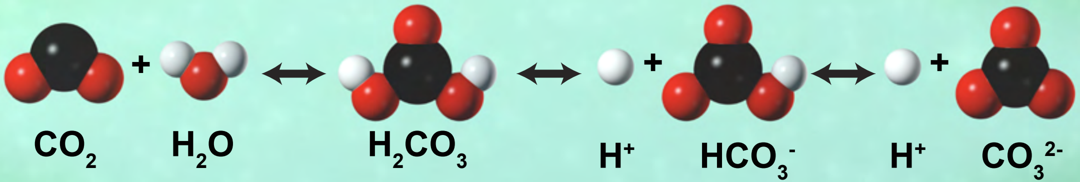
Prepared as part of the U.S. Geological Survey
Coastal and Marine Geology Program and Coral Reef Ecosystem Study

Chemical and Biological Consequences of Using Carbon Dioxide Versus Acid Additions in Ocean Acidification Experiments



Scientific Investigation Report 2012–5063

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Cover image. Depiction of the inorganic carbon species and reactions affected by ocean acidification that occurs when atmospheric CO_2 is absorbed by seawater at the ocean's surface. Artwork by Betsy Boynton, U.S. Geological Survey.

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Contents

Figures

Tables

Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
kilometer (km)	0.5400	mile, nautical (nmi)
meter (m)	1.094	yard (yd)
	Area	
square meter (m^2)	0.0002471	acre
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
	Mass	
kilogram (kg)	2.205	pound avoirdupois (lb)
	Pressure	
kilopascal (kPa)	0.009869	atmosphere, standard (atm)
	Density	
gram per cubic centimeter (g/cm^3)	62.4220	pound per cubic foot (lb/ft^3)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32

Concentrations of chemical constituents in seawater are given either in millimoles per kilogram (mmol kg^{-1}) or micromoles per kilogram (µmol kg^{-1}).

Abbreviations

ATP	adenosine triphosphate
CDIAC	Carbon Dioxide Information Analysis Center
dbar	decibar
DOS	Disk Operating System
EPOCA	European Project on Ocean Acidification
G	net calcification rate
µatm	microatmospheres
µM	micromolar
mL	milliliters

µmol	micromoles
mmol	millimoles
N	Normality
OCB	Ocean Carbon and Biogeochemistry
pCO_2	partial pressure of carbon dioxide
pH_T	total pH scale
pmol	picomoles
ppm	parts per million
STP	standard atmospheric temperature and pressure
SW	seawater
TA	total alkalinity
TCO_2	total carbon
Ω	mineral saturation state
Ω_A	aragonite saturation state
Ω_{Ca}	calcite saturation state

Chemical Abbreviations

$CaCO_3$	calcium carbonate
CO_2	carbon dioxide
CO_3^{2-}	carbonate
Ca^{2+}	calcium
H^+	hydrogen ions (or acid)
HCl	hydrochloric acid
H_2CO_3	carbonic acid
HCO_3^-	bicarbonate
$KHSO_4$	sulfate
Na^+	sodium
Na_2CO_3	sodium carbonate
OH^-	hydroxyl ions (or base)
P	total phosphate
Si	silicate
SW	seawater

Chemical and Biological Consequences of Using Carbon Dioxide Versus Acid Additions in Ocean Acidification Experiments

By Kimberly K. Yates, Christopher M. DuFore, and Lisa L. Robbins

Abstract

Use of different approaches for manipulating seawater chemistry during ocean acidification experiments has confounded comparison of results from various experimental studies. Some of these discrepancies have been attributed to whether addition of acid (such as hydrochloric acid, HCl) or carbon dioxide (CO_2) gas has been used to adjust carbonate system parameters. Experimental simulations of carbonate system parameter scenarios for the years 1766, 2007, and 2100 were performed using the carbonate speciation program CO2SYS to demonstrate the variation in seawater chemistry that can result from use of these approaches. Results showed that carbonate system parameters were 3 percent and 8 percent lower than target values in closed-system acid additions, and 1 percent and 5 percent higher in closed-system CO_2 additions for the 2007 and 2100 simulations, respectively. Open-system simulations showed that carbonate system parameters can deviate by up to 52 percent to 70 percent from target values in both acid addition and CO_2 addition experiments. Results from simulations for the year 2100 were applied to empirically derived equations that relate biogenic calcification to carbonate system parameters for calcifying marine organisms including coccolithophores, corals, and foraminifera. Calculated calcification rates for coccolithophores, corals, and foraminifera differed from rates at target conditions by 0.5 percent to 2.5 percent in closed-system CO_2 gas additions, from 0.8 percent to 15 percent in the closed-system acid additions, from 4.8 percent to 94 percent in open-system acid additions, and from 7 percent to 142 percent in open-system CO_2 additions.

Introduction

Climate change and ocean acidification could have profound impacts on coastal and oceanic ecosystems ranging from the shallowest tidal pools to the deepest abyssal plains (Bindoff and others, 2007). Many of these ecosystems are intimately tied to the long-term viability of society, providing economic, recreational, and aesthetic value from which communities thrive. For example, coral reefs provide protection to coastlines from storm waves, essential fish nurseries and habitat, and production of sand for beaches and are the center of trade, recreation, and tourism in many tropical coastal communities. Previous research indicates that elevated atmospheric carbon dioxide (CO_2) and decreased seawater pH reduce rates of skeletal formation by corals and other calcifying species (Gattuso and others, 1999; Kleypas and others, 1999; Langdon and others, 2000, 2003; Jokiel and others, 2008; De'ath and others, 2009; Doney and others, 2009; Silverman and others, 2009) and increase dissolution of carbonate sediments (Yates and Halley, 2006; Andersson and others, 2007; Anthony and others, 2008; Silverman and others, 2009). Whether coral reefs will continue to grow their three-dimensional structure fast enough to keep up with rising sea level, or whether they will begin to erode or be overgrown by opportunistic non-calcifying species such as sponges and algae (Kleypas and Yates, 2009) is unknown. Continental shelves are home to the commercial fishing industry that contributes approximately $34 billion to the U.S. economy each year (Cooley and Doney, 2009). Recent research (Feely and others, 2008) indicates that ocean acidification is increasing the areal extent of corrosive waters that are seeping onto the continental shelves of western North America. These corrosive waters may impede the ability of shellfish larvae to produce their calcium carbonate shells (Gazeau and others, 2007) and may have toxic effects on marine bony and cartilaginous fish species (Ishimatsu and others, 2004). Wooten and others (2008) estimate up to a 40 percent decrease in calcifying organisms in coastal waters of the State of Washington due to decreasing seawater pH based on 8 years of monitoring data for physical-chemical parameters and species dynamics. This observed decrease in seawater pH was likely due to a combination of increased atmospheric CO_2 and changes in biological productivity and upwelling. Cooley and Doney (2009) used atmospheric CO_2 trajectories and results from laboratory studies on the effects of increasing CO_2 on mollusks to examine the potential economic consequences of ocean acidification. Cooley and Doney (2009) suggest that mollusk harvests may decrease by up to 25 percent in the next 50 years. The surface waters of the deep ocean are home to 50 percent of the world's primary productivity through the production of plankton that supports much of marine life as the base of the trophic chain (Longhurst and others, 1995; Carr and others 2006). Calcifying plankton is responsible for production of approximately 40 percent of the world's calcium carbonate sediments (Milliman, 1993; Milliman and Droxler, 1996). Coccolithophores, foraminifera, and pteropods account for most of the export of calcium carbonate ($CaCO_3$) from the upper ocean to the deep sea (Fabry and others, 2008). Recent studies on the effects of ocean acidification on plankton indicate that some species may be adversely affected while others may benefit (Doney and others, 2009). However, it is likely that even small disturbances to ocean productivity will have profound effects on marine food webs and sediment production. Studies such as those described above have been instrumental in demonstrating the potential magnitude of climate change impacts on ocean and coastal ecosystems; however, there are still many unknowns about the effects of ocean acidification on biological processes.

Heightened scientific interest in climate change and ocean acidification issues has prompted much experimentation by researchers with very diverse expertise who may have limited knowledge of carbonate chemistry (Gattuso and Lavigne, 2009). Much of this experimentation has focused on manipulating seawater chemistry using a variety of techniques to examine effects of predicted future climate scenarios on biological organisms. However, use of different experimental approaches, measurement techniques, and data reporting standards often makes it difficult to compare results from different studies. Recent efforts have been made by the European Project on Ocean Acidification (EPOCA; accessed August 2011, at *http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/*), the U.S. Ocean Carbon and Biogeochemistry Program (OCB) - Ocean Acidification Subcommittee (*http://www.whoi.edu/courses/OCB-OA/*), and the Carbon Dioxide Information Analysis Center (CDIAC) of the U.S. Department of Energy (Dickson and Goyet, 1994; Dickson and others, 2007; Riebesell and others, 2010) to standardize carbonate system parameter measurement techniques and approaches to ocean acidification experiments and to educate the research community on these topics.

