially important aquatic life. As in the open ocean, the carbonate system in coastal waters consists of the major forms of inorganic carbon present in seawater, which are carbon dioxide, bicarbonate and carbonate. Although there are numerous groups interested in monitoring pH or other carbonate parameters in coastal waters, there is little available guidance on how these groups can best utilize or expand their existing capabilities.

Coastal acidification differs somewhat from ocean acidification, which is a global process that involves a reduction in the pH of the ocean (see section below on the seawater carbonate system). It is caused primarily by carbon dioxide from the atmosphere entering the ocean. Coastal acidification is a more localized, further reduction in pH. It is primarily driven by high levels of respiration (typically by bacteria involved in decomposition), which releases carbon dioxide into the water. Coastal acidification is often fueled by nutrients entering the water from land, stimulating phytoplankton blooms that subsequently decompose on or near the seabed. Coastal acidification happens in coastal waters because that is where high nutrient levels and algal blooms occur [http://www.necan.org/].

In the past few decades, only half of the $CO_2$ released by human activity, including fossil fuel emissions, land use change and cement production, has remained in the atmosphere; of the remainder, about 30% has been taken up by the ocean and 20% by the terrestrial biosphere (Khatiwala et al., 2009; Sabine et al., 2004). The evidence for decreasing pH in the open ocean is unequivocal (Caldeira and Wickett, 2003; Doney et al., 2009), as is the evidence for negative effects on many marine organisms when these chemical changes are simulated under controlled laboratory conditions (e.g., Kroeker et al., 2013; Talmage and Gobler, 2009). However, scientists are just beginning to test the severity of these effects in ocean and coastal ecosystems where an organism's chemical environment is only one of many ecological factors affecting its fitness.

There are many clear cases of extreme biological sensitivity to acidification among economically important coastal organisms such as shellfish and corals, but the biological responses of many other species are variable and difficult to predict (Kroeker et al., 2010). For example, many types of marine plants and algae may be harmed by lower pH (i.e., higher acidity) but may also benefit from increases in the carbon dioxide they require for photosynthesis (Riebesell, 2004). Thus, although species composition may change in the future, neither the details nor the ecosystem level consequences (e.g., food production) are predictable (Grear et al., 2017). The continued study of these effects needs to be accompanied by a clear understanding of how coastal carbonate chemistry varies through space and time. A number of methods have been described for the coastal current and upwelling zones of the US west coast (e.g., McLaughlin et al., 2014; McLaughlin et al., 2013). While coastal upwelling occurs on the east coast, deep water upwelling does not strongly influence acidification in the short term (Wang et al., 2013). Thus, observations from the mid- and outer-shelf may be less comparable to the

inshore environment than on the west coast. Moreover, many coastal organisms have sensitive estuarine and nearshore life stages that coincide with mid and late summer extremes in dissolved oxygen, pH, and other characteristics of the carbonate system and are thus expected to be especially vulnerable (Wallace et al., 2014). These issues raise concern about coverage in the nearshore environments that fall outside of areas covered by the major federal observing programs (e.g., ECOMON and GOMECC) and which tend to be too infrequent to capture either seasonal or more frequent excursions in carbonate chemistry.

The decrease in pH in the open ocean during the industrial age has been on the order of 0.1 to 0.2 pH units per century (Caldeira and Wickett, 2003), which translates to more than a 25% increase in the concentration of hydrogen ions. Relative to coastal environments, pH in the open ocean is generally less dynamic in terms of diurnal and seasonal variations (Hofmann et al., 2011), which has made open ocean trends easier to distinguish from background variability. In addition, the ocean is extremely important to the global carbon cycle, so scientists have been taking highly precise measurements of the carbonate system in the open ocean for decades. However, due to greater variability of pH in the coastal environment, a trend of similar magnitude would require a larger number, and longer time-series, of samples to detect (Keller et al., 2014). This creates a unique challenge for coastal monitoring because current best practices for handling and analyzing samples for carbonate system parameters are expensive, and therefore possibly not feasible for the high frequency and spatially extensive sampling that would be necessary to detect decadal and spatial trends in the coastal environment. For example, while pH is easy to measure with handheld meters or multi-function autonomous sensors that use glass membrane pH electrodes, chemical oceanographers often question the value of these measurements for the study of carbonate chemistry, including acidification (Re´rolle et al., 2012). Although this criticism is sometimes unwarranted because of differences in study goals (e.g., see the "climate vs. weather quality" discussion below; Newton et al., 2014), accepted protocols are unlikely to change without an improved understanding of coastal acidification, and until issues relating to appropriate pH scales, calibration standards, instrument drift, and indirect pH estimation are further refined or agreed upon by the research community.

These guidelines are meant to be a resource for learning about and performing measurements of the seawater carbonate system, especially as they relate to coastal acidification. The intended audience includes scientists in academic, government, and non-government organizations including those involved in citizen science and shellfish management. Many such organizations are already monitoring or beginning to monitor components of the seawater carbonate system that may be partially or completely sufficient for assessing coastal acidification. For example, specific organizations in the northeast are examining coastal acidification as a potential cause for recent declines in shellfish abundance. Other organizations study coastal carbonate chemistry and acidification as part of a broader interest in coastal carbon cycles. Clearly, there is a wide diversity of rationales and capabilities for monitoring acidification in the coastal environment.

Numerous publications exist in the peer-reviewed and online "gray" literature that describe recommended practices for measuring and calculating the various components of the seawater carbonate system. Most of these resources are written by and for oceanographic researchers and place less emphasis on informing, for example, the expansion of an existing shellfish or nutrient monitoring program to include coastal acidification parameters. In addition, the available resources (e.g., Dickson et al., 2007; McLaughlin et al., 2014; Riebesell et al., 2010) tend to be generalized to accommodate a wide variety of instrument and laboratory

configurations. This creates a challenge for the new investigator and slows the rate at which new monitoring efforts can be implemented. Thus, this document will attempt to address the unique issues and some of the available solutions for measuring the seawater carbonate system in coastal and estuarine environments. This includes alternatives and clarifications of existing best practices that will make them suitable for typical environments of the US east coast. Because the study of ocean and coastal acidification is changing rapidly, these guidelines are not meant to be prescriptive, but are intended to facilitate the development of compatible datasets for sharing insights and experiences from the present community of investigators interested in coastal acidification. Although these guidelines are intended to apply throughout the east coast, much of the nearshore research in the eastern US has been conducted in the northeast. There are likely to be solutions that we have not covered here, so continued communication through acidification monitoring networks will be critical.

Before describing specific methods, we provide an overview of coastal acidification, the seawater carbonate system, and ecological considerations that normally affect study design. There is a vast literature on these topics for the ocean and a growing one for the coasts. For a recent overview of the state of the science in the US northeast, see Gledhill et al. (2015) and other resources on the Northeast Coastal Acidification Network (NECAN) website (http://www.necan.org). For other eastern US coastal regions, see ongoing developments on the SOCAN (http://secoora.org/socan) and MACAN websites (http://midacan.org). Although the seawater carbonate system is described later in greater detail, our overview begins with the summary in Figure 1-1.

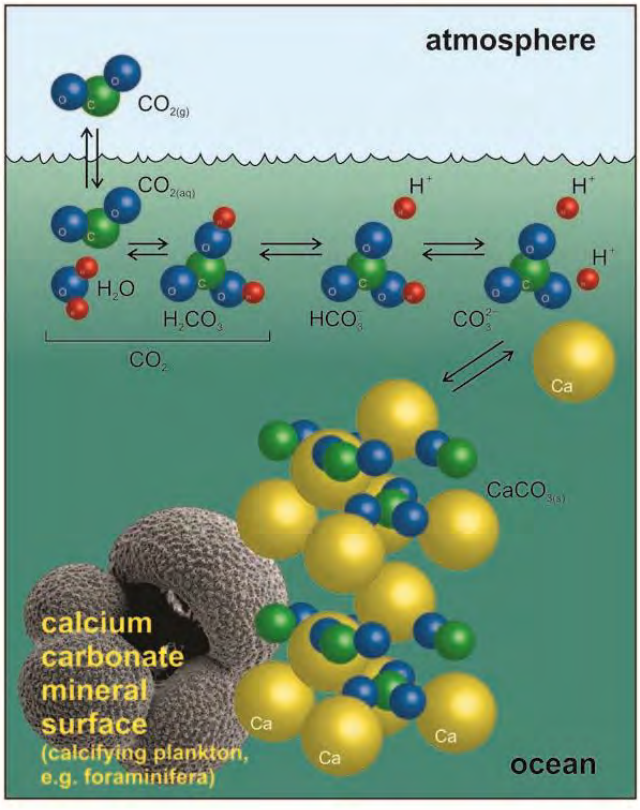


**Figure 1-1: Carbonate system equilibrium reactions and relationship to calcification** (Barker and Ridgwell, 2012)**.**

## 2. COASTAL VS OCEAN ACIDIFICATION

Although the fundamental factors driving carbonate chemistry are essentially the same in ocean and coastal waters, the relative importance of each of these factors differs a great deal. This results in large differences in spatial and temporal variability, with nutrient dynamics, primary production, and respiration being especially important in coastal areas. In nutrient-poor surface waters of the open ocean, for example, equilibration of carbon dioxide concentration with the atmosphere via air-sea exchange is less significantly perturbed by short-term variations in biological activity and terrestrial run-off events. Biological fixation and eventual removal from surface waters of carbon favors further uptake of carbon dioxide from the atmosphere by the oceans, but the large magnitude of these processes per unit area in coastal environments result in much more dynamic systems both temporally and spatially (Johnson et al., 2013). This is why productive estuaries exhibit much greater diurnal and seasonal variation in pH than open-ocean systems (Hofmann et al., 2011). In addition, although the net effects of atmospheric nitrogen and sulfur dioxide deposition on ocean pH are thought to be small, they are potentially more important in coastal ecosystems (Doney et al., 2007).

A pH probe placed in the upper water column of a productive estuary will typically indicate rising pH (lower acidity) to levels sometimes exceeding >8.4 during the day and then declining into the <7 range during the night. Similarly, pH tends to be higher during the growing season than in the fall. These fluctuations occur in estuaries because pH change is determined primarily by the amount of carbon dioxide that enters and leaves the water column, although other acid species (e.g., organic acids) can significantly affect water pH in coastal oceans. The key sources for carbon dioxide in the water column include absorption from the atmosphere, respiration by marine organisms, and inputs from other systems, such as rivers, wetlands, and sediments. Conversely, when photosynthetic organisms (e.g., phytoplankton) produce oxygen, they consume carbon dioxide. These ecosystem processes explain why daily and seasonal patterns of rising and falling pH and $pCO_2$ often mirror trends seen in oxygen measurements. The sensitivity of the local carbonate system to these processes also depends on alkalinity (the ability of substances in seawater to react with the addition of a strong acid and convert it to an uncharged species) and buffer capacity, which in turn is sensitive to nearby ocean characteristics as well as the limnological and lithological characteristics of the watershed. These various processes interact to control pH and carbonate ion availability (Cai et al., 2011; Feely et al., 2010; Mucci et al., 2011; Wallace et al., 2014; Wang et al., 2013).

Although strong diurnal and seasonal variability can be exacerbated by human activity, there is an expectation that coastal organisms have evolved under highly variable conditions and may be less sensitive than oceanic biota to long-term changes in carbonate chemistry. Although differences in acclimation and adaptation potential among subpopulations from environments with differing pH variability have been observed, neither the specific characteristics of pH variability that affect organisms – i.e., daily minimum pH, total amplitude, mean pH, breeding season pH, etc., nor the phylogenetic traits that confer tolerance or adaptive capacity are well known, so the sensitivity of most coastal biota remains unclear.

# 3. FACTORS CONTRIBUTING TO ACIDIFICATION ON THE US EAST COAST

Against a background of increasing carbon dioxide concentrations in the atmosphere due primarily to fossil fuel combustion, there are several factors contributing to geographical variation in coastal acidification that are particularly important on the US east coast (Salisbury et al., 2008; Wang et al., 2013). Consideration of these factors will help readers to identify survey design issues, areas of possible collaboration with other scientists, and coordination with existing monitoring efforts. This section gives an overview of these factors.

## 3.1 Eutrophication

One of the most important factors driving acidification of coastal and estuarine waters is nutrient loading. Local input of nutrients, especially $NH_3$ and $NO_3^-$, can be increased due to urbanization, wastewater input, and agricultural/urban runoff. The northeast US has been shown to host some of the largest nutrient loading rates in the world (Anderson and Taylor, 2001; Howarth, 2008). These nutrient inputs can lead to enhanced primary production in estuarine and coastal systems by phytoplankton (Beman et al., 2005; Carpenter et al., 2008; Feely et al., 2010; Newton and Van Voorhis, 2002; Simonds et al., 2008), which eventually senesce and sink to the bottom waters where their microbial decay consumes oxygen and produces carbon dioxide. As already noted, this microbial respiration and remineralization of organic matter, whether from in situ phytoplankton production or from watershed loading of organic matter, can increase localized $CO_2$ concentrations in aquatic ecosystems (Figure 3-1; Cai et al., 2011). While this can occur in any part of the water column, these conditions can be exacerbated in bottom waters due to reduced vertical mixing thus enhanced respiration/decomposition during periods of stratification (Feely et al., 2010; Gobler and Baumann, 2016; Wallace et al., 2014). As a result, bottom waters especially, can be affected by lower pH and carbonate ion concentration (Feely et al., 2010), often expressed as a reduction in the saturation state of the calcium carbonate minerals aragonite and/or calcite. Through turnover and mixing events, usually in early fall, these undersaturated waters can also make their way to the surface layer.

