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# Corals on Acid

## An Inquiry-based Activity Leading Students to a Better Understanding of Ocean Acidification Impacts

BY CASEY L. BOLEMAN, PHILIP M. GRAVINESE, ELLEN N. MUSE,  
ANDREA E. MARSTON, AND JOHN G. WINDSOR

### PURPOSE

The objective of this inquiry-based lesson is for students to gain an understanding of how increasing ocean acidity can affect the calcification of marine organisms. During this activity, students: (1) design an experiment to quantify the  $\text{CaCO}_3$  concentration of two invertebrate skeletal samples, one that has been soaked in normal seawater and another in a low pH solution, and (2) use critical thinking and discussion to evaluate possible explanations for the difference in the skeletal  $\text{CaCO}_3$  compositions. Our lesson focuses on exploring the activity before ocean acidification is introduced to provide a common conceptual framework to engage students.

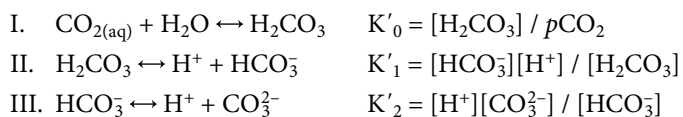
### AUDIENCE

The lesson is designed for undergraduate introductory chemistry or chemical oceanography courses.

### BACKGROUND

The ocean has absorbed more than one-third of the atmospheric  $\text{CO}_2$  released since the Industrial Revolution (Fabry et al., 2008). Current atmospheric  $\text{CO}_2$  levels are approaching 400 ppmV (Hilmi et al., 2012). Oceanic surface waters absorb excess atmospheric  $\text{CO}_2$ , resulting in decreased ocean pH via a series of chemical reactions (see below). This process is known as ocean acidification (Caldeira and Wickett, 2003). When  $\text{CO}_2$  dissolves into the ocean, carbonic acid forms (I). Carbonic acid

dissociates in water, forming  $\text{H}^+$  and bicarbonate ions (II). The release of  $\text{H}^+$  ions (II) results in lower ocean pH (pH is defined as  $-\log [\text{H}^+]$ ). The bicarbonate ( $\text{HCO}_3^-$ ) formed (II) dissociates into  $\text{H}^+$  ions and carbonate ions ( $\text{CO}_3^{2-}$ ) (III). The reaction of  $\text{H}^+$  with carbonate lowers the concentration of  $\text{H}^+$ , serving to buffer the system and minimize changes in ocean pH.



The equilibrium relationships between total dissolved inorganic carbon (i.e., the relationship between the species in brackets) are represented by  $K$  and are provided to the right of each equation (Dickson, 2011). When  $\text{CO}_2$  is added to seawater, the reactions take place in equilibrium. The equilibrium constants are a function of temperature, salinity, and atmospheric pressure of  $\text{CO}_2$  (Dickson, 2011). The increase in anthropogenic release of  $\text{CO}_2$  leads to increasing  $\text{CO}_2$  concentration in the ocean and results in a reduction of carbonate ions needed by marine calcifiers (e.g., clams, oysters, corals) to make their exoskeletons/shells (EUR-OCEANS, 2007; Ries et al., 2009). Under current rates of fossil fuel release, models forecast a 178% increase in carbonic acid concentrations, which is projected to reduce surface seawater pH from 8.1 to 7.7 by the end of the century (Intergovernmental Panel on Climate Change, 2013; Fabry et al., 2008).

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Figure 1. Materials used may include dolomite, pure calcium carbonate, or crushed shells from oyster, mussel, shrimp, pencil urchin, coral, and cuttlebone.

Decreases in ocean pH pose problems for calcifying marine organisms and could negatively impact marine food webs. For example, the ability of calcifying plankton (foraminiferans and pteropods) to maintain their shells significantly declines with increasing acidity due to the lower availability of carbonate ions (Feely, 2006). Many of these planktonic organisms are important food sources for fish, invertebrates, and mammals. Severe changes in planktonic populations may alter the community structure and could negatively impact trophic cascades (i.e., primary and secondary consumers; Kleypas et al., 2006).

Diminishing availability of carbonate ions ( $\text{CO}_3^{2-}$ ) can reduce development, growth, and survival throughout larval development (Ries et al., 2009; Gazeau et al., 2010; Barros et al., 2013; Kroeker et al., 2013). For example, a recent meta-analysis demonstrated that molluscs show significant reductions in calcification (40%), survival (34%), growth (17%), and development (25%), with larvae being the most sensitive to lower pH scenarios (values are effect sizes, see Kroeker et al., 2013). Reductions in calcification and growth during larval stages could lead to potential bottlenecks that result in decreased ecological fitness for the species (Langenbuch and Portner, 2004). As a result, early life history stages could be physiologically constrained in their ability to adapt to climate stressors.