The chemical and biological consequences of experimental approaches used to study the effects of ocean acidification and the impact these approaches may have on results have only recently come to the attention of the scientific community. Recent studies suggest that drastically different experimental outcomes may have resulted from the use of acid additions in some experiments and CO_2 gas additions in others or from experiments that did not discriminate impacts from changes in individual carbonate system parameters (Iglesias-Rodriguez and others, 2008; Jury and others, 2010).

Purpose and Scope

The purpose of this paper is to demonstrate the geochemical consequences of adding CO_2 versus acid for ocean acidification perturbation experiments and the potential variation in biological response resulting from choice of approach. The carbonate speciation program CO2SYS (Pierrot and others, 2006) was used for conceptual development and simulation of manipulative experiments and as an example of how this program can be used to facilitate development of experimental strategies. Input conditions from a similar exercise by Gattuso and Lavigne (2009) that describes use of the program Seacarb (Lavigne and Gattuso, 2011) were used to provide a direct comparison of the differences in logical approach and programming mechanics required to achieve similar results in CO2SYS. The results of this simulation were applied to experimentally determined responses of a few key marine organisms to show the significant impact experimental approaches may have on biological responses in ocean acidification perturbation experiments.

Approach

Decreases in seawater pH associated with ocean acidification are driven by uptake of carbon dioxide into the surface waters of the ocean, reaction of that carbon with seawater, and the resulting shift in the equilibrium of dissolved inorganic carbon species (eqs. 1 and 2). The equilibrium chemistry associated with these reactions and the effects on pH and carbonate mineral saturation state have been extensively described in previous literature (Dickson and Goyet, 1994; Millero, 1995; Zeebe and Wolf-Gladrow, 2001). Essentially, CO_2 reacts with seawater to form a weak acid called carbonic acid (H_2CO_3). As CO_2 increases in the atmosphere (Denman and others, 2007; Keeling and others, 2008), more CO_2 is added to seawater, pH decreases (Byrne and others, 2010), the concentration of carbonate ($[CO_3^{2-}]$) decreases, and the concentration of bicarbonate ($[HCO_3^-]$) increases due to the shift in carbonate speciation from CO_3^{2-} to HCO_3^- resulting from the decreased pH. This also results in a decrease in carbonate mineral saturation states (Ω) that are dependent upon $[CO_3^{2-}]$ as shown in equation 3. When pH is equivalent to pK1, then $[CO_2] = [HCO_3^-]$. When pH is below pK1, then $[CO_2] > [HCO_3^-]$. The same relation holds true for pK2, $[HCO_3^-]$, and $[CO_3^{2-}]$ (Zeebe and Wolf-Gladrow, 2001).

$$CO_2(g) + H_2O \leftrightarrow HCO_3^- + H^+$$

pK1 @ 25ºC, salinity 35, 1 atm = 5.8457 (1)

$$\mathrm{HCO3–} \leftrightarrow H^+ + CO_3^{2-}$$

pK2 @ 25ºC, salinity 35, 1 atm = 8.9454 (2)

Ω = ion concentration product (ICP)/mineral solubility product = $[Ca^{2+}][CO_3^{2-}]/K'sp$ (3)

Equilibrium constants, pK1 and pK2, are from Mehrbach (1973) refit by Dickson and Millero (1987). Predicted changes in seawater carbonate system parameters due to elevated atmospheric CO_2 and ocean acidification have been summarized in Feely and others (2009).

Carbonate system parameters can be manipulated for experimental purposes in several ways. The practical and mathematical definitions of the carbonate system parameters, as well as the relations among them, must be well understood prior to performing experiments. Riebesell and others (2010) provide a detailed guide of best practices for ocean acidification research and experimental approaches that describes the benefits and limitations of various approaches used to manipulate seawater chemistry in perturbation experiments that investigate the effect of elevated partial pressure of carbon dioxide (pCO_2) and ocean acidification on biological response. These approaches include manipulating the carbonate chemistry of seawater using: CO_2 gas bubbling, addition of high CO_2 seawater, addition of CO_3^{2-} and(or) HCO_3^-, addition of strong acids or bases, addition of strong acid plus CO_3^{2-} or HCO_3^-, and manipulation of the calcium concentration. Some of the most common manipulations have been summarized in table 1. These manipulations can be combined as multiple steps or performed in open or closed systems to achieve target seawater conditions. The carbonate chemistry of starting seawater in all cases should be well characterized, reasonable targets for all carbonate system parameters identified, and careful monitoring of seawater should be performed during manipulation and use in experiments. Manipulated seawater will need to be maintained in controlled systems (for example, pH–stat, pCO_2–stat, and so forth) in most cases to prevent deviation from intended target chemistry.

Carbonate speciation programs have been developed to simplify the traditionally tedious “hand calculation” of carbon system parameters and manipulations thereof. Lewis and Wallace (1998) developed CO2SYS, a carbonate speciation program first written in Microsoft QuickBASIC, which was able to run on any platform using the Disk Operating System (DOS). Later versions have been released that operate in Microsoft Excel (Pierrot and others, 2006, used for this study) and MATLAB (van Heuven and others, 2009). Seacarb (Lavigne and Gattuso, 2011) is a carbonate speciation program that was developed in a free cross-platform programming, statistics, and graphics language known as R (R Development Core Team, 2008). Seacarb, version 2.0, was updated in 2008 to include programming functions that assist with development of ocean acidification perturbation experiments (Lavigne and others, 2007; Gattuso and Lavigne, 2009). Each of these programs calculates carbonate system parameters, dissolved inorganic carbon speciation, and carbonate mineral saturation states based on input of two of four carbonate system parameters: total alkalinity (TA), total carbon (TCO_2), pH, and pCO_2. Program users can select equilibrium constants and pH scales appropriate to their specific environmental and experimental needs. Both programs are widely used by chemists, biologists, geologists, and oceanographers for carbonate system data analysis, modeling, and experimentation.

The effect of experimental approach on cellular physiology must also be considered. Prokaryotic and eukaryotic cell physiology is driven by a system of molecular and ionic pumps, chemical gradients that regulate cell nutrition (uptake and metabolism of food, release of energy in cells, and elimination of waste products), and response to chemical and physical aspects of the environment (Geise, 1962). For example,

Table 1. Procedures for manipulating seawater chemistry.

[TA, total alkalinity; TCO_2, total carbon; pCO_2, partial pressure of CO_2; H+, acid; OH–, base; HCO_3^-, bicarbonate; CO_3^{2-}, carbonate; CO_2, carbon dioxide; Δ, the difference in concentration between starting and target seawater concentrations for the parameter indicated; mol kg^{-1} SW, moles per kilogram of seawater]

Seawater manipulation	Procedure	Calculations[a]
Increase or decrease TA	Add H+ to increase or OH– to decrease	ΔTA = moles acid or base added
Increase TCO_2 and pCO_2	Bubble with CO_2 gas	ΔTCO_2 = moles carbon added See eq. 5 and 6 = liters CO_2 gas added
Increase TA and TCO_2	Add HCO_3– and/or CO_3^{2-}	ΔTCO_2 = total moles of C added $\Delta TA - \Delta TCO_2$ = moles of CO_3^{2-} added $\Delta TCO_2 - (\Delta TA - \Delta TCO_2)$ = moles HCO_3– added
Decrease TCO_2[b]	Purge CO_2 by bubbling with CO_2–free air	Monitor TCO_2 closely during manipulation to reach target TCO_2
Decrease TCO_2 and TA[b]	Add acid followed by purging with CO_2–free air	ΔTA for target TA, monitor TCO_2 to reach target TCO_2
Decrease TCO_2 and increase TA[b]	Add acid followed by purging with CO_2–free air, followed by addition of OH– in closed system	Monitor for target TCO_2, ΔTA = moles OH– added

[a] Δparameter = refers to the difference between starting and target seawater concentrations for that parameter. Standard units for these parameters are mols kg^{-1} SW.

[b] These manipulations require particularly careful monitoring and measurement of carbonate system parameters to ensure that target conditions are acquired.

the driving force for production of adenosine triphosphate (ATP), the primary energy source for cellular metabolism of all living organisms, is production of a proton motive force, or H^+ electrochemical gradient across cellular membranes. The primary food source for photosynthetic cells/organisms is inorganic carbon, primarily in the form of CO_2 and HCO_3^-, which must be transported into cells from the external environment for photosynthetic fixation. Transport of ions such as calcium (Ca^{2+}) and sodium (Na^+) across cell membranes plays a vital role in many physiological aspects of cells, including production of energy-generating electrochemical potentials, transport of inorganic carbon across cell membranes, and maintenance of highly regulated intracellular chemical environments. These basic cellular processes in all organisms are defined by reactions involving the same chemical species of concern in ocean acidification studies (CO_2, HCO_3^-, CO_3^{2-}, Ca^{2+}, and H^+). Fabry and others (2008) summarized potential physiological impacts that may result from exposure of organisms to elevated CO_2 and decreased pH. These impacts include hypercapnia (increased diffusion of CO_2 across cells), changes in the buffering capacity that regulates pH in bodily fluids, and changes in ion transport mechanisms and acid-base regulation through bicarbonate accumulation. These processes can affect both calcifying and non-calcifying aquatic and marine organisms; however, the impacts these changes have on cellular physiology and calcification are not well quantified.