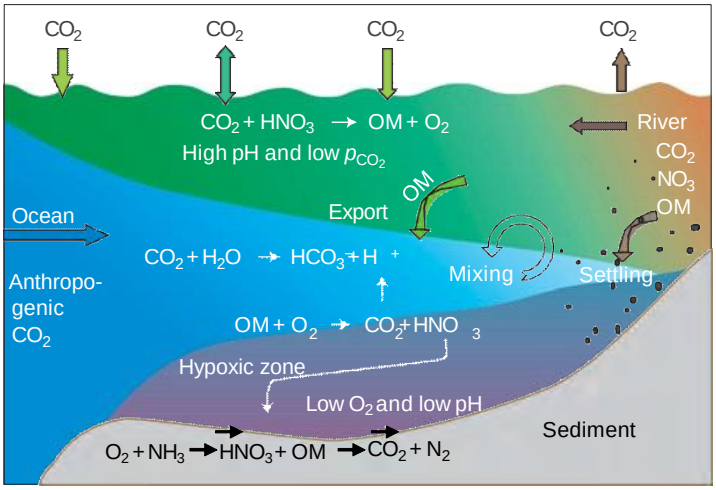


**Figure 3-1. A conceptual model for a large river plume eutrophication and subsurface water hypoxia and acidification.** From Cai et al. (2011) by permission from Springer Nature: Macmillan Publishers, Nature Geoscience.

### 3.2 Nitrification

Nitrification is a biochemical process carried out primarily by sediment bacterial communities and results in the oxidation of ammonium to nitrate. Changes in total alkalinity may accompany nitrification because carbonate species are consumed in the conversion of ammonia to nitrite (Wolf-Gladrow et al., 2007). Nitrification can occur in freshwater, brackish and marine systems. During nitrification, the oxidation of ammonium to nitrate produces two moles of $H^+$ ions for every mole of ammonium oxidized, with a corresponding decrease in alkalinity.

$$2\ NH_4^+ + 3\ O_2 = 2\ NO_2^- + 4\ H^+ + 2\ H_2O$$

$$2\ NO_2^- + O_2 = 2\ NO_3^-$$

### 3.3 Denitrification

Denitrification is a biochemical process, in which nitrate is reduced to molecular nitrogen. The process results in the consumption of a proton and production of carbon dioxide (Brenner et al., 2016; Drtil et al., 1995). The overall net effect on pH that can be attributed to both nitrification and denitrification will be small in a well-buffered system because these processes tend to co-occur in time and space (Rysgaard et al., 1996).

### 3.4 Stratification

Due to temperature and salinity differences between bottom and surface waters and between riverine and oceanic waters, it is common for stratification (i.e., layering) to occur in estuarine systems. Stratification tends to be strongest in the summer, especially during periods of reduced wind-driven mixing and tides (i.e., neap tides) and in systems with sufficient surface water heating to form a thermocline. Seasonal changes in precipitation and runoff are additional factors affecting stratification because of their effect on water buoyancy via salinity. Sinking of organic matter during periods of stratification causes these materials to accumulate in bottom waters and near the sediment layer where they fuel $CO_2$ production due to microbial respiration. Since mixing is reduced under strong stratification, $CO_2$ builds up in the bottom waters, increasing acidification. In some systems, especially in late summer and early fall, bottom water $CO_2$ concentrations can greatly exceed atmospheric concentrations (Frankignoulle, 1988) and remain high until temperature, tidal, or wind-driven mixing occurs during the fall and winter months (Gledhill et al., 2015; Wallace et al., 2014; Wang and Cai, 2004).

### 3.5 Regional lithology, aquatic geochemistry, carbon import/export, and climate

The geologic and land use makeup of the watershed can have a pronounced effect on the pH, dissolved inorganic carbon (DIC) concentration, alkalinity and buffering capacity of the freshwater entering an estuary. Rivers that drain upland areas with exposed bedrock (especially carbonate rocks like limestone or marble) generally have higher carbonate content, pH and buffering capacity. Rivers originating in coastal plains may have lower pH and buffering capacity and higher amounts of $CO_2$ and also may contain high concentrations of humic, fulvic and tannic acids (Cai and Wang, 1998; Suchet et al., 2003). Changes in precipitation amounts and timing in coastal watersheds have the potential to change the alkalinity and thus the buffering capacity of receiving waters. Such watershed effects were invoked as a potential explanation of the lower buffering capacity observed in shelf waters off of New England (Wang et al., 2013).

Carbonate chemistry can be strongly influenced by oxidation of organic matter that originates from the watershed (Cai et al., 2011). In addition, Wang et al. (2016) showed that coastal marshes are a significant source of DIC and alkalinity and reported estimated extremely high annual DIC exports to coastal waters from the salt marsh at their Massachusetts study site.

### 3.6 Calcification and dissolution

Calcifying organisms extract $Ca^{+}$ and carbonate system ions from the water surrounding them in order to build shell. Although the calcification process is complex and variable among species (Jury et al., 2010; Leung et al., 2017; Riebesell et al., 2010), many marine organisms directly use carbonate ions through either passive or active uptake. Because this often involves active transport of protons across the cell membrane to maintain pH balance, dissolution or a slowing of calcification due to energetic costs occur when pH either alters the proton gradient across the membrane or otherwise affects calcification physiology. Calcification and dissolution reactions can also affect observed carbonate chemistry (e.g., Alling et al., 2012), especially through abiotic reactions at the sediment-water interface (Soetaert et al., 2007).

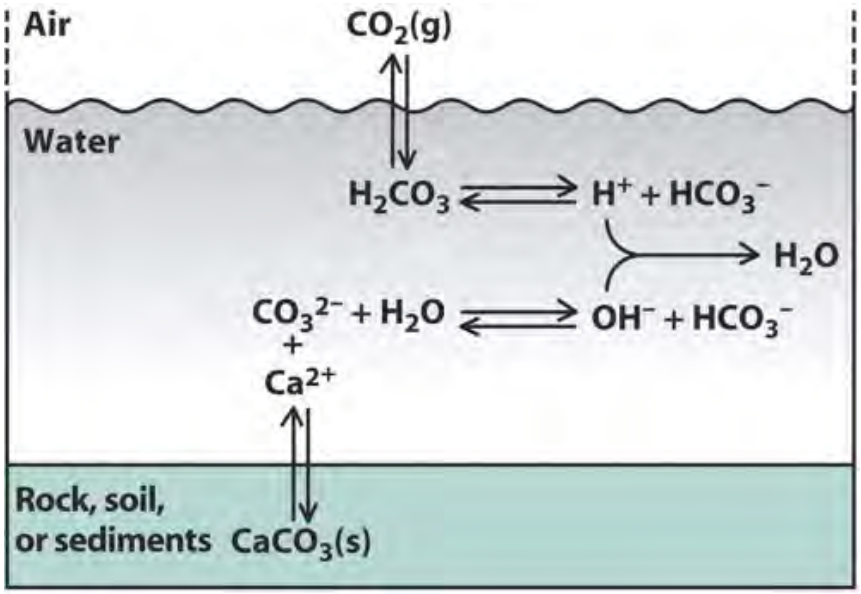


**Figure 3-2. A conceptual model describing the interactions between carbonate system species and associated atmospheric and sediment fluxes.** (Image from Patricia Shapley http://butane.chem.uiuc.edu/pshapley/GenChem1/L26/3.html).

In the ocean, $CaCO_3$ is found in sediments. While $CaCO_3$ exists in equilibrium with its constituent ions (see Figure 3-2), the equilibrium is shifted toward dissolution as the concentration of $CO_2$ increases. Dissolution occurs via the following reaction:

$$CaCO_3 + CO_2 + H_2O = Ca_2^{+} + 2HCO_3^{-}$$

While these precipitation and dissolution processes are of interest in studies of ecosystem processes governing DIC pools (e.g., Alling et al., 2012), the concentration of carbonate ions tends remain near 10% of the DIC pool via equilibrium reactions over large spatial and temporal scales. However, it is important to note that riverine calcium concentration is more variable than in the ocean, so the solubility coefficients for carbonate minerals may also be less stable in coastal environments.

### 3.7 Sediment diagenesis

Sediment diagenesis describes a diverse set of mechanisms that transform sediment following deposition and may significantly affect alkalinity (Jahnke and Jahnke, 2004; Krumins et al., 2013). These processes are complex and can work over timescales from hours to hundreds of thousands of years. Some of these processes can be biologically mediated by bottom-dwelling communities that ingest, burrow and otherwise transform the sediment, altering the sediment-water interface. Most research on ocean acidification has focused on surface waters and does not consider sediment processes, so this is seen as a major science gap in the coastal environment where the effect of sediments on alkalinity should be considered.

# 4. THE SEAWATER CARBONATE SYSTEM

## 4.1 Overview

Concise overviews of the seawater carbonate system are provided in Dickson et al. (2007) and Riebesell et al. (2010), both of which are available online. Seawater carbonate chemistry consists of a system of chemical states and reactions by which $CO_2$ gas, whether originating from the atmosphere, water column or sediment is taken up and transformed in the seawater. $CO_2$ and its dissociation products $HCO_3^-$ (bicarbonate ion) and $CO_3^{2-}$ (carbonate ion) are further utilized in other important chemical and biological processes. $CO_2$ entering the water column via atmospheric exchange or produced during respiration combines with $H_2O$ to form carbonic acid, which immediately dissociates into $HCO_3^-$ and $H^+$; when $HCO_3^-$ further dissociates into $CO_3^{2-}$ another $H^+$ ion is released (Figure 1-1). In addition to being short lived, carbonic acid only constitutes 0.1% of dissolved $CO_2$ and thus, is not considered biologically or chemically significant (Riebesell et al., 2010). With increasing concentrations of dissolved $CO_2$, dissociation reactions increase the concentration of $H^+$ in the water, decreasing pH. If $CO_2$ concentrations decrease, these reactions go in the opposite direction, decreasing the concentration of $H^+$ in the water and increasing pH. The equilibrium expressions below in Figure 4-1 show the major stoichiometric reactions that control $CO_2$ speciation in water.

1. $CO_2(g) = CO_2(aq)$
2. $CO_2^-(aq) + H_2O(l) = H^+(aq) + HCO_3^-(aq)$
3. $HCO_3^-(aq) = H^+(aq) + CO_3^{2-}(aq)$

**Figure 4-1: These equilibrium expressions are based on A. Dickson's chapter in Riebesell et al. (2010), with the letters g, l, and aq denoting gas, liquid, and aqueous solution, respectively.**

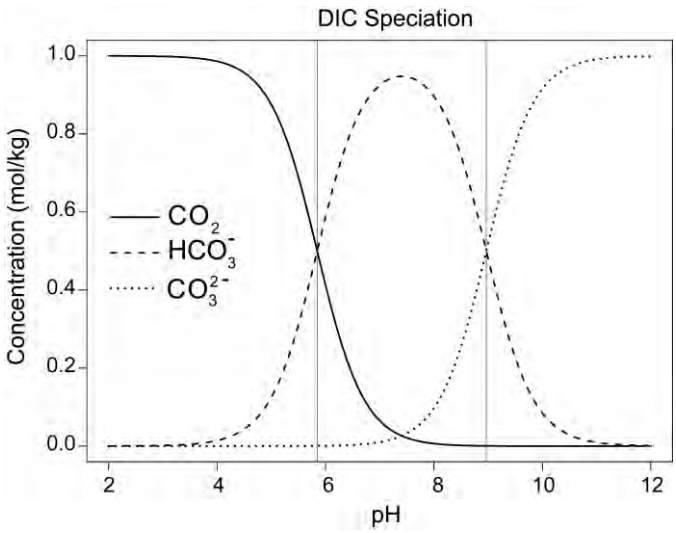


**Figure 4-2. Concentration of inorganic carbon species changes as a function of pH.** The above Bjerrum plot was created using the seacarb package in R (Lavigne et al., 2011; R Core Team, 2017). Dissolved $CO_2$ is the predominant species at low pH but at pH values above 5, the concentration of dissolved $CO_2$ rapidly decreases. Conversely, the concentration of $CO_3^{2-}$ is near zero at low pH, and it becomes the predominant species at pH values above 9.

Acidity is determined by the concentration of hydrogen ions. In seawater, hydrogen ion concentrations are buffered (i.e., maintain a nearly constant concentration) by the interconversion of bicarbonate and carbonate and concomitant release or consumption of a hydrogen ion (Figure 4-1, reaction 3); consequently, hydrogen ion concentration is largely controlled by the ratio of bicarbonate to carbonate. However, the buffer capacity of seawater is finite and increasing concentrations of carbon dioxide will ultimately increase the concentration of hydrogen ions. The relative abundance of bicarbonate and carbonate over a range of pH values is shown in Figure 4-2. In typical seawater, as hydrogen ion concentration increases (i.e., pH decreases), carbonate ion concentration decreases and bicarbonate ion concentration increases.

Additional information on the seawater carbonate system can be found in chemical oceanography textbooks (e.g., Pilson, 2013). Concise summaries can also be found in Dickson et al. (2007), Riebesell et al. (2010) and Zeebe (2012). In addition, we find Wolf-Gladrow et al. (2007) discussions of processes affecting alkalinity especially helpful. A simplified visual representation of ocean acidification is given at http://www.nature.com/scitable/knowledge/library/ocean-acidification-25822734, part of which is also shown in Figure 1-1.

