**Abstract**

This hands-on lab allows students to explore concepts and quantify effects of ocean acidification. Many laboratory activities simplify ocean acidification through computer simulations or dripping acid on non-living materials (e.g., sea shells) but do not provide adequate opportunities for students to measure, inquire, or see real consequences for living organisms. Thus, we developed this low-cost, easily accessible experiment to imitate ocean acidification on living, calcifying organisms.

**Key Words:** ocean acidification, climate change, biomineralization, calcification; Chara

**Introduction**

Rising atmospheric CO₂ due to fossil fuel consumption has widespread effects on climate and ocean chemistry, which in turn has cascading ecological impacts (Archer, 2005). The oceans act as “sinks” for CO₂, absorbing at least 30 million tons of CO₂ every day (Hardt & Safina, 2010). On a positive note, absorption of CO₂ by the oceans slows the accumulation of CO₂ in the atmosphere, slowing global warming. However, the downside of this CO₂ absorption is the phenomenon of ocean acidification caused by the dissolved CO₂ reacting with water to form carbonic acid. Rising levels of dissolved CO₂ in the oceans over the last 250 years have subsequently increased hydrogen ion concentration in ocean waters by 30%, changing the pH from 8.25 to 8.14 (Jacobson, 2005; Meehl et al., 2007). The resulting acidification is associated with weak, brittle shells (Wang et al., 2017), coral bleaching (Hoegh-Guldberg, 1999), lower reproduction rates in multiple marine species (Hardt & Safina, 2010), and reduced growth of spines and shells of organisms (Pfister et al., 2016; De Wit et al., 2018). These impacts on growth and reproduction disrupt marine food webs (Ullah et al., 2018). This lab activity allows students to explore effects of elevated CO₂ and decreased pH on a living, calcifying organism. The lab aligns with the NGSS 3 Dimension Learning by providing opportunities to use and refine practices (e.g., developing/using models; planning and carrying out investigations; analyzing/interpreting data; and using computational thinking). It addresses crosscutting concepts (e.g., cause and effect; scale, quantity, and system models; stability and change) and core concepts in science (e.g., ecosystems).

**Learning Objectives**

Upon completion, students will be able to

- predict the effect of rising carbon dioxide on the pH of water,
- predict the effects of pH on calcification of organisms,
- measure changes of pH in solution, and
- use qualitative and quantitative observations to draw conclusions about the impact of pH on calcification.

**Background**

Carbon dioxide (CO₂) absorbed by the ocean reacts with water (H₂O), producing carbonic acid (H₂CO₃) that dissociates to hydrogen ions (H⁺) and bicarbonate ions (HCO₃⁻). As H⁺ concentration increases due to an increase in dissolved CO₂, the pH of the water decreases. Seawater is typically basic (pH >7.0) with concentrations of H⁺ low enough to allow organisms to readily combine carbonate (CO₃²⁻) with calcium ions (Ca²⁺) forming calcium carbonate (CaCO₃), a crucial compound for forming spines, tests, shells, and exoskeletons. However, under acidic conditions, the excess H⁺ ions react more with CO₃²⁻, resulting in more HCO₃⁻ and less CaCO₃ (Figure 1). Reduced CaCO₃ production and/or increased cost on organisms to raise pH for calcification decreases an organism’s ability to build calcified structures (Liu et al., 2018). Investigating acidification of water and its real-time effects on organisms in a classroom can be complicated and expensive, so we developed this lab to allow students to explore these concepts in a relatively simple and inexpensive way. We chose Chara, a genus of freshwater algae, because these species are easy to grow and manipulate. Chara and other charophyte algae have been shown to use calcification to elevate...
their photosynthetic output by increasing access to protons (H+) and CO$_2$ for photosynthesis (McConnaughey, 1998). In addition, many marine organisms may use rapid calcification to generate protons that can be pumped out of cells in exchange for other nutrients (e.g., potassium, nitrate, iron, magnesium; McConnaughey, 1998).