Methods

The Microsoft Excel version of the carbonate speciation program CO2SYS (Pierrot and others, 2006) was used to simulate experimental variations in pCO_2 and pH from CO_2 and acid additions to seawater. Experimental conditions targeted seawater carbonate system parameters that approximate values within the range of pre-industrial levels of pCO_2, approximately 270 parts per million (ppm), to levels predicted for the year 2100 of approximately 800 ppm (Meehl and others, 2007). Results of these simulations provided starting conditions for these seawater chemistry perturbation experiments and do not reflect the effect of biological organisms or calcification on seawater chemistry. Closed-system, acid-additions simulated adjustment of the pH and inorganic carbon species and were performed using addition of known quantities of a strong monoprotic acid or base such as hydrochloric acid or sodium hydroxide, respectively. Closed-system, CO_2–additions simulated adjustment of pCO_2 and were performed using the addition of CO_2 gas to achieve target pCO_2 concentrations. Open-system, acid-additions, and open-system, CO_2-additions, simulated the effect of allowing experiments to equilibrate with atmospheric pCO_2 after chemical manipulations as examples of what could happen if an open system experiment is not properly monitored or controlled.

Target values for carbonate system parameters, temperature, salinity, total phosphate (P), and total silicate (Si) for all simulations are from scenarios calculated using Seacarb for the years 1766, 2007, and 2100 by Gattuso and Lavigne (2009) and were analyzed again using CO2SYS (table 2). Seacarb scenarios calculated by Gattuso and Lavigne (2009) used equilibrium constants of Lueker and others (2000). Pressure was held constant at 0 decibars (dbars), representing surface ocean calculations in CO2SYS, and a calcium concentration of 10.12 mmol kg^{-1} SW was used. Starting seawater chemistry for all experimental manipulations was approximated at pre-industrial conditions with a TCO_2 of 1,994 µmol kg^{-1} SW, TA of 2,326 µmol kg^{-1} SW, pCO_2 of 267 µatm, and pH of 8.2 (table 2). These parameters are considered baseline conditions for seawater chemistry and are an important end-member target condition for many ocean acidification experiments. The CO2SYS program was used to calculate carbonate system parameters for all simulation experiments using dissociation constants for the carbonate system, K1 and K2, from Mehrbach and others (1973) refit by Dickson and Millero (1987), for sulfate ($KHSO_4$) from Dickson (1990), and using the total pH scale (pH_T). The difference in parameters calculated using Seacarb with dissociation constants from Lueker and others (2000) is negligible for the purpose of this exercise. Choice of equilibrium constants depends upon the pH scale used for measuring pH, whether natural or artificial seawater was used in experiments, and which carbonate system parameters are measured versus calculated, among other factors. A good general discussion on choice of equilibrium constants is provided in Zeebe and Wolf-Gladrow (2001).

Table 2. Target values and experimental parameters for CO2SYS experimental simulations.

[Target values from Gattuso and Lavigne (2009) using CO2SYS. T, temperature in degrees celsius; P, total phosphate; S, total silicate; TA, total alkalinity; pCO_2, partial pressure of carbon dioxide; HCO_3-, bicarbonate; CO_3^{2-}, carbonate; CO_2, carbon dioxide; ΩA, aragonite saturation state; mol kg^{-1} SW, moles per kilogram of seawater]

Year	T (°C)	Salinity	Total P*	Total S*	TA*	pCO_2 (µatm)	TCO_2*	pH	[HCO_3-]*	[CO_3^{2-}]*	[CO_2]*	Ω_A
1766	18.3	34.9	0.66	7.35	2,326	267	1,994.4	8.194	1,753.5	231.9	9.05	3.59
2007	18.9	34.9	0.63	7.35	2,325	384	2,064.4	8.065	1,863.6	187.0	12.8	2.90
2100	21.4	34.7	0.55	7.35	2,310	793	2,162.1	7.792	2,019.4	118.1	24.7	1.85

*10^{-6} mol kg^{-1} SW.

Closed-System, Acid-Additions

Closed-system, acid-additions simulated three experimental conditions whereby hydrochloric acid (HCl), 0.1 (Normality) N, was added to a closed system to adjust the pH from 8.194 to 8.065 and 7.792, and TCO_2 was held constant at 1994.4 µmol kg^{-1} SW because no carbon entered or escaped the experimental system. Input parameters for these experiments were entered on the data page of the CO2SYS macro file (fig. 1). Salinity, temperature, pressure, total P, and total Si were entered under their respective columns on rows 4, 5, and 6 of the data input spreadsheet using experimental conditions from table 2. Since TCO_2 was held constant, at 1994.4 µmol kg^{-1} SW, this value was entered under the TCO_2 column for each of these three rows. Values for pH of 8.194, 8.065, and 7.792 were entered under the pH column of the spreadsheet, and data columns for TA, fCO_2, and pCO_2 were left blank for this simulation because they were not used as input parameters. Results for input conditions for this simulation are shown in figure 2 (rows 4, 5, and 6). Output condition results were identical to input condition results because input and output conditions were the same and are, thus, not shown.

The volume of acid needed to achieve experimental conditions was calculated using the difference in TA from starting seawater conditions and the TA value at the target pH to determine the number of moles of H^+ needed to balance the total alkalinity change and then dividing by the concentration (normality) of stock acid (0.1N HCl) (eq. 4 and table 1).

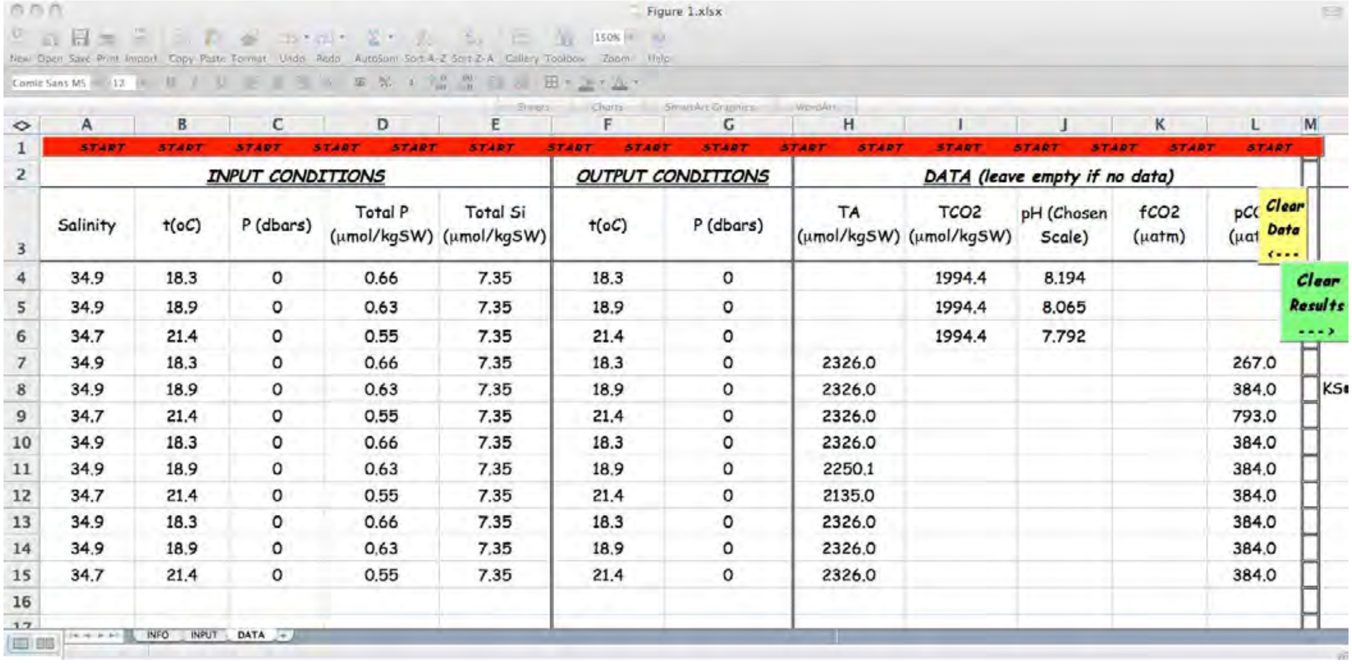
$$\text{Liters of HCl added (L kg}^{-1}\text{ SW)} = \Delta\text{TA (mols kg}^{-1}\text{ SW)}/[\text{H}^+]\text{ (mol L}^{-1}) \qquad (4)$$

This calculation can be made because it is a closed system, and no other constituents besides $[H^+]$ that affect total alkalinity are added (for example, refer to the definition of TA in Dickson, 1981; Zeebe and Wolf-Gladrow, 2001). Experimental parameters and the volume of acid added to each system are listed in table 3.

