



# **CO<sub>2</sub> system Calculation Part 2**

**Using CO<sub>2</sub> system calculations  
for experimental CO<sub>2</sub> manipulations**

# Introduction

- Impact of changing seawater chemistry as a result of OA on marine organisms is not well constrained
- Experimental data are needed to understand the consequences
- Perturbation experiments of seawater chemistry are key approaches to investigating biological responses to increasing pCO<sub>2</sub>
- We will examine several techniques carried out in closed and open cell vessels and discuss pros and cons of these approaches

# Main approaches to manipulate CO<sub>2</sub>-system chemistry

## Change TCO<sub>2</sub> at constant TA

- Gas bubbling
- Add high-CO<sub>2</sub> sea water
- Add CO<sub>3</sub><sup>2-</sup> and/or HCO<sub>3</sub><sup>-</sup> followed by strong acid

## Change TA at constant TCO<sub>2</sub>

- Add strong acids and base

## Change TA & TCO<sub>2</sub>

- Add CO<sub>3</sub><sup>2-</sup> and/or HCO<sub>3</sub><sup>-</sup>

## Manipulate [Ca<sup>2+</sup>]

# We will follow modified Gattuso et al. 2009 examples

also section 2 of Guide to Best Practices

## Assumptions:

Seawater pCO <sub>2</sub>	= 384 μatm (Year = 2007)
Target pCO <sub>2</sub>	= 793 μatm (Year = 2100)
Salinity	= 34.9
Temperature	= 18.9°C
Depth	= 0 m
Phosphate & Silicate	= 0 μmol/kg SW
TA	= 2325

*Except we will use Lueker et al. 2000 K<sub>1</sub>, K<sub>2</sub>, Dickson 1990  
KHSO<sub>4</sub> constants ; Use pH-total scale ; Use Uppstrom et al., 2010  
Boron constant*

# **Bubbling gas in a vessel is fairly easy effective way to manipulate CO<sub>2</sub>- system chemistry**

**Systems that have been used:**

**control atmospheric CO<sub>2</sub> and then bubble that  
into container**

**pH-stat- pH is monitored continuously and  
controller opens or closes valves when pH  
goes above or below the target value**

# Lets get started “bubbling”

- Scenario: You have a seawater sample that has the following conditions-

Sal = 34.9    T = 18.9C    pH = 8.2    TA = 2300  $\mu\text{mol/kg}$

Nutrients were not measured

To “mimic” year 2100, you bubble in a CO<sub>2</sub> –air mixture of 793  $\mu\text{atm}$ – what is your final pH?

.....

*Constants:  $K_1, K_2$ : Leuker,  $K_{SO_4}$ : Dickson,  $pH_{tot}$ ,  $B_t$ : Uppstrom*

# 8.2 starting point

## Sample Results

Input Conditions

Computed Constants

### Physical Parameters

Salinity

34.900

Temperature (°C)

18.900

Pressure (dbars)

0.000

Density (kg m<sup>-3</sup>)

1024.971

### Nutrient Data

Total P (μmol/kgSW)

0.000

Total Si (μmol/kgSW)

0.000

### Air-Sea CO<sub>2</sub> Flux

Flux (mmol/m<sup>2</sup>/d)

### Carbonate

TA (μmol/kgSW)

2300.000

TCO<sub>2</sub> (μmol/kgSW)

1963.773

pH (chosen scale)

8.200

fCO<sub>2</sub> water (μatm)

259.093

pCO<sub>2</sub> water (μatm)

259.987

CO<sub>3</sub> (μmol/kgSW)

234.509

### Calcium

Calculated (μmol/kgSW)

10255.185

User Value

### Auxiliary Results

HCO<sub>3</sub> (μmol/kgSW)

1720.601

CO<sub>2</sub> (μmol/kgSW)

8.664

B Alk (μmol/kgSW)

104.941

OH (μmol/kgSW)

5.447

P Alk (μmol/kgSW)

0.000

Si Alk (μmol/kgSW)

0.000

Revelle

9.106

xCO<sub>2</sub> (dry @ 1 atm)  
(ppm)

265.598

Ω Ca (Using:

Ca from Salinity

CO<sub>3</sub> from Carbonates)

5.607

Ω Ar (Using:

Ca from Salinity

CO<sub>3</sub> from Carbonates)

3.633

Ω Ca (Using User Values)

Ω Ar (Using User Values)

## Calculation Preferences

CO<sub>2</sub> Constant: Lueker et al., 2000

pH Scale: Total scale (mol/kg-SW)

KHSO<sub>4</sub>:

Dickson, 1990

# At 793 $\mu$ atm pH=7.789

## Sample Results

### Input Conditions

### Computed Constants

#### Physical Parameters

Salinity

34.900

Temperature ( $^{\circ}$ C)

18.900

Pressure (dbars)

0.000

Density ( $\text{kg m}^{-3}$ )

1024.971

#### Nutrient Data

Total P ( $\mu\text{mol/kgSW}$ )

0.000

Total Si ( $\mu\text{mol/kgSW}$ )

0.000

#### Air-Sea CO<sub>2</sub> Flux

Flux ( $\text{mmol/m}^2/\text{d}$ )

#### Carbonate

TA ( $\mu\text{mol/kgSW}$ )

2300.000

TCO<sub>2</sub> ( $\mu\text{mol/kgSW}$ )

2168.661

pH (chosen scale)

7.789

fCO<sub>2</sub> water ( $\mu\text{atm}$ )

790.273

pCO<sub>2</sub> water ( $\mu\text{atm}$ )

793.000

CO<sub>3</sub> ( $\mu\text{mol/kgSW}$ )

107.519

#### Calcium

Calculated ( $\mu\text{mol/kgSW}$ )

10255.185

User Value

#### Auxillary Results

HCO<sub>3</sub> ( $\mu\text{mol/kgSW}$ )

2034.717

CO<sub>2</sub> ( $\mu\text{mol/kgSW}$ )

26.425

B Alk ( $\mu\text{mol/kgSW}$ )

48.150

OH ( $\mu\text{mol/kgSW}$ )

2.112

P Alk ( $\mu\text{mol/kgSW}$ )

0.000

Si Alk ( $\mu\text{mol/kgSW}$ )

0.000

Revelle

14.284

xCO<sub>2</sub> (dry @ 1 atm)  
(ppm)

810.116

$\Omega$  Ca (Using:  
Ca from Salinity  
CO<sub>3</sub> from Carbonates)

2.571

$\Omega$  Ar (Using:  
Ca from Salinity  
CO<sub>3</sub> from Carbonates)

1.666

$\Omega$  Ca (Using User Values)

$\Omega$  Ar (Using User Values)

## Calculation Preferences

CO<sub>2</sub> Constant: Lueker et al., 2000

pH Scale: Total scale (mol/kg-SW)

Total Boron: Uppstrom, 1974

KHSO<sub>4</sub>: Dickson, 1990

Air-Sea Flux:



# Now using a pH-stat system

- Scenario: You have a seawater sample that has the following conditions-

Sal = 34.9    T = 18.9C    pH = 8.2    TA = 2300  
μmol/kg

Nutrients were not measured

Now, you want to keep pH at 7.3 target – what [CO<sub>2</sub>] do you bubble in?

# pCO2 of water should be = 2648.554

CO2Calc v4.0.4

## Sample Results

Input Conditions

Computed Constants

### Physical Parameters

Salinity

34.900

Temperature (°C)

18.900

Pressure (dbars)

0.000

Density (kg m<sup>-3</sup>)

1024.971

### Nutrient Data

Total P (μmol/kgSW)

0.000

Total Si (μmol/kgSW)

0.000

### Air-Sea CO2 Flux

Flux (mmol/m<sup>2</sup>/d)

### Carbonate

TA (μmol/kgSW)

2300.000

TCO2 (μmol/kgSW)

2332.795

pH (chosen scale)

7.300

fCO2 water (μatm)

2639.448

pCO2 water (μatm)

2648.554

CO3 (μmol/kgSW)

37.863

### Calcium

Calculated (μmol/kgSW)

10255.185

User Value

### Auxillary Results

HCO3 (μmol/kgSW)

2206.673

CO2 (μmol/kgSW)

88.258

B Alk (μmol/kgSW)

16.966

OH (μmol/kgSW)

0.686

P Alk (μmol/kgSW)

0.000

Si Alk (μmol/kgSW)

0.000

Revelle

16.714

xCO2 (dry @ 1 atm)  
(ppm)

2705.719

Ω Ca (Using:  
Ca from Salinity  
CO3 from Carbonates)

0.905

Ω Ar (Using:  
Ca from Salinity  
CO3 from Carbonates)

0.587

Ω Ca (Using User Values)

Ω Ar (Using User Values)

TCO<sub>2</sub> increased

TA constant

# Summary: Bubbling of CO<sub>2</sub> gas

**Technique:** Bubble CO<sub>2</sub> gas mixtures in seawater

- (1) air and CO<sub>2</sub>
- (2) CO<sub>2</sub>-free air and CO<sub>2</sub>
- (3) CO<sub>2</sub>-free air, air and CO<sub>2</sub>

