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Carbon manipulations and measurements for a changing ocean

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Oceanography

by

Emily E. Bockmon

Committee in charge:

Professor Andrew G. Dickson, Chair
Professor Andreas J. Andersson
Professor Clifford P. Kubiak
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Professor Todd R. Martz

2014

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Chair

University of California, San Diego

2014

DEDICATION

I dedicate this dissertation to my Grandmother. She is an unending source of inspiration and love. I strive to be as curious, passionate, and thoughtful as my life continues.

EPIGRAPH

“If the world were merely seductive, that would be easy. If it were merely challenging, that would be no problem. But I arise in the morning torn between a desire to improve (or save) the world and a desire to enjoy (or savor) the world. This makes it hard to plan the day.”

– E.B. White

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LIST OF ABBREVIATIONS

AIRICA	—	Automated infrared inorganic carbon analyzer
A_T	—	Total Alkalinity
CaCO_3	—	calcium carbonate
CRM	—	Certified Reference Material
C_T	—	Total dissolved inorganic carbon
CTD	—	conductivity, temperature, depth
GEOSECS	—	Geochemical Ocean Sections
GOA-ON	—	Global Ocean Acidification Observing Network
IAPSO	—	International Association for the Physical Sciences of the Oceans
JGOFS	—	Joint Global Ocean Flux Study
MSEAS	—	Multiple Stressor Experimental Aquarium at Scripps
NBS	—	National Bureau of Standards (now National Institute of Standards and Technology)
NDIR	—	non-dispersive infra-red (sensor)
NOAA	—	National Oceanographic and Atmospheric Administration
$p(\text{CO}_2)$	—	partial pressure of carbon dioxide
PID	—	proportional-integral-derivative
SIO	—	Scripps Institution of Oceanography
UNESCO	—	United Nations Educational, Scientific, and Cultural Organization
SOMMA	—	Single-operator multiparameter metabolic analyzer
VAM	—	Valid Analytical Measurement
VINDTA	—	Versatile instrument for the determination of total inorganic carbon and titration alkalinity
WOCE	—	World Ocean Circulation Experiment

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Chapter 2, in full, is a reprint of the material as it appears in *Limnology & Oceanography: Methods*. E. E. Bockmon and A. G. Dickson, 2014. A seawater filtration method suitable for total dissolved inorganic carbon and pH analyses. *Limnology and Oceanography: Methods*, 12: 191–195 [doi:10.4319/lom.2014.12.191]. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in full, is a reprint of the material as it appears in *Biogeosciences*. Bockmon, E. E., C. A. Frieder, M. O. Navarro, L. A. White-Kershek, and A. G. Dickson, 2013. Technical Note: Controlled experimental aquarium system for multi-stressor investigation of carbonate chemistry, oxygen saturation, and temperature. *Biogeosciences*, 10: 5967–5975 [doi:10.5194/bg-10-5967-2013]. The dissertation author was the primary investigator and author of this paper.

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ABSTRACT OF THE DISSERTATION

Carbon manipulations and measurements for a changing ocean

by

Emily E. Bockmon

Doctor of Philosophy in Oceanography

University of California, San Diego, 2014

Professor Andrew G. Dickson, Chair

In this dissertation, I present several tools to assist the community in making accurate and precise manipulations and measurements of carbonate chemistry parameters, which are essential for understanding, interpreting, and predicting the anthropogenic impact on the chemistry of our oceans. As the frequency of carbonate chemistry measurements increases with interest in the ocean's response to climate change, there is a continued need for confidence in the measurements to ensure data quality and consequent data usefulness. First, I explain the results of an international inter-laboratory comparison of various carbonate chemistry measurements. The majority of the results exhibit agreement within 0.5% of the assigned value for total alkalinity and total dissolved

inorganic carbon, with significantly more variability in pH measurements. In many cases there is evidence of significant loss of CO₂ from the seawater samples, a particularly alarming bias given how critical these measurements are to the understanding of increasing anthropogenic carbon in our oceans. Carbonate chemistry measurements can also be compromised when taken from environments such as coastal and estuarine seawater, as well as laboratory cultures and aquaria, containing large numbers of suspended biogenic particles. The presence of these particles in a seawater sample may alter the results of the analysis for carbonate chemistry parameters including total alkalinity, total dissolved inorganic carbon, and pH. In this dissertation, I present the verification of a filtration method using a peristaltic pump and enclosed filter housing, which does not alter the dissolved CO₂ content of the seawater sample, and thus is suitable for filtration of samples before analysis. Finally, manipulation of carbonate chemistry in the laboratory is a crucial tool for studying the impacts of increasing CO₂ on organisms and communities, however is not always straightforward. I developed a carefully controlled aquarium system capable of manipulating the carbonate chemistry, oxygen levels, and temperature of seawater. The multi-stressor nature of the control is critical, particularly regarding the investigation of coastal ocean conditions, which are unique in the magnitude of range and temporal variability possible in these parameters. The aquarium system is dynamically controlled such that variability in pH may be introduced across many time scales. The novel tools presented in this dissertation for the manipulation and measurements of carbonate chemistry will assist in ensuring greater accuracy and understanding of the impacts of changes in carbon dioxide on our oceans.

INTRODUCTION

As the oceans continue to absorb a significant proportion of the anthropogenic carbon dioxide that has been released into the atmosphere, there is an increasing desire to document and understand the consequent physical, chemical, and biological changes. While the basic chemistry of CO₂ absorption into the oceans is well understood, a phenomenon commonly known as ocean acidification, many questions remain regarding the magnitude and spatial distribution of changes, as well as their impact on organisms. In this dissertation, I present several tools that can be used to help the ocean acidification community gain a clearer understanding of some of the anthropogenic carbon impacts on the oceans in the context of global change.

The chemistry of inorganic carbon in seawater can be described using known equilibrium constants given salinity, temperature, and pressure, together with two other measured parameters. Total alkalinity (A_T), total dissolved inorganic carbon (C_T), the partial pressure of CO₂ ($p(\text{CO}_2)$), and pH are the parameters usually measured in seawater, however direct measurement of the carbonate ion concentration is also possible. The addition of anthropogenic carbon dioxide (CO₂) to the atmosphere causes an increase in the CO₂ of the oceans as the surface waters try to reach equilibrium. This increase in CO₂ directly increases the $p(\text{CO}_2)$ and C_T of seawater, with the resulting decrease in pH and carbonate ion concentration a consequence of the addition of CO₂. A_T is a conservative property, based on mass-balance relationships, and is not altered by the addition or removal of CO₂ from seawater. In addition to ocean acidification, other anthropogenic impacts such as warming, nutrient runoff, and increased freshwater inputs

can also alter the carbonate chemistry of seawater, as well as typical biological modification such as photosynthesis, respiration, calcification and dissolution.

Our understanding of the carbonate chemistry in the oceans, and of anthropogenic ocean acidification, is informed by measurements of these carbonate parameters made all over the world. Global scale projects such as CLIVAR make carbonate chemistry measurements on repeat hydrography cruises across oceans. On a regional scale, efforts like the California Current Acidification Network (C-CAN) seek to use a variety of platforms and approaches such as autonomous measurements on moorings and quarterly coastal ocean and shore monitoring efforts. Many additional measurements are made by individual laboratories investigating local environments and ecosystems of interest. This expansion of measurements offers the opportunity for an increased understanding of carbon dynamics in the oceans and the associated biogeochemical cycling. However, there continues to be a need for great care when performing measurements and evaluating the resultant data quality. Measurements are neither simple nor inexpensive to make (Dickson 2010) and to be useful they must be sufficiently accurate and precise. Laboratories can make good measurements, aided by the availability of certified reference materials and the recent emergence of commercial instrumentation designed for making these measurements. However, many factors can complicate measurements including gas exchange, temperature effects, particle loading, and the direct influences of organisms on the water chemistry.

Samples with high CO_2 content can be particularly difficult to measure well. Examples include older seawaters and oxygen minimum zones which often have increased C_T from remineralization of organic matter (Paulmier et al. 2011), estuaries and

coastal oceans (Andersson and Mackenzie 2012; Frieder et al. 2012), as well as samples from experimental aquaria enriched with CO₂ so as to investigate the impacts of ocean acidification on organisms in a laboratory setting. The $p(\text{CO}_2)$ of such samples is significantly out of equilibrium with the atmosphere. This large difference between the atmosphere and seawater will result in outgassing of CO₂ from the seawater to the atmosphere. This complicates measurement of the sample, as gas exchange must be minimized to ensure that CO₂ is not lost. At the same time, measurement of high-CO₂ samples is becoming increasingly frequent with the growing interest in ocean acidification. Another concern regarding the accuracy of carbonate measurements is the propensity for suspended particles to interfere with the measurement. Current methods for characterizing the carbonate chemistry of seawater were generally developed for the open ocean, where seawater usually has low levels of particulates. However, environments such as estuaries and productive coastal regions are increasingly of interest, and can have high levels of CO₂ and biogenic particulates. Similarly, aquariums and experimental cultures can often be densely populated resulting in significant amounts of suspended matter.

Also interesting in these coastal environments is how dynamic the carbonate chemistry is, complicating our understanding and ability to characterize it. Different regions of the ocean are not expected to respond to increasing anthropogenic carbon in the same way (Duarte et al. 2013). In some environments such as coral reefs, $p(\text{CO}_2)$ already often exceeds levels expected from equilibration with the atmosphere (Andersson and Mackenzie 2012). Fluctuations in carbonate chemistry on short time scales in various coastal environments have been observed around the world (Hofmann et al. 2011). For

example, in coral reefs systems daily $p(\text{CO}_2)$ fluctuations can be quite large due to net ecosystem calcification and production (Bates et al. 2010). Such environmental variability complicates our understanding of how organisms may respond to ocean acidification.

In the past decade a significant amount of work has been completed investigating the impact of changes in carbonate chemistry on organisms (Hofmann et al. 2010; Kroeker et al. 2013). Experimental studies in the laboratory are an important part of this research, helping us to predict strengths and weaknesses of organisms, as well as mechanisms of change and adaptation. While we have gained significant insight from laboratory experiments, we have also learned that these questions about biological response are not simple to answer, and that appropriate control of the experimental conditions can be challenging. Although the field has come a long way in a short time, gaps in knowledge and in the execution of some studies still exist (Gattuso and Lavigne 2009). Only recently has any emphasis been placed on naturally elevated or naturally variable chemical environments and how to correctly study and mimic these environments in the laboratory (McElhany and Busch 2013). Additionally, multi-stressor interactions have gained a lot of attention recently, as researchers have begun to realize the need to examine the many simultaneous impacts that climate change will have (Boyd 2011; Pörtner et al. 2005; Wernberg et al. 2012). Along with changes to the carbonate chemistry, other expected effects of climate change on the oceans include increasing temperatures (Lyman et al. 2010), and decreasing oxygen levels as the oceans warm, stratify, and absorb carbon (Keeling et al. 2010; Shaffer et al. 2009). Ocean acidification effects are often dependent on these other climate variables, and predictions of the

consequences of climate change on the oceans must consider interactions between them and the changing inorganic carbon parameters.

Chapter 1 of this dissertation (which has been submitted for publication in *Marine Chemistry*) details an inter-laboratory comparison of seawater CO₂ measurements undertaken with the goal of evaluating and documenting the current quality of such measurements within the marine science community. Test samples were sent to sixty-six laboratories, which measured C_T , A_T , and/or pH. While many labs showed reasonable quality control over their measurements for C_T and A_T , few were able to achieve results within 2 $\mu\text{mol kg}^{-1}$. This study is particularly relevant to the discussion of anthropogenic climate change and naturally variable oceanic environments, because it included a test sample of high-CO₂ seawater. Laboratories typically performed more poorly on this enriched seawater. Suspected loss of CO₂ from samples often gave results too low for C_T and too high for pH. With the increased frequency of seawater CO₂ measurements being made, regular checks on data accuracy and the associated uncertainties are an important part of quality control.

Chapter 2 (published as Bockmon and Dickson 2014) presents a filtration method, which aims to help reduce potential analytical errors in complex environments caused by suspended particles such as sand, debris, biogenic CaCO₃, and even phytoplankton, all which have the potential to interfere with carbonate measurements. This filtration method removes the particles without otherwise altering the carbon content of a seawater sample. The need to filter samples before analysis by alkalinity titration has been discussed in the past (Chanson and Millero 2007; Kim et al. 2006), but the potential for gas exchange to alter a C_T or pH sample discouraged filtration using available methods. The method

presented here uses a peristaltic pump and a 47 mm polycarbonate filter holder, with a replaceable 0.45 μm filter. Both ambient and high- CO_2 seawater were tested to show that carbon was not lost from or added to the samples during filtering. Additionally, two scenarios were tested to ensure that the method successfully filtered out biogenic particles: seawater with high concentrations of phytoplankton, and seawater with CaCO_3 particles added. Both of these scenarios would be expected to produce artificially altered measurement results. This filtration method will improve the accuracy of carbonate measurements made in a variety of natural and laboratory environments as the method can be employed easily and routinely.

Chapter 3 (published as Bockmon et al. 2013) outlines a method for the manipulation of carbonate chemistry in a laboratory aquarium environment, intended for the study of biological responses to climate change in the ocean. The aquarium chemistry is controlled by the equilibration of a carefully created gas mixture with the seawater. By taking extra care to correctly manipulate and characterize the carbonate chemistry in an aquarium setting, it will likely be easier to elucidate and interpret the biological signal of interest. In addition to controlling the CO_2 content, the system also independently controls the oxygen level and temperature of the seawater, providing the ability to create a more realistic environment for the study organism. This also allows for experiments involving manipulations of multiple variables simultaneously, as might be experienced with eutrophication or the deoxygenation of the oceans that is accompanying anthropogenic warming and acidification. This simultaneous control is particularly relevant along nearshore upwelling-influenced environments, such as the California Current, where pH and oxygen levels are tightly coupled and tend to fluctuate in unison

(Frieder et al. 2012; Nam et al. 2011), and is particularly interesting because low oxygen and pH are expected to affect species synergistically (Pörtner 2012).

Chapter 4 improves on the method for aquarium control presented in Chapter 3 and extends the applicability of the aquarium system to a more natural environment, by including the ability to mimic variability in the carbonate chemistry. Many successful and respected studies examining the effects of ocean acidification on organisms come with the caveat that they were performed in an environment with relatively constant chemistry, one that organisms are unlikely to experience in the ocean (Andersson and Mackenzie 2012). Although carbonate chemistry is often relatively constant in the open ocean on short timescales, in some environments, particularly coastal environments such as those that experience upwelling, or environments dominated by photosynthesis and respiration, carbonate chemistry can vary significantly over short timescales (Duarte et al. 2013; Hofmann et al. 2011). Mimicking this natural variability in the laboratory, while at the same time adding the anthropogenic contribution we expect to see in the future, can provide a clearer picture of how organisms that live in variable environments may respond to climate change. Semi-diurnal variability, for example, can ameliorate ocean acidification effects on bivalve larvae observed under constant low pH (Frieder et al. 2014). The aquarium system is designed so that the chemistry can be varied as desired: either repeatably, giving the same variation day after day, or so that it reproduces natural variability observed over time in the field, which may change from day to day.

Accurate and high quality measurements of seawater CO₂ parameters are essential as research into ocean acidification, global change, and carbon cycling grows. This dissertation contributes both to the community's ability to make accurate CO₂

measurements and to approaches to control CO₂ in a laboratory aquarium environment. It will aid in the characterization of anthropogenic oceanic climate change and carbon cycling, as well as in understanding the organismal responses to the human-caused changes we are seeing in our oceans.

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CHAPTER 1

An inter-laboratory comparison assessing the quality of seawater carbon dioxide measurements

Seawater CO₂ measurements are being made with increasing frequency as interest in the ocean's response to changing atmospheric CO₂ levels and to climate change grows. The ultimate usefulness of these measurements is dependent on the data quality and consistency. An inter-laboratory comparison was undertaken to help evaluate and understand the current reliability of seawater CO₂ measurements. Two seawater test samples of different CO₂ content were prepared according to the usual method for creation of seawater reference materials in the Dickson Laboratory at Scripps Institution of Oceanography. These two test samples were distributed in duplicate to more than 60 laboratories around the world. The laboratories returned their measurement results for one or more of the following parameters: total alkalinity (A_T), total dissolved inorganic carbon (C_T), and pH together with information about the methods used and the expected uncertainty of the measurements. The majority of laboratories reported A_T and C_T values for all their measurements that were within 10 $\mu\text{mol kg}^{-1}$ of the assigned values (*i.e.* within $\pm 0.5\%$), however few achieved results within 2 $\mu\text{mol kg}^{-1}$ (*i.e.* within $\pm 0.1\%$), especially for C_T . Results for the analysis of pH were quite scattered, with little suggestion of a consensus value. The high-CO₂ test sample produced results for both C_T and pH that suggested in many cases that CO₂ was lost during analysis of these

parameters. This study thus documents the current quality of seawater CO₂ measurements in the various participating laboratories, and helps provide a better understanding of the likely magnitude of uncertainties in these measurements within the marine science community at the present time. Further improvements will necessarily hinge on adoption of an improved level of training in both measurement technique and of suitable quality control procedures for these measurements.

1.1 Introduction

Carbonate chemistry measurements of seawater have become routine in recent decades. Large-scale, regular observations of CO₂ parameters began in the 1970s with the Geochemical Ocean Sections (GEOSECS) program. However, disagreement in total alkalinity (A_T) and total dissolved inorganic carbon (C_T) was sometimes greater than 1% of the ambient values, requiring large adjustments to create complete data sets for comparison (Feely et al., 2001). In 1988 an intercomparison of CO₂ measurements (A_T , C_T , pH, and $p(\text{CO}_2)$) was undertaken. Seawater at four different salinities, prepared by the IAPSO Standard Seawater Service, was distributed to 14 laboratories for analysis. Although precision within each laboratory was quite high, the accuracy of the measurements was low. The results disagreed considerably, with differences in mean A_T and C_T of 20–30 $\mu\text{mol kg}^{-1}$ for seawater with salinities in the range appropriate to the open ocean (Poisson et al., 1990). Another intercomparison of 14 laboratories who were using the extraction/coulometric procedure for determination of C_T was carried out beginning in 1990 and showed similar disagreement (Dickson, 1992). The desired accuracy of these measurements for the Joint Global Ocean Flux Study (JGOFS) and

World Ocean Circulation Study (WOCE) programs was $\sim 1 \mu\text{mol kg}^{-1}$ (UNESCO, 1992), far smaller than the agreement found, prompting a call for suitable reference materials to help increase measurement accuracy (Poisson et al., 1990; UNESCO, 1990).

The Dickson lab has been producing seawater-based reference materials for C_T since 1990 (Dickson, 2001), and began to certify them for A_T in 1996 (Dickson et al., 2003). In 2012, the lab began to measure the pH of these reference materials using a spectrophotometric technique using purified *m*-cresol purple (Liu et al., 2011). This reference material project began originally as a response to the need to standardize CO_2 measurements made during the JGOFS program (Dickson, 2001) and has grown to a process that distributes nearly 10,000 bottles of reference material every year, sending them to approximately 250 laboratories around the world. Since the introduction of these reference materials, there has been substantial improvement in the quality of seawater CO_2 measurements. For example, where in the past C_T measurements made on deep seawater of intersecting cruises might have disagreed by $15\text{--}20 \mu\text{mol kg}^{-1}$, high-quality measurements now often agree within $2 \mu\text{mol kg}^{-1}$ (Dickson, 2010).

Various efforts were made in the early 1990s to improve the accuracy of carbon measurements across the community, including the creation of CO_2 in seawater reference materials (Dickson, 1992; UNESCO, 1991) and the documentation of CO_2 measurement techniques (DOE, 1994). This continues to be especially relevant now that many more researchers have become interested in the implications of the ocean's response to increasing atmospheric carbon dioxide concentrations, and the use of seawater reference materials to calibrate instruments for carbonate chemistry measurements has increased significantly. There are an increasing number of laboratory manipulations with high CO_2

treatments, as well as an emergence of interest in and monitoring of coastal upwelling zones experiencing low oxygen and low pH seawater (Feely et al., 2008). Each of these research themes is growing and each requires frequent measurement of seawater samples with high total dissolved inorganic carbon (C_T) and low pH. This has made it desirable to have a seawater reference material with a high CO_2 content so as to enable a reduction in carbonate chemistry measurement uncertainties (Hoppe et al., 2012). If a reference material with high CO_2 content is used in conjunction with an “ambient level” reference material to calibrate or evaluate an instrument, much more information can be learned about the performance of the instrument.