Closed-System, Carbon Dioxide-Additions

Simulations of closed-system, CO_2-additions assumed pH and carbonate system parameters were adjusted by addition of 100 percent CO_2 gas bubbled into seawater. This experimental approach is the preferred methodology because it most closely resembles the effect of elevated atmospheric pCO_2 equilibrating with surface seawater (Gattuso and Lavigne, 2009). Gattuso and others (2010) describe precautions and appropriate methodologies that should be taken when using this approach to achieve accurate results. The CO_2 bubbling can be achieved by either bubbling seawater with standardized CO_2 gas at the desired pCO_2 concentration and allowing carbonate system parameters to equilibrate or by adding a discrete amount of concentrated CO_2 gas to a closed tank and equilibrating seawater to achieve the desired CO_2 and total carbon concentrations. Calculations for this simulation were based on the latter approach.

Total alkalinity of seawater is a conservative property and does not change with the addition or subtraction of CO_2.



	A	B	C	D	E	F	G	H	I	J	K	L
1	START	START	START	START	START	START	START	START	START	START	START	START
2	INPUT CONDITIONS					OUTPUT CONDITIONS		DATA (leave empty if no data)				
3	Salinity	t(oC)	P (dbars)	Total P (µmol/kgSW)	Total Si (µmol/kgSW)	t(oC)	P (dbars)	TA (µmol/kgSW)	TCO2 (µmol/kgSW)	pH (Chosen Scale)	fCO2 (µatm)	pC (µat
4	34.9	18.3	0	0.66	7.35	18.3	0		1994.4	8.194		
5	34.9	18.9	0	0.63	7.35	18.9	0		1994.4	8.065		
6	34.7	21.4	0	0.55	7.35	21.4	0		1994.4	7.792		
7	34.9	18.3	0	0.66	7.35	18.3	0	2326.0				267.0
8	34.9	18.9	0	0.63	7.35	18.9	0	2326.0				384.0
9	34.7	21.4	0	0.55	7.35	21.4	0	2326.0				793.0
10	34.9	18.3	0	0.66	7.35	18.3	0	2326.0				384.0
11	34.9	18.9	0	0.63	7.35	18.9	0	2250.1				384.0
12	34.7	21.4	0	0.55	7.35	21.4	0	2135.0				384.0
13	34.9	18.3	0	0.66	7.35	18.3	0	2326.0				384.0
14	34.9	18.9	0	0.63	7.35	18.9	0	2326.0				384.0
15	34.7	21.4	0	0.55	7.35	21.4	0	2326.0				384.0
16												

Figure 1. Data input page and input data for CO2SYS experimental simulations calculated in the Microsoft Excel version of the CO2SYS program (Pierrot and others, 2006).

Table 3. Volume of acid or CO_2 gas added to experimental simulations.

[Acid, 0.1 N hydrochloric acid; mL kg^{-1} SW, milliliters per kilogram of seawater; CO_2 gas, 100 percent carbon dioxide gas]

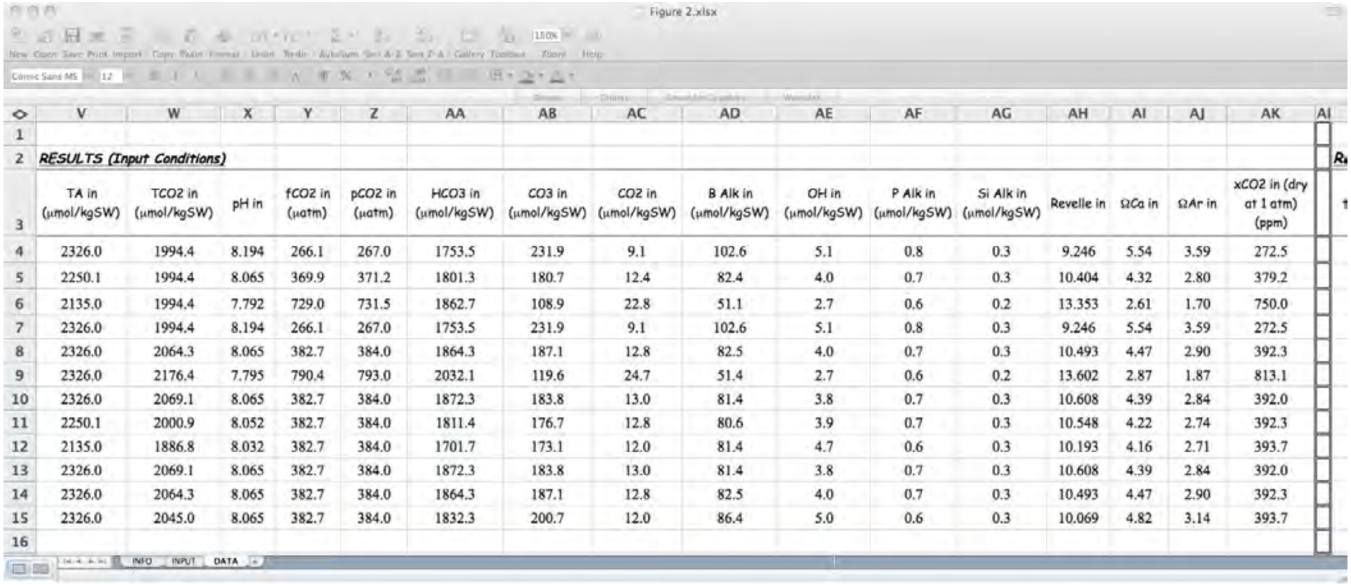
Experimental set	Row number (see Fig. 1)	Acid added (mL kg^{-1} SW)	CO_2 gas added (mL kg^{-1} SW)
Closed system, acid-addition, 1766	4	0.0	–
Closed system, acid-addition, 2007	5	0.759	–
Closed system, acid-addition, 2100	6	1.910	–
Closed system, CO_2-addition, 1766	7	–	0.0
Closed system, CO_2-addition, 2007	8	–	1.701
Closed system, CO_2-addition, 2100	9	–	4.430

Therefore, total alkalinity in all of these experiments was held constant at the starting seawater TA of 2,326 µmol kg^{-1} SW, and this value was entered on rows 7, 8, and 9 under the TA column heading in the CO2SYS spreadsheet (fig. 1). Salinity, temperature, pressure, total P, and total Si were entered on these three rows under their respective columns in the spreadsheet as previously described, and target values for pCO_2 (table 2) were entered under the pCO_2 column of the spreadsheet. Data columns for TCO_2, pH, and fCO_2 were left blank for this simulation (fig. 1). Results for closed-system, CO_2-additions are shown in figure 2.

Discrete volumes of CO_2 gas added to each experiment (table 3) were calculated by multiplying the difference in TCO_2 (in g C kg^{-1} SW) between starting seawater conditions and the TCO_2 of seawater at the target pCO_2 value times the molecular weight of CO_2 and then dividing by the density of CO_2 gas (1.808 g CO_2 L^{-1}) at standard atmospheric temperature and pressure (STP) (eq. 5 and table 1). For use of certified CO_2 gas concentrations lower than approximately 100 percent (for example, 1,000 ppm, or 0.001 L CO_2/L air), the density of CO_2 gas to use in equation 5 can be calculated as a simple dilution factor (eq. 6).

$$\text{Liters of } CO_2 \text{ gas added at STP } kg^{-1} \text{ SW} = \Delta TCO_2 \text{ (mols } kg^{-1} \text{ SW) x } 44.01 \text{g } CO_2 \text{ mol}^{-1}/1.808 \text{ g } CO_{2(g)} \text{ L}^{-1} \quad (5)$$

$$\text{Density of } CO_2 \text{ gas at 1,000 ppm } CO_2 = 0.001 \text{ L } CO_2 \text{ L}^{-1} \text{ air x } 1.8080 \text{ g } CO_2 \text{ L}^{-1} = 0.001808 \text{ g } CO_2 \text{ L}^{-1} \quad (6)$$



	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK
1																
2	***RESULTS (Input Conditions)***															
3	TA in (µmol/kgSW)	TCO2 in (µmol/kgSW)	pH in	fCO2 in (µatm)	pCO2 in (µatm)	HCO3 in (µmol/kgSW)	CO3 in (µmol/kgSW)	CO2 in (µmol/kgSW)	B Alk in (µmol/kgSW)	OH in (µmol/kgSW)	P Alk in (µmol/kgSW)	Si Alk in (µmol/kgSW)	Revelle in	ΩCa in	ΩAr in	xCO2 in (dry at 1 atm) (ppm)
4	2326.0	1994.4	8.194	266.1	267.0	1753.5	231.9	9.1	102.6	5.1	0.8	0.3	9.246	5.54	3.59	272.5
5	2250.1	1994.4	8.065	369.9	371.2	1801.3	180.7	12.4	82.4	4.0	0.7	0.3	10.404	4.32	2.80	379.2
6	2135.0	1994.4	7.792	729.0	731.5	1862.7	108.9	22.8	51.1	2.7	0.6	0.2	13.353	2.61	1.70	750.0
7	2326.0	1994.4	8.194	266.1	267.0	1753.5	231.9	9.1	102.6	5.1	0.8	0.3	9.246	5.54	3.59	272.5
8	2326.0	2064.3	8.065	382.7	384.0	1864.3	187.1	12.8	82.5	4.0	0.7	0.3	10.493	4.47	2.90	392.3
9	2326.0	2176.4	7.795	790.4	793.0	2032.1	119.6	24.7	51.4	2.7	0.6	0.2	13.602	2.87	1.87	813.1
10	2326.0	2069.1	8.065	382.7	384.0	1872.3	183.8	13.0	81.4	3.8	0.7	0.3	10.608	4.39	2.84	392.0
11	2250.1	2000.9	8.052	382.7	384.0	1811.4	176.7	12.8	80.6	3.9	0.7	0.3	10.548	4.22	2.74	392.3
12	2135.0	1886.8	8.032	382.7	384.0	1701.7	173.1	12.0	81.4	4.7	0.6	0.3	10.193	4.16	2.71	393.7
13	2326.0	2069.1	8.065	382.7	384.0	1872.3	183.8	13.0	81.4	3.8	0.7	0.3	10.608	4.39	2.84	392.0
14	2326.0	2064.3	8.065	382.7	384.0	1864.3	187.1	12.8	82.5	4.0	0.7	0.3	10.493	4.47	2.90	392.3
15	2326.0	2045.0	8.065	382.7	384.0	1832.3	200.7	12.0	86.4	5.0	0.6	0.3	10.069	4.82	3.14	393.7
16																