The current rapid ocean acidification does not allow time for slow-growing organisms, such as corals, to acclimate. For instance, resistance to bleaching and disease may decrease as corals re-allocate energy toward overcoming reductions in skeletal production. Diminishing coral habitat will adversely impact coastal communities that rely on reefs for protection from storm surge and erosion (Kleypas et al., 2006). In addition, a deteriorating reef ecosystem is likely to negatively impact important economic fisheries (e.g., grouper, snapper, and lobsters) and tourism, resulting in a loss of billions of dollars for these industries (National Oceanic and Atmospheric Administration, 2008).

## RESEARCH QUESTIONS AND HYPOTHESES

During this activity, students choose one type of animal skeleton (e.g., crab, shrimp, urchin, coral) and design an experiment to compare the  $\text{CaCO}_3$  content of the skeletal samples from two different environmental scenarios (control pH vs. low pH solutions). Prior to class, the instructor prepares the skeletal materials by soaking them in solutions of differing pH. Students will then generate their own hypotheses and design procedures to determine the  $\text{CaCO}_3$  content of their samples. For example, students might hypothesize that they can capture and quantify the amount of  $\text{CO}_2$  gas released from the reaction of HCl with  $\text{CaCO}_3$  in their unknown samples. The balanced equation and mole ratios could then be used to estimate the amount of  $\text{CaCO}_3$  in their samples. Research questions for this activity include: (1) How can we calculate the  $\text{CaCO}_3$  content of the samples? and (2) What might have led to the variation in calcium carbonate content between the two treatments? Prior to experimentation, instructors should have students explain the rationale for their hypotheses and critique their experimental designs.

## MATERIALS

### Main Activity

- Organism information table (Table 1)
- Samples of cuttlefish bone, coral, and shells from crab, shrimp, clam, mussel, snail, and oyster. Some shells may be found at craft stores; shrimp, clam, and oyster shell samples can be obtained at seafood markets or grocery stores.
- Gloves, goggles, aprons
- Vinegar (or dilute HCl) for instructor to prepare “low pH condition” samples
- HCl (3 M solution)
- Thin-stem pipettes (~ 4 ml)
- Electronic balance (precision of  $\pm 1$  mg)
- Mortar and pestle
- Beakers of various sizes (50 ml, 250 ml)
- Graduated cylinders (10 ml, 50 ml)
- Fume hood
- Watch glasses
- Balloons
- Drying paper
- Rubber spatula
- Stirring rod
- Filter paper and weigh paper
- Funnel

### Post-Activity Demonstration

- Clear plastic cups
- Straws
- Freshwater pH indicator solution (for aquarium) with color pH scale
- Tap water

## MAIN ACTIVITY | Determining the CaCO<sub>3</sub> Composition of Shells

*SUGGESTED CLASS TIME | ~ 2.5 hours (may vary due to the open-ended nature of the inquiry activity)*

*INSTRUCTOR PREPARATION IN ADVANCE OF LESSON | One day (or more) prior to planned class activity, split up the skeleton or shell samples and soak half of each in two treatments, control pH (regular seawater, ~ 8.0 pH) and low pH (vinegar or dilute HCl). Soak in treatment overnight. Remove samples from solutions and allow them to dry. If you plan on crushing the samples, you may prefer to crush them after this step.*

During this activity, student groups (two or three per group) are challenged to design an experiment to compare the calcium carbonate content of two shell samples from one type of marine invertebrate. The students can choose from whatever types of skeletons or shells are available from the instructor. After completing the experiment, students compare their results and discuss the differences they found between the two skeletal samples. Ocean acidification is introduced as a potential contributor to the deterioration of calcium-based structures in marine organisms. This activity is a revision of a guided inquiry activity investigating the CaCO<sub>3</sub> composition of eggshells (Lechtanski, 2000).