The aim of this study is to document the current quality of seawater CO_2 measurements (A_T , C_T , and pH) in the various participating laboratories. It will provide a better understanding of the current likely uncertainties in these measurements, which are central not only to research into the changing marine carbon cycle, but also which underpin our understanding of ocean acidification and its implications for marine organisms.

1.2 Materials and methods

1.2.1 Preparation of the sample materials

Two batches of seawater (Batches A and B) were prepared according to the standard technique for preparation of seawater reference materials in the Dickson Laboratory at the Scripps Institution of Oceanography (UC San Diego). For each batch a large volume of seawater was filtered, poisoned with mercury(II) chloride and bottled in 500 mL Corning Pyrex[®] reagent bottles sealed with greased ground-glass stoppers and

leaving ~1% headspace. These bottles were cleaned by first baking in an annealing oven (at 590 °C) and then rinsing thoroughly with 18 MΩ water (UNESCO, 1991).

Batch A had a lower $p(\text{CO}_2)$ typical of a normal reference material batch; Batch B was modified to have a higher CO_2 by bubbling with CO_2 gas. Bubbling with CO_2 to increase C_T modifies the seawater in a way that mimics the expected future conditions of the ocean, since it does not change the A_T , while it does increase $p(\text{CO}_2)$ and decrease pH. The bubbling took place three days before the bottling was to occur, during normal recirculation of the reference material seawater, which allowed time for complete mixing. The $p(\text{CO}_2)$ of the seawater was monitored during this recirculation using a CONTROS HydroC™ CO_2 FT sensor and temperature was monitored using a DirecTemp™ surface thermistor (Model # DTU6022).

During the several hours that it took to transfer the reference material seawater from the large container into individual bottles, the CO_2 level of the headspace in the large container was controlled dynamically to match the $p(\text{CO}_2)$ in the seawater. This control ensured that the seawater did not change its CO_2 concentration during the bottling process, even with the elevated CO_2 of Batch B. The appropriate gas mixture for the headspace was calculated from the initial $p(\text{CO}_2)$ measured by the CONTROS, A_T measured on a discrete sample taken on the first day of recirculation, and the instantaneous temperature of the seawater in the jug, which can change over the course of the day. This headspace gas mixture was created dynamically by combining N_2 , O_2 , and CO_2 gas streams, using three mass flow controllers managed by software written in LabVIEW™ (National Instruments), and was introduced into the large container at the top, at a rate slightly above the water removal rate during bottling.

1.2.2 Assignment of values to the sample materials

It is essential to ensure both homogeneity and stability of a batch of reference materials. The homogeneity was assured by initial thorough mixing of the seawater, together with the dynamic control of the headspace outlined above and was confirmed by subsequent analysis. The stability was confirmed by a series of analyses made over a three month period, as is usual for the certification of the Scripps CO₂ in seawater reference materials. Such reference materials have been shown to be stable for at least three years with respect to changes in A_T and C_T . The measurements performed on these sample materials were identical to those that are performed on each new batch of reference material.

C_T was assayed by the vacuum extraction/manometric procedure originally developed in Dr. C. D. Keeling's laboratory and based on the work of Wong (1970). A weighed sample is acidified with phosphoric acid; the CO₂ evolved is then extracted under vacuum and condensed in a trap cooled by liquid nitrogen. The water and CO₂ are separated from one another by sublimation and the CO₂ is transferred into an electronic constant-volume manometer. There its pressure, volume, and temperature are measured and the amount of CO₂ separated is computed from a virial equation of state.

A_T was determined by a two-stage, potentiometric, open-cell titration using coulometrically analyzed hydrochloric acid. A weighed sample of reference material is acidified to a pH between 3.5 and 4.0 with an aliquot of titrant. The solution is stirred while bubbling with air to allow the evolved CO₂ to escape. The titration is then continued to a pH of about 3.0 and the equivalence point evaluated from titration points

in the pH region 3.0–3.5 using a non-linear least squares procedure that corrects for the reactions with sulfate and fluoride ions (Dickson et al., 2003).

Values for pH at 25 °C were estimated spectrophotometrically (on the total hydrogen ion concentration scale) using purified *m*-cresol purple indicator dye (supplied by Dr. DeGrandpre, U. Montana), the equations of Liu et al. (2011), and a procedure similar to that described by Carter et al. (2013).

The results for these various analyses are reported in Table 1.1 as means and standard deviations, and these means are used as the assigned values for this inter-laboratory comparison. The methods for C_T and A_T have been evaluated for overall uncertainty (unpublished work), and we believe that the assigned values are within 2 $\mu\text{mol kg}^{-1}$ of their “true” values (95% confidence). The assigned pH is, as yet, an *operational* value (based on a particular dye and a particular calibration of that dye) and its likely uncertainty is not well known, though we believe it to be within 0.01 in pH of the “true” value (unpublished work).

Table 1.1. Assigned values for total alkalinity, total dissolved inorganic carbon, and pH (25 °C; total scale) for the test samples. Values are expressed as *mean ± standard deviation (number of analyses)*.

	Batch A	Batch B
Salinity	33.190	33.186
Total alkalinity	2215.08 ± 0.49 (24) $\mu\text{mol kg}^{-1}$	2216.26 ± 0.52 (18) $\mu\text{mol kg}^{-1}$
Total dissolved inorganic carbon	2015.72 ± 0.74 (9) $\mu\text{mol kg}^{-1}$	2141.94 ± 0.37 (6) $\mu\text{mol kg}^{-1}$
pH (25 °C; total scale)	7.8796 ± 0.0019 (18)	7.5541 ± 0.0020 (18)

1.2.3 Distribution and collection of results

We are very grateful to the CO₂ chemistry community for their participation in this inter-laboratory comparison. Every effort was made to include as many laboratories as wished to participate. Test samples were shipped to 66 participants: 30 in the United

States and 36 in 18 other countries. We received results back from 59 participants (Table 1.2). All laboratories were assured anonymity and thus their results are presented without any designation. Several interested laboratories were not able to participate due to constraints of time, instrumentation, and/or budget.

Laboratories were responsible for their own sample handling and data reporting. Several laboratories did not perform measurements of all parameters. Thus, the total number of results reported for each parameter differs, and furthermore separate results for a parameter may in fact reflect work done in the same lab, but on different instruments. Only two laboratories measured $p(\text{CO}_2)$ directly; their results are not included in this report.

Participants were asked to provide final, calibrated, measurement results for each of the parameters they determined, noting whenever more than one analysis for a particular parameter was done out of a single bottle. Additionally, laboratories were asked to indicate how the measurements were made (including equipment); how the measurement data were calibrated and an assessment of the uncertainty; and finally, if any adjustment was made to the data prior to reporting.

It is generally not a good idea to try to measure both C_T and pH from the same sample as some CO_2 transfer may occur before the second analysis is done, and the results will be sensitive to this. Of the laboratories that made multiple measurements of C_T and pH, some took multiple sub-samples out of the same bottle, while others purchased separate sets of bottles for each instrument. Any consequent errors, while possibly small, will be reflected in the results reported.

Table 1.2. Participating Organizations. Several labs from a single organization may have participated.

Country	Organization
Australia	Australian Institute of Marine Science CSIRO - Marine and Atmospheric Research (Hobart) Geoscience Australia Southern Cross University University of Tasmania
Belgium	Université de Mons Université Libre de Bruxelles
Canada	Institute of Ocean Sciences
China	Xiamen University
France	Station Biologique de Roscoff
Germany	Alfred Wegener Institute GEOMAR Helmholtz Centre for Ocean Research Kiel Leibniz Institute for Baltic Sea Research
Italy	Istituto di Scienze Marine - Consiglio Nazionale delle Ricerche Istituto Nazionale di Oceanografia e di Geofisica Sperimentale
Japan	Japan Agency for Marine - Earth Science and Technology Meteorological Research Institute, Japan Meteorological Agency Tokyo University of Marine Science and Technology
Netherlands	Royal Netherlands Institute for Sea Research, NIOZ
New Zealand	University of Otago National Institute of Water and Atmospheric Research
Norway	Institute of Marine Research University of Bergen
South Africa	Council for Scientific and Industrial Research Department of Environmental Affairs, Oceans and Coasts
Spain	Instituto de Investigaciones Marinas de Vigo - CSIC
UK	National Oceanography Centre Plymouth Marine Laboratory
USA	California State University, Northridge NOAA - Alaska Fisheries Science Center, Auke Bay Laboratories NOAA - Alaska Fisheries Science Center, Kodiak Laboratory NOAA - Atlantic Oceanographic & Meteorological Laboratory NOAA - Northeast Fisheries Science Center, Milford Laboratory NOAA - Northeast Fisheries Science Center, Sandy Hook Laboratory NOAA - Northwest Fisheries Science Center NOAA - Pacific Marine Environmental Laboratory Oregon State University Sunburst Sensors, LLC Texas A&M University Texas A&M University - Corpus Christi U.S. Geological Survey - St. Petersburg Coastal and Marine Science Center University of California, San Diego - Scripps Institution of Oceanography University of California, Santa Barbara University of Georgia University of Hawaii University of Miami University of Montana University of New Hampshire University of Washington - Friday Harbor Laboratories Woods Hole Oceanographic Institution

1.3 Results

Altogether 59 separate groups participated; 61 sets of results were returned for A_T , 58 for C_T , and 33 for pH. For each of these parameters, the difference between the average measured value reported and the assigned value for the test samples (Table 1.1) has been plotted in Figs. 1.1–1.3. Whenever more than a single value was reported for a bottle, the average was taken and used to represent the value for the bottle, unless instructed otherwise by the lab returning results. Nearly all laboratories reported using reference materials provided by Prof. Dickson's laboratory to calibrate the A_T and C_T instrumentation or results, however a few used no correction.

While most laboratories reported their pH results on the total scale and at 25 °C, there were some exceptions. Seven sets of results were reported at a different temperature, often near 20 °C. For those results, CO2calc (Robbins et al., 2010) was used to adjust the results to 25 °C using the assigned A_T value and the reported pH. Also, an adjustment of –0.14 pH units was applied to the results from the two laboratories that reported their results on the conventional (NBS) pH scale (Dickson, 1984). Although each of these adjustments to the reported pH values is subject to uncertainty, the magnitude of such uncertainty is likely small compared to the range of deviations displayed in Figure 1.3.

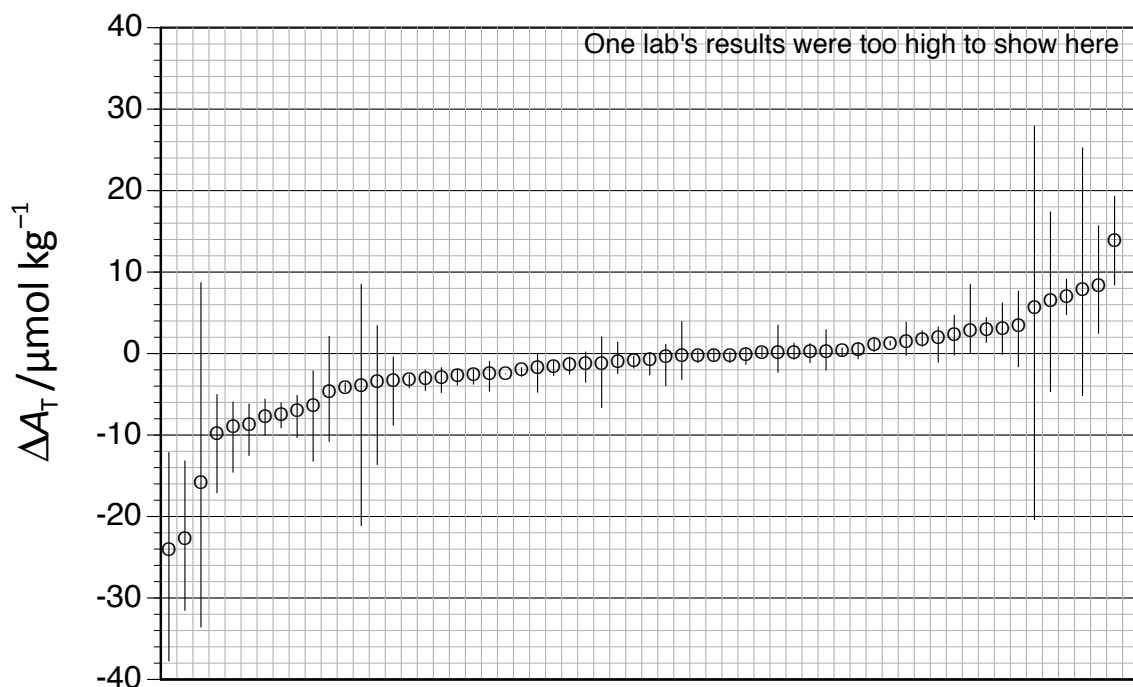


Figure 1.1. Differences between the total alkalinity values reported by the participating laboratories and the assigned values for the test samples (Table 1.1). The open circles indicate the average difference, the lines the range of such differences. Differences for both Batch A and Batch B have been combined, as the two batches were so similar to each other.

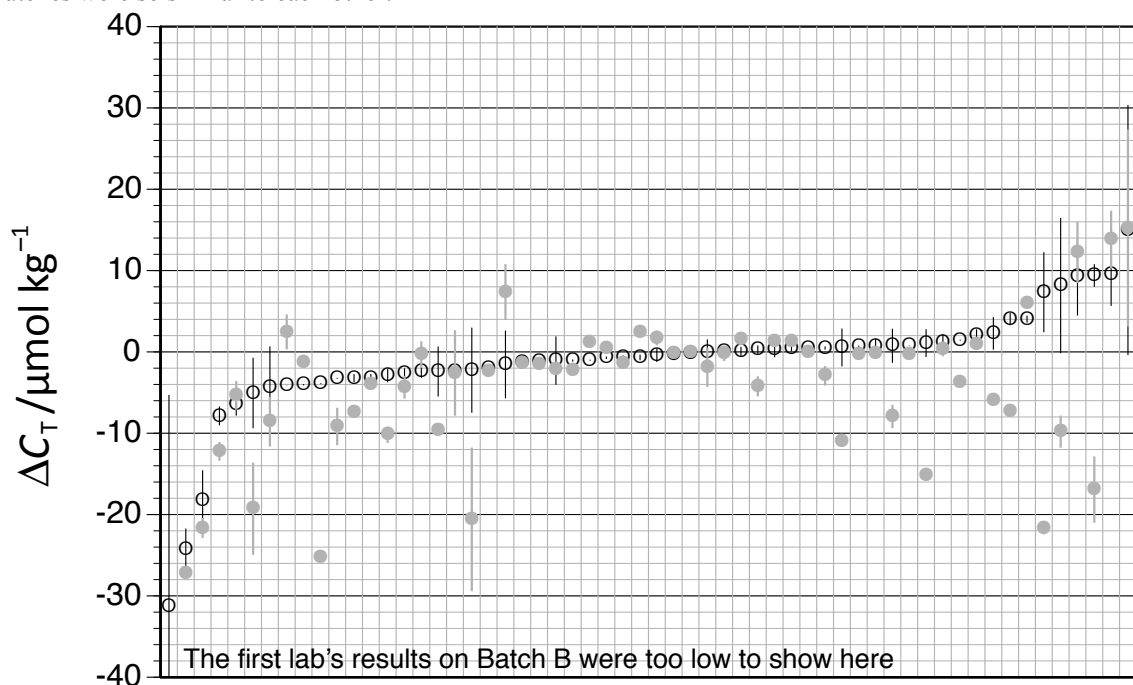


Figure 1.2. Differences between the total dissolved inorganic carbon values reported by the participating laboratories and the assigned values for the test samples (Table 1.1). The open circles indicate the average difference for Batch A, the corresponding black lines the range of such differences; the filled grey circles indicate the average difference for Batch B, the corresponding grey lines the range.

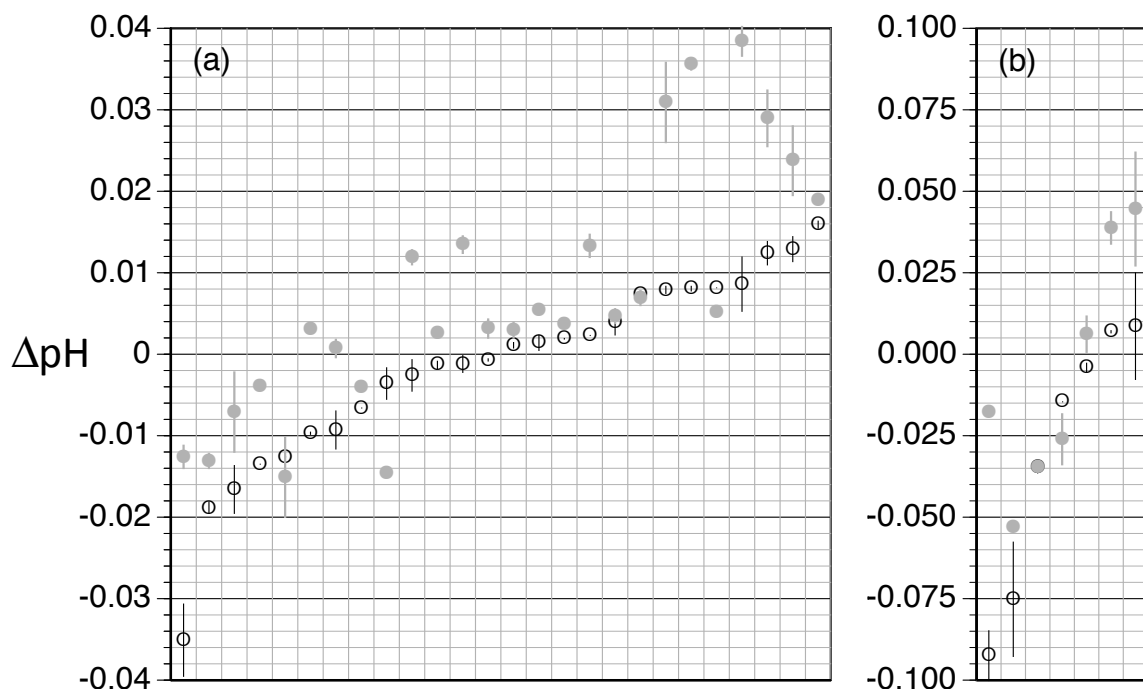


Figure 1.3. Differences between the pH values (25 °C; total scale) reported by the participating laboratories and the assigned values for the test samples (Table 1.1). The open circles indicate the average difference for Batch A, the corresponding black lines the range of such differences; the filled grey circles indicate the average difference for Batch B, the corresponding grey lines the range. The laboratories grouped in panel (a) used spectrophotometric techniques to measure pH; those grouped in panel (b) used electrometric techniques.

1.3.1 Brief overview of the methods of determination

There was some variety in the instrumentation used for the analyses. In recent years, commercially available instruments have emerged capable of measuring A_T and C_T . Many measurements are still made using instrumentation custom developed by or for an individual laboratory, especially for A_T . Figure 1.4 illustrates the distribution of reported instrumentation for A_T and C_T measurements.

Alkalinity measurements were almost exclusively done by open cell titration, including four participants who performed micro-titrations on sample sizes less than 20 mL. The most common instrument for A_T analysis was the VINDTA (Models 3S and 3C) by Marianda, followed by a significant proportion of custom or in-house designs. Other

commercially available instruments used for open cell titration include systems from Apollo SciTech, Metrohm, Mettler Toledo, and Kimoto. A couple of labs used spectrophotometric methods for measuring A_T including in one case a system built by Nippon ANS Inc., and one lab did a closed cell titration.