Figure 2. Results page and data for CO2SYS experimental simulations calculated in the Microsoft Excel version of the CO2SYS program (Pierrot and others, 2006).

Open-System, Acid-Additions

Open-system, acid-addition simulations demonstrate the changes that occur to acid-addition experiments if the system is left open to the atmosphere and not controlled with a pH-stat after making chemical adjustments to the system. Input parameters for these simulations were derived from the TA results of closed-system, acid additions and the assumption that each experiment was allowed to re-equilibrate with an assumed atmospheric pCO_2 of 384 µatm. Salinity, temperature, pressure, total P, and total Si were entered on rows 10, 11, and 12 under their respective columns in the CO2SYS spreadsheet as in sets 1 and 2 (fig. 1). Values obtained for TA results in closed-system, acid additions were entered as input data under the TA column of the spreadsheet on rows 10, 11, and 12. A pCO_2 value of 384 µatm was also entered on each of these three rows. Data columns for TCO_2, pH, and fCO_2 were left blank for this simulation (fig. 1). Results for open-system, acid-additions are shown in figure 2 (rows 10, 11, and 12).

Open-System, Carbon Dioxide-Additions

Simulations of open-system, CO_2-additions demonstrate the changes that occur to CO_2 bubbling–perturbation experiments that are left open to the atmosphere and allowed to re–equilibrate with atmospheric pCO_2 after making chemical adjustments to the system. Total alkalinity for these experiments was again held constant at the total alkalinity value from closed–system, CO_2–additions (2,326 µmol kg^{-1} SW), and atmospheric pCO_2 of 384 µatm was assumed. Input parameters for salinity, temperature, pressure, total P, and total Si from closed–system, CO_2–additions were entered on rows 13, 14, and 15 under their respective columns in the spreadsheet (fig. 1). Total alkalinity of 2,326 µmol kg^{-1} SW and pCO_2 of 384 µatm were entered on each of these three rows under their respective columns; data columns for TCO_2, pH, and fCO_2 were left blank (fig. 1). Results for open–system, CO_2–additions are shown in figure 2 (rows 13, 14, and 15).

Assessment

The CO2SYS results for carbonate system parameters from all simulations are shown in figure 2. Differences between calculated carbonate system parameters from each simulation and target values from table 2 are shown in table 4. There are no differences between target values and calculated values for 1766 results in closed-system simulations because target parameters for 1766 were used as the starting point for seawater manipulations simulating conditions during the years 2007 and 2100. Likewise, input parameters for each simulation that were the same as target values for each year showed no difference in table 4.

Chemical Consequences

Data for 2007 and 2100 scenarios from closed-system, acid-additions indicate that calculated carbonate system parameters are approximately 3 percent and 8 percent lower, respectively, than target values for all carbonate system parameters. While target pHs are acquired through acid addition, the lower values for dissolved inorganic carbon species and mineral saturation states result from not adding inorganic carbon to the closed system that would have simulated the increase in TCO_2 that occurs naturally as CO_2 is added. This can be remedied by adding HCO_3^- and/or CO_3^{2-} to increase TCO_2 and TA in the system or avoided by adding acid and inorganic carbon concurrently in appropriate amounts to achieve target levels for all carbonate system parameters. For example, using equations from table 1 and results from table 5, the concentration of dissolved inorganic carbon that needs to be added can be calculated from ΔTCO_2, 70 µmol kg^{-1} SW for the 2007 experiment and 167.7 µmol kg^{-1} SW for the 2100 experiment. The $[CO_3^{2-}]$ to add can be calculated from $\Delta TA - \Delta TCO_2$, and the $[HCO_3^-]$ to add can be calculated from $\Delta TCO_2 - (\Delta TA - \Delta TCO_2)$. The 2007 experiment should receive 4.9 µmol kg^{-1} SW of CO_3^{2-} and 65.1 µmol kg^{-1} SW of HCO_3^-. The same calculation can be performed for the 2100 experiment.

Differences between target and calculated carbonate system parameters in closed-system, CO_2-additions are very small, less than or approximately 1 percent for the 2007 experiment, and less than 5 percent for the 2100 experiment. This experimental approach most closely resembles the natural process of CO_2 equilibration with seawater and the resulting changes to seawater chemistry that occur as TCO_2 increases and pH decreases. TA, TCO_2, HCO_3^-, CO_3^{2-}, and mineral saturation states are slightly higher than target values in the 2100 experiment because TCO_2 was elevated above target levels in a closed system as carbon was added in the form of CO_2, and none was lost through venting to the atmosphere. TA can be reduced to target values by addition of a small amount of acid calculated from ΔTA (table 1), and TCO_2 can be reduced by allowing carbon to off–gas as CO_2 during the acid addition. The amount of acid to add for the 2100 experiment (from table 4) is 16 µmol kg^{-1} SW. Results of closed–system simulations demonstrate the differences in carbonate system parameters from using different seawater manipulation approaches and from target values (particularly in the case of acid additions), even when similar pH conditions are achieved using acid/base additions and CO_2 bubbling approaches.

Inadequate control and monitoring of experimental conditions are common errors in perturbation experiments. Results from open–system simulations show how drastically seawater chemistry can deviate from target values if manipulated seawater conditions are not closely monitored and are allowed to re-equilibrate with atmospheric pCO_2 because they have been left open to the atmosphere after adjustment. These simulations demonstrate the importance of maintaining a controlled system (usually through use of pH or pCO_2-stats) and careful monitoring of chemical parameters through the

Table 4. Difference between target and experimental simulation values for carbonate system parameters.

[TA, total alkalinity; TCO$_2$, total carbon; pCO$_2$, partial pressure of CO$_2$; H+, acid; HCO$_3$-, bicarbonate; CO$_3$, carbonate; CO$_2$, carbon dioxide; Ω_{CA}, calcite saturation state; Ω_A, aragonite saturation state; µmol kg^{-1} SW, micromoles per kilogram of seawater; µatm, microatmospheres]

Experimental simulation	Year	TA in	TCO$_2$ in[a]	pH in	pCO$_2$ in[2]	HCO$_3$ in[a]	CO$_3$ in[a]	CO$_2$ in[a]	Ω_{CA} in	Ω_A in	xCO$_2$ in[c]
Target conditions	1766	2,326.0	1,994.4	8.194	267.0	1,753.5	231.9	9.1	5.5	3.6	272.5
	2007	2,325.0	2,064.4	8.065	384.0	1,863.6	187.0	12.8	4.5	2.9	392.3
	2100	2,310.0	2,162.1	7.792	793.0	2,019.4	118.1	24.7	2.8	1.8	813.1