The well-studied seawater carbonate system of the open ocean and, to some extent, nearshore waters, can be fully described by measuring only two of its four major (directly measurable) parameters along with temperature and salinity. This often allows effort to be focused on high quality measurements and equipment for two parameters. The equilibrium constants used in making these calculations are critically dependent on high quality measurements of temperature, salinity and pressure. Millero (2010) and Riebesell et al. (2010) provide detailed descriptions of these constants and where/when they should be used (also see section below on software packages that include documentation of the constants). Careful selection and reporting of the units of scale for all parameters is also critical. pH, for example, can be measured on a variety of scales, each of which has a different meaning and usage in the carbonate system calculations. DIC, total alkalinity and dissolved $CO_2$ can all be expressed in gravimetric (micromoles per kilogram) or volumetric (micromoles per liter) units. In order to minimize the chance for reporting or calculation error, all measured carbonate variables, with the exception of pH, temperature and salinity, should be reported in micromoles per kilogram. This straightforward conversion will be possible because sample salinity and temperature will be known.

### 4.2 Dissolved inorganic carbon (DIC)

DIC, sometimes referred to as total $CO_2$, $TCO_2$, or $\Sigma CO_2$, is the sum of the inorganic carbon species that are dissolved in a solution.

$$DIC = [CO_2(aq)] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

The majority of DIC in seawater exists as bicarbonate and carbonate ions. At surface, open-ocean equilibrium conditions, the DIC pool consists of carbon dioxide: $CO_{2(aq)}$* (~1%), carbonate ion: $CO_3^{2-}$ (~10%) and bicarbonate ion: $HCO_3^-$ (~89%). DIC is typically measured using instruments that incorporate infrared, coulometric, and spectrophotometric detection (see section on analyzing bottle samples for details).

### 4.3 Carbon dioxide ($CO_2$)

Under equilibrium conditions aqueous $CO_2$ ($CO_{2(aq)}$*) may only make up approximately 1% of the total DIC pool but is the most labile inorganic carbon species. The mole fraction of $CO_2$ ($XCO_2$) can be directly measured using a non-dispersive infrared gas analyzer after equilibration of gas and liquid phases in the measurement system (Frankignoulle et al., 2001; Hales et al., 2004; Wanninkhof and Thoning, 1993). $pCO_2$ and $fCO_2$ can be calculated from $XCO_2$ with other related parameters (e.g., temperature and pressure) [1], and are often reported in micro-atmospheres. [$CO_2$*aq] can be calculated from $fCO_2$ via Henry's Law (Dickson et al., 2007).

### 4.4 Total alkalinity (TA)

The measurement of alkalinity quantifies the ability of substances in seawater to react with the addition of a strong acid and convert it to an uncharged species. For this reason, it is sometimes informally denoted as "buffering" or "acid buffering capacity". At its simplest, TA is the excess of proton acceptors over proton donors (Wolf-Gladrow et al., 2007) though it is defined in many ways[2]. The TA of a system is dependent on a large number of seawater constituents and can be expressed as follows:

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] - [H^+]_f - [HSO_4^-] - [HF] - [H_3PO_4] \ldots \text{(other undefined acid-base species)}$$

Alkalinity tends to be pseudo-conservative with salinity (i.e., alkalinity does not dilute linearly with salinity because there are biogeochemical processes that can add or remove alkalinity). Generally, higher salinity waters (containing a greater concentration of salt and carbonate ions) will have higher alkalinity (Millero et al., 1998) and a greater ability to neutralize acidic inputs. In estuarine and near-shore systems, the contribution to total alkalinity by organic alkalinity (derived from dissolved organic matter) can be significant (Cai and Wang, 1998; Yang et al., 2015). TA can be measured through the use of acidimetric titration using potentiometric or colorimetric methods (Dickson et al., 2007; Gran, 1952).

### 4.5 pH

Water molecules dissociate into a hydrogen ion [$H^+$] and a hydroxyl ion [$OH^-$]. These hydrogen ions can exist freely in solution or combine with water ions. pH is a negative logarithmic numeric scale used to specify the acidity of an aqueous solution.

$$pH = -\log_{10}[H^+]$$

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[1] Aqueous $CO_2$ concentration is proportional to $CO_2$ fugacity ($fCO_2$) via dissolution of $CO_2$ gas into water (Henry's Law). Values of $fCO_2$ are similar to, but not the same as partial pressure of $CO_2$ ($pCO_2$), that would be exerted by the $CO_2$ if it existed at the observed temperature in isolation from other gasses (i.e., ideal gas) in equilibrium with seawater. $pCO2$ is the product of mole fraction of $CO_2$ ($XCO_2$) and atmospheric pressure, accounting for the interaction with other gases in air. The major difference between $pCO_2$ and $fCO_2$ is that the $fCO_2$ definition incorporates the non- ideal nature of $CO_2$ gas, making $fCO_2$ a more precise measure (Schuster et al. 2009b). In practical terms, differences between $pCO_2$ and $fCO_2$ are often not discernible. In typical estuarine conditions the difference is a few tenths of a percent, which is in the range of and often smaller than measurement uncertainty except on state-of-the art equipment.

[2] Dickson et al., 2007 defines seawater TA as "the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant $K \leq 10–4.5$ at 25°C and zero ionic strength) over proton donors (acids with $K > 10–4.5$) in 1 kilogram of sample".

Because the pH scales are logarithmic, a one unit change in pH represents a tenfold change in hydrogen ion concentration. In oceanic, coastal and estuarine systems, surface seawater pH averages 8.1 ± 0.1 (Millero, 2007). pH is a parameter that can be directly measured using potentiometric or colorimetric methods.

There are four pH scales that are commonly used in seawater pH measurement, and the lack of a consistently agreed upon scale can make interpretation between studies difficult (Marion et al., 2011; Orr et al., 2009). Dickson (1993) provides an excellent overview of the major pH scales and the suitability of each. They are determined by the method of calibration and are a critical piece of reporting. The four scales are the NBS (National Bureau of Standards) also known as IUPAC (International Union of Pure and Applied Chemistry) scale ($pH_{NBS}$), seawater scale ($pH_{SWS}$), free hydrogen scale ($pH_F$) and total hydrogen scale ($pH_T$). The NBS scale measures hydrogen ion activity; the seawater, free hydrogen and total hydrogen scales measure hydrogen ion concentration but differ from each other by incorporating measurements of different dissociation protons. These scales can be converted from one to the other, but the calculations can introduce error. Choosing the correct scale is important and can make a large difference in the final pH values, e.g. for a seawater sample at a salinity of 35 and a temperature of 25°C the difference between pH values using the $pH_{SWS}$ and $pH_T$ scales can be as much as 0.1 units, a sizeable difference on a logarithmic scale (Marion et al., 2011). The difference in values between $pH_{NBS}$ and $pH_T$ can range from hundredths to tenths of a pH unit, but more importantly, these differences are rarely consistent or predictable. Conversions from $pH_{NBS}$ to $pH_T$ are sometimes possible but require rigorous calculations and values for activity coefficients and liquid junction potential of the electrode (Pilson, 2013). Moreover, the relationship between the total pH scale and the NBS scale typically used in handheld meters and sondes is not conservative or straightforward because the two methods measure differing chemical properties.

For carbonate chemistry measurements/calculations in oceanic and estuarine systems using established stoichiometric models, the total hydrogen scale is the most suitable partly because of the high sulfate concentration in seawater. The total hydrogen scale ($pH_T$) includes the concentration of the free hydrogen proton as well as the proton that dissociates from hydrogen bisulfate ($HSO_4^-$).

The NBS (or IUPAC) scale is optimized for glass membrane electrodes and uses NBS or similar buffers. This method measures the free hydrogen ion activity, but the low ionic strength buffers may not be suitable for non-freshwater systems (Dickson, 1984). In any case, preparation of calibration buffers with ionic strengths that are close to those of the expected sample is an important consideration in pH measurement (see ANALYZING BOTTLE SAMPLES). The free hydrogen scale accounts only for the free hydrogen ion concentration. The chemical composition of seawater makes calibration when using the free hydrogen scale difficult by neglecting to account for the hydrogen ion from hydrogen bisulfate, and is not recommended for seawater. Both the seawater scale and total hydrogen scale measure the $H^+$ and $HSO_4^-$concentration. The seawater scale additionally incorporates the concentration of HF in the solution. Due to the relatively small differences between $pH_T$ and $pH_{SWS}$, it is clearer to treat fluoride explicitly as a minor acid-base species when needed rather than to incorporate it implicitly into the definition of pH (Dickson, 1993). While this document recommends using the total hydrogen scale whenever possible and especially when high-quality data are the goal, one must be mindful that the constants used in the calculation match the intended scale. Using incorrect constants can contribute systemic error in calculations and reporting (Riebesell et al., 2010).

### 4.6 Bicarbonate

The bicarbonate ion is the result of the first dissociation reaction of carbonic acid into seawater. At a pH ~8 bicarbonate makes up 89% of the inorganic carbon species constituting the DIC pool. Bicarbonate is not currently directly measured, but is calculated using measurements of at least two other parameters of the carbonate system along with salinity and temperature.

### 4.7 Carbonate

The carbonate ion is the result of the second dissociation reaction of carbonic acid into seawater. At a pH ~8 carbonate makes up ~10% of the inorganic carbon species constituting the DIC pool. Carbonate is not typically measured directly, but is calculated from measurements of at least two other parameters of the carbonate system along with salinity and temperature.

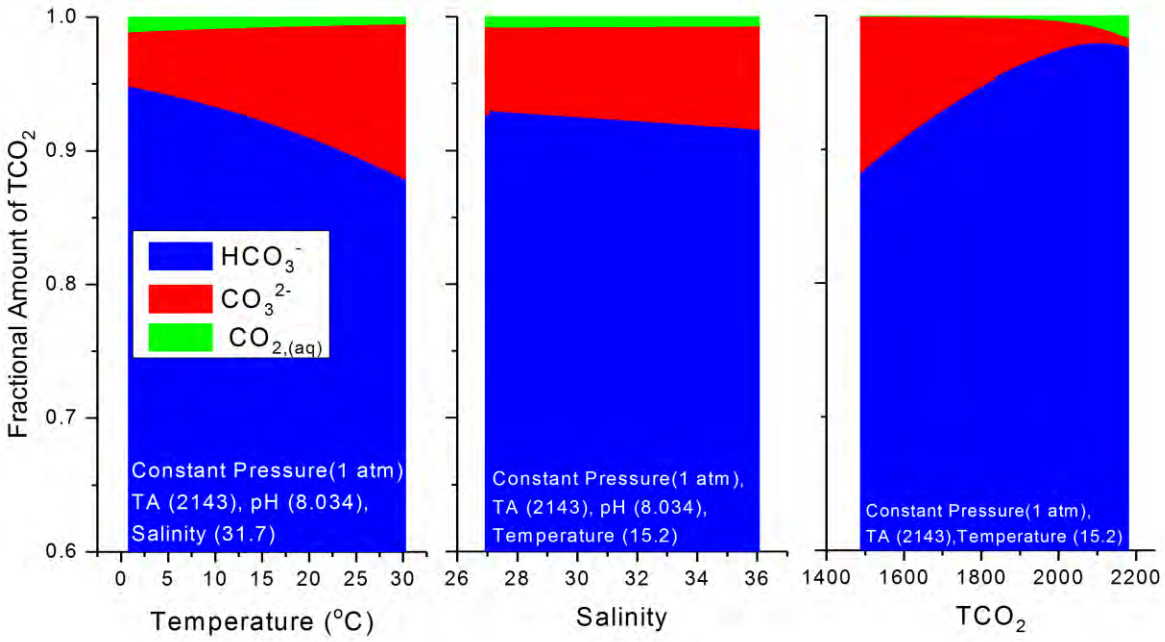


**Figure 4-3. Partitioning of the carbonic acid system as a function of temperature, salinity, and DIC ($TCO_2$) generated using CO2SYS** (see "CARBONATE SYSTEM CALCULATIONS AND REPORTING"). Note that as DIC is increased either by atmospheric exchange (i.e. ocean acidification) or by respiration, the relative proportion of carbonate ion decreases. Temperature and salinity also impart important controls on carbonate ion availability (Dwight Gledhill, personal communication).

## 4.8 Calcite and aragonite saturation states

This metric refers to the actual concentrations of calcium and carbonate ions relative to their expected concentrations at equilibrium with their respective allotropic form of calcium carbonate. Solubility product constants are used to describe saturated solutions of ionic compounds of relatively low solubility. A saturated solution is in a state of dynamic equilibrium between its constituent ions and the undissolved solid. For calcium carbonate, the solubility product is expressed as follows:

$K_{sp} = [Ca_2^{+}][CO_3^{2-}]$

We refer to the degree to which seawater is saturated with respect to allotropic form of calcium carbonate as saturation state, which is represented by Ω (omega).

$\Omega = [Ca_2^{+}][CO_3^{2-}]/K_{sp}$

Saturation states less than 1.0 indicate "undersaturation" while saturation states greater than 1.0 indicate "supersaturation" and are thus of interest for evaluating potential biological and ecological effects, especially for calcifying organisms. Calcium carbonate dissolution is thermodynamically favorable in undersaturated waters and calcium carbonate formation is favored in supersaturated waters (note that effects are sometimes seen at saturation states well above 1.0). Saturation states are calculated from measurements of at least two other parameters of the carbonate system along with salinity and temperature measurements (see "CARBONATE SYSTEM CALCULATIONS AND REPORTING").

# 5. COLLECTING BOTTLE SAMPLES

Sample collection and preservation is often an overlooked part of water quality chemistry but is an area where standardization is important and achievable. General standard operating procedures (SOPs) are available and commonly used, but there remains considerable flexibility within those SOPs that could affect sample quality (e.g., maximum time between sample collection and sample preservation). This section discusses recommended sample containers, collection techniques, and the pros and cons of varying preservation techniques. We recommend use of the standard operating procedures described in Dickson et al. (2007) including those for sample collection and preservation. Here, we provide only brief summaries of those methods and focus on additional considerations for coastal sampling.