The coulometric method for determination of seawater C_T was adapted by Johnson et al. (1985) from a standard method for CO_2 determination and then automated and calibrated (Johnson et al., 1987). The resulting instrumentation developed at the University of Rhode Island has become known as the SOMMA system and has been used extensively in combination with a UIC Coulometer over the past few decades to monitor C_T in the oceans. Various other instruments are now commercially available based on this method of acidification and coulometric determination including the VINDTA (Models 3D and 3C) by Marianda and an instrument by Nippon ANS Co. In addition, several labs have devised their own custom systems based on a similar coulometric approach. Other C_T systems use a similar acidification technique, but quantify the resulting CO_2 gas using a nondispersive infrared sensor (NDIR – commonly the LiCOR 7000) rather than coulometry. The Apollo SciTech DIC analyzer (AS-C3) was the most common instrument of this type used, followed by custom systems and the AIRICA by Marianda. These NDIR based instruments use a much smaller sample size than the original SOMMA and VINDTA, which can be an advantage if water availability is limited, with some potential trade off in terms of precision and accuracy. Two laboratories used a SEAL Analytical QuAAtro Segmented Flow Analyzer to colorimetrically determine C_T . Several other unique methods of analysis were reported and are classified with those laboratories that failed to report their method in “Other” (Figure 1.4).

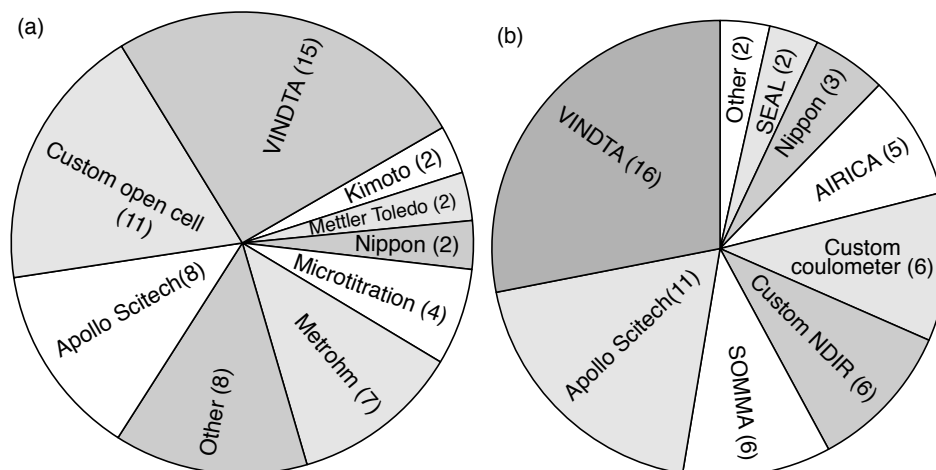


Figure 1.4. Types of analytical systems used for (a) total alkalinity determinations; and (b) total dissolved inorganic carbon determinations.

Most pH measurements were performed spectrophotometrically, although six laboratories used electrodes and one measured pH with an optode (Figure 1.3b). The spectrophotometric measurements are grouped in Figure 1.3a, and are generally more accurate than those done with a glass electrode (note the different y-axis scales). Although commercially available instruments that perform the entire pH measurement do not exist, there was some consistency in the spectrophotometer and dye used. Spectrophotometers made by Agilent were the most common, followed by spectrophotometers purchased from Cary, Ocean Optics, and Shimadzu. Most laboratories used *meta*-cresol purple indicator dye, but three reported using thymol blue. In the last couple of years, attention has been brought to the impurities present in commercially available *m*-cresol purple (Yao et al., 2007). Methods for purification have been published (Liu et al., 2011; Patsavas et al., 2013) and equations for calculating pH from absorbance values obtained using such purified dye are available (Liu et al., 2011). While a number of labs had access to purified dye for this study, most used commercially

available *m*-cresol purple indicator dye. A cell path length of 10 cm was by far the most commonly used, although 2 and 3 cm cells were also reported.

1.4 Discussion

In our discussion below, we make some necessarily subjective assessments of the overall data quality, and speculate as to the reasons that underlie it. In most cases, there is no clear relationship between measurement quality and the choice of analytical instrumentation. Some laboratories using a given, commercially available, system obtained excellent agreement with the assigned values, while others using apparently identical equipment did not. This suggests, unsurprisingly, that a significant contribution to the overall uncertainty of measurement can be ascribed to the operator and to the exact laboratory procedure, including quality control practices, used for the analysis.

Results from this inter-laboratory study thus show that many of the participating laboratories are able to make reasonable measurements for CO₂ in seawater. The majority of laboratories reported A_T and C_T values within $\sim 10 \mu\text{mol kg}^{-1}$ of the assigned values (*i.e.* within $\pm 0.5\%$). However, few laboratories were capable of achieving results within $2 \mu\text{mol kg}^{-1}$ of the assigned values (*i.e.* within $\pm 0.1\%$), especially for C_T . Results for the analysis of pH were quite scattered, with little suggestion of a consensus value (especially for the lower pH sample), probably reflective of the fact that there is currently no widely available certified reference material for this parameter.

It is difficult to judge the “quality” of analytical measurements without first clarifying the scientific application that they are required for and the maximum uncertainty that is considered appropriate for that application. A report that is in

preparation (Newton et al., 2014) describing plans for a Global Ocean Acidification Observing Network (GOA-ON) articulates two such applications: a “weather” goal and a “climate” goal, and recognizes that in all cases it is desirable to fully characterize the seawater CO₂ system by measurement.

The “weather” goal is defined as measurements of quality sufficient to identify relative spatial patterns and short-term variations, supporting mechanistic response to and impact on local, immediate ocean acidification dynamics. The GOA-ON report proposes that this objective requires the carbonate ion concentration (used to calculate CaCO₃ saturation states) to have a relative standard uncertainty of $\leq 10\%$. This, in turn, implies an uncertainty of ~ 0.02 in pH; of $\sim 10 \mu\text{mol kg}^{-1}$ in measurements of A_T and C_T ; and a relative uncertainty of about 2.5% in $p(\text{CO}_2)$.

The “climate” goal is defined as measurements of quality sufficient to assess long-term trends with a defined level of confidence, supporting detection of the long-term anthropogenically driven changes in hydrographic conditions and carbon chemistry over multi-decadal time scales. This objective is far more demanding and requires that a change in the carbonate ion concentration be estimated at a particular site with a relative standard uncertainty of 1%. This, in turn, implies an uncertainty of ~ 0.003 in pH; of $\sim 2 \mu\text{mol kg}^{-1}$ in measurements of A_T and C_T ; and a relative uncertainty of about 0.5% in $p(\text{CO}_2)$. The discussion below is thus framed in terms of these quality objectives, and tries to evaluate whether current laboratory quality control approaches are adequate to meet either of these objectives reliably.

1.4.1 Total alkalinity

The A_T results seem perhaps the most encouraging (Figure 1.1). However, the two batches of seawater used for this inter-laboratory comparison had nearly identical A_T values (2215.08 and 2216.26 $\mu\text{mol kg}^{-1}$). This is also very similar to the A_T of the seawater reference materials typically provided by our laboratory and which many of the participants used to calibrate their measurements. Consequently, this study is a less than ideal investigation into a laboratory's ability to make A_T measurements and leaves open the question of whether laboratories that showed themselves capable of getting the correct value at this A_T would necessarily be capable of getting the correct answer at significantly lower or higher A_T . Despite this, A_T results were not uniformly perfect. A number of laboratories failed on one or more of their analyses to reproduce the assigned value to within 10 $\mu\text{mol kg}^{-1}$. In addition, about a quarter of the laboratories had a range in their reported values larger than 10 $\mu\text{mol kg}^{-1}$, and more than 10% had a range greater than 20 $\mu\text{mol kg}^{-1}$, suggesting that the measurement technique was not operating reproducibly.

A careful examination of Figure 1.1 shows that the majority of participants reported alkalinities that were lower than the assigned values for the samples. It is difficult to be sure of the reason for this, but it seems in part to be associated with the common use of simpler end point determinations (as opposed to equivalence point determinations based on the use of full equilibrium expressions for seawater acid-base chemistry). In addition, systems that used very small sample sizes (less than 20 mL) had considerable uncertainties associated with their results.

1.4.2 Total dissolved inorganic carbon

The results reported for C_T (Figure 1.2) for Batch A are generally encouraging, though again the test sample is similar in composition to that of the seawater reference materials we distribute. Of the 58 results returned for the C_T of Batch A, only a small number of them were further than $10 \mu\text{mol kg}^{-1}$ from the assigned value. However, for Batch B (with the higher CO_2 level) a significant number of additional laboratories did not meet this standard. Furthermore, in most cases, the reported values for Batch B tended to be lower than the assigned value whereas for Batch A they were more evenly distributed, both lower and higher. The mean difference of reported values from the assigned values is $-1.17 \mu\text{mol kg}^{-1}$ for Batch A and $-4.95 \mu\text{mol kg}^{-1}$ for Batch B. The exact reason for this discrepancy is not known and may reflect an unidentified calibration problem for some instruments. However, the consistent sign of the discrepancy, even with different instruments, is suggestive of a loss of CO_2 at some unidentified point in the analytical process. Such a loss might be expected to be concentration dependent to some extent, and it may well be that some loss also occurred for measurements on Batch A, but that the use of a similar reference material for instrument calibration adjusted for it. Also, the loss could be worse for even higher CO_2 levels, such as might be found in water samples from certain environments and from intentionally modified seawater intended for ocean acidification studies.

It is not straightforward to use this exercise to distinguish between problems inherent to instrument design, and problems that are due to use of suboptimal analytical procedures or to operator inexperience. For example, measurements made using SOMMA systems did not exhibit such discrepancies. However, SOMMA instruments

have been extensively used starting with the WOCE/JGOFS Hydrographic Survey in the 1990s when standard operating procedures for use (and quality control) of these instruments were developed as a group effort (DOE, 1994). Furthermore, the laboratories have typically owned their instruments for many years, and their operators are very experienced in making high-quality C_T measurements. Other instruments (when viewed as a group) usually lack one or more of these desirable characteristics for optimal operation.

1.4.3 pH

Results for pH were quite variable (Figure 1.3) without a clear consensus value. Of the 34 sets of results returned, 27 were measured using a pH indicator dye with a spectrophotometer (Figure 1.3a); the others were measured with pH probes (Figure 1.3b): 6 using glass electrode pH cells, and 1 using an optical pH sensor from PreSens[®]. While all measurements performed on a spectrophotometer using an indicator dye were within 0.04 units of the assigned value (Figure 1.3a), results using a pH probe were as far away as 0.10 pH units. The reasons for the larger range of discrepancies found with pH probes probably reflects a combination of calibration difficulties (including inadequate temperature control) together with sample handling problems. Our remaining discussion will focus on the spectrophotometric pH measurements.

Most laboratories used *m*-cresol purple as the indicator dye for the spectrophotometric measurements. Seven laboratories reported having access to purified *m*-cresol purple indicator dye, and the subsequent results were typically (though not invariably) within 0.004 pH units of the assigned value. Even using impure dyes, the

work of Yao et al. (2007) and of Liu et al. (2011) suggest that discrepancies due to the impurities alone are unlikely to be larger than about 0.015 pH units and often smaller. One approach to correct for this (for a number of commercially available dyes) uses measured discrepancies (reported in Figure 2a of Liu et al., 2011) as an adjustment to the pH provided by the Liu et al. calibration equation (see Carter et al., 2013). However, the reliability of this approach has not been verified.

Figure 1.3 also clearly shows that most of the participants had a more positive deviation from the assigned value for Batch B than they did for Batch A, consistent with the suggestion previously made for the C_T results that CO_2 is being lost from the high- CO_2 sample prior to measurement. Again, this may well be due to handling difficulties that could also affect the uncertainty of the results for the lower- CO_2 sample. However, a recent publication from our laboratory (Bockmon and Dickson, 2014) shows that (with care) it is possible to filter this high- CO_2 sample without apparently increasing the pH significantly. Also, some laboratories, despite showing a substantial discrepancy, had very repeatable results, so we wonder if there is an additional as yet unidentified source of bias in some of the measurements here.

One factor that probably contributes to the lack of reproducibility is that the conventional spectrophotometric method described in Dickson et al. (2007) and based on the original work of Clayton & Byrne (1993) is not automated in any way, and requires some customization to achieve adequate temperature control. Thus, it is easy to imagine that each laboratory may be implementing a slightly different procedure without any awareness of possible biases. This is further exacerbated by the lack of a reliable supply of suitable reference material for pH measurements. Given the typically good

repeatability of many labs on these test samples, it may be that our widely distributed CO₂ in seawater reference materials could be used by laboratories to confirm that their pH repeatability can be extended to longer time-frames.

Nevertheless, although it is clear that more work needs to be done before we can have high confidence in pH values, many of the laboratories using a spectrophotometric procedure were able to achieve results within 0.02 pH units of the assigned value, *i.e.* achieving the “weather” objective specified by GOA-ON (see above).

1.5 Conclusions

The ability of laboratories to perform carbonate chemistry measurements has clearly advanced significantly since the last such study (UNESCO, 1990). This is likely due to three factors: the widespread availability and use of seawater reference materials for A_T and C_T calibrated by our laboratory; the existence of a published Guide describing suitable analytical methods (Dickson et al., 2007); and growing commercial availability of instrumentation for making such measurements.

In this exercise, more than half of the participating laboratories achieved A_T values that agreed with the assigned values to within $\pm 5 \mu\text{mol kg}^{-1}$ for every one of the analyses they reported, with more than two-thirds agreeing within $\pm 10 \mu\text{mol kg}^{-1}$. For C_T , nearly three-quarters of the participating laboratories reported all their values within $\pm 5 \mu\text{mol kg}^{-1}$ of the assigned value for analyses on Batch A, but only half were within $\pm 5 \mu\text{mol kg}^{-1}$ for all their analyses on Batch B. For pH more than half the labs were within 0.01 pH units of the assigned value for all their analyses on Batch A, but only about a third were within 0.01 pH units for their analyses on Batch B.

Almost all participating laboratories used reference materials traceable to the Dickson laboratory for the calibration of their A_T and C_T values. Thus, participants tended to do better on samples similar to the typical reference materials (Batch A & Batch B for A_T ; Batch A for C_T). However, the repeatability on A_T measurements was noticeably worse than for C_T measurements (median difference for measurements of $A_T \sim 4 \mu\text{mol kg}^{-1}$; for measurements of $C_T \sim 1.5 \mu\text{mol kg}^{-1}$), and so a smaller proportion of participating laboratories consistently achieved $\pm 5 \mu\text{mol kg}^{-1}$ for A_T (Batch A and Batch B) than did for C_T (Batch A) despite such calibration. Although there are no widely distributed reference materials for pH measurement, the repeatability was typically very good (median ~ 0.002 in pH). (The Dickson lab has started to provide an information value for the pH of their seawater reference materials; obtained using the procedure described here.)

As noted above, for many laboratories there is a clear suggestion that CO_2 has been lost in the analysis of Batch B (when compared to the results for the same laboratory for Batch A). This is apparent in the results for C_T , where twice as many labs underestimate the value for B (relative to A) as overestimate it; for pH the proportion that overestimate B (relative to A) is about 5:1. Of course, in a number of these cases, the difference is within the likely analytical reproducibility, and in others, it may reflect a calibration problem. Nevertheless, these results – taken at face value – lead us to believe that C_T or pH measurements on samples with high CO_2 content are typically more uncertain than those with levels closer to current atmospheric values.

Clearly there is a need for more than one reference material for seawater CO_2 properties so that laboratories can better confirm the quality of their measurement

procedures, and thus minimize apparent sample handling problems. In the future, our laboratory will try to supply additional such materials. However, we do note that there is presently too high a reliance on CO₂ in seawater reference materials not only for quality control, but also for calibration purposes. Whenever a reference material is used for calibration, it inherently limits its usefulness as a quality control check. Unless the laboratory is independently confident that the instrument is operating correctly, and with a stable reproducibility (verified, perhaps, over time using a laboratory's own stable test solution), use of a certified reference material for calibration can be misleading.

We also suggest that there has been, as yet, insufficient appreciation of the likely overall uncertainty of these measurements. Although many of the laboratories participating in this study showed themselves capable of achieving the “weather” objective of GOA-ON, few were capable of achieving the “climate” objective. Almost certainly, the seawater CO₂ measuring community would benefit from a more nuanced discussion of what constitutes an acceptable measurement uncertainty for achieving particular scientific goals. We also suspect that for most laboratories, their reported overall uncertainty is likely underestimated, typically either conflating or confusing precision and uncertainty – see *e.g.* the discussion in De Bièvre (2008).

So, how should the CO₂ measurement community work to improve matters? In 1989, the UK Department of Trade and Industry set up its “Valid Analytical Measurement” program. It articulated six principles (Sargent, 1995) that should be emphasized by the marine CO₂ measuring community (Table 1.3). We plan to do our part.

Table 1.3. Six principles of Valid Analytical Measurement developed by the Laboratory of the Government Chemist and the National Physical Laboratory in the UK as part of the Valid Analytical Measurement (VAM) programme set up by the UK Department of Trade and Industry. See the UK National Measurement System Chemical and Biological Metrology Website: <http://www.nmschembio.org.uk/GenericListing.aspx?m=108>

Valid Analytical Measurement (VAM) Principles
<ol style="list-style-type: none"> 1. Analytical measurements should be made to satisfy an agreed requirement. 2. Analytical measurements should be made using methods and equipment which have been tested to ensure they are fit for purpose. 3. Staff making analytical measurements should be both qualified and competent to undertake the task. 4. There should be a regular independent assessment of the technical performance of a laboratory. 5. Analytical measurements made in one location should be consistent with those elsewhere. 6. Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

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Chapter 1, in full, has been submitted for publication of the material as it may appear in *Marine Chemistry*. Bockmon, E. E., and A. G. Dickson. An inter-laboratory comparison assessing the quality of seawater carbon dioxide measurements. The dissertation author was the primary investigator and author of this paper.

CHAPTER 2

A seawater filtration method suitable for total dissolved inorganic carbon and pH analyses

High biomass and heavy particle loads may interfere with carbonate chemistry analyses of samples from experimental aquaria and cultures used to investigate the impact of ocean acidification on organisms, as well as from biologically productive coastal regions. For such samples, a filtration method is needed that does not change the dissolved CO₂ content, and consequently does not alter the total dissolved inorganic carbon and pH of the sample. Here, a filtration method is presented in which the sample seawater is pumped by a peristaltic pump through a replaceable 0.45 μm filter in a 50 mm polycarbonate filter holder and then into the sample bottle. Seawater samples of known carbonate composition were filtered to confirm that the filtration method did not alter the CO₂ content, and compromise the subsequent sample analysis and data usefulness. Seawater samples with added phytoplankton concentrations in the range of 1–5 × 10⁵ cells mL⁻¹ were also filtered successfully. Finally, seawater with added biogenic CaCO₃ was tested to prove that the method could successfully filter out such particles and produce dependable results. This approach will help to ensure more consistent and reliable carbonate chemistry measurements in coastal environments and from ocean acidification aquaria and cultures, by providing a well-tested method for sample filtration.

2.1 Introduction

Inorganic carbon measurements of seawater are becoming increasingly common as we attempt to better understand the oceanic carbon cycle and future anthropogenic changes. As a result, there is a need to make high-quality CO₂ measurements on samples with significant particle loading such as those collected from coastal and estuarine environments or even from experimental mesocosms. Published standard operating procedures (Dickson et al. 2007), developed for open-ocean studies, typically assume that filtration is not necessary. Indeed filtration is often seen as potentially problematic as it affords additional opportunity to exchange CO₂ between the sample and the atmosphere, thus changing the sample's total dissolved inorganic carbon level, and hence other CO₂-related parameters such as pH, $p(\text{CO}_2)$, or calcium carbonate saturation state. For this reason, authors have avoided filtration of samples intended for these analyses (Kim and Lee 2009; Koeve and Oschlies 2012).

Nevertheless, the existence of particles in samples can compromise seawater CO₂ measurements in a variety of ways (Gattuso et al. 2010; Hydes et al. 2010). Particles of CaCO₃ can be dissolved by the addition of acid as in total alkalinity (A_T) titrations, or in many methods for the determination of total dissolved inorganic carbon (C_T) thus increasing the apparent levels of these parameters in the sample. Kim et al. (2006) found that particulate organic matter in the form of phytoplankton and bacterial cells contributed significantly to A_T titrations in laboratory samples, suggesting that filtration was necessary prior to analysis. Chanson and Millero (2007) later confirmed that filtration was unnecessary in open-ocean conditions where particulate organic carbon values are low. Spectrophotometric techniques for pH measurement (Clayton and Byrne

1993; Liu et al. 2011) and spectrophotometric estimates of carbonate ion concentration (Byrne and Yao 2008; Martz et al. 2009) may also be susceptible to interference by particles.