Human-induced changes to climate and ocean pH started >200 years ago (Abrams et al., 2016), but widespread concern about the effects of acidification have increased only recently (IPCC, 2019). While survey data indicate that parents want their children to be taught about climate change, the subject is often not included in curricula (Kamenetz, 2019). Perhaps the challenges of modeling climate change in the classroom contribute to this disparity. Testing climate impacts on calcifying organisms may be more tractable in a classroom, allowing teachers to address documented misconceptions (Danielson & Tanner, 2015). The widespread effects of ocean acidification on individuals, populations, and ecosystems necessitate informing students about this process (Schoedinger et al., 2006). Indeed, mass extinction events on Earth have generally been associated with rapid changes in climate along with rapid ocean acidification (Veron, 2008). Exploring the human impacts and individual and collective actions that can be taken to improve ocean health are fundamental components of the Ocean Literacy Framework (http://oceanliteracy.wp2.coexploration.org/). Ocean acidification is likely to be a persisting multigenerational problem due to the expected slow rate of recovery (Hardt & Safina, 2010). Fortunately, young children have strong beliefs in their ability to create global change. Young students retain more knowledge through hands-on experiences such as labs (Taber & Taylor, 2009), thus, a deeper understanding of ocean acidification through inquiry and hands-on activities can drive understanding and positive change.

Virtual labs on ocean acidification and hands-on labs using nonliving materials made of CaCO$_3$, such as shell, eggshell, antacid, or pure calcium carbonate (e.g., Kelley et al., 2015), have been accessible for some time. We were unable to find an easily implemented model of ocean acidification that used living organisms outside of facilities limited to universities with marine science programs. Thus, we designed this lab to provide a realistic model of ocean acidification using a living calcifier, Chara. Compared to an animal model, algae are much easier to maintain in a classroom. This lab can be performed using a variety of methodologies for manipulating pH and observing and measuring calcification, but we will focus on our preferred method of using CO$_2$ for acidification and for water displacement to measure calcification. This lab can be modified for various grade levels and emphasizes components of the Next Generation Science Standards, A Framework for K–12 Science Education, and the Ocean Literacy Framework.

○ Engage

Before lab, students can research calcifying marine organisms and predict how particular ecosystems (e.g., coral reefs), organisms, or businesses (e.g., oyster farming) could be impacted by a decrease in calcification.

Next, students can predict what kind of environment (e.g., high or low pH) would result in reduced calcification. This may provide an opportunity to drip acids on inanimate calcified structures, such as shells, to observe the breakdown and release of CO$_2$.

Finally, students can employ different methods to explore altering the pH of a solution independent of a calcifier. Many options exist, including adding an acid (e.g., HCl, vinegar, citric acid), adding a base (e.g., NaOH, KOH, ammonia), and adding CO$_2$ to water. Bubbling CO$_2$ through solution (e.g., baking soda and acid, exhaled CO$_2$, compressed CO$_2$) provides the closest model of actual conditions. Our preferred method for CO$_2$ production is to combine a weak citric acid solution with sodium bicarbonate. Relatively inexpensive reactors, often used for aquarium plants, quickly allow the combination of citric acid and sodium bicarbonate and provide a valve for controlling CO$_2$ flow, simplifying this process (Figure 2).

○ Experiment

Timeframe

- Total: 4 days to several weeks
- Initial weighing and creating solutions: 1–2 hours
- Daily checks and pH adjustment: 10–15 minutes
- Final analysis (weighing, observing, and collecting CO$_2$): 1–2 hours.

Materials List for Initial Setup

Basics

- Spring water
- Live Chara (attached organisms such as planaria and snails removed), which can be ordered from many biological supply companies

**Figure 1.** Calcification of Chara in presence of low and high amounts of CO$_2$. Under conditions of high CO$_2$ and low pH, less CaCO$_3$ is produced. Arrow thickness represents net movement of reactions, with thicker arrows representing higher net movement compared to thin arrows.
The pH alteration is a buffer; if it is added, it will make it more difficult to store-bought spring water had a near-neutral pH. Note that calcium water we used already contained trace calcium. We found that do not add >0.1 g calcium/1000 mL. We chose not to add a supple-imentary source of calcium in our experiment because the spring water we used already contained trace calcium. We found that store-bought spring water had a near-neutral pH. Note that calcium carbonate is a buffer; if it is added, it will make it more difficult to alter the pH.

**Figure 2.** A simple, inexpensive aquarium CO₂ reactor can be used to quickly make CO₂ bubbles to acidify a solution. Squeezing the bottle of citric acid will force the fluid into the sodium bicarbonate, liberating CO₂ that can be controlled and released through a diffuser into the Chara container.