## 5.1 Sample containers

Sample containers should be chosen based upon the intended analysis, volume of sample required for analysis, length of anticipated storage and collection method. Important considerations in bottle choice include volume, leaching of bottle material, gas permeability, opening size, neck size, and sealing (see sample storage sections below).

Whenever possible, volume should be collected in a sufficient quantity to perform the expected analysis with additional volume for rinsing pipettes, instruments etc. Headspace should be kept to a minimum while allowing for sample expansion. See the section below on storage methods for additional information.

## 5.2 Cleaning

To minimize contamination of carbonate samples, the analyst must ensure that everything that comes in contact with the sample (sample collection devise, sample bottles, pipettes) is rigorously cleaned. The cleaning, rinsing and filling of sampling devices and sample containers should be done in a standard and reproducible way in order to minimize inconsistencies that can reduce precision. Ideally, they should be thoroughly cleaned with a 5-10% (v/v) solution of 36% hydrochloric acid and then thoroughly rinsed (at least 3 rinse cycles) with deionized water to remove acid residue (i.e., “acid washed”). Careful, thorough rinsing is an important step as residual acid can greatly effect carbonate chemistry of the sample. Detergents are not preferred but may be an acceptable alternative cleaning agent; however, their effects on sample quality should be assessed beforehand (e.g., via analysis of sample blanks). Containers/caps should be inverted and allowed to air-dry and capped or covered to minimize contamination. As with most water quality sampling, nitrile or latex gloves should be worn to further minimize contamination. For large pieces of equipment such as large rosette units, acid washing of equipment between sampling stations is not feasible, so rinsing with sample water from the sample location becomes especially important.

## 5.3 Preferred order of sample collection

The dissolved $CO_2$ concentration in the water sample will rapidly equilibrate with the atmosphere once a sample is collected; consequently, samples will change over time. The rate of change is dependent on factors such as the sample volume, surface area, surface area to volume ratio, $CO_2$ gradient between the seawater and atmosphere, and biological activity. For this reason, the minimization of atmospheric exchange during sample collection and storage is paramount in obtaining accurate measurements of carbonate parameters. Some carbonate parameters are more sensitive to atmospheric gas exchange compared to other measured

parameters. Due to the low concentration of $CO_2$ found in seawater (typically about $2x10^{-5}$ mol $kg^{-1}$), dissolved $CO_2$ measurements are extremely sensitive to atmospheric exchange. For this reason, it is useful to collect samples in decreasing order of their sensitivity to atmospheric exchange, which is $pCO_2$ > pH > DIC > TA. In order to minimize the level of atmospheric exchange, Niskin, Go-Flow and Van Dorn sampling devices are common choices. After retrieval, the device should be vertically oriented with its outlet lying below the vented top surface, to prevent bubbles from entering the device. Flexible silicon, FEP or Teflon© tubing is typically used to fill and transfer samples from the bottom outlet of the sampling device to the sample bottles. The tubing should be rinsed with sample seawater prior to introducing it into a sample bottle. Skin contact with the portion of the tube that enters the bottle should be avoided, even in cases where it will be rinsed.

### 5.4 Filling sample bottles

Prior to filling, sample bottles cleaned as above should then be rinsed and emptied 3 times with the sample seawater. The flexible tube is then placed with its opening at the bottom of the sample container and allowed to fill at a rate that minimizes visible turbulence in the bottle. The sample bottle should be filled and allowed to overflow for approximately twice the time needed to fill the container to the top (e.g., if it requires 5 seconds to fill the bottle, the overflow process should continue for 10 seconds). If bubbles enter the sample, the entire overflow process should be repeated.

The tube can be slowly withdrawn from the sample bottle and the sample can be closed with a screw cap, ground glass stopper or conical displacement cap. Normally, a headspace of about 1% of the total sample volume is left to allow for sample expansion. This can be achieved by pinching the tube before it is completely withdrawn.

Samples should not be taken for $pCO_2$, pH and DIC when the water volume in the sampling device falls to ~25% of the total device volume, due to the potential of atmospheric exchange significantly altering the sample. In some cases, and for certain purposes, bucket collection of seawater will be the only option. However, the same principles of rinsing and reducing surface exposure and turbulence apply.

### 5.5 Long-term storage bottles

The preferred method for long-term storage (>1 months) of samples uses a narrow mouth borosilicate glass necked bottle with a ground borosilicate glass stopper (Dickson et al., 2007). Apiezon high vacuum grease (or similar) is applied in a thin layer on the stopper which is inserted into the bottle and turned to completely coat the surfaces where the stopper contacts the bottle. To further ensure there is no gas exchange nor stopper displacement the stopper can be secured with positive pressure (e.g., using an elastic band or plastic strap).

### 5.6 Short-term storage bottles

For short-term storage of samples (<1 months) borosilicate glass screw thread sample vials are a suitable storage option. Huang et al. (2012) demonstrated that DIC concentration of samples preserved and stored in a specific type of screw cap vial vs. borosilicate stoppered bottles were not statistically different over the 148-day study period. These vials are available in a variety of volumes and can be ordered with caps that are tailored for the expected analysis. Solid caps with Teflon © liners retard gas exchange with the atmosphere.

## 5.7 Sample preservation and shelf life

A saturated mercuric chloride solution (in DI water) is the standard preservative used for seawater carbonate system sampling. Preserving a sample is sometimes also referred to as "fixing" a sample. Immediate preservation is advised in SOPs, but there is no clear guidance on how quickly samples degrade after collection and what exactly constitutes immediate preservation (i.e., delays less than seconds, minutes, or hours). This will be addressed in future versions or additions to these guidelines but is largely related to the relative productivity of the waters. Water samples with high levels of nutrients and organic matter probably require more immediate fixing. Freezing is not an acceptable method of preserving samples. In any case, it has been suggested that a well preserved sample in a high quality sealed bottle has a shelf life of at least one year (e.g., Tris samples: http://www.whoi.edu/fileserver.do?id=53806&pt=2&p=58666).

## 5.8 Sampling without preservation

In some cases, if the samples are to be analyzed quickly (i.e., within a few hours), preservation of properly handled samples may be unnecessary. Proper handling of samples can include keeping them cool and dark after collection to slow down biological activity. We recommend storing samples in a refrigerator (Dickson et al., 2007) or a cooler with ice or cold packs.

## 5.9 Transport

Sample containers should be stored in a cool, dark environment (e.g., a cooler). Shipping of samples preserved with mercuric chloride or other toxic chemicals requires special handling and labeling. Mercuric chloride preserved samples fall under Department of Transportation Hazard Class 6.1; poisonous materials must be transported in compliance with 49 CFR 173.132. Refer to these Department of Transportation guidelines for further information.

## 5.10 Other sampling equipment

As noted above, latex or nitrile gloves should be worn when sampling to prevent contamination of the water sample. If the sample is to be fixed with a hazardous preservative, personal protective equipment (PPE) that is appropriate for working with that preservative should be worn. This typically includes nitrile gloves, eye protection and a lab coat.

## 5.11 Filtration

Instruments with small diameter syringes and injection ports or combustion columns or optically-based measurements may provide improved results if samples are pre-filtered. Filtering, however, can have a deleterious effect on carbonate measurements for $pCO_2$, pH and DIC. Samples are subjected to pressure changes and increased turbulence in most filtering apparatus. Benchtop and syringe filtering using glass fiber filters should not be used because their use will lead to atmospheric exchanges and, thus, alter the sample. Bockman and Dickson (2014) described a method using a peristaltic pump in conjunction with a membrane filter to remove phytoplankton and $CaCO_3$ particles from a sample without altering the carbonate chemistry of the sample. This method may be useful when trying to characterize systems high in biological and inorganic particulate matter but, if used, details need to be carefully reported.

In-line barrel filters, such as those used in groundwater sampling (0.45 μm), properly filled with the water being sampled are sometimes used in the bottle-filling process when filtering large volumes of water. Effects on sample quality are not known so we do not

recommend this method. In cases where water is so turbid that such filtering is necessary, other issues emerge that would only be addressed by advanced chemistry labs equipped for "over determination" of the carbonate system.

### 5.12 Analyte-specific sampling techniques

$pCO_2$: Borosilicate glass should be used along with a cap that is impermeable to gas exchange. As already noted, $pCO_2$ samples should be collected with a headspace approximately 1% of the bottle volume. This is to prevent possible breakage due to the expansion of the sample if its temperature increases. Required sample volume is instrument and analysis dependent. Systems for measuring discrete $pCO_2$ samples are not common and are often custom made in research laboratories (Hales et al., 2004). Recent evaluations of a commercially available $CO_2$ probe can be found in Moran et al. (2010) and Pfeiffer et al. (2011). More commonly, $pCO_2$ measurements are taken using a flow-through system. Flow-through systems present a separate set of protocols to be followed to ensure that a representative environmental sample is measured.

pH: In general, there is no accepted method for preservation of pH samples, so many investigators opt for direct in situ measurement or immediate analysis of collected samples (i.e., within seconds or minutes). A recent study (Chou et al., 2016) examined the viability of preserving pH samples in the field for later lab analysis, so this method deserves further consideration. pH samples should be collected with minimal headspace and should be stored in a borosilicate glass container with a cap that is impermeable to gas exchange. The amount of sample volume that is necessary to collect is instrument and analysis dependent. Samples collected for analysis using the potentiometric method must be collected with minimal headspace and the volume should be sufficient to allow the electrode to be immersed. Due to gas exchange driven pH changes and ion consumption by the electrode, a larger volume of sample collected will potentially give more stable and precise readings. When performing a measurement in an open container, the surface area to volume ratio of the sample should be minimized (to reduce gas exchange) by choosing smaller diameter/tall vessels vs. larger diameter/short vessels. Electron starvation and gas exchange become more of an issue when there is large variation between samples and thus longer times for stabilization of the reading.

DIC: Each DIC sample bottle should be filled as described above and stored in a borosilicate glass container with a cap that is impermeable to gas exchange; samples with high $pCO_2$ are especially susceptible to gas exchange concerns. The amount of sample volume that is necessary to collect is instrument and analysis dependent although most commercially available systems require a sample volume of 5-25 mL. The need for splitting samples or repeat analysis is also a factor.

Total Alkalinity: Samples for total alkalinity can be collected in borosilicate glass or HDPE sample containers. Borosilicate glass is preferable to "softer" glasses for sample storage. Storing samples in soda lime glass can cause significant increases in alkalinity concentrations as seawater can leach sodium and other compounds from the glass over time (Huang et al., 2012). Alkalinity samples are less sensitive to gas exchange than DIC samples so it is less critical but still advisable to collect them in gas impermeable containers. The amount of sample volume that is necessary to collect is instrument and analysis dependent. A minimum volume of 40 mL is recommended although accuracy will be greater with a larger sample volume.

# 6. ANALYZING BOTTLE SAMPLES

All of the measured carbonate parameters discussed below can be analyzed using different protocols and procedures. There are existing comprehensive resources that go into great detail regarding carbonate system measurement principles and method SOPs (Dickson et al., 2007; Martz et al., 2015; Schuster et al., 2009a). For most of these parameters, there exists an array of instruments ranging from highly customized "research instruments" to off-the-shelf commercial instruments. Martz et al. (2015) provides a good overview regarding the availability and intended usage of instruments measuring the seawater carbon system. For all analysis, standard or certified reference materials (CRMs) are recommended to quantify sample precision and accuracy.

## 6.1 Certified reference materials (CRMs)

The importance of CRMs for carbonate system analysis cannot be overstated. Careful use of certified reference materials can dramatically improve measurement precision and accuracy. Martz et al. (2015) noted that establishment of the CRM program reduced residual errors in DIC from ~14 µmol $kg^{-1}$ during GEOSECS (Bradshaw et al., 1981) to ~3 µmol $kg^{-1}$ during WOCE/JGOFS/OACES (Lamb et al., 2002). Andrew Dickson's Marine Physical Laboratory at the University of California at San Diego provides CRMs that currently serve as the industry standard for the traceable QA in inorganic carbon system analyses. These CRMs are provided for DIC and alkalinity in sterilized natural seawater; uncertified reference materials for $pH_T$ are made in artificial seawater. These CRMs have been widely used in oceanographic research beginning around 1990. DIC and alkalinity concentration values and salinity are provided along with the concentration of phosphate, silicate, nitrite and nitrate (all values are in micromoles per kilogram). Salinity is usually close to 33 PSU so care must be taken when using these oceanic reference materials in low ionic strength estuarine systems (i.e., < 20 PSU). Detailed information on each batch and storage instructions can be found at (http://cdiac.ornl.gov/oceans/Dickson_CRM/batches.html). Some investigators prepare inorganic carbon or borate solutions in seawater and then analyze their DIC and alkalinity against the CRMs. These can then be used as secondary standards for monitoring drift during an analysis session, thereby reducing the use of CRMs and their associated waste stream. However, we recommend that all DIC and alkalinity sessions include a minimum of at least two CRM determinations (beginning and end). If the electrode system is affected by switching salinity between samples, it may be beneficial to try measuring a diluted (by weight) CRM as well as an off-the-shelf one to confirm that such a problem does/or does not exist (Dickson, personal communication).

## 6.2 Alkalinity

Relative to the inorganic carbon parameters, total alkalinity tends to remain stable during short term exposure to the atmosphere during sample collection and analysis. However, alkalinity measurements require careful determination of salinity and temperature. Care must be taken to use an instrument that incorporates these parameters into the measurement or one must record these parameters using calibrated sensors and incorporate them into final calculations. Commercial alkalinity measurement equipment is available and can be purchased for approximately $10,000 to $20,000, depending on extra features (Riebesell et al., 2010; also see

IOCC instrument list). See Table A-1 for typical precision goals. Two methods are described below.