-----

- (1) pH-stat or pCO<sub>2</sub>-stat
- (2) bubbling with premixed gases (purchased or made with mass flow controllers or gas mixing pumps)

In both cases, TCO<sub>2</sub> increased and TA remained same

**Advantages:** TA unaffected

**Disadvantages:** Requires daily calibration; Can cause coagulation of organic matter, or unwanted turbulence; changes in alkalinity because of precipitation or dissolution of CaCO<sub>3</sub> not accounted for

**seacarb:** pgas

TCO<sub>2</sub> increased  
TA constant

# Bubbling of CO<sub>2</sub> gas

**Example:** Estimate the volume of CO<sub>2</sub> gas needed to adjust pCO<sub>2</sub> from 384 to 793 μatm

@STP:

$$\text{L of CO}_{2(g)} = \Delta\text{TCO}_2 \text{ (mol kg}^{-1} \text{ SW)} * \text{Mol. Wt. CO}_2 / \text{density of CO}_{2(g)}$$

$$\begin{aligned} \text{L of CO}_{2(g)} &= (0.000126 \text{ mol kg}^{-1} \text{ SW} * 44.01 \text{ g CO}_2 \text{ mol}^{-1}) / 1.808 \text{ g L}^{-1} \\ &= 0.00443 \text{ L kg}^{-1} \text{ SW} \\ &= 4.43 \text{ mL kg}^{-1} \text{ SW} \end{aligned}$$

***In reality, this calculation is not very useful.  
The best way to determine volume of gas needed is to  
experiment with it!***

TCO<sub>2</sub> increased  
TA constant

# Adding high-CO<sub>2</sub> water

**Technique:** Mixing of two water masses

(T=18.9°C; S=34.9; TA=2325 μmol kg<sup>-1</sup>)

**Water mass #1:** pCO<sub>2</sub>=10<sup>6</sup> μatm (CO<sub>2</sub>-saturated)

**Water mass #2:** pCO<sub>2</sub>=384 μatm

**Desired mass:** pCO<sub>2</sub>=793 μatm

*Q: What proportions of water masses #1 and #2 should be mixed to achieve the desired concentration?*

TCO<sub>2</sub> increased  
TA constant

# Adding high-CO<sub>2</sub> water

**Technique: Mixing of two water masses**

(T=18.9°C; S=34.9; TA=2325 μmol kg<sup>-1</sup>)

**Water mass #1:** pCO<sub>2</sub>=10<sup>6</sup> μatm → TCO<sub>2</sub> = 35666 μmol kg<sup>-1</sup>

**Water mass #2:** pCO<sub>2</sub>=384 μatm → TCO<sub>2</sub> = 2063 μmol kg<sup>-1</sup>

**Desired mass:** pCO<sub>2</sub>=793 μatm → TCO<sub>2</sub> = 2190 μmol kg<sup>-1</sup>

TCO<sub>2</sub> increased  
TA constant

# Adding high-CO<sub>2</sub> water

Technique: **Mixing of two water masses**

(T=18.9°C; S=34.9; TA=2325 μmol kg<sup>-1</sup>)

**Water mass #1:** pCO<sub>2</sub>=10<sup>6</sup> μatm → TCO<sub>2</sub> = 35666 μmol kg<sup>-1</sup>

**Water mass #2:** pCO<sub>2</sub>=384 μatm → TCO<sub>2</sub> = 2063 μmol kg<sup>-1</sup>

**Desired mass:** pCO<sub>2</sub>=793 μatm → TCO<sub>2</sub> = 2190 μmol kg<sup>-1</sup>

**Basic Mixing Equation:**

$$\text{Conc}_1 \times \text{Vol}_1 + \text{Conc}_2 \times (\text{Vol}_2 - \text{Vol}_1) = \text{Conc}_3 \times (\text{Vol}_2)$$

$$35666 \mu\text{mol kg}^{-1} \times (V_1) + 2063 \mu\text{mol kg}^{-1} \times (1 - V_1) = 2190 \mu\text{mol kg}^{-1} \times (1)$$

**Vol<sub>1</sub> = 0.00378 kg of high pCO<sub>2</sub> seawater**

**1 - V<sub>1</sub> = 0.99622 kg of normal pCO<sub>2</sub> seawater**

TCO<sub>2</sub> increased  
TA constant

# Adding high-CO<sub>2</sub> water

## Technique: Mixing of two water masses

1. Water with normal values (e.g. pCO<sub>2</sub>=373 uatm)
2. Water that has been saturated with CO<sub>2</sub> (pCO<sub>2</sub>=10<sup>6</sup> uatm)