1. The instructor provides students with 0.5 g each of one type of shell or skeleton from the “control” and “low pH” prepared samples. Students are asked to design an experiment to compare the CaCO<sub>3</sub> content from the two samples of crushed skeletons or shells.
2. If students do not have strong chemistry backgrounds, instructors can hint/suggest using hydrochloric acid and provide the following chemical equation for the reaction that will take place:



3. Instructors review and approve student procedures before experimentation begins. Experimental design flaws often overlooked by students include remembering to record the sample mass before/after experimentation, use of proper filtering techniques, and recording the filter paper mass before use. The experimental design and experiment take about two to three hours. Address safety concerns before experimentation. Assume standard temperature and pressure.
4. Methods developed by students (not provided as “choices”) used to determine percent calcium carbonate composition of the samples are outlined below (Figure 2):
  - a. Method #1 (most common): Add 3 M HCl to the skeleton or shell (whole or crushed) and stir until the bubbling stops (need to do this exercise for each of the two samples). Filter the mixture and determine the mass of the remaining skeleton after drying. The mass of undissolved solid equals the mass of the skeleton that is **not** CaCO<sub>3</sub>. Percent composition can then be calculated:

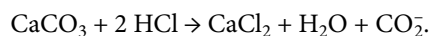
$$\% \text{ composition} = \frac{\text{mass CaCO}_3}{\text{initial mass of skeleton}}$$

Table 1. Descriptions of organisms’ key features. Information provided can be presented to the students via picture description cards (previously made by the instructor). *From Clarke and Wheeler (1922)*

| Organism      | Taxonomy                                   | Shell Composition  | Characteristics  |
|---------------|--|--|--|
| Cuttlefish    | Phylum: Mollusca<br>Class: Cephalopoda     | Chitin, 85% CaCO <sub>3</sub>  | Soft body, internal shell, cuttlefish bones implode between 200 and 600 m, most live near seafloor |
| Pencil Urchin | Phylum: Echinodermata<br>Class: Echinoidea | Thin shell, 60% CaCO <sub>3</sub> , phosphate and silica   | Spines, grazes on algae, spherical body, live in reef habitat                                      |
| Oyster        | Phylum: Mollusca<br>Class: Bivalvia        | Shell 90% CaCO <sub>3</sub> , organic material, some manganese, iron, aluminum, sulfate, and magnesium | Sessile, two shells, filter feeders, live cemented to bottom, mostly in bays and estuaries         |
| Coral         | Phylum: Cnidaria<br>Class: Anthozoa        | Secreted skeleton, 99% CaCO <sub>3</sub> , some sulfate  | Soft gelatinous-like body, lives in large colonies in warm clear water, slow growing               |
| Shrimp        | Phylum: Arthropoda<br>Class: Crustacea     | Exoskeleton composed of carbohydrate chitin, 20% CaCO <sub>3</sub>                                     | Nocturnal, thin exoskeleton with thickest part near the head, scavengers                           |

Percent CaCO<sub>3</sub> content calculation errors occur when students measure the mass of the sample before it is completely dry or before the reaction has completed.

- b. Method #2 (second most common): This method follows the same procedures as the first method, but rather than measuring mass, students evaporate the water in the filtrate. After evaporation, CaCl<sub>2</sub> remains. Students use stoichiometry to determine the CaCO<sub>3</sub> percent composition by using the mole ratio of CaCl<sub>2</sub> to CaCO<sub>3</sub> in the following reaction:



Students can determine mass of CaCO<sub>3</sub> from the mass of CaCl<sub>2</sub> as follows:

$$\begin{aligned} \text{Mass of CaCO}_3 &= \\ \frac{\text{mass of CaCl}_2}{1} \times \frac{1 \text{ mol CaCl}_2}{110.0 \text{ g CaCl}_2} \times \frac{1 \text{ mol CaCO}_3}{1 \text{ mol CaCl}_2} \times \frac{101.1 \text{ g CaCO}_3}{1 \text{ mol CaCO}_3} \\ &= \text{g of CaCO}_3 \end{aligned}$$

Students can then calculate percent composition of CaCO<sub>3</sub>. Instructors should note that this method takes longer to complete than the previous method.

- c. Method #3 (less common): Students may decide to measure the volume of the CO<sub>2</sub> gas produced using a water displacement method (Figure 3). Students approximate the relationship between the quantity of gas released during their experiment and percent CaCO<sub>3</sub> composition of their sample. After determining the volume and type of gas produced, students write a balanced chemical equation for the reaction. Knowing the volume of gas collected allows students to solve for moles of CO<sub>2</sub> using the Ideal Gas Law (Table 2)  $PV = nRT$  ( $P = 1 \text{ atm}$ ,  $V = \text{volume}$

of CO<sub>2</sub> collected,  $n = \text{moles of CO}_2$ ,  $R = 0.0821$ , and  $T = \text{temperature of water the gas was collected over}$ ). After determining moles of CO<sub>2</sub>, students can estimate percent CaCO<sub>3</sub> composition by using mole ratios to determine the moles of CaCO<sub>3</sub>. Mass of CaCO<sub>3</sub> can then be calculated. Remind students that temperature and pressure affect gas solubility (i.e., as temperature of a solution increases, gases become less soluble; as pressure increases, gases become more soluble). Be sure the gas collection apparatus is tightly sealed to prevent gas escape. This method yields poor analytical results due to gas loss.