Potentiometric method: A seawater sample is placed in a thermally jacketed, open container. A known amount of a hydrochloric acid titrant solution is added to the sample. During titration, this acid titrant reacts with the alkaline species present and drives the pH down below 3. Once pH decreases past the second equivalence point, the alkalinity is back calculated based on the sample weight, amount of hydrochloric acid titrant solution added, corrections for preservative effects and the molarity of the titrant solution (typically requiring a Tris calibration; see Dickson et al., 2007; Gran, 1952). The molarity of the hydrochloric acid titrant solution can be determined using Tris [tris (hydroxymethyl) aminomethane, an organic compound with the formula $(HOCH_2)_3CNH_2$)] buffers (Marion et al., 2011) prepared in an NaCl solution of similar salinity (approximately) to that of the samples being analyzed. During the alkalinity titration, the temperature of the sample should be held constant and near to 25°C. This can be done using a jacketed beaker plumbed to a temperature-controlled water circulator. Continuous mixing using a magnetic stir bar or similar is necessary to ensure that the titrant is uniformly dispersed and the electrode reading is representative.

Potentiometric method considerations

- Benefits: High precision (±0.1% at 2000 μmol $kg^{-1}$). Widely used and well-documented method. Good analytical stability (Riebesell et al., 2010).
- Disadvantages: In samples with high nutrient and/or dissolved organic matter concentrations (e.g. eutrophic estuarine systems), interpreting alkalinity titrations can be difficult, refer to Riebesell et al. (2010).
- Careful determination of titration reagent molarity is required.
- Temperature control (±0.1°C) is required for high precision.
- Samples should be kept in a temperature-controlled bath prior to analysis, and titration should be carried out in a temperature-controlled jacketed beaker.
- Acid addition should proceed slowly (0.01 mL/second) so that evolved $CO_2$ gas can escape solution. Gas exchange can be facilitated through the gentle bubbling of air (or nitrogen).
- CRM: Dickson seawater reference material.

Colorimetric method: The colorimetric method of determining total alkalinity was commonly used for environmental samples in the past and there is ample documentation from USGS and EPA (USEPA, 1974) on this method. Colorimetric alkalinity measurements work on the principle of using a reagent that contains a known quantity of acid and a color indicator, which is typically methyl orange. The color change that occurs when a known amount of sample water is added to the reagent is used to calculate the alkalinity. The acid-methyl orange indicator works under the principle that "any addition of alkalinity causes a loss of color directly proportional to the amount of alkalinity. This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The applicable range is 10 to 200 mg/L as $CaCO_3$" (USEPA, 1974). There is an autonomous system currently under development using bromocresol purple indicator dye as the color reagent and calculating alkalinity via a spectrophotometer (Spaulding et al., 2014).

Colorimetric method considerations

- Disadvantages: Slow analysis time (individual sample time is > 10 minutes). High turbidity can interfere with analysis. Interpreting alkalinity can be difficult (Riebesell et al., 2010).
- This method is not currently widely used in monitoring environmental samples.
- High purity indicator dye must be used.
- CRM: Dickson seawater reference material.

### 6.3 Dissolved inorganic carbon

DIC measurement requires careful determination of salinity and control of temperature. DIC can be measured using either infrared or coulometric methods. Because of its shorter processing time, the infrared $CO_2$ analysis method is more commonly used. As with alkalinity, quality assurance is much improved through the use of CRMs (see Certified Reference Materials CRMs). For both methods, samples are preserved, stored and prepared in the same way. Inorganic carbon standards are sometimes prepared or purchased but this practice is rare because of the lack of traceability and the unknown sources of error it may introduce. Commercial dissolved inorganic carbon measurement equipment is available and can be purchased for roughly $50,000 (Riebesell et al., 2010).

Infrared method: When measuring DIC, a known quantity of a water sample is dispensed by the instrument into a stripping chamber. The water sample is then acidified, typically with phosphoric acid, to a pH below 3.0. By decreasing the pH to less than 3.0, all of the inorganic carbon species in solution ($H_2CO_3$, $HCO_3^-$, $CO_3^{2-}$) are converted to $CO_2$, which is then removed from the acidified solution by sparging with a high purity, $CO_2$ free, inert carrier gas ($N_2$ is commonly used). This $CO_2$-containing carrier gas is passed through a drying column, to remove water vapor, and then is transferred to the built-in non-dispersive infrared gas analyzer for measurement of $CO_2$ concentration. CRMs are used for calibration of the DIC analyzer.

Infrared method considerations

- Benefits: Small sample volume needed (>1mL). Short measurement time (~10-15 minutes). High precision (0.05-0.1% CV). One reagent needed, and smaller amount of hazardous waste produced.
- Disadvantages: Expensive (but similar cost to coulometric). Calibration is less stable than coulometric and thus more frequently required. Instrument drift must be evaluated using periodic check standards (i.e., every five or ten samples).
- Temperature control (±0.1°C) is required for high precision.
- Samples should be kept in a temperature-controlled bath prior to analysis and temperature-controlled jacketed beakers should be used for the reagents and samples.
- CRM: Dickson seawater reference material.
- CRM stability is subject to headspace equilibration. Portion large CRM bottle into many smaller, gas-tight containers.

Coulometric method: The preliminary sample manipulation steps for determining DIC coulometrically are similar to the infrared DIC method (i.e. acidifying, sparging, drying). The difference lies in how the $CO_2$ entrained in the carrier gas is measured. The amount of $CO_2$ in the carrier gas is measured by trapping the $CO_2$ in an absorbent containing ethanolamine and

coulometrically titrating the hydroxyethylcarbamic acid that is formed. Further information on coulometric methods can be found in Lindberg (1978) and Johnson et al. (1985).

Coulometric method considerations

- Benefits: High Precision (± 1 μmol $kg^{-1}$). Longer instrument stability following calibration.
- Disadvantages: Expensive but similar to infrared; greater number of chemical reagents needed and larger volume of waste produced. Sample run time is longer than IR method.
- Preferred CRM: Dickson seawater reference material.
- CRM stability is subject to headspace equilibration. Portion larger CRM bottles into many smaller, gas-tight containers.

## 6.4 pH

Water naturally dissociates into its constituent positive ($H^+$) and negative ($OH^-$) ions. pH measurements are an attempt to quantify hydrogen ion ($H^+$) activity or concentration. However, as discussed above, there are varying scales on which pH is measured and expressed. Seawater pH measurement using the potentiometric method can be accomplished using a number of different electrode configurations along with newer technologies such as solid state sensors. Potentiometric pH measurement requires periodic calibration with a carefully selected calibrant buffers suited to the measurement method, pH scale to be used and expected environmental pH and ionic strength. Unlike the potentiometric method, spectrophotometric pH measurement (Clayton and Byrne, 1993) employs a pH-dependent dye and does not require calibration with a buffer. However, we recommend periodic checks using a standardized Tris buffer solution. For both the potentiometric and spectrophotometric methods, temperature needs to be carefully recorded or controlled and salinity needs to be recorded. Because pH is affected by $CO_2$ flux between the sample and atmosphere, laboratory pH measurements should be carried out in closed containers. When measuring pH directly in a water body using a portable probe, this is not applicable.

Colorimetric/spectrophotometric method: In spectrophotometric pH measurements, which measure total pH, a known quantity of purified m-Cresol purple, a sulfonphthalein acid-base indicator, is added to a seawater sample (Clayton and Byrne, 1993; Dickson et al., 2007; Easley and Byrne, 2012; Liu et al., 2011). Mosley et al. (2004) discusses applications to a wide salinity range. Absorbances are measured with a spectrophotometer at the maximum absorption wavelengths corresponding to the different forms of the indicator dye, which dissociates into acid and base forms with distinct light absorbance spectra. This dissociation, and hence the absorption ratio, depends on the pH of the sample (including the effect of the sulfate ion). The calculation to determine total pH is made from the measured absorbance ratios, blank correction, pH perturbation (due to dye addition), temperature, salinity, pressure and dissociation constant of the indicator dye. Temperature must be tightly controlled (±0.1°C) because of its effect on pH, so jacketed cells are often used. Dye impurities are known to affect the accuracy and precision of measurements, so highly purified dye (e.g., from R. Byrne's lab at the University of South Florida) are typically required for high quality measurements. Mosley et al. (2004) found that precision using this method was better at salinities greater than 30 (±0.0005) as opposed to salinities under 5 (±0.002). Additionally, correcting the dye pH to match expected sample pH will be more difficult in highly variable estuarine systems.

Colorimetric/spectrophotometric method considerations

- Benefits: High precision (±0.002 to ±0.0005) can be one or two orders of magnitude better than glass electrode method. Measures $pH_T$, the preferred scale for seawater.
- Disadvantages: Required equipment is more expensive and less portable than pH electrodes. Sample analysis time greater than with handheld electrode. Limited availability of purified m-Cresol dye, no batch tracking or certification. Making Tris buffers can be time consuming and difficult.
- Temperature control (±0.1°C) is required for high precision.
- Samples should be kept in a temperature-controlled bath before analysis and a jacketed, temperature-controlled optical cell should be used in the spectrophotometer.
- Dye pH is subject to change, prior to use it should be adjusted to 8.1 ±.05 (e.g., using HCl or NaOH). Some investigators argue that the dye should be close in pH to the samples, so that the perturbation effect of the dye on the sample is small.
- Dye perturbation analysis (measuring the pH change caused by the addition of dye alone) should be carried out at least once per week (R. Byrne, personal communication).
- Preferred CRM: Tris buffers are available as reference materials from the Dickson lab at Scripps (not certified) or can be prepared in artificial seawater with considerable effort and potential for error (Nemzer and Dickson, 2005). Tris buffer salinity should be adjusted to match sample salinity.

Potentiometric method: In general, potentiometric pH measurement utilizes electrodes to measure electromotive force, i.e., the difference in voltage measured between two points with different electrical potential values. Electrodes for pH measurement must be ion selective for the $H^+$ ion and will consist of a reference and a glass electrode. These electrodes can be combined (typical in handheld pH meters) or separate. The three main types of pH electrodes in use are hydrogen, metal, and glass. Of these, glass electrodes are the most frequently used (the term glass electrode is a description of the membrane material, i.e., glass membrane, rather than of the material used to construct the electrode). Combination glass/reference pH electrodes are commonly used but measurement precision can be improved by employing separate glass and reference electrodes (Dickson et al., 2007). The reference electrode is contained in a medium that provides a constant electrical potential value that is not affected by the pH of the sample, while the glass electrode returns an electrical potential value that is dependent on the hydrogen ion activity in the sample. These two electrical potential values determine the pH based upon the premise that the system provides a Nernstian response. Because the electrochemical potential measured by a glass electrode system is dependent upon an external liquid junction potential, which is sensitive to changes in the seawater environment (e.g., salinity, temperature), frequent re-calibration (weekly or more frequently; Easley and Byrne, 2012) is necessary to ensure that the electrodes are not exhibiting non-Nernstian behavior. Dickson et al. (2007) describes the methodology for obtaining accurate total hydrogen scale pH measurements from glass electrodes. This method is more complex and requires more involved and frequent calibration compared to the spectrophotometric method, but the equipment cost is smaller.

We generally do not recommend measurement of pH on the NBS scale, except in very limited circumstances (e.g., educational settings, monitoring of qualitative patterns to compliment other carbonate measurements). Within stable water conditions, some investigators will calculate a regression between a $pH_{NBS}$ and a $pH_T$ determination to allow conversion, but this is only reliable if the biological and chemical composition of the sample source is similar to

the samples used for the regression. There are several different pH buffers that are either commercially available or can be prepared in-house. NBS (or IUPAC) buffers are available from a variety of manufacturers. These buffers are suited for calibrating electrodes on the NBS scale only; if using NBS buffers it is recommended to use those in individual foil packets. pH buffers will start to degrade when opened, whereas unexpired buffers in foil packets ensures fresh buffers for each calibration (after dispensing into suitable beakers). Researchers measuring ocean pH are more likely to use Tris buffers (Marion et al., 2011). Tris has a pKa of 8.07, which makes it an effective buffer in the pH range of 7 to 9 and can be prepared in artificial seawater at a variety of ionic strengths (matching the ionic strength of the intended sample is necessary to minimize residual liquid junction errors [Dickson et al., 2007]). Calibrating with Tris buffers is acceptable for the total hydrogen pH and free hydrogen pH scales (Millero, 1986), but low quality Tris preparations may introduce as much error as the methods such as the regression approach described above. A detailed description of Tris buffer use, including information on stability preparation can be found in (Nemzer and Dickson, 2005). Glass electrodes can also be calibrated to total pH using spectrophotometric pH measurements (Easley and Byrne, 2012). More discussion of the varying pH scales in included in the section on the seawater carbonate system.

Potentiometric method considerations

- Benefits: Equipment is easy to use in field conditions. Fast response time ~30 seconds. Comparatively inexpensive. Many commercial systems can be set up to continuously monitor and record pH measurements.
- Disadvantages: Precision is not as good as spectrophotometric method. Requires frequent calibration. Making Tris buffers can be time consuming and difficult. The availability of inexpensive meters contributes to insufficient calibration practices and misinterpretation of data.
- Electrodes must be periodically monitored for stability/degradation and non-Nernstian behavior.
- Preferred CRM: Tris buffer in artificial seawater.
- If the only available buffers are NBS, opt for those sold in single use foil packets and try to calibrate at the temperature specified in the buffer label.