Several researchers have reported filtering samples for analysis of total dissolved inorganic carbon (Brading et al. 2011; Czerny et al. 2009; Kline et al. 2012; Krug et al. 2011; Miller et al. 2009). Recently Hansen et al. (2013) published a new method for C_T analysis of extremely small volume samples, which includes sample filtration and showed that it only marginally affected the measured C_T . Here, we describe a procedure for filtering seawater samples, and demonstrate that the filtration has no detectable influence on the sample composition even when using state-of-the-art analytical techniques for the analysis of total alkalinity, total dissolved inorganic carbon, and pH. The proposed filtration technique is relatively straightforward and suitable for routine application whenever filtering is required.

2.2 Materials and procedures

A peristaltic pump is used to transfer the seawater from the sampling reservoir, through the filter and into the sample bottle. For this method, a Pall Life Sciences 47 mm Polycarbonate In-Line Filter Holder (1119) was used. This screw-type filter holder allowed us to use replaceable 0.45 μm Durapore Membrane Filters (Millipore Cat. No. HVLP04700). The filter holder also has a white screw cap on the top that serves as a vent for trapped air, important to ensure that no gas exchange occurs between the sample and the atmosphere. Tubing (Tygon R-3603 14-169-1G 3/16 inch ID with 1/16 inch wall

thickness) was secured to barbs on either end of the filter holder and the inlet end run through a Manostat Simon Varistaltic Pump (Model 72-310-000).

Before collecting sample, the tubing and filter need to be rinsed with the sample water and all bubbles removed from the filter holder. To do this, the peristaltic pump is turned on at a slow speed, which fills the tubing and filter housing with sample seawater. Any seawater passing through during this stage should go to waste. Tapping on the side of the filter holder helps to dislodge bubbles that are trapped, and the small white cap on the filter holder can be removed so that bubbles may escape. With the peristaltic pump still running slowly, replace the white cap. The tubing and filter housing should now be empty of air. Preparing the tubing and filter housing in this way should use roughly 0.1 L seawater. Once ready, a filtered sample can be collected. The speed of the peristaltic pump is then increased; a reasonable flow is approximately 0.8 L min^{-1} when a filter is inline. The exact flow speed while filtering will depend on the mass of particulates collected on the membrane filter; as the filter clogs, the flow will slow. Seawater is pumped through the filter and into the sample bottle.

The membrane filter must be changed periodically between samples, depending on the particle load of the seawater passed through. To do this, the filter holder is unscrewed, and the membrane filter replaced. Each time the holder is opened the bubble removal technique must be repeated. If the same membrane filter is used, then seawater of the new sample must be run through to waste, replacing the previous sample. This also requires $\sim 0.1 \text{ L}$, however the volume will be dependent on the length and diameter of tubing used.

A filter membrane size of 0.45 μm was chosen for this study. Particulate matter is operationally defined as that collected on a 0.45 μm filter (Pickering 1978), making it an appropriate choice. In addition, use of 0.45 μm maintains consistency with previous studies done on seawater filtration and carbonate chemistry (Chanson and Millero 2007; Kim et al. 2006). Several larger filter sizes (0.6, 1.0, and 5.0 μm) were initially tested, and all were found to produce reproducible results between filtered and unfiltered samples.

2.3 Assessment

Experiments were performed both to demonstrate that the described filtration method does not alter the seawater carbonate chemistry independent of any particle load, and to show that it could adequately remove undesired contributions to C_T and A_T caused by cells and CaCO_3 particles suspended in the samples. For this study, 250 mL borosilicate glass bottles (Corning 1500-250) were used to collect the filtered samples for analysis of C_T and A_T , or for analysis of pH and A_T . For high-quality measurements from a bottle, it is not usually a good idea to try to measure both C_T and pH from the same bottle as some CO_2 transfer may occur before the second analysis is done. The bottles were allowed to fill and overflow, a 1% headspace was created by removing seawater with a syringe, and a greased stopper with rubber band and clip was used to close the bottle.

Total dissolved inorganic carbon was measured using a SOMMA-Coulometer system (Dickson et al. 2007; Johnson et al. 1993). Samples for pH were analyzed spectrophotometrically (Carter et al. 2013) at 25°C using pure *m*-cresol purple dye and

the equations of Liu et al. (2011). A_T samples were taken from all bottles previously sampled for C_T and pH, and were analyzed using the open-cell method described by Dickson et al. (2003). Certified reference materials were used in conjunction with the sample analyses to ensure the systems were functioning properly, and to provide a calibration for the coulometric C_T measurements.

2.3.1 Filtration does not alter the carbonate chemistry

Seawater samples that had been originally prepared as certified reference materials (CRMs) for oceanic CO_2 measurements (Dickson 2001) were filtered and analyzed in replicate to show that the method does not alter the C_T , pH, or A_T of samples. This is of particular concern due to the potential for gas exchange to alter C_T and pH. CRMs were useful for this test, as they have a known certified value, and the seawater has been previously filtered. Twenty-four 500 mL bottles of CRM Batch 124 were filtered using the method described directly into 250 mL sample bottles. No significant difference in C_T , pH, or A_T was found between these filtered sub-samples of CRM Batch 124 and the original certified values (Table 2.1).

This experiment was repeated using CRM Batch 125, a seawater intentionally modified to a $p(\text{CO}_2)$ of approximately 1250 μatm by bubbling with CO_2 gas. Again, no difference was found between the measured values of the filtered samples and the certified values (Table 2.1). Demonstrating that the method performs well under high CO_2 conditions assures us that this filtration method is useful in the context of ocean acidification experiments and coastal monitoring where sample seawaters often have high $p(\text{CO}_2)$, and are thus prone to loss of CO_2 to the atmosphere during sample handling.

2.3.2 Filtration successfully removes phytoplankton

Experiments were also conducted to show that the proposed method is equally effective for seawater samples with significant suspended biomass. Concentrated aliquots of the cultured diatom *Phaeodactylum tricornutum* were added to bottles of CRM Batch 124 to bring the cell concentrations to $1-5 \times 10^5$ cells mL⁻¹. These bottles of seawater were then filtered into 250 mL bottles as described above, and analyzed for either C_T or pH, as well as A_T . Filtration successfully removed the phytoplankton from the seawater, and the values for the filtered seawater again matched the certified values for CRM Batch 124 (Table 2.1).

The average A_T of the unfiltered samples of CRM Batch 124 with this amount of phytoplankton added was increased by $\sim 3 \mu\text{mol kg}^{-1}$ (Table 2.1). This matches the contribution to A_T found by Kim et al. (2006) for phytoplankton bloom conditions. Filtration returned the observed A_T value to the expected, certified value. Unfiltered samples were consistently slightly elevated in C_T , but by less than $1 \mu\text{mol kg}^{-1}$ and thus not obviously different than the average filtered or certified value. Similarly, no difference in the average pH of the unfiltered seawater samples was detected at this concentration of cells. However, our tests suggested an increasing variability in spectrophotometric pH with increasing cell concentrations, as might be expected. Samples with denser cell concentrations may be filtered; although the filter was found to clog once approximately 2×10^8 cells were collected. Thus, for denser cultures, smaller sample volumes or larger filter areas are recommended. Alternatively, the filter could be changed during the filtration of a single sample, but this will add additional opportunities for error.

2.3.3 Filtration successfully removes CaCO_3 particles

Another study was done to show that the filtration method successfully removes CaCO_3 particulates from seawater. Shell collected from La Jolla Shores was crushed using a mortar and pestle and added to a polyethylene carboy of seawater to mimic suspended biogenic CaCO_3 . Use of CRMs as a baseline for this test was not considered helpful because the addition of finely ground CaCO_3 can alter the carbonate chemistry of seawater samples by either dissolution or precipitation. Replicate samples of both filtered and unfiltered seawater were taken from the carboy and again analyzed for either C_T or pH as well as A_T . The added CaCO_3 represented a large load of particulates, which substantially altered both the observed C_T and A_T of the unfiltered seawater samples (Table 2.1). The observed standard deviations of the unfiltered measurements of C_T and A_T show the significant added variability such particles cause. After filtration of the seawater, results were again reproducible, giving confidence that all of the particles were removed by the filtration. For the pH measurements, we found no real difference between filtered and unfiltered samples, suggesting either that the particles settled out of the light path in the cell, or that the background correction adequately accounted for any scattering effects.

Table 2.1. Measured results for C_T , pH, and A_T for the various experiments reported here. The certified values for CRM Batches 124 and 125 are included for comparison. In each case, values are expressed as mean \pm one standard deviation (number of analyses).

	C_T ($\mu\text{mol kg}^{-1}$)	pH (Total scale)	A_T ($\mu\text{mol kg}^{-1}$)
Measurements of CRM Batch 124			
Batch 124 certified values	2015.72 \pm 0.74 (9) [*]	7.8796 \pm 0.0019 (18) [†]	2215.08 \pm 0.49 (24)
Filtered samples	2016.18 \pm 0.93 (12)	7.8799 \pm 0.0006 (12)	2215.40 \pm 0.76 (24)
Measurements of CRM Batch 125			
Batch 125 certified values	2141.94 \pm 0.37 (6) [*]	7.5541 \pm 0.0020 (18) [†]	2216.26 \pm 0.52 (18)
Filtered samples	2141.19 \pm 1.07 (12)	7.5569 \pm 0.0020 (12)	2216.30 \pm 0.78 (24)
Measurements of CRM Batch 124 with <i>P. tricornutum</i>			
Batch 124 certified values	2015.72 \pm 0.74 (9) [*]	7.8796 \pm 0.0019 (18) [†]	2215.08 \pm 0.49 (24)
Filtered samples	2015.79 \pm 0.61 (7)	7.8807 \pm 0.0007 (7)	2215.53 \pm 0.89 (14)
Unfiltered samples	2016.25 \pm 0.98 (7)	7.8799 \pm 0.0012 (7)	2218.45 \pm 0.68 (14)
Measurements of seawater with CaCO ₃ particles			
Filtered samples	2000.17 \pm 0.49 (7)	7.8860 \pm 0.0006 (7)	2201.74 \pm 1.07 (13)
Unfiltered samples	2071 \pm 11 (7)	7.8855 \pm 0.0007 (7)	2457 \pm 145 (14)

^{*}The certified C_T values for reference materials were not measured using the SOMMA-Coulometric system used for the other measurements reported here. They were measured using a more involved vacuum extraction/manometric method.

[†]The assigned pH values for reference materials were measured by the same technique used here over a period of 4 months.

2.4 Discussion

This study describes a simple and relatively low-cost method for sample filtration, which can eliminate the potential for errors in carbonate chemistry measurements resulting from particles such as biogenic CaCO₃ or particulate organic matter. If there is any concern over the seawater quality due to the presence of particles, then care should be taken to filter samples intended for C_T , pH, or A_T analysis. It is important to ensure removal of particulate inorganic carbon (CaCO₃) from samples, so that reported values of C_T and A_T are not misleadingly elevated. It is unclear how often this error may already occur in recorded datasets, but it is easily avoidable in the future.

Our results confirm the conclusions of Kim et al. (2006) in that we found A_T could be elevated by the presence of cells. We also concur with the observation of Chanson and Millero (2007) that there is likely to be no significant interference when measuring clean seawater. The simple filtration methods employed in those studies, although suitable for A_T analysis, will likely change C_T and pH. The method described

here, using a peristaltic pump, ensures no observable effect of filtration on the CO_2 content, even at the high sensitivity of current state-of-the-art ocean-measuring techniques. Furthermore, filtration will eliminate potential interference by particles or organisms on any of the sample analyses for CO_2 parameters.

2.5 Comments and recommendations

The method described here has been tested thoroughly in a laboratory environment, and shown to successfully filter out particles that could otherwise interfere with analyses for C_T , pH, and A_T . Samples can be easily taken and filtered from laboratory experimental aquaria, where a peristaltic pump might easily be used as a reliable sampling technique. We have also used this filtering method at sea, with samples taken directly from Niskin bottles on a CTD rosette. Sampling in other environments while in the field should be possible, as the filtration setup is transportable, but it may be complicated by the need for power to run the peristaltic pump.

Although this method has been tested on relatively large volume samples so as to allow for analysis using standard open-ocean techniques (typically using about 450 mL seawater to collect a 250 mL sample), it is likely that the same technique could be successfully adapted to a smaller sample using a smaller filter and smaller diameter tubing. Hansen et al. (2013) used a peristaltic pump together with a $0.2 \mu\text{m}$ syringe filter to filter samples of <10 mL, and found only minor alteration of the C_T , though with a less precise approach than that described here. This adaptation should be of particular interest to scientists working with high-density cultures, or in other situations with a significant particle load but limited seawater for sampling. We expect that our filtration method can

easily be adapted for other environments and scenarios not discussed here, but where filtering of samples for CO₂ measurement is necessary. The use of a peristaltic pump and enclosed filter assembly ensures that samples collected will not be compromised by gas exchange, and will be suitable for high quality analyses for C_T , pH, and A_T .

2.6 References

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2.7 Acknowledgments

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Chapter 2, in full, is a reprint of the material as it appears in *Limnology & Oceanography: Methods*. E. E. Bockmon and A. G. Dickson, 2014. A seawater filtration method suitable for total dissolved inorganic carbon and pH analyses. *Limnology and Oceanography: Methods*, 12: 191–195. The dissertation author was the primary investigator and author of this paper.

CHAPTER 3

Technical Note: Controlled experimental aquarium system for multi-stressor investigation of carbonate chemistry, oxygen saturation, and temperature

As the field of ocean acidification has grown, researchers have increasingly turned to laboratory experiments to understand the impacts of increased CO₂ on marine organisms. However, other changes such as ocean warming and deoxygenation are occurring concurrently with the increasing CO₂ concentrations, complicating the understanding of the impacts of anthropogenic changes on organisms. This experimental aquarium design allows for independent regulation of CO₂ concentration, O₂ levels, and temperature in a controlled environment to study the impacts of multiple stressors. The system has the flexibility for a wide range of treatment chemistry, seawater volumes, and study organisms. Control of the seawater chemistry is achieved by equilibration of a chosen gas mixture with seawater using a Liqui-Cel[®] membrane contactor. Included as examples, two experiments performed using the system have shown control of CO₂ at values between approximately 500 and 1400 μatm and O₂ at values from 80 to 240 μmol kg⁻¹. Temperature has been maintained to 0.5 °C or better in the range of 10–17 °C. On a weeklong timescale, the system has achieved variability in pH of less than 0.007 pH units and in oxygen concentration of less than 3.5 μmol kg⁻¹. Longer experiments, over a month in duration, have been completed with control to better than 0.08 pH units and 13

$\mu\text{mol kg}^{-1} \text{O}_2$. The ability to study the impacts of multiple stressors in the laboratory simultaneously, as well as independently, will be an important part of understanding the response of marine organisms to a high- CO_2 world

3.1 Introduction

The amount of inorganic carbon in the oceans is increasing and the pH decreasing, a process commonly known as ocean acidification (Caldeira and Wickett, 2003). This is a consequence of the oceans taking up a proportion of the anthropogenic carbon dioxide emissions added to the atmosphere each year. In the past decade, ocean acidification has received increasing attention from the scientific community, particularly the impact of the expected changes in carbonate chemistry on organisms (Doney et al., 2009a).

Experimental studies in the laboratory are an important part of this research (Doney et al., 2009b). While significant insight has been gained from these laboratory experiments, it has also become clear that the questions are not simple to answer, and that reasonable control of the experimental conditions can be challenging.

In 2010 EPOCA published a *Guide to best practices for ocean acidification research and data reporting* (Riebesell et al., 2010), which includes several chapters that are relevant to setting up experimental aquaria with the intent to control carbonate chemistry. Various options for modifying carbonate chemistry are suggested, which change the total dissolved inorganic carbon (C_T) of the seawater, the total alkalinity (A_T), or both simultaneously. A variety of experimental systems have been designed and used, with varying degrees of success, by researchers interested in controlling seawater carbonate chemistry in the laboratory. For example, several experiments have been

performed by bubbling seawater directly with gas mixtures created by combining pure CO₂ with ambient or CO₂-stripped air to create an elevated partial pressure of CO₂: $p(\text{CO}_2)$ (Miller et al., 2009; Talmage and Gobler, 2009). One published system bubbles a custom gas mixture while monitoring $p(\text{CO}_2)$ (Fangue et al., 2010); yet another uses acid additions to create constant pH seawater as determined by spectrophotometric measurements (McGraw et al., 2010). Some discussion has surrounded the various accepted methods of manipulation, and the differences in carbonate chemistry have been evaluated and found to be small (Gattuso and Lavigne, 2009; Schulz et al., 2009). Nevertheless, bubbling is often recommended as the “first choice” because it “exactly mimics carbonate chemistry changes occurring in the years to come” (Gattuso et al., 2010). However, direct bubbling with gas can lead to difficulties in sustaining phytoplankton cultures (Shi et al., 2009), and hence header tanks are often used for equilibration to eliminate the impact of bubbling on the experimental organism.

In addition to changes in the carbon parameters, other impacts of anthropogenic climate change on the ocean are expected. Temperatures in the upper 300m of the oceans are rising (Lyman et al., 2010), and changes in stratification have occurred (Palacios et al., 2004). Also, oxygen saturation is expected to decrease as the oceans warm, stratify, and absorb carbon (Keeling et al., 2010; Shaffer et al., 2009). Predictions of the consequences of ocean acidification must consider synergistic effects between changing inorganic carbon parameters and changes to these other variables. Multi-stressor, or multi-variable, interactions have gained a lot of attention recently as researchers have begun to examine the many simultaneous impacts that climate change will have on organisms (Boyd, 2011; Pörtner et al., 2005). However, a review of marine climate

change papers found that most were single-factor experiments, most often focusing on acidification alone (Wernberg et al., 2012). In particular, there is a need for experiments focusing on the combination of CO₂ and O₂ (Melzner et al., 2012; Pörtner et al., 2005). Changes in oxygen and pH are strongly, positively correlated in systems dominated by photosynthesis and respiration, as has been documented recently in coastal upwelling systems (Frieder et al., 2012; Paulmier et al., 2011); the implications for organisms simultaneously experiencing low pH and low oxygen levels in seawater are just starting to be investigated. The ability to modify CO₂ and O₂ levels independently in an experimental laboratory, in addition to temperature, will be critical to understanding the response of organisms that live in natural environments with these multiple stressors.

The Multiple Stressor Experimental Aquarium at Scripps (MSEAS) presented here is designed to enable such experiments and to facilitate the study of organisms under future ocean scenarios. The system is capable of independent manipulation and control of the inorganic carbon chemistry, oxygen levels, and temperature of the seawater in each tank. Additionally, the system is designed with flexibility so that it may be adapted for a variety of marine organisms and life stages. Finally, chemical data from two separate experiments are used to illustrate the stability and accuracy of the system.

3.2 Methods

3.2.1 Carbon parameter control

Achieving good control in an experimental laboratory setting is difficult, in part because the carbonate chemistry is complicated. Factors affecting control of the carbonate chemistry include gas exchange, temperature influences, and direct

modification by the study organisms through processes such as photosynthesis, respiration, and calcification. Another obstacle is that measurement of the carbon parameters is neither simple nor inexpensive (Dickson et al., 2007; Dickson, 2010). Acid-base chemistry in clean seawater can be described using known equilibrium constants (given salinity, temperature, pressure, and the total boron / salinity ratio), together with two other measured parameters (typically from A_T , C_T , $p(\text{CO}_2)$, and pH). (This is only strictly true when the other minor acid-base systems in the seawater (e.g., phosphate, silicate, ammonia, organic bases, etc.) do not contribute significantly to the alkalinity.) Consequently, for a particular seawater at a given temperature and salinity, it is only necessary to control two of these parameters to achieve control over the carbonate chemistry.