- d. Method #4 (less common): Students can also collect the CO<sub>2</sub> released from the reaction by attaching a balloon on top of an Erlenmeyer flask and calculating the mass of CO<sub>2</sub> trapped in the balloon. Make sure the reaction has enough time to complete and minimize gas escape when sealing the balloon onto the flask. Using the same balanced equation and mole ratios described above, students can estimate the mass of CaCO<sub>3</sub>.
5. Discussion of results. After experimentation, students evaluate their results (graphical representations) by making comparisons to the known CaCO<sub>3</sub> concentrations (percent CaCO<sub>3</sub>), and they explain discrepancies between experimental and known values, as well as differences between the two samples taken from different environments. Instructors should direct discussions focusing on experimental design improvements. Example topics for discussion could include: *Describe how you determined the composition of your sample. Explain your hypothesis in reference to your results. Identify possible sources of error that may have caused inaccurate measurements and explain them. What would you modify if*

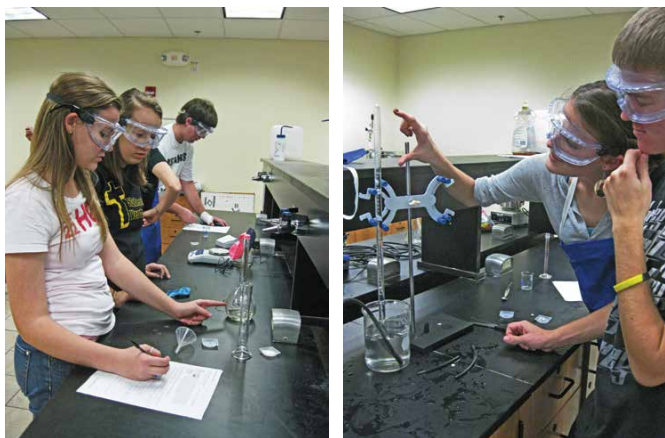


Figure 2. Students use methods selected by them to determine calcium carbonate content from skeletal debris.

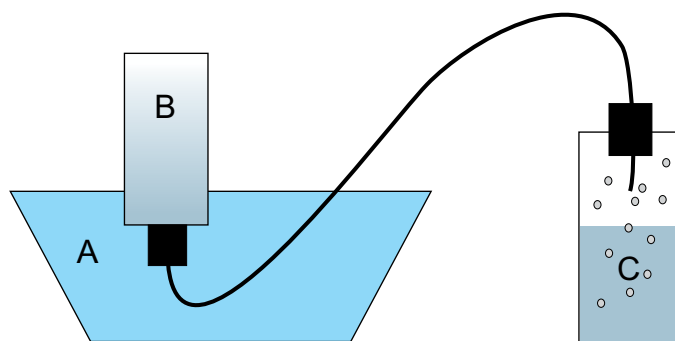


Figure 3. Representation of the water-filled displacement apparatus (pneumatic trough, A). Tubing (black) attaches to the inverted water-filled gas collection bottle (B) and the reaction bottle (C) allowing transport of CO<sub>2</sub> gas bubbles from (C) to (B).

you repeated the experiment? What might explain the differences between the two samples, given that they are from the same species? What difference in environmental conditions might lead to the given results?

- Allow students to come up with pH as a potential reason for the differences in samples. Before getting too involved in the discussion of reduced pH in the ocean, the instructor can challenge students to explore their “theories”/reasons by performing the demonstrations described in the Post-Activity Demonstrations section below.

## POST-ACTIVITY DEMONSTRATION | Observing How CO<sub>2</sub> Lowers Solution pH

SUGGESTED CLASS TIME | 10 minutes

Students have not been introduced to the term ocean acidification. However, after the experiment they conducted, they will likely understand that acids dissolve calcium-based products. The bubble-blowing activity is used as an analogy to allow students to visualize how an invisible gas resulting from respiration reduces the pH of the solution. The link to ocean acidification is explained later with CO<sub>2</sub> emitted from fossil fuel combustion being the air we breathe out in the activity and the solution in the container being the ocean. The bubble-blowing activity is used to demonstrate how the dissolution of CO<sub>2</sub> into the ocean lowers pH.