Solid state sensors: All solid state pH sensors are constructed using metal oxide semiconductor field effect transistors and are typically referred to as ion-sensitive field-effect transistor (ISFET) instruments. When the metal oxide semiconductor is exposed to an aqueous solution, the exchange of protons produces an interfacial potential on the ion selective electrode. The voltage difference between the ion selective electrode and either the internal or external reference electrode is converted to a pH measurement (Bresnahan et al., 2014). These sensors are used in a wide variety of chemical, industrial and environmental uses. Martz et al. (2010) and Bresnahan et al. (2014) describe best practices of sensor conditioning and calibration. ISFET instruments designed for handheld use and autonomous application are commercially available. As of this writing, Honeywell Durafet probe and meter cost is $2500-$3000.

Solid state method considerations

- Benefits: Easy to use and requires minimal training. Fast response time ~30 seconds. Many commercial systems can be set up in a continuous monitor and record pH measurements. Can remain stable for multiple months when continuously deployed in seawater (Bresnahan et al., 2014). Precision of well calibrated instrument comparable to spectrophotometric method.
- Disadvantages: Expensive compared to potentiometric method. Lengthy time period required for sensor conditioning and calibration (Bresnahan et al., 2014).
- Not intended for use with bottle samples.
- Deployed systems subject to biofouling and/or sedimentation.
- Preferred CRM: paired, lab analyzed samples.

### 6.5 *p*$CO_2$

Membrane/infrared method: For instruments that utilize infrared detection, the first step in measuring *p*$CO_2$ is to extract the $CO_2$ dissolved in the water sample into the gas phase. This can be accomplished using headspace equilibration (Frankignoulle, 1988; Frankignoulle and Borges, 2001; Hales et al., 2004). A $CO_2$ free gas (usually $CO_2$ free air) is introduced into a gastight chamber with the water sample. The water and air is agitated together for a period of time (~1 min) until the $CO_2$ concentration in the water and the headspace are equivalent. Alternatively, many commercial instruments use a gas permeable membrane that is immersed in seawater and $CO_2$ can passively diffuse across this membrane. Once the $CO_2$ in the gas phase, the gas is transferred to an optical cell for measurement via non-dispersive infrared analysis (NDIR). The infrared absorbance measurement of the $CO_2$ gas sample is compared to the reference measurement, which does not contain $CO_2$, that allows calculation of a *p*$CO_2$ value because sensor absorbance response complies with the Beer-Lambert law. Alternatively, a set of calibrated reference gases can be used to generate a standard curve. Most commercial *p*$CO_2$ measurement systems are in situ or flow through systems. The measurement of *p*$CO_2$ is extremely sensitive to changes in temperature and pressure; consequently, this analysis is well suited to an in situ application.

Membrane/infrared method considerations

- Benefits: Continuous measurement of *p*$CO_2$ over weeks/months. Does not require frequent calibration.
- Disadvantages: Accurate sensors are expensive. Many of these systems are of the homemade variety, extensive documentation regarding calibration and usage may not be available.
- Not intended for use with bottle samples.
- Deployed systems subject to biofouling and/or sedimentation.
- Preferred CRM: Calibrated gas cylinder.

# 7. DIRECT IN SITU MEASUREMENTS AND AUTONOMOUS SENSORS

In situ and autonomous instrumentation is a rapidly developing area of research and will be covered in greater detail in future versions or additions to these guidelines. Although commercial options are available, many customized instrument configurations are being developed and used in autonomous applications.

There is commercial instrumentation suitable for extended periods of data collection for both pH and $pCO_2$. Martz et al. (2015) and Byrne et al. (2010) provide good overviews of the commercially available autonomous sensors and principles of measurement. Autonomous DIC analysis is a developing field and as a result, instrumentation tends to be custom built by individual research groups. Liu et al. (2013) describes an autonomous instrument that uses spectroscopy to measure ambient DIC. Bell et al. (2011) describes a deployable membrane inlet mass spectrometry instrument that can be used for DIC analysis. For alkalinity measurement, Spaulding et al. (2014) describes a SAMI-ALK system that is in development and is suitable for autonomous determination of alkalinity for a period of up to one month. Those readers interested in autonomous and in situ instrumentation will have to consider the rapid developments in this field and monitor new developments. Jones et al. (2016) recently reported good results using autonomous measurements of pH and an alkalinity approximation from sea surface temperature and salinity for their California coastal site.

Getting high quality, precise carbonate system measurements from in situ and autonomous instrumentation has several challenges. The instrumentation used will need to be carefully calibrated either in the lab prior to deployment or autonomously, to provide the best data. Several of the commercially available sensors are factory calibrated but the majority of users surveyed in Martz et al. (2015) conducted their own calibrations using CRM's or paired, lab-analyzed samples. Biofouling is a persistent issue with any deployed instrumentation. The severity of biofouling can vary between environments and is affected by fouling species present and seasonal differences in settling rates. Biofouling resistance is understood to limit the performance of the instrument, especially as deployment time increases (Byrne et al., 2010). For carbonate chemistry the biofouling effect includes the changes in $pCO_2$ (and thus pH and DIC) that can be caused by respiring organisms.

# 8. CARBON SYSTEM CALCULATIONS AND REPORTING

Unmeasured carbonate system parameters can often be calculated from measured parameters. For the open ocean, it is widely accepted that the measurement of any two carbonate parameters can be used with salinity, temperature and pressure to "constrain" the carbonate system and calculate the remaining parameters. This depends on a number of tightly constrained thermodynamic constants that suit only specific environmental conditions and which govern the dissociation reactions in the seawater carbonate system. The computations have been implemented in a number of open source software tools, each with good documentation on how to select the appropriate constants before performing calculations (ten such packages were reviewed in Orr et al. 2015). In some cases, these constants are less appropriate for coastal monitoring. Currently, the $k_1$ and $k_2$ constants developed by Millero (2010) are often considered suitable for carbonate system calculations at estuarine salinity. The Dickson and Riley (1979) option for $k_f$, and the Dickson (1990) option for $k_s$, are available for most calculation packages and are shown to be suitable over a large salinity range (Millero, 2010; Orr et al., 2015). In addition, CRMs used for quality control are prepared in full strength seawater and are considered inappropriate at low salinities. Because of this, and since there appears to be no consensus on the best pair of carbonate parameters to monitor in the coastal environment, many investigators "overdetermine" the carbonate system by measuring at least three parameters. Over-determination allows identification of cases where one pair of parameters produces a different picture of the carbonate system than a different pair. If measurement error or instrument interferences (e.g., biofouling; colored dissolved organic materials effects on optical measurements and non-carbonate alkalinity) can be ruled out, such an outcome suggests that the open ocean stoichiometry may be poorly suited to the study site and that higher level calculations (e.g., aragonite saturate state) should be performed with caution.

When reporting these higher level calculations (e.g., aragonite saturation state), it is possible and useful to incorporate the uncertainty in the underlying parameters. As noted in Martz et al. (2015), error propagation from measurement to final calculations would be a useful addition to available software (see "Carbonate System Calculations"). This allows attribution of the dominant sources of uncertainty, which informs selection of the set of carbonate parameters that will most effectively minimize uncertainty in monitoring programs (see Table A-1).

While some investigators will have the opportunity to overdetermine the carbonate system by measuring three or more parameters, others may be limited to only one parameter. As monitoring data accumulate, there is the possibility that proxies will be identified that can make such data useful, so these efforts should not be discouraged. For example, alkalinity can sometimes be approximated from pre-existing alkalinity-salinity regressions (Carter et al., 2016; Gledhill et al., 2015; Lee et al., 2006; Millero et al., 1998), and $pCO_2$ or $TCO_2$ can sometimes be crudely predicted using production/respiration calculations or proxies thereof, such as changes in dissolved oxygen concentrations (e.g., Sunda and Cai, 2012).

# 9. OVERVIEW OF SAMPLING CONCEPTS AND DOCUMENTING UNCERTAINTY

## 9.1 Precision and accuracy

Uncertainty is a critical component of all environmental data reporting and is usually expressed in terms of accuracy and precision. These terms are sometimes incorrectly used as though they are interchangeable. However, they have very specific and different meanings that are important to keep in mind when planning, collecting, or interpreting observations (Figure 9-1).

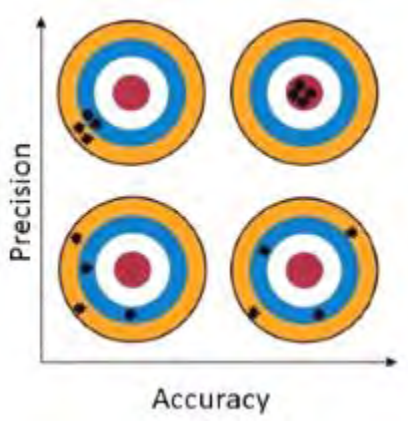


**Figure 9-1: Precision vs. accuracy.**

Accuracy refers to how close a given observation or measurement is to the true value, whereas precision refers to how close multiple measurements are to each other (i.e., repeatability). An instrument that is always biased away from the true value would be considered inaccurate but if this bias is extremely consistent, the instrument would also be considered highly precise. An instrument that rarely returns the true value but which, after many randomly varying measurements, produces an average value that is close to the true value, would be considered imprecise but accurate when a sufficient number of measurements is averaged. Imprecise methods are sometimes useful, accessible and/or inexpensive, but are problematic if replication is too low. These same principles of accuracy and precision apply to environmental sampling methods. For example, repeated measurements of pH at a small number of fixed locations in a large water body may be extremely consistent (i.e., high precision), but accuracy of the mean and variance estimates depends on whether the sample times and locations are representative of the point, water body, or parcel about which generalizations are being made. Calculation of final sample precision and accuracy involves detailed methods for combining error sources into a single estimate (Ellison and Williams, 2012) but is beyond the scope of these guidelines.

The sources of uncertainty affecting a set of samples can be lumped into observation error and process variability. Process variability is the result of deterministic processes (e.g., biogeochemical reactions, spatial heterogeneity). Since these processes can operate on tiny scales of space and time, it is theoretically impossible to sample a process more than once before it changes state. Nonetheless, duplicate sampling is an important component of quality assurance because it helps to identify sources of error. Samples intentionally taken over wider

intervals or spaces serve to describe process variability over the sampling domain or water parcel from which the final reported values are averaged.

Sample handling, analyst skill, preparation and storage of reagents used in instrument calibration, and instrument error are some of the obvious contributors to observation error, but as described above, the total variance of a set of samples also includes the effect of process error. Thus, a well-designed sampling program will include quality control measures to assess and constrain these sources individually. As already noted, sample duplication is one example, where multiple samples are collected simultaneously (to minimize the effect of process error) and compared to assess observational precision (i.e., repeatability). Samples may also be split and repeatedly analyzed to assess instrument precision. Many instruments perform this repetition internally. Analysis of "blanks" (e.g., deionized water) are often used to assess contamination during sampling, handling and analysis. And as discussed below, analyses of the carbonate system should be referenced to certified materials to assess accuracy.

Although the dissection of total variance into observation error and process error is rarely reported, it can be extremely useful for improving and fine tuning sampling programs. For example, if observation error can be estimated through methods like the ones discussed above, the remaining error is conventionally attributed to process error. The investigator can then focus sampling design on capturing or constraining the effect of this process noise on inferences and calculated endpoints. Although these are well established concepts in sampling design, our sense is that the study of coastal acidification could benefit from more systematic application of these principles.

As already noted, $pH_T$ typically varies much more in the coastal environment than in the open ocean. When designing a sampling program, there is usually a trade-off between accuracy and precision that depends on this variability and the purpose of the study. For example, the number of samples required to characterize a well-mixed or homogeneous water body is smaller than for a highly dynamic system. Conversely, in a heterogeneous system, a larger number of samples enabled by cheaper (i.e., lower accuracy) measurements may produce a more representative estimate than a single high accuracy measurement. In other cases, such as long term moored time series, high precision makes subtle trends more detectable if key assumptions about background variability are met. A key point is that these trade-offs should always be considered and, in some cases, can be quantitatively optimized when goals of monitoring are clear (e.g., using power analyses, etc.).

Many measurements are involved in the determination of individual carbonate parameters. Determination of total alkalinity by titration, for example, involves measurements of temperature, salinity, sample mass, titrant concentration, titrant volume, and voltage. Uncertainty in each of these measurements propagates to uncertainty in the final alkalinity determination. When known, these uncertainties can be combined into a single estimate of measurement uncertainty using standard methods (Ellison and Williams, 2012). This allows the relative contribution of each source to be assessed so that areas needing improvement can be identified. However, for reporting purposes, the repeatability (precision) and accuracy (determined using CRMs) are usually sufficient. In all cases, ID numbers of reference materials should be included. Often, publications will report "+/-" bounds for a measured quantity, without defining how these bounds were determined and whether they are standard error, standard deviation, or confidence limit. Without such information, the uncertainty estimate is virtually useless. Additional information and reporting templates for ocean acidification data are

described in Jiang et al. (2015) and Ellison and Williams (2012) and provide methods for both spreadsheet calculation and Monte Carlo simulations of combined standard uncertainty for chemical measurements.

## 9.2 Weather vs. climate data quality benchmarks

There are many criteria and study goals that might be considered in designing a monitoring effort, two of which are captured by the Global Ocean Acidification Observing Network (GOA-ON.org) as "climate" and "weather" data quality objectives. The following definitions are from Newton et al. (2014).

Climate

- Defined as data of quality sufficient to assess long term trends with a defined level of confidence.
- With respect to OA, this is to support detection of the long-term anthropogenically-driven changes in carbon chemistry over multi-decadal timescales.

Weather

- Defined as data of sufficient and defined quality used to identify relative spatial patterns and short-term variation.
- With respect to OA, this is to support mechanistic interpretation of the ecosystem response to and impact on local, and immediate OA dynamics.