Experimental simulation	Year	ΔTA[d]	ΔTCO2[d]	ΔpH[d]	ΔpCO2[d]	ΔHCO3[d]	ΔCO3[d]	ΔCO2[d]	ΔΩCa[d]	ΔΩAr[d]	ΔxCO2[d]
Closed-system, acid-addition	1766	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	% Diff.	–	–	–	–	–	–	–	–	–	–
	2007	–74.9	–70.0	0.0	–12.8	–62.3	–6.3	–0.4	–0.2	–0.1	–13.1
	% Diff.	3.2	3.4	0.0	3.3	3.3	3.4	3.1	4.5	3.5	3.3
	2100	–175.0	–167.7	0.0	–61.5	–156.7	–9.2	–1.9	–0.2	–0.1	–63.1
	% Diff.	7.6	7.8	0.0	7.8	7.8	7.8	7.7	7.1	5.4	7.8
Closed-system, CO$_2$-addition	1766	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	% Diff.	–	–	–	–	–	–	–	–	–	–
	2007	1.0	–0.1	0.0	0.0	0.7	0.1	0.0	0.0	0.0	0.0
	% Diff.	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	2100	16.0	14.3	0.003	0.0	12.7	1.5	0.0	0.1	0.1	0.0
	% Diff.	0.7	0.7	0.0	0.0	0.6	1.3	0.0	3.5	5.4	0.0
Open-system, acid-addition	1766	0.0	74.7	–0.13	117.0	118.8	–48.1	4.0	–1.1	–0.8	119.5
	% Diff.	0.0	3.7	1.6	43.8	6.8	20.7	43.6	19.9	22.3	43.8
	2007	–74.9	–63.5	–0.01	0.0	–52.2	–10.3	0.0	–0.3	–0.2	0.0
	% Diff.	3.2	3.1	0.2	0.0	2.8	5.5	0.0	6.7	6.9	0.0
	2100	–175.0	–275.3	0.240	–409.0	–317.7	55.0	–12.7	1.4	0.9	–419.4
	% Diff.	7.6	12.7	3.1	51.6	15.7	46.6	51.4	49.4	48.7	51.6
Open-system, CO$_2$-addition	1766	0.0	74.7	–0.13	117.0	118.8	–48.1	4.0	–1.1	–0.8	119.5
	% Diff.	0.0	3.7	1.6	43.8	6.8	20.7	43.6	19.9	22.3	43.8
	2007	1.0	–0.1	0.0	0.0	0.7	0.1	0.0	0.0	0.0	0.0
	% Diff.	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	2100	16.0	–117.1	0.273	–409.0	–187.1	82.6	–12.7	2.0	1.3	–419.4
	% Diff.	0.7	5.4	3.5	51.6	9.3	70.0	51.4	70.6	70.3	51.6

[a] units in µmol kg^{-1} SW.

[b] units in µatm.

[c] units in ppm, dry at 1 atm.

[d] Δparameter = difference in concentration from target values for each year ([value] – [target]).

duration of experiments. These results also demonstrate how small changes in pH may be accompanied by very large shifts in other carbonate system parameters. Carbonate system parameters in open-system, acid-additions and open-system CO_2-additions differed from target values by up to approximately 52 percent and 70 percent, respectively. Open-system, acid-addition results show that pH differed by only 1.6 percent, 0.2 percent, and 3.1 percent and TCO_2 differed by only 3.7 percent, 3.0 percent, and 12.7 percent for the 1766, 2007, and 2100 experiments, respectively. Despite the small difference in pH and TCO_2, pCO_2 differed by approximately 44 percent, and $[CO_3^{2-}]$ and mineral saturation states differed by 21 percent in the 1766 experiments. The pCO_2 differed in the 2100 experiment by 52 percent, while mineral saturation states and $[CO_3^{2-}]$ differed by 47 percent. The $[HCO_3^-]$ concentration in all open-system, acid-additions varied by less than 16 percent. Results from open-system, CO_2-additions are similar with small differences in pH and TCO_2 (less than 5.5 percent), resulting in large differences in the remainder of the carbonate system parameters for the 1766 and 2100 experiments (table 4). These differences result from uptake of CO_2 from the atmosphere to the experimental system in simulations with initial seawater pCO_2 less than atmospheric values or release of CO_2 in systems with initial seawater pCO_2 greater than atmospheric values. Data for 2007 in both simulations differed only slightly from target values because manipulated seawater conditions and target conditions for 2007 were very similar. TCO_2 and pCO_2 values were too high for the 1766 targets, and too low for the 2100 targets (fig. 2 and table 4). The TA values for years 2007 and 2100 were too low in open-system, acid-additions and too high in open-system, CO_2-additions (fig. 2 and table 4). Other differences in carbonate system parameters for these sets are shown in table 4. In these cases, multiple adjustments to seawater chemistry are required to re-adjust carbonate system parameters to target values (see table 1). These experimental simulations demonstrate the sensitivity of carbonate chemistry to experimental approach, the significant differences in chemical parameters that may result due to various approaches, and the need to carefully plan and monitor experiments.

Biological Consequences

All experimental simulations were based on inorganic equilibrium chemistry and do not include the effects that biological metabolism or calcification have on seawater chemistry. The effects of biological metabolism on carbonate chemistry are of equal concern. The purpose of perturbation experiments is to determine the effect of changing seawater chemistry on biological metabolism, and metabolic rate responses are the primary unknowns. However, there is often basic information available on natural rates of metabolism for most common marine organisms under investigation that should be considered in experimental planning. For example, by knowing previously measured rates of calcification, photosynthesis, and respiration of species under investigation, the biomass of organisms in the experiment, and the volume of the system (tank, incubation chamber), one can estimate potential diurnal fluctuations in pCO_2, total alkalinity, and the remaining carbonate parameters using the known chemical reactions for these processes. These variations in small volume experiments may be large relative to variations in natural, open systems. This type of information can be used to design appropriate experimental systems and to set control values for pH- or CO_2-stat systems to avoid unreasonably extreme experimental conditions.

Only a limited number of experiments have been conducted specifically on the effects of elevated pCO_2 on calcification rate by calcium carbonate secreting organisms; many of these experiments showed varied results (Fabry and others, 2008; Doney and others, 2009). Some species showed increases and others showed decreases in calcification rate with elevated pCO_2 (Doney and others, 2009). Even fewer studies quantify growth rates of marine organisms relative to changes in carbonate system parameters resulting from ocean acidification (Buitenhuis and others, 1999; Langdon and others, 2000; Schneider and Erez, 2006; Lombard and others, 2009). Rate equations developed in studies that relate calcification in a few common species of marine organisms to carbonate system parameters were used to provide examples of how results from the experimental approaches in the previous section may affect experimental outcome with respect to biological response. The exercises provided in the following sections must be considered with caution and only as examples of *potential* consequences. Although different experimental approaches were used in the studies upon which these examples were based and more recent literature provides alternative theories for mechanisms of calcification for some of these organisms, these studies were chosen because (1) carbonate system parameters were well documented enabling comparison among results and (2) empirically tested rate-equations for calcification were provided enabling quantification of the effect of seawater manipulation on calcification rates.

Coccolithophores

Some studies on the coccolithophore *Emiliania huxleyi* show reduced calcification rates with increasing pCO_2 (Riebesell and others, 2000; Zondervan and others, 2001, 2002; Sciandra and others, 2003; Delille and others, 2005; Engel and others, 2005; Zondervan and others, 2007). However, other studies indicate that some species show either no effect or increased calcification rates (Langer and others, 2006; Iglesias-Rodriguez and others, 2008; Shi and others, 2009). Iglesias-Rodriguez and others (2008) observed that at 750 ppm CO_2, rates of calcification doubled for *E. huxleyi*. They suggest differences from previous studies may be due to the use of CO_2 gas rather than acid addition because CO_2 addition causes an increase in dissolved inorganic carbon along with decreased pH and a greater percentage increase in $[HCO_3^-]$ than when the same pH is reached using acid addition. However, Shi and others (2009) experimentally

Table 5. Potential effects of experimental approach on calcification rates (G) of marine organisms for experimental simulations performed at year 2100 scenarios.

[G, net calcification rate; C, carbon; HC_3–, bicarbonate; CO_3^{2-}, carbonate; CO_2, carbon dioxide; μmol kg^{-1} SW, micromoles per kilogram of seawater]

Organism (unit for G)	Experiment (year 2100)	$[HCO_3-]$ (μmol kg^{-1} SW)	$[CO_3^{2-}]$ (μmol kg^{-1} SW)	G	Percent difference of G from target
Emiliania huxleyi (pmol C cell-1 d-1) via Buitenhuis and others 1999	Target	2,019.4	–	2.272	–
	Closed-system, acid-addition	1,862.7	–	2.114	7.0
	Closed-system, CO_2-addition	2,032.1	–	2.284	0.5
	Open-system, acid-addition	1,701.7	–	1.939	15.0
	Open-system, CO_2-addition	1,832.3	–	2.082	8.0
Biosphere community (mmol CaCO3 m-2 d-1) via Langdon and others 2000	Target	–	118.1	–29.4	–
	Closed-system, acid-addition	–	108.9	–34.1	15.8
	Closed-system, CO_2-addition	–	119.6	–28.7	2.6
	Open-system, acid-addition	–	173.1	–1.7	94.2
	Open-system, CO_2-addition	–	200.7	12.2	141.5
Acropora eurystoma (μmol CaCO3 cm-2 h-1) via Schneider and Erez 2006	Target	–	118.1	0.106	–
	Closed-system, acid-addition	–	108.9	0.096	9.6
	Closed-system, CO_2-addition	–	119.6	0.108	1.6
	Open-system, acid-addition	–	173.1	0.166	57.1
	Open-system, CO_2-addition	–	200.7	0.197	85.8
Globigerinoides sacculifer (μg d-1 mg C-1) via Lombard and others 2009	Target	–	118.1	0.747	–
	Closed-system, acid-addition	–	108.9	0.741	0.8
	Closed-system, CO_2-addition	–	119.6	0.748	0.1
	Open-system, acid-addition	–	173.1	0.783	4.8
	Open-system, CO_2-addition	–	200.7	0.800	7.2

demonstrated that method of acidification had no effect on results; a synthesis of recent studies by Schulz and others (2009) support this conclusion. A synthesis of experimental results by Ridgewell and others (2009) further ruled out that *direction* of the calcification response (increasing or decreasing with elevated pCO_2 and decreased pH) results from the type of chemical manipulation. They suggest that some of the disparity between experimental results may be due to varying irradiance and (or) nutrient levels, units for calcification, use of different strains of organisms, and differing ecological adaptations (natural and/or lab-induced) resulting in intraspecific variability.