- Have students fill a clear cup with tap water. Add 10 drops of freshwater pH indicator solution to the tap water and mix using a straw. Students record the color of the solution.  
\*Warn students: **DO NOT DRINK** the solution!

- Instruct students to blow bubbles in the solution until they see a color change. Compare the pH of the solution before and after they blow bubbles.
- Ask students why the color changed (i.e., why the pH changed), and what gas caused the pH to change?
- The instructor can make the analogy that the solution mimics the ocean and that exhaling CO<sub>2</sub> mimics carbon emissions. This analogy helps students make the connection that higher CO<sub>2</sub> in the atmosphere results in lower pH within the ocean.
- Post-demonstration discussions can also include how CO<sub>2</sub> emissions affect ocean pH. The instructor can lead a discussion on how ocean acidification (lower ocean pH) may affect marine calcifiers (Figure 4 may be used to illustrate the process). Additional discussion comments can include (but should not be limited to): *Apply the bubble blowing demonstration you completed to compose a definition for the term ocean acidification. Identify some causes and effects of ocean acidification. Predict and explain some potential socioeconomic impacts of lower ocean pH.*
- Follow up the discussion with videos and/or additional articles on ocean acidification (see Internet resources).

## INTERNET RESOURCES

VIDEO | *New Google Earth Tour: Ocean Acidification*

International Geosphere-Biosphere Programme, Joint Global Ocean Flux Study Science No. 2, Ocean Biogeochemistry and Global Change, <http://www.igbp.net/multimedia/newgoogleearthtouroceanacidification.5.19b40be31390c033ede80001577.html>.

Table 2. Summary of gas laws used by students to help determine the percent CaCO<sub>3</sub> composition of their samples. Formulas and a general description of the application of each law are provided (Wilbraham et al., 2008).

| Gas Law              | Formula   | Application  |
|----------------------|---|--|
| Boyle's Law          | $P_1V_1 = P_2V_2$   | The volume of a fixed quantity of gas maintained at a constant temperature is inversely proportional to pressure.  |
| Charles's Law        | $V_1/T_1 = V_2/T_2$   | The volume of a fixed amount of gas maintained at constant pressure is directly proportional to its absolute temperature.  |
| Combined Gas Law     | $(P_1V_1)/T_1 = (P_2V_2)/T_2$   | Combination of Boyle's, Charles's, and Gay-Lussac's laws.  |
| Avogadro's Principle | $V = (\text{constant})(n)$<br>when at standard temperature (0.00°C) and pressure (1.00 atm) | The volume of a gas at constant temperature is directly proportional to the number of moles of the gas. Equal volumes of gases at the same temperature and pressure contain equal numbers of molecules. One mole contains $6.02 \times 10^{23}$ molecules. At standard pressure and temperature, any gas will occupy about 22.4 L. |
| Ideal Gas Law        | $PV = nRT$  | "R" represents the ideal gas constant ( $R = 0.0821 \text{ L atm/mol K}$ ). Helps find the number of moles of a gas when pressure, temperature, and volume are known.  |

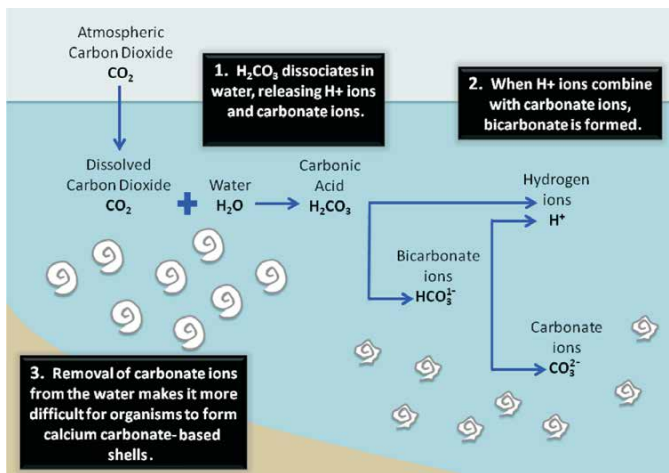


Figure 4. Ocean acidification reactions and the effect of increased acidity in the ocean on calcifying marine organisms. Image adapted from <http://www.chesapeakequarterly.net/V11N1/side1>

#### FAQs AND FACTSHEET | FAQs About Ocean Acidification

<http://www.whoi.edu/OCB-OA/FAQs>

#### LINKS AND ACTIVITIES | Understanding Ocean Acidification

Hands on Demos and Activities, Open Educational Resources, <http://www.oercommons.org/courses/hands-on-demos-and-activities-from-acidocean-org/view>

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