# APPENDIX A: METHOD COMPARISONS AND SAMPLING CHECKLIST

Table A-1. Comparison of methods and uncertainty for carbonate system analyses.

| Parameter | Preferred Method of Measurement | Reference Materials[a] | Expected Measurement Uncertainty[b] | Relative Uncertainties (%) in Calculating $[CO_3^{2-}]$ when Paired with: [b] | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | pH(1) | pH (2) | $pCO_2$ | DIC | ALK |
| $pH_T(1)$ | Spectrophotometric /colorimetric (m-Cresol Purple dye) | Tris buffer in synthetic seawater http://andrew.ucsd.edu/co2qc/ | ±0.005 | NA | NA | 5.7 | 4.2 | 3.7 |
| $pH_T(2)$ | Solid-state pH sensor (Durafet) | Factory calibration, or calibrated to a single independent measurement | ±0.03 | NA | NA | >5.7 | >4.2 | >3.7 |
| $pCO_2$ | Commercially available systems (in situ or flowthrough) | Factory calibration, calibrated gas cylinders http://www.esrl.noaa.gov/gmd/ccgg/refgases/stdgases.html | ~2 µatm | 5.7 | >5.7 | NA | 4.1 | 3.3 |
| | Custom headspace equilibration system / infrared detection | Calibrated gas cylinders http://www.esrl.noaa.gov/gmd/ccgg/refgases/stdgases.html | Design-dependent | ? | ? | NA | ? | ? |
| DIC | Acidification / gas stripping / infrared detection | Sterilized natural seawater http://andrew.ucsd.edu/co2qc/ | 2-3 µmol $kg^{-1}$ | 4.2 | >4.2 | 4.1 | NA | 1.7 |
| | Acidification / gas stripping / coulometric detection | Sterilized natural seawater http://andrew.ucsd.edu/co2qc/ | 2-3 µmol $kg^{-1}$ | 4.2 | >4.2 | 4.1 | NA | 1.7 |
| ALK | Potentiometric determination | Sterilized natural seawater http://andrew.ucsd.edu/co2qc/ | 2-3 µmol $kg^{-1}$ | 3.7 | >3.7 | 3.3 | 1.7 | NA |

[a] Information on reference materials from Riebesell et al. (2010) except for solid state pH, which is from Bresnahan et al. (2014).

[b] Measurement uncertainty and relative uncertainties adapted from table found in Riebesell et al. (2010), except solid state pH, for which uncertainty is reported as accuracy in Bresnahan et al. (2014). Assumed analytical procedures used are those found in Dickson et al. (2007), performed by an experienced laboratory with quality assurance programs and reference materials.

# Bottle Sampling and Preservation Checklist

Containers

- ☐ Narrow mouth borosilicate glass bottle
- ☐ Gas impermeable cap (ground glass stopper or Teflon © lined screw cap)

Preservation

- ☐ No preservation necessary if analysis occurs within 4 hours of collection
- ☐ Mercuric chloride for long term preservation (10 µL saturated mercuric chloride solution per 50 mL of sample volume)

Preparation for Sampling

- ☐ Rinse three times all sample bottles and sampling devices with a 5-10% hydrochloric acid solution
- ☐ Allow sample bottles and sampling devices to air dry and cap/cover to keep clean
- ☐ Collect personal protective equipment, preservatives and coolers/ice

Ready to Sample?

- ☐ Rinse three times all sample bottles and sampling devices with sample water
- ☐ Use a sampling device that minimizes exchange with atmosphere
- ☐ Fill sample container from the bottom in a controlled manner using flexible tubing
- ☐ Allow to overflow by at least twice the time needed to fill the container to the top (e.g., if it requires 5 seconds to fill the bottle, the overflow process should continue for 10 seconds)
- ☐ Allow 1% of bottle volume for headspace
- ☐ Add and gently disperse pickling agent if desired
- ☐ Tightly cap sample bottle
- ☐ Place in a cool, dark location (cooler) for transport back to lab

(continued on next page)

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Sample Storage

- ☐ Samples must not be frozen
- ☐ Samples should be stored tightly capped and in a dark, refrigerated space

Carbonate Chemistry Sampling Tips

- ☐ Order of collection is important. Carbonate system parameters are unequally affected by atmospheric gas exchange
- ☐ Order of collection: $pCO_2$ > pH > dissolved inorganic carbon > total alkalinity
- ☐ Samples should not be taken for $pCO_2$, pH and dissolved inorganic carbon when the water volume in the sampling device falls to ~25% of the total device volume
- ☐ Always collect environmental metadata (location, time of sample, depth of sample, salinity, water temperature, atmospheric pressure)

# APPENDIX B: EXAMPLE LABORATORY OPERATING PROCEDURES

## B.1 Essential elements

Many of the available SOPs, as well as the narrative descriptions in these guidelines lack many of the details needed for practical application. However, documentation of these details is a critical piece of quality assurance, reporting, and sharing of expertise within and between laboratories. To ensure consistency, such documentation takes the form of "Laboratory Operating Procedures" (LOPs) which, ideally, are adapted from SOPs (e.g., Dickson et al., 2007) to suit the equipment and process details of a specific laboratory. LOPs version identifiers can then be referenced in data sheets or lab notebooks to document the specific procedures in effect on the date of the analysis. This section provides examples of LOPs to demonstrate typical content.

## B.2 Example LOP: Adapting a total alkalinity SOP to a specific laboratory setting

DRAFT Determination of Total Alkalinity in Seawater by Titration Laboratory Operating
Point of Contact:
US Environmental Protection Agency
27 Tarzwell Drive
Narragansett, RI 02882

Researcher: _____ QA Officer: _____
SHEMP Manager: _____

DISCLAIMER: This procedure was written to meet the needs of the research program at the U.S. EPA Atlantic Ecology Division. It is not a U.S. EPA standard method and must not be referred to as such. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1. OBJECTIVES

This procedure is intended for use with a Metrohm Titrino 877 Plus automated titration unit for the determination of total alkalinity in seawater and estuarine waters. This titration occurs quickly and can be considered a closed cell titration. This is a lab-specific procedure based on the general approach in Dickson et al. (2007).

2. MATERIALS AND EQUIPMENT

Metrohm Titrino 877 Plus and accessories
Stir plate
Combined pH electrode
Temperature-controlled circulating water bath
Jacketed sample beaker or holder plumbed to circulating water bath
1000 mL volumetric flask
Concentrated hydrochloric acid (reagent grade or better)
Sodium chloride (reagent grade or better)
Sodium carbonate (reagent grade or better)
Indicating soda lime

Tris [tris (hydroxymethyl) aminomethane]
pH calibration solutions (pH 4.01, 7.00, 10.01)
250 mL beakers
50 mL beakers
125 mL sample bottles
Type 1 ultrapure water
Stir bars
Solvent wash bottle
Kimwipes
Metrohm storage solution (p/n 6.2323.000)
3 M potassium chloride solution
Silicone tubing
USB storage device
Analytical balance(s)
Graduated pipette
Pipette bulb

3. PROCEDURE

3.1. Instrumental Setup and Reagent Preparation

3.1.1. Titrino 877 Plus Startup

a) […*details on instrument startup*…]
b) Flush the reagent syringe. Navigate to Menu>>Manual Control>>Dosing, choose prep from the lower menu and press OK. Repeat this step two more times.

3.1.2. Calibration of Combination pH electrode

Calibrate the pH electrode daily by following this procedure:

a) Navigate to Method>>Cal_pH and choose load from the bottom menu using the arrow keys. Press OK.
b) Press start. The unit will proceed through a 5 point calibration (4.01, 7.00, 10.01, 7.00, and 4.01). Follow the on-screen instructions. Remember to always rinse the pH electrode (collect rinse water in a beaker) between calibration solutions and wick away droplets with a Kimwipe. NEVER dry the electrode's glass membrane.
c) Place the pH electrode back in storage solution after completion of the calibration and between uses. NEVER leave the electrode exposed to the air for any period of time.

3.1.3. Preparation of Titrant Solution

a) Weigh an empty 1000 mL volumetric flask on a calibrated analytical balance. Record weight, air temperature, humidity and ambient air pressure.
b) Add about 700 mL of Type 1 Water to volumetric flask.
c) Draw 8.4 mL of concentrated hydrochloric acid (~37% w/w) from a small aliquot of the main container with a graduated pipette, dispense to volumetric flask and mix thoroughly. Dispose excess acid immediately and safely.
d) Add 35 g of sodium chloride, or appropriate amount to match ionic strength of media to be titrated, and mix until dissolved completely.
e) Fill volumetric flask to 1000 mL and mix thoroughly.
f) Weigh filled flask and record weight. Be sure outside of flask is completely dry.
g) Determine solution density in kg $L^{-1}$.

h) Transfer to titration vessel and label accordingly.
i) Prep syringe as described in startup procedure.

3.1.4. Calibration of Titrant Solution with Tris and Determination of Titer

a) In an oven dry about 5 g Tris for 2 h at 105°C and cool and store in a desiccator.
b) Dissolve precisely 0.940 g of Tris in 250 mL of Type 1 water (with NaCl added as above to achieve the same ionic strength as the titrant). Record exactly the weight measured.
c) Multiply the exact weight measured by 0.16. This is the sample weight.
d) Using a graduated pipette, measure 40 mL of Tris solution and dispense into a 50 mL beaker. Add a small stir bar and insert combination pH electrode and dosing tip into solution.
e) Load "Tris" method from the Titrino menu, enter sample weight from step C, and press start. Titration of buffer will proceed.
f) When endpoint is reached, remove electrode and dosing tip.
g) Repeat procedure 5 times total.
h) Statistics will be generated by the Titrino unit and Titer value displayed. The Titer value is the result of the following calculation which provides a correction factor by which the concentration of the titrant solution must be adjusted to determine the actual concentration.

Titer = C00 / (C01 * C02 * EP1)
C00 = sample weight in mg
C01 = 121.4 g $mol^{-1}$ Tris
C02 = 0.1 mol $L^{-1}$ titrant concentration
EP1 = titrant consumption in mL
Titrant (mol $L^{-1}$) *Titer = Actual Titrant (mol $L^{-1}$)

3.2. Sample Analysis

a) In a tared clean beaker, add the sample and record the sample weight in grams.
b) Place the sample in the jacketed beaker or holder.
c) Load the SW_TotalALK method (this is the instrument file containing titration parameters such as initial dose, mL per subsequent dose, etc.). Enter the sample name.
d) Press start. The unit will perform the titration and determine the equivalence points by the Fortuin method and export the results to the USB drive with the sample name as the filename.

3.3. Total Alkalinity Calculation

a) At the endpoint of the titration (pH = 3.0) the solution Alkalinity can be described by.

$$\frac{mC - m_0 A_T}{m_0 + m} = [H^+]_F + [HSO_4^-] + [HF] + [H_3PO_4] + [H_2PO_4^-]$$

Where:
$m$ is the mass of acid consumed during titration
$m_0$ is the starting mass of sample
$C$ is the concentration of acid in mol $kg^{-1}$
$A_T$ is the total alkalinity in mol $kg^{-1}$ Where:

This equation can be solved with a customized computer program or an existing software function that accepts titration volumes, millivolt readings, titrant molarity, temperature, and salinity as input arguments ((e.g., the AT function in the R package 'seacarb', Lavigne et al., 2011).

4. QA/QC

Replicate samples should be run to ensure data quality. At least one set of duplicates should be run every ten samples, and the absolute difference recorded. After an initial dataset of at least 12 duplicates is assembled a control chart should be produced. See SOP 22 in Dickson et al. (2007) for specific recommendations on preparation and interpretation of control charts.

5. TROUBLE SHOOTING

Instruments should only be evaluated by experienced personnel. All other instrumental troubleshooting should be performed under the guidance of the manufacturer or qualified service personnel.

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7. APPENDIX

7.1. Alkalinity titration parameters

[This section should document specific instrument settings such as start conditions, end condition, initial titrant dose, titrant dosage increments, stir bar speed, etc.]

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**B.3 Example LOP: Measuring DIC using an NDIR-based instrument**

DRAFT Determination of Dissolved Inorganic Carbon Laboratory Operating Procedure
Point of Contact:
US Environmental Protection Agency
27 Tarzwell Drive
Narragansett, RI 02882

Researcher: _____ QA Officer: _____
SHEMP Manager: _____

DISCLAIMER: This procedure was written to meet the needs of the research program at the U.S. EPA Atlantic Ecology Division. It is not a U.S. EPA standard method and must not be referred to as such. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

1. OBJECTIVES

This procedure is intended for use with Apollo SciTech AS-C3 dissolved inorganic carbon analyzer for the determination of total dissolved inorganic carbon (DIC) in seawater and estuarine waters. This analysis takes approximately 15 minutes per sample (if using 5 injections per sample). For greatest precision, temperature must be carefully controlled at 25°C for reagents and samples. This is a lab-specific procedure based on the general approach in Schumacher and Smucker (1983) and on specific instrument instructions provided in the AS-C3 User Manual.

2. MATERIALS AND EQUIPMENT

Apollo SciTech Model AS-C3 and accessories
Temperature control recirculating water system
40-60 mm diameter silicon or tygon tubing
4-6 mm diameter silicon or tygon tubing
Jacketed beaker for acid (9.5 cm diameter)
Jacketed beaker for sample (9.5 cm diameter)
$N_2$ regulator suitable for precise operation from 5-15 mL/min
Titanium diffuser
Silicone stopper with two 5 mm ports
5 mL syringe with luer lock fittings
Replacement rubber o-rings
Replacement 0.20 µm, hydrophobic PTFE filters with luer lock fittings
250 mL beakers
50 mL beakers
24-40 mL sample bottles
100 mL graduated cylinder
1000 mL volumetric flask
1000 mL glass stoppered bottle
$N_2$ gas supply
Andrew Dickson Scripps certified reference material
Concentrated phosphoric acid (reagent grade or better)
Sodium chloride (reagent grade or better)
Indicating soda lime
Magnesium perchlorate drying agent
DI wash bottle
Kimwipes
USB storage device
Analytical balance(s)

3. PROCEDURE

3.1. Instrumental Setup and Reagent Preparation

3.1.1. Preparation of 5% Phosphoric Acid Reagent

a) Fill a 1000 mL volumetric flask with 500 mL of milliQ water.
b) Measure out 59 mL of 85% phosphoric acid into a graduated cylinder.
c) Slowly add phosphoric acid to the volumetric flask with milliQ water and gently stir to mix.
d) Add 100 grams of sodium chloride and stir to dissolve.
e) Finally fill to the 1000 mL line of the volumetric flask with milliQ water.
f) Transfer to a stoppered or screwcap bottle.