- Clear containers that can be sealed - we used 8.5 oz. clear, square Nalgene bottles with screw-caps
- Scale (for small samples, a scale accurate to 0.01 g is ideal)

**Water acidification**
- Sodium bicarbonate
- 30% citric acid solution (preferred) or 3% vinegar
- Aquarium diffusers to increase CO₂ dissolved in water

**Water alkalization**
- Household ammonia (preferred) or NaOH or KOH solutions

**Testing pH of solution**
- pH probe or pH paper (range 5–8)

**Lab Activities**

First, label containers by pH (high/low or 5.5/7.5, etc.) and method of pH alteration (e.g., vinegar, CO₂). Next, blot Chara dry and weigh two or more equal quantities per student group (we often use ~2 g), depending on the number of variations you wish to test. Then place Chara into each container and cover with spring water. (If using soft/deionized water, supplementary calcium may be needed, but do not add >0.1 g calcium/1000 mL. We chose not to add a supplementary source of calcium in our experiment because the spring water we used already contained trace calcium.) We found that store-bought spring water had a near-neutral pH. Note that calcium carbonate is a buffer; if it is added, it will make it more difficult to alter the pH.

Optionally, students can choose to add a calcium source to water low in calcium (e.g., soft/deionized water), such as pure CaCO₃, crushed egg/sea shells, chalk, or antacid pills to see if it affects calcification rates.

**Alkalization**

Next, measure the initial pH of each solution and alkalize some solutions to pH 7–8 by adding household ammonia via a dropper bottle. Check the pH regularly.

**Acidification**

Bubbling CO₂ through water is a simple way to achieve a pH ≤6. Our preferred way to generate CO₂ is by using an inexpensive aquarium CO₂ generator (e.g., Anself CO₂ Generator System Kit), but note that you can achieve the results by mixing solutions in a simple squeeze bottle. To use the reactor, start by creating citric acid and sodium bicarbonate solutions in 2 L bottles. Label one 2 L bottle “Citric Acid” and add 200 g of powdered citric acid to 600 mL of water, then mix until it dissolves. Label a second 2 L soda bottle “Sodium Bicarbonate” and combine 200 g baking soda to 200 mL water, then mix until it dissolves. Screw each bottle into the reactor so that squeezing the “Citric Acid” bottle will force citric acid into the “Sodium Bicarbonate” bottle. The acid will react with the sodium bicarbonate, resulting in CO₂ release. The CO₂ gas will make the pressure in the bottle increase. Opening the valve on the reactor will allow control of gas to flow out of the tube and into the solution with the Chara. If a diffuser is attached to the outflow tube, more CO₂ will go into solution (Figure 2). Check the pH of the solution at regular intervals using a pH probe or pH paper. Continue bubbling CO₂ until a pH of 5.5–6.5 is reached. Alternatively, use compressed CO₂ (e.g., a soda or keg carbonator or a paintball gun canister) or add a spoonful of sodium bicarbonate to a squeeze bottle with a short tube attached and pour vinegar or 30% (0.05 M) citric acid solution over the baking soda and immediately close the bottle, forcing the liberated CO₂ into the tube and through the solution. If CO₂ generation is not possible, an acid solution (e.g., 3% vinegar, 30% citric acid, or 0.5 M HCl) can be added directly to the Chara water. We were able to obtain good results by adding acids to the solution.

Once the desired pH has been achieved, seal the containers and place the Chara in a well-lit but not sunny or hot location. Each day, check and adjust pH to desired levels as needed, using the same methods. We recommend running the experiment at least four days, but it can be run for weeks. We suggest having at least four groups of students run the experiment with a minimum of one high-pH and one low-pH flask for each group. It may be desirable to also have groups produce an unmanipulated control flask.

**Qualitative Comparisons of Chara Calcification**

Students may observe color changes from nearly white to dark green as Chara grows under different pH conditions, which may be sufficient evidence for those who will not do quantitative analysis. When the experimental period is complete, dry the Chara and view calcium crystals on the plant under a hand lens or dissecting scope. Record the results in Table 1. CaCO₃ crystals on Chara form under basic conditions, resulting in a whiter and more rigid structure, while those grown at lower pH typically lack crystals (Figure 3). Students can use the crystals and color comparisons to draw conclusions about calcification at different pH.
Materials

- Mortar and pestle
- Filtering flask
- Rubber tubing
- Rubber stoppers
- Ring stand
- Burette clamp
- Eudiometer, graduated cylinder, burette, or water bottles
- Optional: CO₂ gas sensor

Methods

For more detailed measurements, remove Chara samples from solutions, blot dry, and weigh to determine wet masses. Next, dry Chara samples completely in a dehydrator or simply air dry. Once dry, weigh samples to determine dry mass. Comparison of dry and wet masses can be used to estimate calcification differences.