Controlling two parameters at once is not accomplished easily. However, by assuming a constant A_T in the aquarium, only a single parameter needs to be explicitly controlled (Dickson, 2010). Constant A_T is a reasonable assumption for the seawater system that supplies MSEAS; over a three-year period, the observed range of A_T for the seawater system was $50 \mu\text{mol kg}^{-1}$ (Figure 3.1). This would change pH calculated from A_T at a constant $p(\text{CO}_2)$ by less than 0.01 pH units. More often A_T is similar from one day to the next, showing large changes over months, rather than days. Consequently, the assumption of constant A_T only leads to small errors in the understanding of the carbonate chemistry when paired with controlled $p(\text{CO}_2)$, and thus serves as a reasonable assumption for this experimental system.

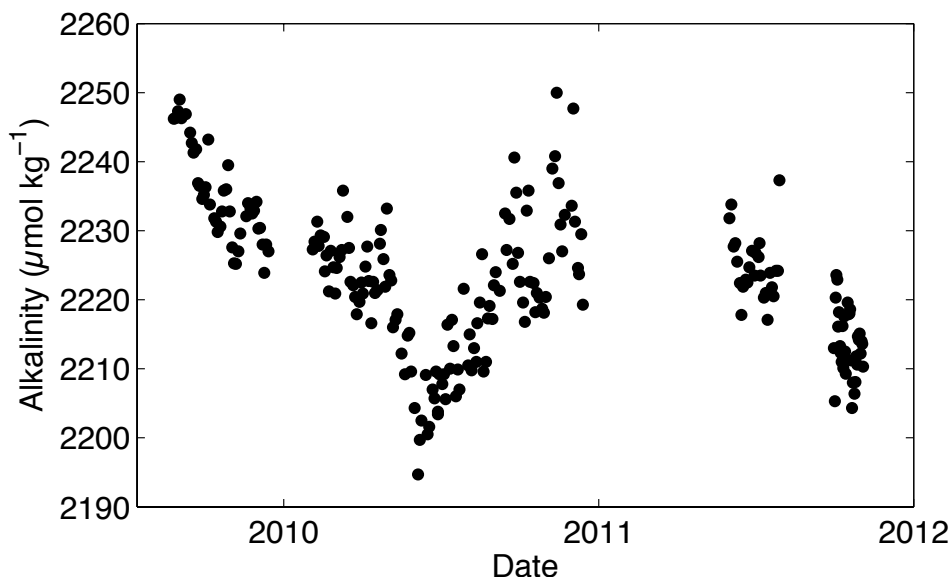


Figure 3.1. Measured total alkalinity ($\mu\text{mol kg}^{-1}$) in the Scripps Institution of Oceanography seawater system over several years. Discrete samples were poisoned for later analysis. The mean is 2223 and the standard deviation is $11 \mu\text{mol kg}^{-1}$.

However, some care must be taken to ensure that the assumption of constant A_T remains true in an aquarium setting. Organisms continually modify the chemistry of their environment. For A_T , this usually means calcification or the assimilation and remineralization of other nutrients and ions (for further discussion, see Wolf-Gladrow et al., 2007). To maintain A_T levels throughout the duration of the experiment, some action must be taken to counteract these modifications. The most straightforward solution is the continual addition of new seawater to the tanks, which replenishes A_T . Sufficient seawater replacement, depending on the organisms and their respective biomasses in the aquaria, will guarantee that the A_T in each tank reflects the assumed constant A_T of the seawater supply system.

Alkalinity is particularly useful as a control variable for carbonate chemistry in an ocean acidification experimental laboratory. Not only is it conservative with respect to

mixing and not affected by changes in temperature, but also the addition or removal of CO₂ gas from seawater does not change A_T. This allows for modification of the total amount of CO₂ (and of O₂) in the seawater as a means to control a second parameter without invalidating the assumption of constant A_T. In MSEAS, the second parameter is controlled by reacting a gas of a particular CO₂ and O₂ content with seawater using a membrane contactor, which allows a desired $p(\text{CO}_2)$ and oxygen percent saturation to be achieved. This direct equilibration of a known gas with seawater has the same effect on carbonate chemistry as if bubbling had been used to modify the seawater sample.

3.2.2 Apparatus

To achieve the desired chemistry in MSEAS, a gas mixture is equilibrated with seawater using a Membrana Liqui-Cel® 2.5 × 8 Extra-Flow membrane contactor for each aquarium (Figure 3.2). The desired gas composition (N₂, O₂, CO₂) is mixed from individual gas cylinders using Omega® mass flow controllers (FMA 5418 0–5 SLM; FMA 5411 0–2 SLM; and FMA 5402 0–10 sccm, respectively). The mass flow controllers are operated by a laptop running NI LabVIEW™ software with communication using a voltage generating NI 9265 4-Channel Analog Output Module™ combined with an NI USB-9162 Single Module Carrier™. Mass flow controller function is monitored using a NI USB-6210 Multifunction DAQ™ (Figure 3.2). Mixing individual gases gives the user complete control over both the CO₂ and O₂ concentrations. After the three gases mix in the desired proportions, the line is split, providing an identical gas mixture to two or more replicate tanks. Currently the system is designed with two sets of three mass flow controllers, allowing for two independent treatment levels.

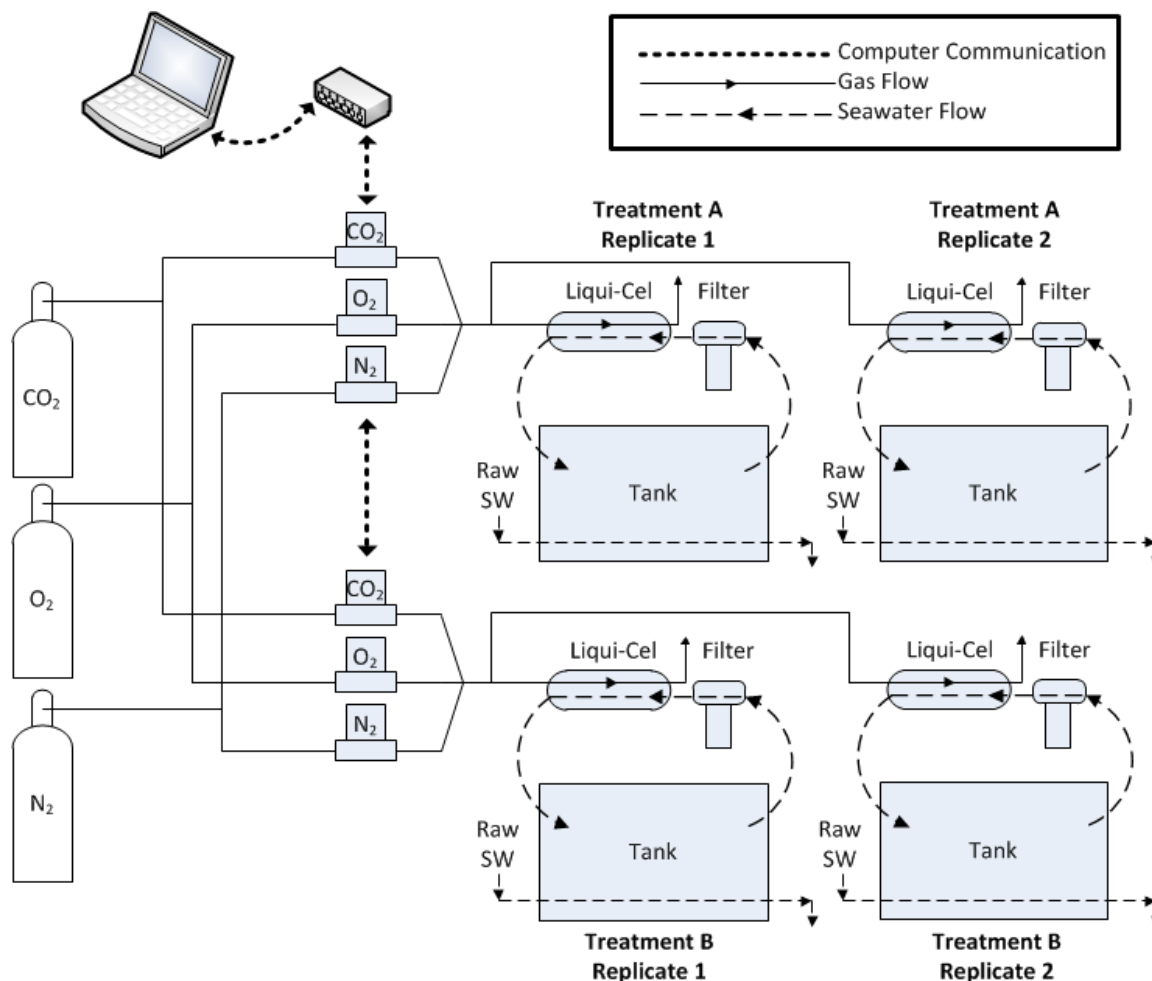


Figure 3.2. Schematic of the aquarium control system. Solid lines indicate gas tubing and flow, and thin dashed lines indicate seawater tubing and flow. Thick dashed lines represent data communication between instruments.

A submerged MARINELAND® Maxi-Jet 1200 Power Head pumps seawater from each treatment tank through a 5 μm filter and then through the tank's associated Liqui-Cel membrane contactor. The gas mixture is introduced to the Liqui-Cel in the opposite direction, enhancing equilibration. The seawater is returned to the corresponding treatment tank, and the gas flows to waste (Figure 3.2). Despite this continuous recirculation of the treatment seawater, it is not expected that the seawater will equilibrate perfectly with the gas phase. Fluctuations in temperature, flow rates, and in the degree of

disequilibrium brought about by changes in the composition of the seawater in the tank due to gas exchange or biological processes all work against achieving complete equilibrium. Nevertheless, as will be seen, a reasonable degree of control can be achieved.

The temperature of each tank is maintained with a titanium coil through which temperature-controlled water flows from a Thermo Scientific NESLABTM RTE 7 Refrigerated Bath. The system design allows the seawater in each aquarium to be exchanged in a flow-through mode, where raw seawater is added continually at a slow rate, maintaining A_T levels. The excess seawater overflows, removing organism waste. This rate of overturning must be optimized, and will be organism and biomass dependent. Although the system was originally designed with a large, 50 L tank in mind, the size of the treatment tank is easily exchangeable. Several of the experiments performed have used much smaller volumes to fit the experimental organism better and to maximize control over the chosen parameters. The size of the tank, and therefore the volume of water needing to be equilibrated, must be chosen for each experimental organism and desired biomass. Smaller volumes of water will recirculate through the membrane and interact with the gas more often, leading to better control.

Equilibration between the gas and seawater using the Liqui-Cel is done independently for each replicate aquarium tank, so there is no mixing of treated seawater. The only shared part of any replicate is the gas composition and the original source water. An often-used technique in ocean acidification experiments involves modifying the composition of a header tank that then provides identical source water to many replicate tanks. In these systems, the actual composition of an individual tank can diverge from

that of the source water due to gas exchange or biological processes. By modifying the composition of individual tanks directly, we hope to mitigate such divergences and achieve better control.

One of the primary advantages of MSEAS, is that it allows the user to choose a desired CO₂ and O₂ composition, within a large range. The CO₂ level of the gas mixture can be chosen by the user to be any value between 0 and 5000 ppm, although the maximum $p(\text{CO}_2)$ of any experiment performed to date is approximately 1500 μatm . Barry et al. (2010) includes suggestions of CO₂ levels for ocean acidification laboratory experiments; the recommendation for a two treatment system is for one treatment near a “present-day” (mid-2008) atmospheric value of 385ppm and the other at a “future” value of 750 ppm. MSEAS is well suited to perform experiments at these specified values, but also has the advantage of flexibility in terms of its target CO₂. This is especially valuable, in that it easily allows investigation of environments that are not at equilibrium with the atmosphere. For example, the coastal region of western North America experiences upwelling events, in which seawater already elevated in CO₂ flows onto parts of the continental shelf (Feely et al., 2008).

3.3 Assessment and discussion

The usefulness and capability of this system is demonstrated by the experiments performed to date. Two of these experiments are described below as examples of the stability that can be maintained – one a week long, the other lasting longer than a month. Both experiments modified the CO₂ and O₂ of the seawater for each treatment and were performed at different temperatures. Discrete samples for A_T, pH, and O₂ were taken

daily during the experiments and analyzed at Scripps Institution of Oceanography. A_T samples were poisoned with saturated mercuric chloride and stored for later analysis which was done by open-cell titration (Dickson et al., 2007). Discrete pH samples were analyzed spectrophotometrically (Dickson et al., 2007) on the same day as sampling. Values are reported at the in situ temperature and on the total pH scale. Discrete oxygen samples were pickled immediately and analyzed within a few days by Winkler titration (Dickson, 1996). Temperature was monitored every five minutes in all tanks by HOBO Pendant™ Temperature/Light Data Loggers.

3.3.1 Experiment M7

A week-long experiment was performed on mussel larvae (*Mytilus galloprovincialis*) with one treatment of pH and oxygen levels, typical of a present-day California coastal upwelling environment (Frieder et al., 2012), and the other with lower pH and oxygen levels indicative of a future upwelling environment. For this experiment, the treatment tanks were round 7.5L buckets with lids. The larvae were protected from the flowing seawater recirculation by containment in a smaller nested bucket that freely exchanged seawater with the main tank. Both treatments were held at an average temperature of 17.2°C. Alkalinity varied only slightly over the week, and consequent control of pH and oxygen levels were very good (Table 3.1 and Figure 3.3).

Table 3.1. Average \pm standard deviation for chemical parameters during experiment M7. The $p(\text{CO}_2)$, Ω_{calcite} , and $\Omega_{\text{aragonite}}$ reported here were calculated using CO2calc (Robbins et al., 2010) with dissociation constants from Mehrbach et al. (1973) as refit by Dickson and Millero (1987). For most values $n = 8$, except for oxygen for which some samples from each tank were lost (see Figure 3.3).

	Temp ($^{\circ}\text{C}$)	Salinity	Alkalinity ($\mu\text{mol kg}^{-1}$)	$\text{pH}_{(\text{in situ})}$ total scale	Oxygen ($\mu\text{mol kg}^{-1}$)	Calculated $p(\text{CO}_2)$ (μatm)	Calculated Ω_{Calcite}	Calculated $\Omega_{\text{Aragonite}}$
Treatment A								
Replicate 1	17.2 ± 0.3	33.65 ± 0.01	2249.3 ± 6.1	7.924 ± 0.004	230.9 ± 2.2	546.1 ± 5.8	3.09 ± 0.03	1.99 ± 0.02
Replicate 2	17.2 ± 0.2	33.64 ± 0.01	2250.4 ± 5.9	7.905 ± 0.007	227.7 ± 1.3	574.6 ± 9.5	2.96 ± 0.05	1.91 ± 0.03
Treatment B								
Replicate 1	17.0 ± 0.4	33.66 ± 0.01	2254.1 ± 7.0	7.619 ± 0.007	86.2 ± 3.4	1188.4 ± 17.7	1.64 ± 0.03	1.05 ± 0.02
Replicate 2	17.2 ± 0.3	33.65 ± 0.01	2250.5 ± 4.0	7.612 ± 0.005	83.9 ± 2.7	1209.8 ± 15.8	1.62 ± 0.02	1.04 ± 0.01

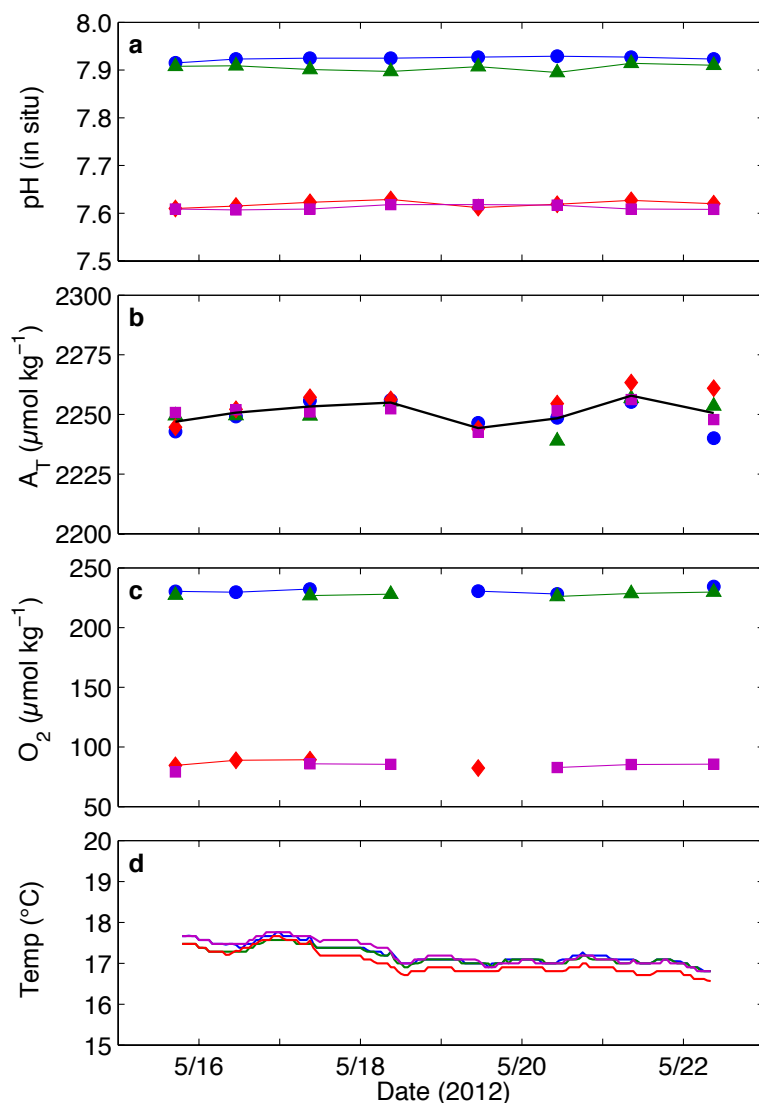


Figure 3.3. Measured data from experiment M7. (a) pH: total pH scale; (b) A_T : the solid black line connects the daily average for all four tanks; (c) oxygen; and (d) temperature. Symbols indicate discrete samples: Treatment A Replicate 1 (blue circles), Treatment A Replicate 2 (green triangles), Treatment B Replicate 1 (red diamonds), and Treatment B Replicate 2 (purple squares).

In addition to the discrete sampling, a Honeywell Durafet® pH sensor was used throughout the experiment to monitor the carbonate chemistry on short timescales, switching between tanks daily. The Durafet sensor data in Figure 3.4 show day-long variability in pH, beginning each day when the sensor's location was changed. Four consecutive examples are given, one from each tank. Some fluctuation is seen, possibly a result of temperature changing throughout the day, or a respiration signal in response to the 12 h light cycle. This fluctuation is possible due to the somewhat passive approach to controlling the carbonate chemistry in the current system design, supplying a constant composition gas mixture to each Liqui-Cel. Such fluctuations could be damped if a more active approach to pH control was taken, using information gathered by chemical sensors in the tanks as a basis to adjust the gas composition supplied to the Liqui-Cels, to compensate for divergences from the desired seawater chemistry. Adjustments to the gas mixture would not only dampen or eliminate the small diurnal signal seen, but would also ensure that large changes in seawater chemistry did not occur over the course of the experiment.

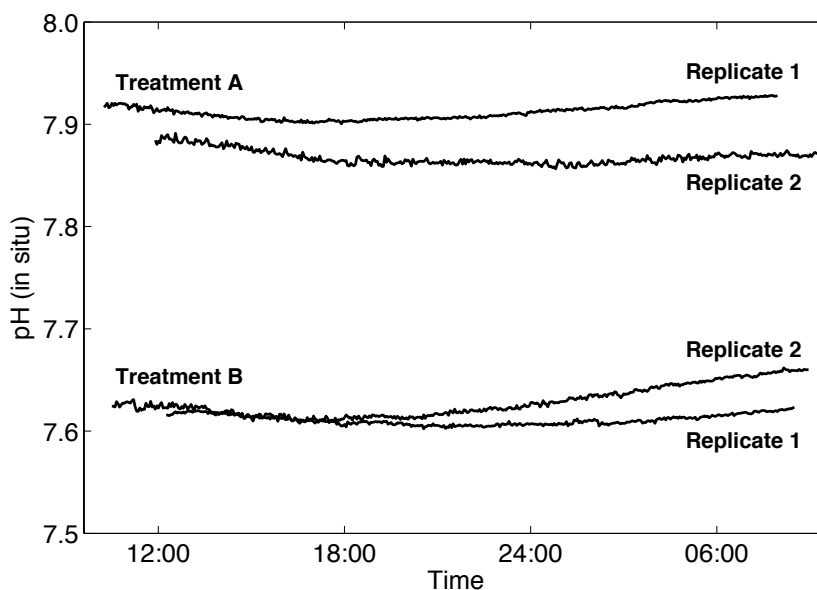


Figure 3.4. pH recorded by a single Durafet sensor. The sensor was used to monitor each of the four tanks on four consecutive days. Data begins and ends at the time the sensor was moved each day, allowing for sensor equilibration once placed in the tank.