3.1.2. Apollo AS-C3 setup

a) […*details on instrument startup*…]
b) Allow the instrument to warm up for at least 60 minutes.
c) Set the temperature-controlled water recirculator and a temperature-controlled water bath to a set temperature, we recommend 25°C for both.
d) Place samples to be run in the water bath and place 2" of DI water into the jacketed water beakers attached to the water recirculator system.
e) Bring phosphoric acid reagent to fume hood and gently bubble for at least 15 minutes with pure $N_2$ gas using a titanium diffuser.
f) After 15 minutes, cap the bottle and transfer to the jacketed beaker to allow the reagent to come up to temperature.
g) Place samples into water bath and allow to come up to temperature.
h) After instrument has warmed up for at least 60 minutes, load DIC analysis program from computer.
i) Insert the silicone cap into acid reagent bottle and attach tubing from port C of the DIC analyzer.
j) Place sample into the second jacketed beaker and place tubing from port B into the sample bottle, leave tubing 2 cm off of bottom.

3.1.3. Calibration of instrument and drift correction

a) Dickson CRM is used in calibration. This can be portioned off from the 500 mL sample bottle to smaller sample vials in order to minimize the amount of change in DIC concentration due to headspace equilibration, as follows:
Using acid washed/DI rinsed, small diameter tygon tubing, create a syphon and slowly gravity fill from a freshly opened CRM bottle into 24 mL borosilicate glass scintillation vials with Teflon© displacement caps (this method will produce ~20 headspace free, daily running standards; not suitable for long term storage).
b) DIC concentration of Dickson CRMs is ~2000 µmol $L^{-1}$. Use the specific batch DIC concentration to bracket the expected DIC concentration of your samples. Run the DIC standard as three separate samples. For example, changing the volume for each sample to solution volumes of 0.8, 1.0, and 1.2 mL will produce a standard curve bracketing samples from 1600 to 2400 µmol $L^{-1}$.
c) Drift will occur during a full day of running the instrument. To account for this run the DIC standard curve at the beginning of the run and end of the run. To further adjust the drift correction, run one volume (1.0 mL) of the standard as an unknown every 5-8 samples.

d) After standardization volumes have been completed, use a linear regression software program to determine the relationship between peak area and DIC. For example, if the standard DIC concentration is 2000 µmol $L^{-1}$ a 1 mL injection is equal to 2 µmol, a .8 mL injection is equal to 1.6 µmol. This can then be converted to DIC concentration per liter by multiplying by 1000.
e) Calculate the DIC concentration of samples by applying the linear model obtained during standardization to peak area of samples.
f) Convert sample concentrations from µmol $L^{-1}$ to µmol $kg^{-1}$ by using a density equation that incorporates temperature, salinity and pressure of the sample.

3.2. Sample Analysis

a) It is possible to perform single sample runs but it is recommended that the analyst uses the batch process function.
b) Open up batch process window and select a measurement "scheme" the software contains 3 schemes to choose from or the analyst can create their own.
c) We recommend that the analyst uses a scheme with 0.1% error allowed within 3 repeats out of a maximum of 5 measurements.
d) Create sample inventory under the sample list tab by adding samples by clicking the "+" button to add lines.
e) Name samples and set injection volume (0.8 – 1.2 mL).
f) Press the "Sample Measurement" button to start the batch measurement.
g) Instruments syringe will automatically begin drawing correct volumes of sample and reagent before sending to the reactor chamber.
h) After ~180 seconds sample will be expelled and instrument will automatically begin next injection (i.e. 2 of 5).
i) Upon sample completion, there will be an audible beeping notification and you will be greeted with a dialogue box asking to run the next sample.
j) If the sample fails the replicate precision parametersµµ specified in the scheme, you will be presented with a dialogue box asking if you want to re-run the sample.
k) Results will be saved within the program and can be viewed by clicking the "Test Result" button.
l) Results will need to be exported prior to closing the program, this can be done through the test result screen.
m) At the end of the run, add a new sample called "DI rinse", set volume to 1.5 mL.
n) Place sample tube and acid reagent tube into DI water and run the sample.
o) Take the tubes out of the DI water and keep in air.
p) Click the "Connect/Disconnect" button, When the prompt appears, press enter.
q) Close down the DIC analysis program.
r) Turn off the power switch for the LI-7000 bottom unit, Apollo AS-C3 upper unit and gas supply.

3.3. Dissolved Inorganic Carbon Calculation Example

Dickson CRM batch #151 standard concentration: 2033.83 ± 0.62 µmol $kg^{-1}$
Density at salinity (33.345), Temperature (25°C): 1022.091 (kg $m^3$)
Dickson CRM batch #151 standard concentration: 2033.83 *(1022.091*$10^{-3}$) = 2078.76 µmol $L^{-1}$

Standard curve:

2078.76 µmol $L^{-1}$ * 0.0008 $L^{-1}$ = 1.663 µmol
1.663 µmol = 11381 raw $CO_2$ peak area

2078.76 µmol $L^{-1}$ * 0.0010 $L^{-1}$ = 2.079 µmol
2.079 µmol = 14246 raw $CO_2$ peak area

2078.76 µmol $L^{-1}$ * 0.0012 $L^{-1}$ = 2.495 µmol
2.495 µmol = 17072 raw $CO_2$ peak area

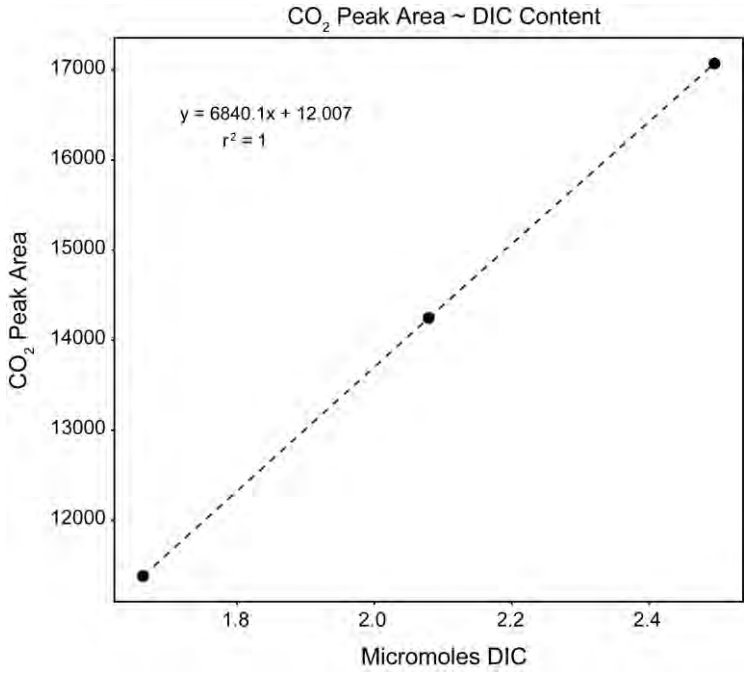


**Figure B-1. Dissolved inorganic carbon peak area as a function of dissolved inorganic carbon concentration.**

Example calculation:
Sample Volume: 1 $mL^{-1}$ (0.001 $L^{-1}$)
Measured Peak Area: 13300
Regression Equation: y(peak area) = 6840.1 * x(DIC) + 12.007
Re-Arranged Regression Equation: x(DIC) = (13300 – 12.007)/6840.1
Calculation: DIC = (13300 – 12.007)/6840.1 = 1.942 µmol $mL^{-1}$
Volume Correction Factor: (Scale to 1 L): 1.942 µmol $mL^{-1}$ / .001 l = 1942 µmol $L^{-1}$

4. QA/QC

As Dickson CRMs are used in the creation of standardization curves, it is important to utilize an independent check standard. This check standard should be made up in artificial seawater with a salinity ±20% expected sample salinity. The check standard DIC concentration should also be ±20% of expected DIC concentration. Replicate samples should be run to ensure data quality. At least one set of duplicates should be run every ten samples, and the absolute difference recorded. After an initial dataset of at least 12 duplicates is assembled a control chart should be produced.

5. TROUBLE SHOOTING

Instruments should only be evaluated by experienced personnel. All other instrumental troubleshooting should be performed under the guidance of the manufacturer or qualified service personnel.

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# APPENDIX C: FOUR EQUIPMENT SCENARIOS

The following scenarios illustrate the range of equipment that can support differing objectives.

## C.1 A water quality research laboratory

EPA's Atlantic Ecology Division (AED) in Narragansett conducts coastal acidification research focusing primarily on land-based drivers. In order to separate these from oceanic drivers and to determine when and where costly methods are necessary, high quality measurements are necessary. The following is a description of equipment used in AED's studies of coastal carbonate chemistry. This work is closely coordinated with nutrient sampling and analysis.

*Sampling*
Research vessels equipped with winches and GPS
Go Flo Bottles and van Dorne samplers with silicon transfer tubes
CTD rosette, from which in situ temperature and salinity is determined (Hydrolab)
Handheld YSI for environmental measurements in shallow waters
400 hundred sample bottles (borosilicate glass-stoppered; borosilicate septum vials, and HDPE)
Auto-pipettors for dispensing preservatives (dedicated sole-purpose to prevent cross-contamination)
Apiezon grease in dispensing syringes
Personal protective equipment
Spill prevention and response kit
Coolers for sample transport

*General support facilities*
Walk-in refrigerator for storage of samples and reference materials
Laboratory glassware cleaning facility
DI and ultra-pure Milli-Q water supply
Waste handling facilities and protocols
Seawater wetlab for testing field instruments

*General laboratory equipment*
General glassware and calibrated cylinders, flask and pipettors
Ventilated storage area (i.e., fume hood) for hazardous reagents (phosphoric acid, hydrochloric acid, mercuric chloride)
Calibrated NIST-traceable digital thermometer for checking water bath controls, instrument sensors, etc.
Drying oven for drying powder/crystal reagents
Personal protective equipment (gloves, eye protection, lab coats)
Eyewash station

*Total alkalinity analysis*
Metrohm Titrino 877 autotitrator with magnetic stir plate, ANOVA cooling/heating water bath, thermometer, combination electrode, jacketed beakers connected to water bath, glassware for acid titrant and Tris preparation, high quality calibrated analytical balance
*DIC analysis*
Apollo-Scitech AS-C3 Dissolved Inorganic Carbon Analyzer; jacketed beaker holders connected to water bath (temperature-controlled), continuous supply of research grade $N_2$ gas, computer for instrument control, moisture and $CO_2$ scrubber columns

*pH determinations*
Perkin-Elmer Lambda 35 dual beam UV-VIS Spectrophotometer with jacketed quartz cell (10 cm path), solid calibration standards, dedicated temperature-controlled water bath, computer, micro-pipettor for dispensing *m*-cresol dye

*Other pH equipment*
Satlantic SeaFET autonomous pH sensor
WTW 3310 pH meters with Sentix probes

*Reagents and reference materials*
Tris (for acid titrant calibration), hydrochloric acid, phosphoric acid, sodium carbonate, bicarbonate, sodium chloride, sodium hydroxide, mercuric chloride, NBS buffers, m-cresol dye
Certified reference materials for DIC, TA
Reference materials for $pH_T$ (Tris buffers)

## C.2 A single-instrument setup in a basic water quality laboratory

As one example, we were recently encouraged by a presentation at the ASLO 2017 meeting by Erin Guyler and Robert Byrne that described a single instrument scenario for full constraint of the seawater carbonate system. Their method used both beams in a single spectrophotometer to obtain $pH_T$ and alkalinity. At low carbonate saturation states, they obtained "climate quality" measurements. Although the TA + $pH_T$ pair has higher uncertainty due the sensitivity of carbonate system determination to error in pH (see Table A-1), the results are likely to be far superior to the use of parameter pairs involving either the salinity-TA regression or low quality pH measurements. When grounded to Tris reference materials for pH and certified reference materials for alkalinity, these measurements could conceivably be used to ground-truth autonomous sensors. Careful consideration of sample holding times and preservation would be required.

## C.3 A monitoring effort with external laboratory support

A growing number of monitoring groups are partnering with water quality research laboratories to obtain ground-truthing of their field measurements. This allows the monitoring group to focus on the sampling design issues (i.e., trade-offs between sample frequency/resolution and sample quality) and operation of autonomous sensors which, at present, are gaps in the application of existing open ocean methods to the coastal zone. See Jones et al. (2016) and Bresnahan et al. (2014).

## C.4 Shellfish growers and hatcheries

A high quality handheld $pH_{NBS}$ meter and probe can be purchased for under $1000. These are useful for monitoring diurnal and spatial patterns with a given growing area, but require calibration and occasional probe replacement. With extensive additional effort and purchase of non-commercial Tris buffers, or purchase of DuraFET sensors ($2500-$3000, with proper power supply), handheld devices can be used for measuring $pH_T$. In any case, our opinion is that growers concerned about low pH zones or seasonal excursions should not be discouraged from taking their own measurements. Additional guidelines regarding the importance of consistent methodology and sample timing would likely be beneficial.

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