## Advantages:

- Natural simulation of future conditions
- No effect on TA

## Disadvantages:

- High pCO<sub>2</sub> waters can easily lose CO<sub>2</sub> due to gas exchange

**Seacarb function:** p<sub>mix</sub>



TCO<sub>2</sub> increased  
TA constant

# Adding CO<sub>3</sub><sup>2-</sup> and/or HCO<sub>3</sub><sup>-</sup> + acid

## Technique:

1. Add CO<sub>3</sub> and/or HCO<sub>3</sub> to obtain desired TCO<sub>2</sub> level
2. Add acid to lower TA to desired TCO<sub>2</sub> level

## Example:

1. Add  $15.3 \times 10^{-6}$  mol kg<sup>-1</sup> of Na<sub>2</sub>CO<sub>3</sub>  
Add  $111.2 \times 10^{-6}$  mol kg<sup>-1</sup> of NaHCO<sub>3</sub>

Note that this elevates DIC by  
126.5 umol  
But also elevates TA by 141.8  
umol

2. Calculate the resulting CO<sub>2</sub>-system chemistry
3. Add 14.18 ml of 0.01N HCl to restore TA to initial value
3. Calculate the final CO<sub>2</sub>-system chemistry

TCO<sub>2</sub> constant  
TA increased

# Adding strong acids, bases

**Technique:** Add strong acids to decrease TA - OR –  
Add strong bases to increase TA

1. In a closed system, DIC remains unchanged
2. In an open system, DIC will change due to air-sea gas exchange

**Example:**

1. *How much acid do you need to change the alkalinity to cause a pCO<sub>2</sub> change from 394 to 793 uatm?*

**Answer 140.8 umol kg<sup>-1</sup>**

2. **Add 14.08 ml of 0.01N HCl to 1 kg seawater**
3. **Calculate the CO<sub>2</sub>-system chemistry in a closed system**

seacarb function: ppH

TCO<sub>2</sub> constant  
TA increased

# Adding strong acids, bases

**Technique:** Add strong acids to decrease TA - OR –  
Add strong bases to increase TA

1. In a closed system, DIC remains unchanged
2. In an open system, DIC will change due to air-sea gas exchange

## **Advantages:**

Relatively easy to do

Can be used in flow-through systems

## **Disadvantages:**

Not a realistic representation of the CO<sub>2</sub> chemistry but depends on your question

TCO<sub>2</sub> changed  
TA changed

# Adding CO<sub>3</sub><sup>2-</sup> and/or HCO<sub>3</sub><sup>-</sup> (no acid addition)

**Technique:** Add CO<sub>3</sub> and/or HCO<sub>3</sub> to obtain desired TCO<sub>2</sub> level

1. Closed system:

TA increases by:  $2 \times \Delta[\text{CO}_3^{2-}]$  and  $1 \times \Delta[\text{HCO}_3^-]$

DIC increases by:  $1 \times \Delta[\text{CO}_3^{2-}]$  and  $1 \times \Delta[\text{HCO}_3^-]$

2. Open system: DIC equilibrates due to air-sea gas exchange

## Advantages:

Can be used to examine physiological responses to different components of the carbonate chemistry

## Disadvantages:

Not recommended

**seacarb function:** pTA

Manipulate  $[Ca^{2+}]$

# Manipulation of $[Ca^{2+}]$

**Technique:** Add or reduce the  $[Ca^{2+}]$  levels

Causes changes in the  $CaCO_3$  saturation states

## **Advantages:**

Can be used to examine the role of  $Ca^{2+}$  in saturation state and its effects on organisms

## **Disadvantages:**

Not recommended

**seacarb function:** pCa

# Summary of Methods

Approach	Target values reached?	Ease of use	Relative cost
Acid addition	No (only pCO <sub>2</sub> )	***	*
Acid addition and restoration of TA	Yes	*	*
Gas bubbling	Yes	***	***
Mixing with high-CO <sub>2</sub> seawater	Yes	**	*
Addition of HCO <sub>3</sub> <sup>-</sup> and/or CO <sub>3</sub> <sup>2-</sup>	No	**	*
Calcium manipulation	No (only Ω)	*	*



## *LAST THOUGHTS*

- ✓ Understand how your choice of approach affects carbonate chemistry
- ✓ The more information you have for planning, the better
- ✓ Choose reasonable target conditions
- ✓ Know your chemistry...don't rely on CO<sub>2</sub>calc (or any other program) to know it for you...this is a VERY common mistake
- ✓ Biological effects on the chemistry must be considered in experimental design
- ✓ Monitor closely - before, during, and after chemical manipulations and throughout experiments