3.3.2 Experiment S32

The system can be used on much longer timescales than a week, demonstrated by a 32-day experiment investigating the impacts of varied pH and oxygen levels on squid embryos (*Doryteuthis opalescens*). The experiment was performed in square 50 L insulated tanks, with the squid egg capsules attached to the bottom. Some turbulence was caused by the recirculation of the seawater for equilibration. Target pH and oxygen levels were chosen based on values recorded at a location near Scripps (Nam et al., 2011). For this experiment, low pH and high oxygen levels were paired in one treatment, and high pH and low oxygen levels in the other, to attempt to understand organismal responses to the individual parameters. This is in contrast to experiment M7, which paired low oxygen and low pH, thus demonstrating the system flexibility and independent control of chosen seawater chemistry. The longer duration of this experiment reflects a growing need in the

scientific community to understand the effects of chronic exposure to low pH on organisms. Results from discrete samples indicate adequate control for a successful biological experiment, even over this extended period (Table 3.2 and Figure 3.5). However, there are clear discrepancies from target values and both gradual and abrupt changes occur during the experiment, some of which are easily explained.

The control of the seawater chemistry in MSEAS is based on the mole fraction of CO₂ and O₂ in the gas that is supplied to the Liqui-Cel for equilibration. Any changes in that mole fraction will be apparent in the resulting seawater chemistry. Throughout experiment S32 several deliberate changes were made to the control parameters: on 11 March 2012, the amount of oxygen in the gas mixture was increased from 5.4% to 6.9% of the total gas flow in Treatment A, and from 19.7 % to 20.1 % in Treatment B. The subsequent increase in the dissolved oxygen content of the seawater on that day is apparent in Figure 3.5. Similarly, the CO₂ fraction in the gas of Treatment B was increased from 1500 ppm to 1600 ppm during the experiment, likely causing the decrease in pH seen. The abrupt increase in seawater temperature of all tanks beginning 23 March 2012 results from a deliberate increase in the temperature setting of the thermostat baths. These changes are reflected in Table 3.2 by the much larger standard deviations for pH, oxygen level, and temperature than were observed during experiment M7. The reason for the significant pH decrease in Treatment A Replicate 2 on 24 March 2012, which is then maintained the rest of the experiment, is unknown, although it may indicate problems with the particular Liqui-Cel that was in use on the tank.

Table 3.2. Average \pm standard deviation for chemical parameters during experiment S32. The $p(\text{CO}_2)$, Ω_{Calcite} , and $\Omega_{\text{Aragonite}}$ reported here were calculated using CO2calc (Robbins et al., 2010) with dissociation constants from Mehrbach et al. (1973) as refit by Dickson and Millero (1987). For most values $n = 29\text{--}32$, except for oxygen for which some samples from each tank were lost (see Figure 3.5).

	Temp ($^{\circ}\text{C}$)	Salinity	Alkalinity ($\mu\text{mol kg}^{-1}$)	$\text{pH}_{(\text{in situ})}$ total scale	Oxygen ($\mu\text{mol kg}^{-1}$)	Calculated $p(\text{CO}_2)$ (μatm)	Calculated Ω_{Calcite}	Calculated $\Omega_{\text{Aragonite}}$
Treatment A								
Replicate 1	11.2 ± 0.5	33.50 ± 0.07	2239.1 ± 5.5	7.923 ± 0.035	86.4 ± 8.3	540.7 ± 48.7	2.49 ± 0.15	1.58 ± 0.10
Replicate 2	11.6 ± 0.5	33.51 ± 0.05	2241.8 ± 4.5	7.908 ± 0.072	83.0 ± 12.9	570.4 ± 107.9	2.46 ± 0.32	1.57 ± 0.21
Treatment B								
Replicate 1	11.3 ± 0.5	33.49 ± 0.07	2241.1 ± 5.8	7.559 ± 0.029	241.1 ± 9.1	1337.3 ± 97.4	1.15 ± 0.06	0.73 ± 0.04
Replicate 2	11.6 ± 0.6	33.51 ± 0.06	2244.2 ± 7.1	7.552 ± 0.026	241.7 ± 7.6	1364.2 ± 88.1	1.15 ± 0.06	0.73 ± 0.04

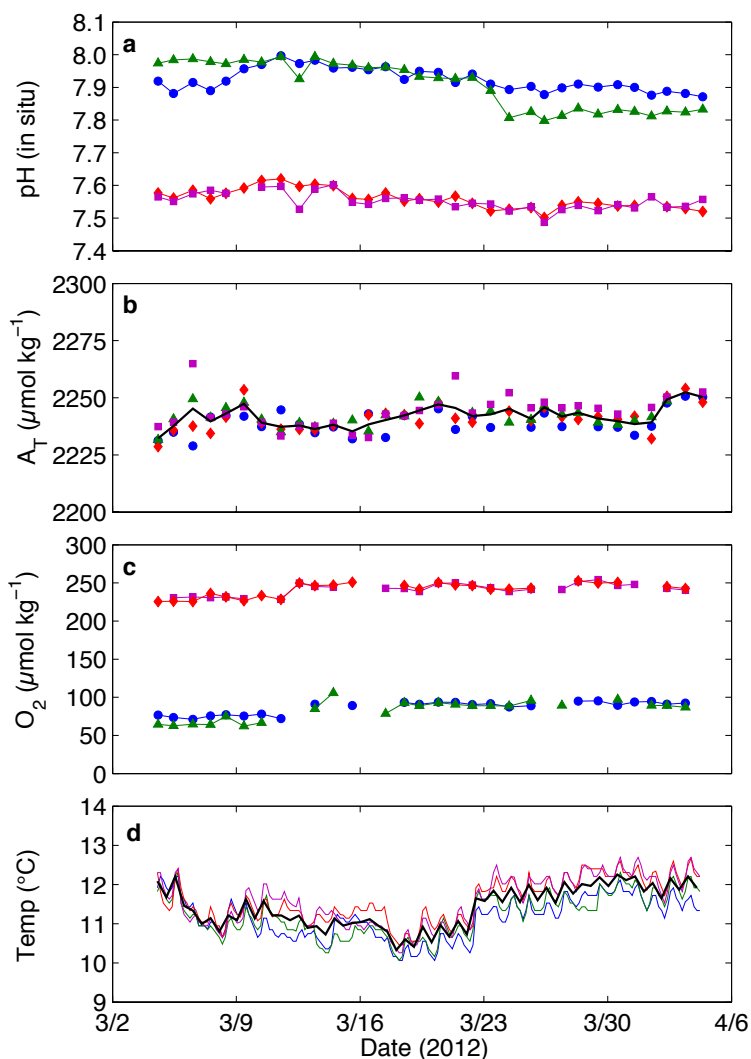


Figure 3.5. Measured data from experiment S32. (a) pH: total pH scale; (b) A_T : the solid black line connects the daily average for all four tanks; (c) oxygen; and (d) temperature – the thick black line is the average of all tanks calculated at 12 h intervals. Symbols indicate discrete samples: Treatment A Replicate 1 (blue circles), Treatment A Replicate 2 (green triangles), Treatment B Replicate 1 (red diamonds), and Treatment B Replicate 2 (purple squares).

Probably the easiest way to improve the consistency of pH and oxygen levels in this system would be to improve the seawater temperature control. In both example experiments, the temperature of the treatment seawater in the tanks was influenced in part by the room air temperature, which was strongly influenced by the San Diego weather. This effect was much stronger in S32, with a daily seawater temperature cycle of approximately 0.5 °C occurring throughout most of the experiment. This likely contributed to the poorer control over the carbonate chemistry compared to experiment M7. Such temperature changes strongly influence the carbonate parameters; a 1°C change in temperature causes about a 0.015 change in pH and a 20–50 μatm change in $p(\text{CO}_2)$. Between experiments S32 and M7, the location of MSEAS was moved from an uninsulated building (located at the Birch Aquarium at Scripps) to a more protected location (the Scripps Experimental Aquarium facility). This change in location likely helps account for the difference in seawater temperature control, as there was less room temperature variability in the second location.

3.4 Conclusions

MSEAS will be useful to help elucidate responses of organisms to expected future ocean scenarios, which involve changes to multiple physical and chemical parameters. The system design allows for manipulation of any one or multiple of the three control parameters: CO_2 concentration, O_2 levels, and temperature. This independent control is a potentially useful experimental approach for investigating drivers underlying organismal responses. The automated prototype presented here is easily scalable to larger numbers of replicates by splitting the gas line (provided one ensures adequate gas flow) and adding

Liqui-Cels for each tank. Implementation of additional simultaneous treatments requires more mass flow controllers for creation of a separate gas composition, in addition to Liqui-Cels and tanks.

The use of Liqui-Cel membrane contactors in the system design allows for rapid equilibration between the gas and seawater. Equilibration by bubbling can be quite slow depending on the volume of seawater needed (Schulz et al., 2009). Membrane contactors also eliminate any concern over the direct impacts of bubbling on the experimental organism, and the continual cycling of the water through the Liqui-Cels allows for well-controlled experiments over a long time period. The system is well suited to convert into one with feedback from chemical sensors in the individual aquaria, thus achieving a more active control over the carbonate chemistry. Such active control of each tank will allow for the transition of the system to one with intentional variability in each of the three controlled parameters. There is a growing need to understand the responses of organisms that live in variable environments, whether weekly, daily, or tidal timescales of variability, and how they may change in the future (Andersson and Mackenzie, 2012; Dufault et al., 2012).

MSEAS has been used successfully to study several organisms, life stages, and parameters. The flexibility of the system design has allowed for experimental organisms ranging from mussel larvae (C. A. Frieder, Experiment M7) to juvenile abalone (White, 2011) to adult oysters (M. Tresguerres, unpublished data, 2011). Experiments on moon jellies have been completed modifying only the oxygen levels in the seawater (Cawood, 2012), while in the experiment on adult oysters, O₂ levels were held steady between treatments while CO₂ concentration and temperature were modified. These examples

indicate the large range of possible biological questions that can be examined using MSEAS, to expose a variety of species to future ocean conditions.

3.5 References

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CHAPTER 4

Carbonate chemistry variability in an experimental aquarium

Recognizing the degree of temporally and spatially dynamic variability in oceanic carbon dioxide conditions has led to questions regarding the anthropogenic impact on the chemistry of these variable environments and on the organisms that reside in them. As researchers turned to the laboratory to investigate the impacts of climate change on organisms, many experiments were performed under constant pH conditions. While useful, these conditions do not match those experienced by many organisms in the ocean. Including carbon dioxide variability in laboratory studies will make experiments more comparable to the in situ environments experienced, and thus will give more accurate insight into the impacts of ocean acidification on organisms. The few previous attempts to study variability in the laboratory have mostly failed to mimic these environments well, often creating an oscillating environment by manually moving the experimental organism back and forth daily between separately controlled treatments of different pH or $p(\text{CO}_2)$ levels. These results could be problematic if organisms have an unrecognized response to this extremely rapid change in environment, which might be attributed to a response to the environmental variability rather than the experimental design. Presented here is an enhancement to the previously described aquarium control system, the Multiple Stressor Experimental Aquarium at Scripps (MSEAS), which controls the pH and the oxygen content of seawater using equilibration with a custom gas mixture. The system is

capable of mimicking natural environmental variability in pH for a variety of magnitudes and timescales characteristic of the diverse oceanic environments around the world. Tests of the control system are shown for different scales and types of variability including diel, semi-diurnal (tidal), upwelling, and slowly changing. This aquarium, with careful carbonate chemistry control, and the ability to mimic environmental variability, will help to elucidate the organismal impacts of this variability and of increasing oceanic carbon dioxide.

4.1 Introduction and motivation

Ocean acidification refers to the general increase of anthropogenic carbon dioxide in our oceans and the resulting decrease in pH. Predictions of this acidification from models based on IPCC scenarios estimate carbonate ion concentration decreases throughout the surface oceans (Rhein et al. 2013). However, it is hard to predict the impact of this carbon dioxide on the varied ecosystems of the oceans, or to understand the timescales on which changes will occur. Model studies have recently become more regionally focused and able to incorporate some chemical variability (Bjork et al. 2014; Hauri et al. 2013; Popova et al. 2014), but usually on longer timescales (seasonal, annual), important to long-term forecasting. Even those predictions that do include variability are limited by the timing or type of variability and the spatial coverage.

Environmental Variability

Organisms often live in localized environments, which can have seawater chemistry that deviates significantly from general expectations for that region. For example, in coral reef systems daily $p(\text{CO}_2)$ fluctuations can be quite large due to net

ecosystem calcification and production (Bates et al. 2010). In fact, dynamic temporal ocean chemistry on scales that are significant to organisms may be pervasive, rather than the exception, in shallow-water coastal environments. A compilation of high-frequency data from around the world by Hofmann et al. (2011) highlights that in addition to average pH varying among ecosystems, the magnitude of daily variability differs substantially. The spatial variability of carbonate chemistry can also be quite remarkable in environments influenced by upwelling. In 2007, across a large portion of the continental North American shelf of the Pacific Ocean, the depth of the saturation horizon varied spatially between the surface and 200 m depending on latitude (Feely et al. 2008). Also, localized undersaturation of surface waters with respect to aragonite, at levels not predicted for the open ocean until 2050, were observed. In this location, as well as in other environments, $p(\text{CO}_2)$ already often exceeds that expected from equilibration with the atmosphere (Andersson and Mackenzie 2012).

There is some discussion as to whether this environmental variability might be an advantage to certain organisms. More research is needed, particularly regarding the physiological tolerance and phenotypic plasticity of organisms, to give an understanding of the capacity for organism acclimatization to ocean acidification conditions (Hofmann et al. 2010). It is speculated that organisms which regularly experience large natural variability will have differential susceptibility to changes in the chemistry of their environment (Hurd et al. 2011; Johnson et al. 2014), or a greater capacity to adapt (Hofmann et al. 2014). These potential responses are particularly important in light of the many results regarding organismal response to ocean acidification coming from studies performed in laboratory experimental aquaria. For the most part these experiments have

been performed with steady, or near-steady CO₂ conditions (McElhany and Busch 2013). While constant conditions are appropriate for some organisms and regions of the ocean, and can be useful for investigating mechanisms of response, they may not be appropriate for many studies. Experiments investigating the impacts of ocean acidification on organisms must reflect the altered environments that have been identified, both in the normal average pH experienced and in the magnitude of regular variability (Andersson and Mackenzie 2012).

The concept of environmental variability and its impact on organisms is not new or unique to carbonate chemistry. Insight into potential biological responses to environmental variability may be obtained by looking to research on thermal fluctuations, which has documented differing biological outcomes when compared to stable conditions (Christiansen and Costlow 1975; Coles 1975; Oliver and Palumbi 2011; Putnam et al. 2010). These results forecast how organisms might respond to pH oscillations. In fact, for organisms particularly well-suited or accustomed to variability in pH, early experiments exposing them to a constant high-CO₂ environment actually overestimated the expected impacts of ocean acidification (Comeau et al. 2014; Dufault et al. 2012), as oscillations allow amelioration of the impacts. Most likely organism response will be some function of the magnitude of pH fluctuation and the duration (Shaw et al. 2013b), both of which are expected to alter with anthropogenic changes.

For any given experiment, determining the drivers of the variability of interest, and which carbonate parameters are thought to be relevant, is critical to the experimental design. Additionally, determining the mean CO₂ levels for study may be complicated. When considering anthropogenic changes to a regularly varying system, some

determination must be made of when a threshold relevant to organisms has been crossed. Although this will probably be organism and ecosystem specific, the idea can be approached generally using conceptual models. Discussing annual variability of pH in the California Current at the surface and 100 m depth, Hauri et al. (2013) define the annual range of variability, or pH “envelope” for preindustrial times, and for the current ocean. Organisms in this environment are used to experiencing the pH of this entire envelope, at least for some part of the year. What they find though is that model predictions of surface pH decrease quickly, leaving the current pH envelope by year 2040, and even sooner in the Southern region and at depth (Hauri et al. 2013). This means that within 30 years, some organisms will experience entirely different carbonate chemistry environments on annual timescales, with lower pH. A similar model can be considered for diurnal fluctuations in pH or $p(\text{CO}_2)$. Given a typical fluctuation in pH and a critical threshold, as the mean pH of the ocean decreases, a growing portion of the typical fluctuation will be below this threshold. Shaw et al. (2013b) define the initial onset, when the natural variability first crosses the critical threshold, the time of non-varying onset, when the environment would have crossed the threshold in the absence of variability, and finally the time of permanent onset. At this point, the fluctuating pH is completely below the threshold for the entire day and organisms are experiencing an entirely new environment. These landmarks help us consider how the exposure time of organisms to harmful CO_2 levels may be just as important as the magnitude of pH variability (Shaw et al. 2013b). In reality, variability may provide something of a relief for organisms, as it maintains their environment above the critical threshold for some part of each day in the near future. On the other hand, the natural variability on the upper limit of a CO_2 tolerance will take an

organism past their threshold on a daily basis, if only for a short time. Whether the exact pH exposure resulting from variability will be a reprieve or harmful will depend on the organism in question and its sensitivity to high CO₂. This has, almost certainly, complicated our current understanding of the biological response of organisms to ocean acidification.

Laboratory Experiments

Experimental aquaria with controlled carbonate chemistry and capable of mimicking natural variability, designed with the intention of performing experiments on organisms, will become more important as the field advances. The contribution of the many laboratory manipulation experiments to the understanding of organism response to ocean acidification and multiple stressors is important. Enabling the ability to vary the parameters of interest makes the chemistry more realistic from an organism's perspective. However, only a handful of experiments have been published to date with the goal of investigating the impacts of semi-diurnal, diurnal or intermittent exposure to low pH, high-CO₂ environments.

The first pH variability experiment published (Dufault et al. 2012) examined calcification and survival in coral recruits. In addition to constant ambient and high-CO₂ treatments, coral recruits attached to coverslips were moved back and forth between these treatment extremes at 7:00 and 19:00 daily. This created an oscillating environment like that might be observed at Hobihu Reef, Nanway Bay where the organisms were originally collected, although with a larger magnitude of oscillation. A recent experiment on adult branching corals was very similar in experimental design, but included an extreme variable treatment: corals were moved between seawaters with $p(\text{CO}_2)$ 400 μatm

and 2000 μatm (Comeau et al. 2014). They found increased resistance to ocean acidification conditions in corals that had experienced these large fluctuations, indicating a habitat-dependent response to ocean acidification. Johnson et al. (2014) found a comparable response in crustose coralline algae, although they only exposed the organism to the elevated $p(\text{CO}_2)$ treatment for 6 hours per day rather than 12. Instead of adjusting the timescale of variability, Alenius and Munguia (2012) varied their pH differences from the stable treatment each day, producing a randomized variable treatment. However, they found increased mortality in their variable treatment, as opposed to the increased resistance or amelioration of negative ocean acidification impacts observed with other oscillations. Similarly, Cornwall et al. (2013) had lower growth in their fluctuating treatments meant to mimic a kelp forest at Karitane, near Dunedin, New Zealand, when compared to the similar stable treatment.