Despite the variability in results from ocean acidification experiments on coccoliths, most studies that examine physiological mechanisms (such as the inorganic carbon substrate used for calcification and photosynthesis) agree that the effect of pH on rates of calcification and photosynthesis in *E. huxleyi* is smaller than the effect of $[CO_2]$ or $[HCO_3^-]$ between pH 7.5 to 8.5 and that calcification rates are independent of $[CO_3^{2-}]$ (Buitenhuis and others, 1999; Berry and others, 2002). Berry and others (2002) results indicate that HCO_3^- is the primary source for coccolith calcite, CO_2 is preferred for photosynthesis when it is available, and HCO_3^- provides the substrate for both calcification and elevation of CO_2 in the chloroplast through conversion of HCO_3^- to CO_2. Rost and others (2008) provide further discussion on the effects of elevated CO_2 on phytoplankton physiology.

Buitenhuis and others (1999) performed a series of experiments to examine inorganic carbon uptake mechanisms in *E. huxleyi*. They measured rates of photosynthesis and calcification for *E. huxleyi* over a $[HCO_3^-]$ range from approximately 500 to 3,500 μM, a $[CO_3^{2-}]$ range from 15 to 492 μM, a $[CO_2]$ range from 1.7 to 27.6 μM , and a pH range from 7.5 to 8.5 and provided equations relating these rates to $[HCO_3^-]$. Carbonate system parameters in their cultures and experiments were adjusted using a combination of acid additions and bubbling with air containing CO_2. It is notable that the purpose of these experiments was to examine physiological mechanisms, *not* the effects of ocean acidification on growth rates. Therefore, some of the experimental sets in this study were performed under conditions that were not representative of natural seawater dissolved inorganic carbon concentrations to isolate variables that may affect carbon uptake mechanisms. Studies, specifically on ocean acidification impacts, should target natural seawater conditions. However, some physiological studies may appropriately warrant use of unusual conditions to isolate response to individual experimental variables. Results of the Buitenhuis and others (1999) study showed that photosynthesis increases with $[HCO_3^-]$ approximately proportionately with an increase in calcification rate (G). The photosynthesis rate that is supported by CO_2 from the medium remained relatively constant between CO_2 concentrations ranging from 10 to 30 µM. Neither photosynthesis nor calcification correlated with $[CO_2]$ above inorganic carbon concentrations of 500 µM, and photosynthesis likely used hydrogen ions generated by calcification to convert HCO_3^- to CO_2 for photosynthesis. The rate dependency equation for calcification is:

$$G\ (\text{pmol C cell}^{-1}\ \text{day}^{-1}) = ([HCO_3^-] - MIN_{HCO3-})*V_{max}/([HCO_3^-] - MIN_{HCO3-}) + K_m \qquad (7)$$

where

$[HCO_3^-]$ is the bicarbonate concentration of the external medium, MIN_{HCO3-} = 506 µM and is the $[HCO_3^-]$ below which calcification rate is zero;

V_{max} = 6.4 pmol C $cell^{-1}$ day^{-1} and is the saturation constant for photosynthesis; and

K_m = 2,750 µM and is the bicarbonate concentration at which V_{max} is reached. K_m and V_{max} were determined using a Michaelis-Menten kinetics curve fit through their experimental data.

Interestingly, the Buitenhuis and others (1999) experiments on *E. huxleyi* strain Ch 24-90 suggest that calcification rates will increase with ocean acidification as $[HCO_3^-]$ increases. A similar effect was observed by Iglesias-Rodriguez and others (2008) in cultures using CO_2 bubbling and by Shi and others (2009) for strain PLY M219 in cultures using acid/base additions. Ridgewell and others (2009) argue that the differences in carbonate system parameters resulting from use of CO_2 as opposed to acid/base additions (namely that CO_2 bubbling produces a slightly larger increase in $[HCO_3^-]$ than acid additions) are not critical; however, calcification responses dominated by $[HCO_3^-]$ may be underestimated in ocean acidification experiments carried out by acid/base manipulations.

The $[HCO_3^-]$ results from the CO2SYS simulations for the year 2100 were applied to equation 7 from Buitenhuis and others (1999). These results, shown in table 5, demonstrate the potential variability in calcification rate that could have occurred due only to the effect of experimental approach on $[HCO_3^-]$. Percent difference calculations are relative to calcification rates calculated using target values $[HCO_3^-]$ from table 2.

These calculations indicate differences between calcification rates at target and experimental $[HCO_3^-]$ for the experimental sets are relatively small, 0.5 percent for the CO_2-addition approach and 7 to 15 percent for the other experimental approaches (table 5). These results support the arguments of Ridgewell and others (2009) that acid/base additions only slightly underestimate ocean acidification impacts. However, it must be considered that even small differences extrapolated over natural habitat dimensions may produce a significant outcome. For example, maximum cell counts for some mesoscale-coccolithphore blooms were measured at approximately 2,000 cells mL^{-1} (Balch and others, 1991). The difference in calcification rate between the target and acid addition value is 0.158 µmol $CaCO_3$ L^{-1} of seawater. Considering a coccolithophore bloom size of 1 km × 1 km × 10 m of seawater (10^{10} liters), a 7 percent difference in calcification rate is equivalent to a difference of 316 kg of $CaCO_3$ production per bloom per day.

Corals and Coral Reef Communities

Experiments on the response of coral calcification to changing carbonate system parameters show varied results (Jury and others, 2010). Some species show large decreases, while others show small decreases in calcification rate with elevated CO_2 (Langdon and Atkinson, 2005), and some show no response (Pelejero and others, 2005). Calcification by corals (and many other marine organisms including coccolithophores) is further complicated because it occurs in fluids that are isolated from ambient seawater and not directly exposed to ambient changes in $[CO_3^{2-}]$ or $[HCO_3^-]$. Cohen and Holcomb (2009) suggest that decreases in calcification due to decreased pH may also be due to the additional energy expended by organisms to keep pH elevated in their calcifying fluids. Jury and others (2010) suggest that some of the discrepancies among experimental results may be due to experimental approach, particularly experiments that do not discriminate among individual effects of carbonate system parameters.

Recent experiments on coral species (Langdon and others, 2000; Schneider and Erez, 2006; Jury and others, 2010) provide good examples of how multiple approaches for manipulating seawater chemistry can be combined to produce experimental conditions that allow investigation of individual effects of carbonate system parameters. Jury and others (2010) examined the individual effects of $[HCO_3^-]$, $[CO_3^{2-}]$, and pH on calcification rates of the coral species *Madracis auretenra*. Seawater chemistry was manipulated in their experiments using a combination of CO_2, acid, base, and $NaHCO_3$ additions. They showed that calcification rates for this species are best explained by $[HCO_3^-]$ and that increases in $[HCO_3^-]$ from ocean acidification may increase calcification rates for this species. Additionally, Jury and others (2010) suggest that calcification by corals may be affected by changes in $[H^+]$ alone since calcifying cells must expel $[H^+]$ produced during calcification to prevent tissue acidosis. However, Langdon and others (2000) showed that net community calcification for coral reef communities consisting of *Porites astreoides*, *Favia fragum*, *Agaricia* sp., *Siderastrea* sp., and *Acropora cervicornis* was linearly correlated with $[CO_3^{2-}]$. They measured rates of coral reef community calcification in the Biosphere-2 in response to saturation state of seawater at pCO_2 ranging from 192 to 1,394 µatm, $[CO_3^{2-}]$ ranging from 87.9 to 422.1 µmol kg^{-1}, and $[Ca^{2+}]$ ranging from 6.84 to 9.49 mmol kg^{-1} SW. Carbonate system parameters were manipulated in these experiments through addition of $NaHCO_3$, Na_2CO_3, and $CaCl_2$. They showed that net community calcification rate responds to manipulations in concentrations of both CO_3^{2-} and Ca^{2+} and that the rate can be described by a linear function of the ion concentration product ($ICP = [Ca^{2+}][CO_3^{2-}]$) used to calculate mineral saturation state:

$$G\ (\text{mmol CaCO}_3\ \text{m}^{-2}\ \text{day}^{-1}) = k[Ca^{2+}]^n[CO_3^{2-}] + C \qquad (8)$$

Best-fit parameters for their experiments were $k = 1.2 \times 10^7$, $n = 0.69$, and $C = -89$. Zero net calcification occurred at $[CO_3^{2-}]$ of 172 µmol kg^{-1}. The parameter n was used to account for the response of calcification to $[Ca^{2+}]$, which shows saturation at approximately 10 mmol kg^{-1} SW of Ca^{2+} (Langdon and others, 2000). When n is less than 1, the change in calcification rate for a given change in calcium concentration decreases as calcium concentration increases. Factor k described the environmental conditions of their experiment including areal biomass and physical conditions such as temperature, light, and flow rate. The relation $-C/k$ is the ion concentration product at which net calcification was equal to zero. The constants C, k, and n will change with different environmental settings, resulting in different absolute values for calcification rates. The Langdon and others (2000) equation consistently described the dependency of calcification on saturation state for both short–term (days) and long–term (months to years) changes in CO_3^{2-}.