To quantify differences in CaCO₃ production, acid can be added to dried and crushed Chara, releasing CO₂ from accumulated CaCO₃. The amount of CO₂ given off is an indicator of how much CaCO₃ from calcification was present in the algae. More CaCO₃ in Chara results in more CO₂ liberated. When vinegar is used, the following reaction occurs:

\[
\text{CaCO}_3 + 2 \text{CH}_3\text{COOH} \rightarrow \text{Ca(CH}_3\text{CO}_2)_2 + \text{H}_2\text{O} + \text{CO}_2
\]

When citric acid solution is used, the following reaction occurs:

\[
3 \text{CaCO}_3 + 2 \text{H}_3\text{C}_6\text{H}_5\text{O}_7 \rightarrow \text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2 + 3 \text{H}_2\text{O} + 3 \text{CO}_2
\]

To use this method, grind dried Chara into a fine powder (note: color differences in the powder can be profound and a useful observation). Use water displacement to quantify CaCO₃ by CO₂ liberation (Figure 4). The following is our preferred setup:

1. Attach an inverted eudiometer filled with water to a ring stand so that the opening of the eudiometer is submerged in a mostly full beaker of water (a burette, graduated cylinder, or water bottle could also be used to capture liberated gas).
2. Add powdered samples to a filtering flask and run tubing from the flask partially up the opening of the eudiometer.
3. Note initial volume in eudiometer.
4. Add 30% citric acid solution or vinegar to flasks so that powdered Chara is covered.
5. Quickly stopper the flask.
6. Let the reaction run, occasionally swirling the mixture.
7. Note the final volume in the eudiometer and determine how much gas was released by the reaction. Higher displacement means more CO₂ liberated and, thus, more CaCO₃ and calcification. Alternatively, we have used Vernier CO₂ probes to measure the CO₂ produced in a flask with powdered Chara and acid.

Table 1. Various measures of the effect of pH on calcification in Chara, with examples of measurements that we have obtained.

| pH          | Starting Mass (g) | Color       | Level of Visible Calcification | Wet Mass (g) | Dry Mass (g) | Volume of Water Displaced (mL) | Volume of CO₂ per Gram Dry Chara (mL/g) |
|-------------|------------------|-------------|-------------------------------|--------------|-------------|------------------|----------------------------------------|
| Low (acidic)| 2.4g             | Green       | Very little                   | 2.20         | 0.65        | 21.5             | 33.08                                  |
| High (alkaline) | 2.3g          | Light green | Covered in white areas        | 2.25         | 0.71        | 57.8             | 81.41                                  |

a Under microscope.
b Powdered.
c Or volume of CO₂ liberated.

Figure 3. Chara under a dissecting scope: (top) Chara grown at pH 5.5–6.5; (bottom) Chara grown at pH 7.5–8.
Color of Chara, mass differences, and amount of CO$_2$ released can all be discussed as evidence for what happened to Chara under different pH conditions. Students can also discuss sources of measurement error and ways the experiment can be improved or expanded. Students should compare their results and determine whether their hypotheses were supported. These experiments build on ideas of climate change and its broader effects on marine ecosystems.

**Evaluate & Expand**

Upon completion, students should ponder how people might reduce ocean acidification. Using knowledge about ocean acidification, students can brainstorm how to reduce CO$_2$. Students can discuss what lifestyle and societal changes might reduce ocean acidification.

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**References**

Abrahms, N.J., McGregor, H., Tierney, J., Evans, M., McKay, N. & Kaufman, D. (2016). Early onset of industrial-era warming across the oceans and continents. Nature, 536, 411–418.

Archer, D. (2005). Fate of fossil fuel CO2 in geologic time. Journal of Geophysical Research–Oceans, 110, C09S05.

Boyer, J.S. (2016). Enzyme-less growth in Chara and terrestrial plants. Frontiers in Plant Science, 7, 866.

Centers for Ocean Science Education Excellence, National Geographic Society, National Oceanic and Atmospheric Administration & College of Exploration (2005). Ocean Literacy: The Essential Principles and Fundamental Concepts of Ocean Sciences [jointly published brochure for grades K–12]. Available at https://www.coexploration.org/oceanliteracy/documents/OceanLitConcepts_10.11.05.pdf.

Danielson, K.I. & Tanner, K.D. (2015). Investigating undergraduate science students’ conceptions and misconceptions of ocean acidification. CBE–Life Sciences Education, 14, ar29.