Of the few systems that report the ability to study pH fluctuations on organisms in the laboratory, many seem to employ a “manual” approach to oscillating pH. During the day the organism is kept in a high pH environment; at night the organism is removed from the high pH treatment and switched to a low pH environment (Dufault et al. 2012; Kim et al. 2013). Even some of the more elegant systems, appear to involve large changes in pH over very short timescales, faster than would be experienced in the environment (Alenius and Munguia 2012; Cornwall et al. 2013). What implications this could have on their experimental results, as the rate of change of pH may influence an organism’s fitness, is unknown. Frieder et al. (2014) is the first known laboratory experiment to include fluctuations in pH on timescales relevant to the natural

environment. That study found alleviation of decreased mytilid mussel developmental rates caused by low pH when semi-diurnal variability was included.

There are some systems capable of including the natural variability characteristic of the local environment. Jokiel et al. (2014) recently published a low-cost method for control of a flow-through mesocosm system that tracks the variability of seawater conditions in Kaneohe Bay, Hawaii, with a fixed offset for an acidified treatment. The system has been used previously for studying coral response to ocean acidification (Andersson et al. 2009; Jokiel et al. 2008; Kuffner et al. 2008) and will have clear applications in future variability studies. In situ manipulations of carbonate chemistry are another important advancement in experimental ocean acidification studies. Originally developed at MBARI, techniques using Free Ocean CO₂ Enrichment (FOCE) allow for controlled CO₂ perturbation experiments directly on the seafloor (Walz et al. 2008). A study by Kline et al. (2012) utilizes a system modified from FOCE, that maintains pH at a given offset from the natural environment on a reef flat. While useful for capturing the natural environment and variability, these systems limit the user to the natural conditions where a lab is located. They also do not provide control over parameters other than pH or $p(\text{CO}_2)$, which while giving the aquaria a natural quality, potentially make results more difficult to interpret.

These early experimental results provide motivation for designing an aquarium system capable of performing oscillations in a more elegant and controlled way, which will allow for more realistic mimicking of the natural environment. The choice of ecologically relevant environmental conditions when performing laboratory manipulations will help elucidate organismal responses to climate change. Researchers

have begun by improvising current aquarium designs, and manually moving organisms between treatments in order to recreate environmental fluctuations. While this provides a reasonable first approximation at mimicking the chemical variability observed in the natural environment, the aquarium system presented in this Chapter offers a more robust and elegant way to make these changes. The user has more control over the carbonate chemistry and oxygen content, and any questions over tank effect, or the impact of moving organisms and their sudden exposure to different conditions, will be alleviated.

4.2 Aquarium design

For this study, the Multiple Stressor Experimental Aquarium at Scripps (MSEAS) control system described in Chapter 3, and published as Bockmon et al. (2013), was used for the initial design. From there, upgrades and changes were made to introduce additional control and variability in the carbonate chemistry. MSEAS achieves the desired chemistry in the aquaria by equilibrating a custom gas mixture with seawater using a Membrana Liqui-Cel[®] membrane contactor. The gas mixture is created from N₂, O₂, and CO₂ gases using mass flow controllers operated from a custom LabVIEW[™] program. The seawater from each individual tank recirculates through the tank's corresponding Liqui-Cel[®] for equilibration with the created gas mixture. The constant recirculation helps to maintain the desired chemistry, or to introduce a change quickly. The system can be operated as either flow-through or static in terms of the seawater turnover. Most often, a flow-through design makes sense, where “fresh” seawater is added to the tank at a constant rate and the replaced water flushes out of the tanks to waste.

The advantage of MSEAS explored in Chapter 3 is the ability to control multiple parameters at the same time (CO_2 , O_2 , and temperature), potentially simulating the natural environment in a more real way, and offering the possibility of multi-stressor experiments. This quality is maintained in this version, with the addition of several exciting new features. First, monitoring of the chemistry in real-time was added by including autonomous sensors in the tanks. The inclusion of these sensors allowed for feedback from the tanks to the gas mixing system, to improve the stability of pH in the tanks. Finally, the ability to control how these parameters change with time was added. Although for now only pH is controlled dynamically providing a wide range of flexibility in the chosen pH levels and the desired variability, it is also possible to vary oxygen using this system design because of the continuous control over the gas mixture passing through the Liqui-Cel[®].

The goal of this aquarium system is to simulate natural environmental variability where the instantaneous carbonate chemistry can be considered to be a function of A_T and pH at a given temperature and salinity. The system is designed to control temperature by the removal and addition of heat using a thermostat system. For MSEAS there is no intent to control A_T but to instead focus on the control of pH. However, as discussed in Bockmon et al. (2013), the assumption of constant A_T is reasonable for this system, as the source water for the aquarium has been shown to have a range of only $50 \mu\text{mol kg}^{-1}$ over a three-year period. More often A_T is similar from one day to the next. Regular measurement of A_T throughout an experiment, such that A_T is assured to be known within $20 \mu\text{mol kg}^{-1}$ will ensure adequate control. This level of uncertainty in A_T produces errors in $p(\text{CO}_2)$ and $[\text{CO}_3^{2-}]$ of less than 1%; $\sim 4 \mu\text{atm}$ at high pH and up to $30 \mu\text{atm}$ at

extremely low pH. With the A_T assumed to be constant and known, pH can be controlled to create the desired chemical environment.

A Honeywell Durafet[®] pH sensor is therefore included in each tank. This gives continuous pH and temperature data, which couples with the known A_T to constrain the carbonate chemistry fully. The inclusion of this sensor also allows for feedback to the gas mixing system from the experimental tanks, so that pH can be controlled more actively and accurately. In Chapter 3, the system set the CO₂ concentration of the custom gas mixture to the desired value and then assumed constant equilibration with the seawater to control the carbonate chemistry. While this worked adequately, it allowed changes beyond the user's control to vary the carbonate chemistry and alter the system from a perfectly constant state. For example, changes in temperature, incomplete equilibration, or modifications by the study organism all potentially caused undesired variability in the seawater pH. For this improved version of MSEAS, the gas control system has been redesigned, so that adjustments to the CO₂ concentration of the gas mixture can be made in real time to counteract any undesired pH deviations. A ΔpH is calculated as the difference between the pH measured by the Durafet and the target pH, and then proportional-integral-derivative (PID) control software is used to eliminate the ΔpH by adjusting the CO₂ level of the gas mixture. Calculations between pH and $x(\text{CO}_2)$ are done at constant A_T , salinity, and the measured temperature, using the program CO2SYS in MATLAB[®] (Van Heuven et al. 2011) running through LabVIEW[™].

This also means that the system is now using pH as the input parameter of control, in contrast to the choice of $p(\text{CO}_2)$ in the previous version of MSEAS. While $p(\text{CO}_2)$ of the seawater is the actual parameter being controlled by changing the $x(\text{CO}_2)$ of the gas

mixture, the choice of pH for the target chemistry has several advantages. The use of the Durafet to continuously monitor the chemistry of the system makes the choice of pH for the control parameter logical. Controlling and measuring the same parameter eliminates any need for calculation of parameters and the assumptions and errors that come with calculation. Compared with available $p(\text{CO}_2)$ sensors, the Durafet has a fast response, is affordable, easy to use, widely available, and communicates easily with the control computer, which make it good choice in this aquarium environment. Most importantly, it has been shown to be relatively stable over months (Martz et al. 2010), meaning that with regular checks the data can be trusted and the sensor does not need to be recalibrated as frequently as a glass electrode would.

4.2.1 Addition of variability in pH

As variability in pH is added to the system, the basic control principles remain the same. However, instead of a constant target pH, the target pH is now changed with time, to produce the desired variability. The sensor feedback remains the same, calculating ΔpH from the measured pH and the target pH at any given time. The desire to change pH quickly will necessitate that the CO_2 of the gas mixture will sometimes be quite high, or even zero, depending on the direction of desired change. The large difference between the seawater $p(\text{CO}_2)$ and the $x(\text{CO}_2)$ of the gas mixture introduced to the Liqui-Cel[®] will ensure that changes to the CO_2 of the seawater, and consequently to the pH, can be made quickly.

For regular sustained oscillations, the system has been designed to create the desired fluctuations automatically. The user inputs the high and low pH for the system to

oscillate between, along with the chosen period of oscillation, and the date and time the system will start and stop. The system then creates linearly changing pH variability, going from the high pH setting to the low pH setting in the designated amount of time, and then repeating indefinitely. This gives smooth, regular changes, useful for study of an organism that experiences changing pH with a tidal cycle, whether diurnal or semi-diurnal, or any other intermediate interval. It will produce something of an idealized fluctuation, likely more smooth and regular than found in nature.

However, the system does not require that the pH vary or fluctuate in a regular way, repeating the same values each day. Instead, the system can reproduce irregular changes in pH that might be more realistic for many natural environments. In this case, the variability in pH is achieved using a user-inputted text file containing columns with the desired pH levels and date and time of change. This might be variability contrived for a specific purpose or data recorded by sensors in the environment of interest. The LabVIEW™ control VI sources the text file and determines whether it is time for a pH change based on the date and time stamp given in the file. Once the date/time on the computer exceeds the date/time indicated by the file, the new pH is read into the program, and the mass flow controllers are adjusted accordingly. Changes in pH should not be set to intervals of less than 15 minutes to allow the system to adjust to the changed gas CO₂ concentration. However, with the large water volume typically used for the tanks, the system takes longer than 15 minutes to reach equilibrium, so there is some delay inherent in the response, especially if the steps in pH are large. Changes can occur at much longer intervals as well, for example daily, for acclimation experiments or other experiments needing a slowly changing pH.

As currently designed, the system cannot mimic changes in the natural environment that occur because of changes in the A_T of the seawater. Since both the carbonate chemistry control and the introduction of variability are done using gas exchange of CO_2 , only the $p(\text{CO}_2)$, pH, and C_T of the seawater are changed. A_T is a conservative property and is not altered by the addition or removal of CO_2 from seawater. This feature is an advantage for the control of the carbonate system in this design, requiring us to control just one parameter. However, it does limit the ability to mimic pH changes in the natural environment induced by a change to the A_T of the seawater. Fortunately, such changes are generally minor, as changing A_T by $50 \mu\text{mol kg}^{-1}$ only changes pH by about 0.01 pH units at constant $p(\text{CO}_2)$. Although not technically ocean acidification, changes in A_T are interesting and important, and should not be overlooked for their control on the organisms' environment or how organisms may be impacted by climate change.

4.3 Carbonate chemistry variability and controls

The primary motivation for this aquarium system is to facilitate better understanding of organism and ecosystem responses caused by the addition of carbon dioxide to our oceans. While the addition of anthropogenic carbon to a stable ocean in equilibrium with the atmosphere is easy to understand and predict, the addition of anthropogenic carbon to an ocean that experiences variability in carbon parameters is not necessarily as straightforward. However, paying attention to the change in variance of ecosystems as well as to the mean change in pH is extremely important. MSEAS is designed with these challenges in mind, with the hope of adequately reproducing the

expected changes. As stated above, MSEAS controls the carbonate chemistry by control of pH at a constant A_T , while the increase in carbon dioxide in our atmosphere actually increases $p(\text{CO}_2)$ and C_T directly, with the resulting decrease in pH a consequence of the addition of CO_2 .

The choice of parameter to vary can have important implications for the chemistry of the system. Because the system is non-linear, addition of anthropogenic CO_2 will not change all parameters by the same magnitude. Fig 4.1 shows a model diel oscillation in pH, between 7.5 and 7.9 pH units and the resulting pH when a constant change is applied to either pH (subtracting 0.3 pH units), $p(\text{CO}_2)$ (adding 1000 μatm), or C_T (adding 100 $\mu\text{mol kg}^{-1}$), at constant A_T . These changes are purely chemical, disregarding any effects of biology on the system. Each model, all producing a reduction in pH of approximately 0.3 pH units at the mean pH, produces a clearly different pattern of variability. As expected, the shape of oscillation for the reduced pH remains the same, while an increase in $p(\text{CO}_2)$ reduces pH oscillation and an increase in C_T magnifies the oscillation. These changes are due to the relationship between the parameters at constant A_T . Temperature changes also plays a role in the shape of these curves, most noticeably with an increase in temperature reducing the pH changes due to the C_T oscillation. At low temperatures, the original and reduced oscillations more closely resemble each other. Due to the logarithmic scale, a change in pH from 7.7 to 7.4 pH units is essentially a doubling of the hydrogen ion concentration – not a small change. This means that when considering different methods of modifying CO_2 levels in seawater, care must be taken, because substantially different results can be produced.

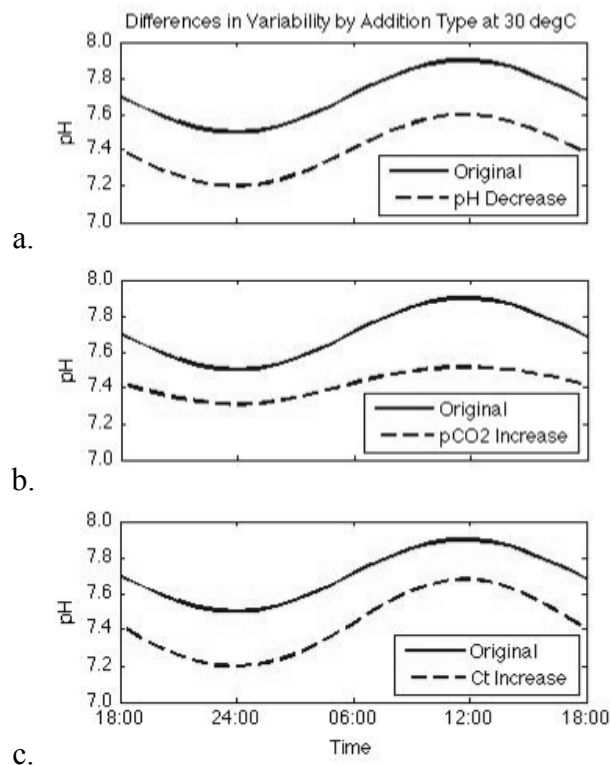


Figure 4.1. Modeled changes to pH at constant A_T and 30 °C when a steady change is applied to a) pH, b) $p(\text{CO}_2)$, and c) C_T .

The pH of seawater is essentially controlled by the ratio A_T/C_T . This is particularly important and useful in the context of MSEAS, where we assume a constant A_T as part of the chemistry control. This simplifies our thinking considerably, as we can ignore A_T and think about variability and ocean acidification in terms of increasing or decreasing C_T , and its effect on the A_T/C_T ratio. Importantly, at higher C_T , any incremental increase in C_T causes a larger resulting decrease in pH (Figure 4.2). This change is the most sensitive near where $C_T=A_T$, decreasing above and below that point (Egleston et al. 2010). This weakening of the buffering capacity of the ocean is also represented by the Revelle factor (Equation 1),

$$R = \frac{\Delta[\text{CO}_2]/[\text{CO}_2]}{\Delta C_T/C_T} \quad (1)$$

a measure of resistivity to the ocean absorbing carbon dioxide. As C_T increases, so does the Revelle factor. With the continued increase of anthropogenic CO_2 in the ocean, the buffering capacity will continue to weaken, resulting in greater variability and more extreme pH and $p(\text{CO}_2)$ environments (Shaw et al. 2013a). Similarly, environments with lower A_T have a lower buffer capacity and consequently greater variability in pH and $p(\text{CO}_2)$ (Schulz and Riebesell 2013). In several instances, this response has also been observed as enhanced pH sensitivity to respiration causing hypoxia. As respiration decreases the pH and oxygen of an ecosystem, the change in pH caused by ocean acidification is magnified (Cai et al. 2011; Melzner et al. 2013).

This discussion of buffering capacity and the control of pH in seawater gives us a context to examine the response of seawater pH to various biological and physical processes. In particular, it will help in examining the drivers of seawater pH variability and the expected changes to the variability with changing ocean chemistry. Furthermore, the changes will be mediated by the impact of ocean acidification and climate change on organisms that control the variability, should it be biologically driven. Predicting changes to the chemistry is difficult without a complete understanding of the organismal response.

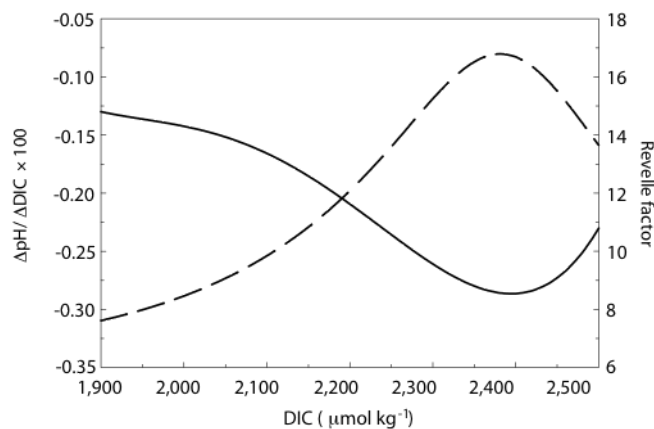


Figure 4.2. Modified from Figure 4 of Cai et al. (2011). Seawater buffering capacity (solid line) and the Revelle factor (dashed line) as a function of C_T .

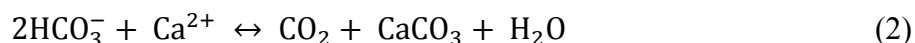
4.3.1 Biological controls of carbonate chemistry

Organisms constantly modify the chemistry of the seawater they reside in. In many cases, this modification itself creates the carbonate chemistry variability of interest. The exact type of modification and its magnitude depends on the particular organism and the environment where it resides. These fluctuations are generally the type we aim to mimic. The processes that control the chemistry in the ocean also will impact the chemistry in a laboratory setting and so must be accounted for or opposed. The constant control of CO_2 levels of this aquarium design, coupled with the pH feedback system allows MSEAS to counteract the undesired changes that organisms can make to the seawater chemistry.

One of the primary ways that organisms modify the CO_2 of seawater is through primary production (generally photosynthesis) and respiration – both autotrophic and heterotrophic. During the day, primary production is generally greater than community respiration, so a drawdown of CO_2 and increase of O_2 is observed. The drawdown of CO_2 decreases $p(\text{CO}_2)$, decreases C_T , and increases pH, creating a more hospitable

environment for calcification (Gattuso et al. 1999). At night, respiration produces CO_2 , lowering pH and the saturation state. While the uptake and release of CO_2 does not itself change the A_T of the seawater, small changes can be observed due to nutrient uptake and release.

Calcification and dissolution also play an important role in producing changes to the carbonate chemistry of an ecosystem. Net ecosystem calcification is often defined as the difference between the production of CaCO_3 and the dissolution. Calcification draws down both C_T and A_T in the proportion 1:2, but in addition releases CO_2 (Equation 2).



This can also have a strong diurnal cycle, partially as a response to the changes in the chemistry due to photosynthesis and respiration. At night, when pH is low from the release of CO_2 during respiration, dissolution increases. Also, calcification has been shown to be on average three times higher in light versus dark in a coral reef community (Gattuso et al. 1999), at least partially because of the higher pH during the daylight hours in response to photosynthesis. With further ocean acidification causing changes to calcification and dissolution processes, projections indicate a net loss of CaCO_3 from coral reef communities in the future (Kleypas et al. 1999). In mesocosm experiments net ecosystem calcification has been shown to be lower under elevated $p(\text{CO}_2)$ conditions (Andersson et al. 2009).

These processes help to produce the variability signal of interest in ecosystems that we would like to mimic, for example diurnal CO_2 oscillations in sea grass beds (Buapet et al. 2013) and coral reefs (Andersson et al. 2009). However, they would also potentially create undesirable variability or fluctuations in an aquarium environment. In

addition to the experimental organism changing the carbonate chemistry in an aquarium, it is also possible for unintended organisms to make their way into the aquaria and to alter the chemistry. MSEAS uses natural seawater pumped from the end of the SIO pier, and so small phytoplankton and bacteria may be present, even though the water is well filtered. Their photosynthesis and respiration, resulting in production and remineralization of organic matter can unintentionally change the seawater chemistry.

4.3.2 Challenges to aquarium control

Organism created

MSEAS is unique because it controls both the dissolved CO_2 and the O_2 of the seawater, which makes it particularly well suited to combat a photosynthesis and respiration cycle in a way that other systems might not be. The feedback system automatically adjusts to any change in CO_2 to keep the desired pH. It also counteracts the changes to $p(\text{CO}_2)$ and C_T caused by calcification and dissolution, but does not oppose changes to A_T in this way as A_T is not altered by the addition or removal of CO_2 gas. Instead, new seawater is introduced to the tanks to restore A_T reduced by calcification. This is particularly important because, as discussed in the methods, the assumption of constant A_T for the chemistry control of MSEAS is critical. This means that the overturning or residence time of the tanks has important implications for the CO_2 control in the aquaria and thus the environment experienced by the organism.