Whether the Langdon and others (2000) equation holds true for other coral reef communities is unknown. However, the $[CO_3^{2-}]$ results from CO2SYS experimental sets for experiments that simulated conditions during the year 2100 were applied to equation 8 from Langdon and others (2000) as an example of potential variation in results from experimental approach. A constant $[Ca^{2+}]$ of 10.12 mmol kg^{-1} SW was assumed. Results of these calculations are shown in table 5. These results indicate dissolution of calcium carbonate in all experimental simulations except for the open–system, CO_2–addition. The dissolution rate for the CO_2–addition experiment was 2.6 percent less than the target value from table 2. However, differences in calcification rate for the remaining simulations were between approximately 16 and 142 percent greater than target calcification rates. The range of calcification rates observed in all of Langdon's experiments was from –8 to 124.7 mmol $CaCO_3$ m^{-2} d^{-1} (or 132 mmol $CaCO_3$ m^{-2} d^{-1}). The range of calculated calcification rates due simply to variation in experimental approach was approximately 42 mmol $CaCO_3$ m^{-2} d^{-1} or 32 percent of the total range of calcification rates observed by Langdon and others (2000). Based on the surface area of the coral reef community of the Biosphere–2 of 750 m^2, a 16 percent difference in calcification rate from the target rate is equivalent to approximately 0.353 kg of $CaCO_3$ d^{-1}.

Schneider and Erez (2006) performed laboratory experiments to separate the effects of different carbonate system parameters on rates of calcification, photosynthesis, and respiration of the hermatypic coral species *Acropora eurystoma*. Experiments were performed at constant TCO_2, constant pH, or constant pCO_2 to examine effects of individual carbonate system parameters. They manipulated carbonate system parameters using acid and base additions, addition of $NaHCO_3$ followed by acid or base addition, and acid or base addition followed by bubbling with ambient air. Schneider and Erez (2006) results indicated that both light and dark calcification rates were positively correlated with $[CO_3^{2}]$ over a concentration range of 117 to 471 µmol kg^{-1} and that the rate was a linear function of $[CO_3^{2-}]$ for both light and dark calcification:

$$G(\mu\text{mol CaCO}_3\ \text{cm}^{-2}\ \text{h}^{-1}) = 1.1 \times 10^{-3}[CO_3^{2-}] - 2.4 \times 10^{-2}\ (\text{light}) \qquad (9)$$

$$G(\mu\text{mol CaCO}_3\ \text{cm}^{-2}\ \text{h}^{-1}) = 1.3 \times 10^{-3}[CO_3^{2-}] - 0.26\ (\text{dark}) \qquad (10)$$

The Schneider and Erez (2006) experiments did not directly examine the effect of $[HCO_3^-]$ concentration on calcification rate. However, their experimental manipulations at constant TCO_2 resulted in a decrease in $[CO_3^{2-}]$ concurrent with increasing $[HCO_3^-]$ as acid or base was added to adjust pH and total alkalinity, while TCO_2 was held constant. Calcification increased linearly in these experiments with increasing $[CO_3^{2-}]$ and decreasing $[HCO_3^-]$. This result was confirmed in their constant pH and pCO_2 experiments where $[CO_3^{2-}]$ and $[HCO_3^-]$ increased concurrently during manipulation of seawater chemistry.

Table 5 shows the results of applying $[CO_3^{2-}]$ from CO2SYS simulation results for the year 2100 to equation 9. Calcification for the closed-system, CO_2-addition experiment was only 1.6 percent higher than the calcification rate calculated for the target $[CO_3^{2-}]$. However, the closed-system, acid-addition experiment was approximately 10 percent lower, while the open-system, acid- and CO_2-addition experiments were approximately 57 and 86 percent higher, respectively.

Foraminifera

The effects of ocean acidification have been studied on only two species of planktonic foraminifera (Doney and others, 2009). Spero and others (1997) and Bijma and others (1999) showed that shell mass of *Globigerinoides sacculifer* and *Orbulina universa* decreased with decreasing [CO_3^{2-}]. Lombard and others (2009) quantified calcification rates for these two foraminiferal species relative to carbonate ion concentrations ranging from 71.9 to 566 μmol kg^{-1} SW and in high- and low-light conditions. The TA and [CO_3^{2-}] in these experiments were manipulated in closed incubation chambers using additions of NaOH and HCl. Results indicated that biomass-normalized rates of calcification decreased significantly with decreasing [CO_3^{2-}] for both species and are also a linear function of [CO_3^{2-}] for *G. sacculifer* and *O. universa* grown in both high- and low-light conditions. The results of the CO2SYS simulations for the year 2100 were applied to the Lombard and others (2009) equation for *G. sacculifer* grown in high-light conditions:

$$G(\mu g\ CaCO_3\ d^{-1}\ \mu gC^{-1}) = 6.5 \times 10^{-4}[CO_3^{2-}] + 0.67 \quad (11)$$

Calcification rates for the closed-system, CO_2-addition and acid-addition experiments were only 0.1 percent higher and 0.8 percent lower from the calcification rate at the target [CO_3^{2-}] for 2100, respectively. Calcification rates for the open-system, CO_2- and acid-addition experiments were 7.2 percent and 4.8 percent higher than the target calcification rate, respectively (table 5). Lombard and others (2009) indicate that calcification rates could decrease by 6 to 13 percent by the year 2100. The differences in calcification rates calculated for each experimental approach seem relatively small; however, they represent a relatively large difference when compared to the total predicted percent change in calcification rate by the year 2100.

Conclusions

Most ocean acidification experiments have focused on one of two different objectives:

1. Investigating biological response to environmental conditions predicted for future years, and
2. Determining the effect of individual chemical parameters on biological response.

Very little empirical data are currently available on the physiological effects of chemical changes due to ocean acidification on marine organisms, thus making it critical to “get the chemistry right” in these types of experiments. The CO2SYS experimental simulations provide very simple examples of the variation in seawater chemistry that can result from use of carbon dioxide (CO_2) versus acid in ocean acidification experiments. Use of CO_2 gas most closely simulates the changes in seawater chemistry resulting from increased atmospheric pCO_2. Use of acid (or base) addition alone results in only small discrepancies (up to approximately 8 percent in our experimental simulations targeting seawater conditions predicted out to the year 2100) from target seawater conditions with respect to carbonate system parameters, even though pH targets are met. Most recent ocean acidification experiments use a combination of techniques for manipulating seawater chemistry to produce accurate target seawater compositions or to produce sets of experimental conditions that examine the effects of individual carbonate system parameters (Schneider and Erez, 2006; Gattuso and Lavigne, 2009; Jury and others, 2010). Acid additions alone have most appropriately been used in the latter to change total alkalinity, pH, and the distribution of dissolved inorganic carbon species while holding TCO_2 constant or combined with aeration to reduce CO_2 levels in experimental media. Acid and base additions can easily be combined with addition of dissolved inorganic carbon (usually in the form of sodium carbonate, Na_2CO_3), or bicarbonate, HCO_3^-) to accurately simulate the changes in pH and TCO_2 that result from CO_2 gas addition.

Even small discrepancies in carbonate chemistry resulting from choice of experimental approach may result in considerable differences in biological response. Only a few studies quantify relations among biogenic calcification rates and carbonate system parameters for marine organisms such as coccolithophores, corals, and foraminifera (Buitenhuis and others, 1999; Langdon and others, 2000; Schneider and Erez, 2006; Lombard and others, 2009). Careful comparison of these empirical relations to the results of CO2SYS experimental simulations for seawater conditions from 1766 to 2100 demonstrates how choice of experimental approach and lack of system control can result in considerable error in results. These results emphasize the need for careful planning, control, and monitoring of experimental systems to ensure that realistic targets are met for seawater media and accurate results are obtained.

Carbonate speciation programs such as CO2SYS (Pierrot and others, 2006) and Seacarb (Lavigne and Gattuso, 2011) are available at no cost through the Internet and are widely used throughout the scientific community to assist with calculation of carbonate system parameters. These programs are valuable tools for conceptualizing and preparing experimental scenarios and chemical manipulations for ocean acidification experiments. The experimental simulations in this study are simple examples of how CO2SYS can be used in this capacity. Gattuso and Lavigne (2009) provide similar methods for use of Seacarb to plan ocean acidification experiments. The use of these programs, however, should not preclude a good understanding of carbonate chemistry. Guides for their use in ocean acidification experiments and appropriate methods for analyzing carbonate system parameters in seawater for ocean acidification experiments are also available from the U.S. Ocean Carbon and Biogeochemistry Program (accessed August 2011 at *http://www.whoi.edu/courses/OCB-OA/*) and the European Project on Ocean Acidification (accessed August 2011 at *http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/*).

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