In addition to mitigating changes in A_T due to calcification, regular overturning of seawater is important because it flushes the organisms' waste from the system. The uptake and remineralization of nutrients and organic matter affect A_T . In a seawater

system, we typically assume that carbonate (and borate) alkalinity represents almost all of the total alkalinity, and therefore can be used to calculate other carbon parameters. However, with significant organic loading, as can be caused by organism waste, this assumption may be invalid and A_T difficult to interpret. Because of these potential modifications or complications of A_T , for most experiments it makes sense to use a flow-through design, although the system can be run either as a flow-through system or with no water exchange.

The regular addition of new filtered seawater to the aquaria, in combination with the feedback of the pH sensors to the CO₂ control of the system, helps to ensure that MSEAS can effectively counteract any deviations to the carbonate chemistry created by organisms. Although not yet implemented, it will be possible to include feedback from oxygen sensors to further minimize any fluctuations in O₂ due to photosynthesis and respiration. Although the O₂ is only controlled passively right now, large oxygen fluctuations have not been observed in the tanks in previous experiments, likely due to the Liqui-Cels that are specifically designed to equilibrate oxygen quickly.

Gas exchange

The most direct way that control over an aquarium can be compromised is through gas exchange between the seawater and the room air in the laboratory. Gas exchange of CO₂ will work to bring the two reservoirs (seawater and atmosphere) towards equilibrium. This process is aided by any regular mixing of the seawater (and of the atmosphere, although it mixes more readily). This is especially of concern when making CO₂ manipulations such that the $p(\text{CO}_2)$ of the seawater is significantly out of equilibrium with the atmosphere, as we would expect with any high-CO₂ experimental

treatment. The larger differential between the atmosphere and seawater will cause more rapid outgassing of CO₂ from the seawater to the atmosphere.

There are several ways to counteract this problem. One approach is to ensure that the carbon dioxide level in the atmosphere above each tank matches the desired content in the seawater. However, having multiple treatments at different CO₂ levels complicates this. In these aquaria, undesirable changes due gas exchange are minimized in two ways: First, care is taken to ensure that there are few places of contact between the seawater and the atmosphere. A fitted lid encloses each treatment tank, and seawater fills the tank completely, so that there is very little, if any, headspace. As the seawater is recirculated from each tank, through the filter, the Liqui-Cel[®], and back to the tank, the seawater line is kept closed so that no gas exchange can occur. A submerged aquarium pump is used, and the seawater inflow tube is immersed as well. The only gas the seawater should contact is the gas mixture supplied to the Liqui-Cel[®]. Secondly, the constant control over the seawater carbonate chemistry, through the constant interaction of the seawater with the gas mixture, works to keep the system at the desired chemistry. This is different than systems that pre-equilibrate the seawater to the desired level, and then supply this water to the treatment tanks, allowing for the possibility of modification during this movement or once water is in the treatment tanks.

Temperature

The solubility of CO₂ is temperature dependent, decreasing with increasing temperature. Additionally, the measured parameters $p(\text{CO}_2)$ and pH are both dependent on the seawater temperature and pressure. Given constant A_T and C_T , $p(\text{CO}_2)$ increases with increasing temperature while pH decreases. Changes due to temperature can be

significant, both in the ocean and in an aquarium setting; a 1°C change in temperature induces approximately a 0.015 change in pH and a 20–50 μatm change in $p(\text{CO}_2)$ (about 4.2 percent per degree) (Dickson 2010).

In previous experiments, problems with temperature control potentially contributed to undesired deviations in the carbonate chemistry. Large daily fluctuations in atmospheric temperature caused a daily signal in seawater temperature. Because of the recirculation of the seawater through the filter, Liqui-Cel, and back to the tank, there is sufficient opportunity for the room temperature to alter the seawater temperature. Measures such as tubing insulation and better control of the room temperature were taken to combat this problem. Active control of the seawater temperature is clearly the best solution, to ensure that the temperature does not fluctuate.

Because the system is designed to maintain a desired pH, any changes to the temperature of the seawater will necessarily result in a change in the chemistry of the seawater. The pH will remain constant, but a 1°C change in temperature will change $p(\text{CO}_2)$ by as much as 10 μatm (with C_T also changing) at a lower pH (See Figure 4.3). At pH near 8.0 there is little change in $p(\text{CO}_2)$, but still a measureable change to C_T . These changes are minimal compared to those found when changing the temperature of a water mass with a given A_T and C_T , but are still best avoided for optimal carbonate chemistry control.

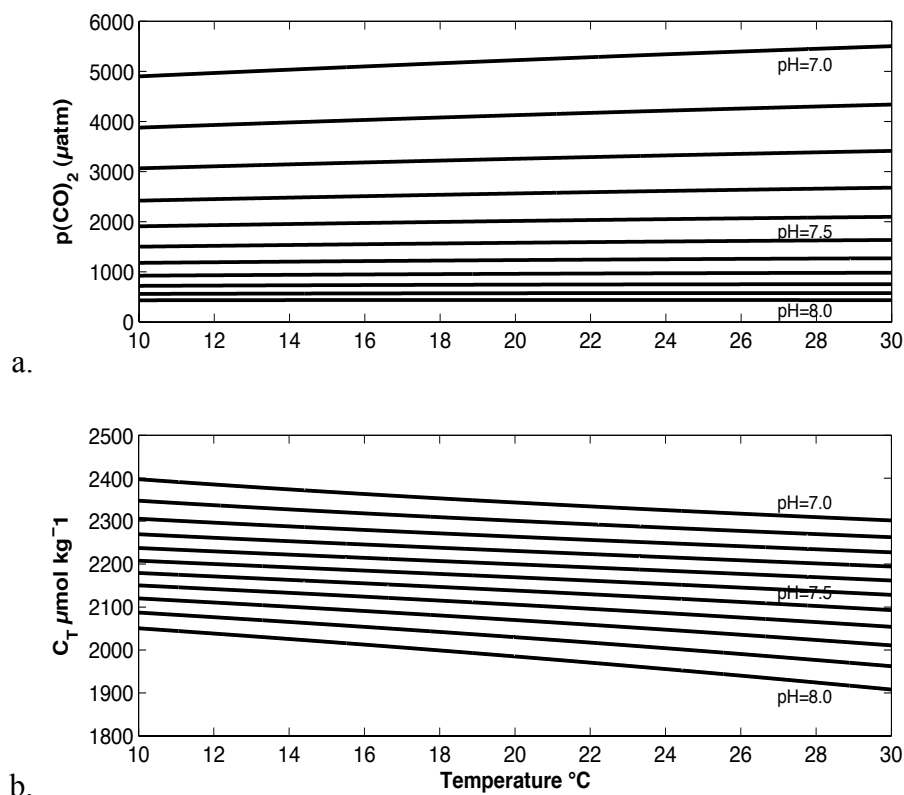


Figure 4.3. Calculated a) $p(\text{CO}_2)$ (μatm) and b) C_T ($\mu\text{mol kg}^{-1}$) across a range of temperatures at constant pH (7.0–8.0) and $A_T = 2200 \mu\text{mol kg}^{-1}$. The calculations were done using CO2SYS (Van Heuven et al. 2011) in MATLAB[®].

Incomplete equilibration

Incomplete equilibration between the seawater and the gas mixture is a common problem in aquaria where the carbonate chemistry is controlled by equilibration. The hydration of CO_2 in seawater is not particularly fast, and thus equilibrium is often not reached. Additionally, as the seawater and gas become more similar in composition (with respect to CO_2 concentration), the equilibration slows. For systems that control the seawater CO_2 by bubbling with gas, the bubble size is very important in addition to the volume of water and gas flow rate. Smaller bubbles allow for quicker equilibration.

The Liqui-Cel[®] Membrane Contactor utilizes microporous hollow fibers to bring the liquid and gas phases in direct contact at an extremely high surface area to volume ratio. This effectively speeds equilibration between the gas and seawater, however it is still not perfect. To some extent, the composition of the seawater is dependent on how often the seawater is exposed to the gas mixture, and therefore the rate of seawater flow through the Liqui-Cel[®]. When using larger aquaria, approximately 50 L, the seawater only recirculates through the Liqui-Cel[®] 2-3 times per hour, limiting the speed of equilibration.

Additionally, the micropores are so small in the Liqui-Cel[®], that they can easily become clogged. This has been a significant problem in previous experiments, particularly because the fouling changes over time, which can cause a slow but noticeable change to the ability of the Liqui-Cel[®] to equilibrate. Also, because the seawater is pumped directly from the tank to the Liqui-Cel[®], any particulates in the tanks become introduced to the Liqui-Cel[®]. Filtering the seawater to 5 μm using a 9.5 cm pleated filter before it is introduced to the Liqui-Cel[®] each time reduces this problem. The system should not be used without these filters in place. Regular cleaning of the Liqui-Cels[®] using hot water and dilute acid restores the membrane contactors to working order.

These challenges with equilibration in the aquarium were particularly important when the aquariums were controlled passively as described in Chapter 3. Because the system set the CO_2 of the gas mixture to a constant level, it was not able to adjust for problems regarding incomplete equilibration. With the addition of the feedback to the gas creation system, incomplete equilibration and changes to the ability for the Liqui-Cels[®] to equilibrate are counteracted. Incomplete equilibration does not influence the carbonate

chemistry control as the aim is a target pH value, not an equilibrium between the gas stream and the water itself.

4.4 Application of the aquarium

The new modifications and methods of MSEAS were tested for a variety of variability, both in amplitude and frequency. The results presented here show both idealized oscillations in pH, as well as attempted mimicking of measured natural environments including a shallow coral reef habitat, coastal San Diego continental shelf at 89 m, and a kelp forest. Although each discussion will focus on the primary process driving the variability observed, in all cases, the ultimate variability in any given ecosystem is complicated by multiple influences on the carbonate chemistry. The consequences of these differences in CO₂ on the organisms being studied in an experimental aquarium are important to consider. Eventually it may be interesting to compare variability in different parameters and the resulting effect on organisms, similar to how different manipulations of the chemistry have been compared in constant environment aquaria (Gattuso and Lavigne 2009; Shi et al. 2009).

4.4.1 Diurnal variability: Ofu Volcanic Island

Shown in Figure 4.4 is a representative week of pH data collected by a SeaFET on a coral reef at Ofu Volcanic Island. A relatively large diurnal cycle (~0.3 pH units) is observed that peaks in the afternoon and goes to a minimum sometime after midnight. This kind of variability is typical of a shallow coral reef environment, where large daily fluctuations in pH and $p(\text{CO}_2)$ are due to high levels of benthic community metabolism.

The balance of net ecosystem production and calcification determines the change to the ratio of A_T/C_T that controls the pH signal.

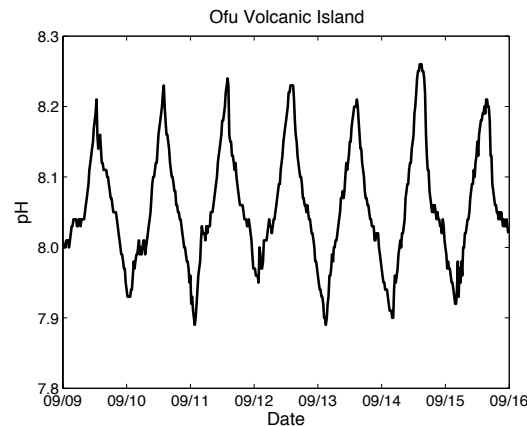


Figure 4.4. pH data collected by Lupita Ruiz-Jones on a coral reef at Ofu Island in 2011. The pH variability is on a diurnal timescale, peaking just after noon each day.

This diurnal biological control and feedback has also been observed in sea grass beds (Buapet et al. 2013), communities dominated by calcareous macroalgae (Bensoussan and Gattuso 2007), and intertidal pools (Christensen et al. 2011). The magnitude of variability in these environments, in addition to the biological feedback, is also affected by tidal flushing or the residence time of seawater on the reef, and by the water depth, with variability decreasing with increasing depth (Middelboe and Hansen 2007). Reproducing the observed diurnal pH cycle in the aquarium will not be a perfect analog to the variability in a coral reef ecosystem. The fluctuation of pH in the aquarium will be produced through the addition/removal of CO_2 gas alone (modifying C_T), while on the reef there will necessarily be some modification to the A_T of the system in addition due to calcification and dissolution. Figure 4.5 shows example diurnal oscillation in pH created by MSEAS in the aquarium. The variability is relatively smooth compared to the

data collected on the Ofu reef, and the magnitude of oscillation is small. Adjustment to the amplitude and frequency of the oscillation is possible depending on the environment of interest.

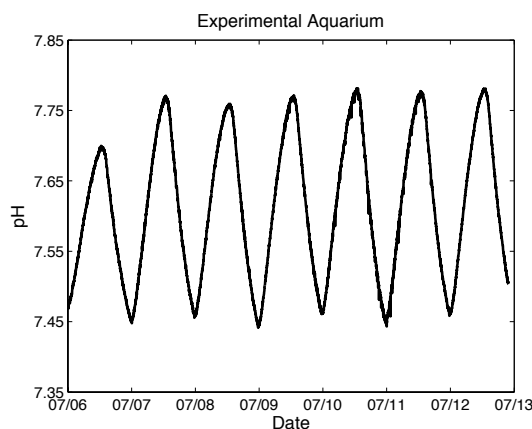


Figure 4.5. An example of regular oscillations in pH produced by MSEAS.

4.4.2 Tidal variability: Del Mar Mooring

Rather than a diurnal cycle in pH, many areas exhibit a twice-daily cycle in pH, controlled by the tides. This example is from the Del Mar Mooring, which is located in 100 m of water on the continental shelf, approximately 3 miles off the coast of Del Mar, CA. A SeaFET pH sensor was located at 89 m depth with a SeaBird SEACAT that measures conductivity, temperature, and oxygen. Figure 4.6 shows a representative week of the pH data. Spectral analysis of the data determined that the major time scales of variability were semi-diurnal, occurring approximately every 12.5 hours, corresponding with the principal lunar semi-diurnal (M_2) tidal constituent. The power spectral density was strong and similar for all parameters measured (oxygen, salinity and temperature), indicating that the primary cause of the variability is physical rather than biological. These semi-diurnal fluctuations in pH have also been observed on coral reefs, when biological and physical processes act together (Price et al. 2012). Although these

changes in pH are most likely not caused primarily by a change in C_T alone, the system can still approximate the variability observed in pH.

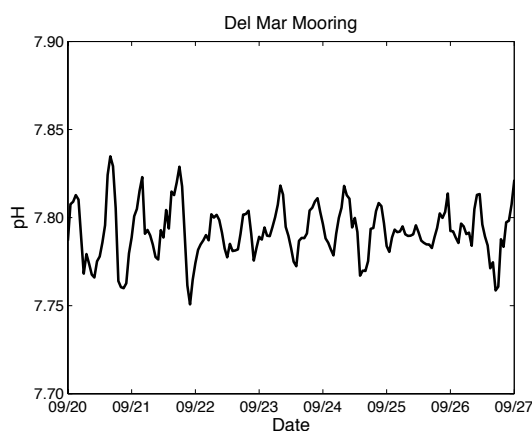


Figure 4.6. pH data collected by the Ocean Time Series Group led by Prof Uwe Send at the Del Mar Mooring in 2011. The pH signal is primarily driven by the semi-diurnal tide.

4.4.3 Irregular variability controlled by mixing: Southern California kelp forest

Variability in CO_2 is often controlled by the physical mixing of waters with characteristically different chemistry. Upwelling of deep seawater that is enriched in CO_2 from the decomposition and remineralization of organic matter has been observed along the continental shelf of western North America (Feely et al. 2008). The intensity, duration, and severity of these upwelling events, which bring low pH and aragonite saturation seawaters near the surface are increasing and will continue to (Hauri et al. 2013). Prolonged upwelling events are predicted to be more detrimental to organisms, as shown by Kim et al. (2013) in a laboratory experiment exposing abalone to mimicked upwelling conditions with both low pH and low oxygen levels. Our ability to predict organism response in these environments is difficult because of the intermittent nature of the episodes. Additionally, these types of environments will often have some tidal or diurnal component to the pH variability as well, particularly in shallow waters. Because

this aquarium can recreate a pH signal inputted from a text file, it can reproduce this combination and irregular variability in pH without having to understand the exact timing and drivers that created the variability. Figure 4.7 gives an example of this kind of variability, showing upwelling that brought low pH, low oxygen, and low temperature seawater up onto the continental shelf. Measurements were made using a SeapHOx pH sensor at 17 m water depth in a kelp forest.

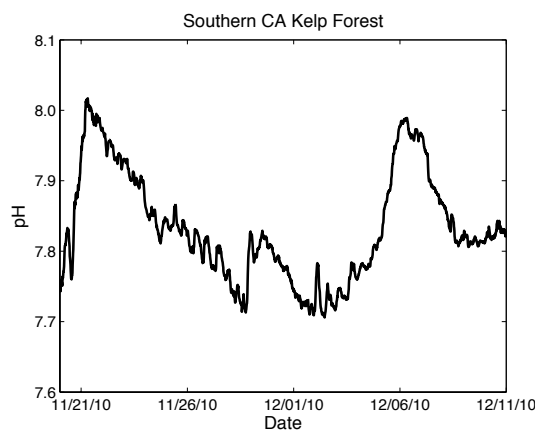


Figure 4.7. pH data collected by Christina Frieder in a kelp forest off La Jolla, CA for three weeks in 2010. Upwelling dominates the control of pH, although pH fluctuations due to tidal influence are also present.

4.4.4 Slow steady changes: acclimation studies

The system can also be used to apply slow, steady changes to the chemistry of the aquaria. With the system operating almost as if in steady state, a slow addition or removal of CO₂ can be made depending on the desired environmental change. Computer control of the gas mixture makes this kind of gradual change straightforward. Slowly changing CO₂ might be akin to the addition of anthropogenic CO₂ to the oceans – although on an accelerated timescale. Prior to laboratory experiments, organisms are sometimes acclimated to the laboratory environment, to recover from any disturbance, preparation or transfer (e.g. Comeau et al. 2013). Adding a slowly changing pH signal could be part of

this acclimatization. A slowly changing pH might also be useful in studying an organism's ability to acclimate to new environmental conditions (Comeau et al. 2014; Hofmann et al. 2014) or to perform experimental evolution to learn about a species' potential for response (Sunday et al. 2014). For example, Form and Riebesell (2012) found differences in a cold-water coral calcification in a multi-month incubation that slowly increased $p(\text{CO}_2)$, compared to a short-term exposure.

4.5 Conclusions

The general understanding of carbonate chemistry dynamics in the coastal oceans is growing. We recognize that $p(\text{CO}_2)$ in the near-shore often already exceeds that expected from equilibrium with current atmospheric CO_2 levels, and even with the levels predicted for the near future. The carbonate chemistry is often complex in these coastal oceans because biological, physical, and chemical processes of control all combine and vary on many timescales. This leads to unique environments that contain different timescales and magnitudes of variability in $p(\text{CO}_2)$ and pH. The expectation is that in the future, this identified variability will change not only in the mean, but also in the frequency, magnitude, and duration of variability depending on the particular ecosystem and its drivers. Predicting these changes to the variability are difficult in the longer term, particularly because of our limited understanding of the effects of ocean acidification and any possible biological feedback.

Quite a variety and large number of organisms inhabit these variable environments with elevated CO_2 . Elucidating their response to climate change will be complicated, as many of these organisms will respond in different ways. Even different

life stages of these organisms have differential susceptibility, and will likely have different exposure to future low pH environments. The addition of variability to aquarium systems used to study ocean acidification is necessary and will significantly advance our ability to understand organismal response to climate change. MSEAS has the ability to perform carefully controlled fluctuations in pH on many timescales, mimicking environmental variability on diurnal, tidal, and even upwelling periods. Getting the expected changes to the variability in CO₂ correct in an experimental aquarium will be necessary to elucidate organismal response.

4.6 References

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