De Wit, P., Durland, E., Ventura, A. & Langdon, C.J. (2018). Gene expression correlated with delay in shell formation in larval Pacific oysters (Crassostrea gigas) exposed to experimental ocean acidification provides insights into shell formation mechanisms. BMC Genomics, 19, article 160.

Hardt, M.J. & Safina, C. (2010, 08). Threatening ocean life from the inside out. Scientific American, 303(2), 66–73.

Hodin, J., Miller, P. & Fauville, G. (n.d.). Our Acidifying Ocean. Retrieved from http://i2sea.stanford.edu/AcidOcean/AcidOcean.htm.

**Figure 4.** Eudiometer setup that uses volume of water displaced to indicate the amount of CaCO$_3$ present in Chara at different pH.

Chara uses CO$_2^-$ and Ca$^{2+}$ to calcify cell walls, forming CaCO$_3$ encrustations (Boyer et al., 2009). The process of rapid calcification frees up H$^+$ that can be used for ATP production, while the stored carbonate can provide carbon dioxide for photosynthesis (McConnaughey, 1998). When the ocean absorbs CO$_2$, the CO$_2$ reacts with water to form H$_2$CO$_3$. The H$_2$CO$_3$ dissociates to HCO$_3^-$ and H$^+$. Thus, as CO$_2$ absorption increases with increased CO$_2$ emissions, there is an increase in H$^+$ concentration and a lower ocean pH. The excess H$^+$ react with CO$_3^{2-}$, forming HCO$_3^-$ rather than CaCO$_3$, leading to a decrease in CaCO$_3$ availability in the ocean. As students work toward completing this experiment, they will see rapid effects of acidification on a live organism. This activity follows the framework for K–12 general science and engineering practices, disciplinary core ideas, and crosscutting concepts like cause and effect. However, the experiment has been used in college courses such as Marine Biology. Introductory Biology, Microbiology, and General Biology. The lab was initially developed as part of an undergraduate research program that supported the work by one of the authors of this paper (K.C.).
Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world’s coral reefs. *Marine and Freshwater Research*, 50, 839–866.

IPCC (2019). *Climate Change and Land: An IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Geneva: IPCC.

Jacobson, M.Z. (2005). Studying ocean acidification with conservative, stable numerical schemes for nonequilibrium air-ocean exchange and ocean equilibrium chemistry. *Journal of Geophysical Research: Atmospheres*, 110(D7).

Kamenetz, A. (2019). Most teachers don’t teach climate change; 4 in 5 parents wish they did. *NPR*, April 22. https://www.npr.org/2019/04/22/714262267/most-teachers-dont-teach-climate-change-4-in-5-parents-wish-they-did? sc=tw.

Kelley, A., Hanson, P. & Kelley, S. (2015). Demonstrating the effects of ocean acidification on marine organisms to support climate change understanding. *American Biology Teacher*, 77, 258–263.

Liu, Y.-W., Eagle, R.A., Aciego, S.M., Gilmore, R.E. & Ries, J.B. (2018). A coastal coccolithophore maintains pH homeostasis and switches carbon sources in response to ocean acidification. *Nature Communications*, 9, article 2857.

McConnaughey, T. (1998). Acid secretion, calcification, and photosynthetic carbon concentrating mechanisms. *Canadian Journal of Botany*, 76, 1119–1126.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, T., Gregory, J.M., et al. (2007). Global climate projections. In S.D. Solomon et al. (Eds.), *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press.

National Research Council (2012). *A Framework for K–12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. Washington, DC: National Academies Press.

NGSS Lead States (2013). *Next Generation Science Standards: For States, by States*. Washington, DC: National Academies Press.

Pfister, C.A., Roy, K., Wootton, J.T., McCoy, S.J., Paine, R.T., Suchanek, T.H. & Sanford, E. (2016). Historical baselines and the future of shell calcification for a foundation species in a changing ocean. *Proceedings of the Royal Society B*, 283, 20160392.

Plass, G., Fleming, J. & Schmidt, G. (2010). Carbon dioxide and the climate. *American Scientist*, 98, 58–67.

Schoedinger, S., Francesca, C. & Jewell, B. (2006). The need for ocean literacy in the classroom. *Science Teacher*, 73(6), 44–52.

Taber, F. & Taylor, N. (2009). Climate of concern – a search for effective strategies for teaching children about global warming. *International Journal of Environmental & Science Education*, 4, 97–116.

Ullah, H., Nagelkerken, I., Goldenberg, S.U. & Fordham, D.A. (2018). Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. *PLoS Biology*, 16, e2003446.

Veron, J.E.N. (2008). Mass extinctions and ocean acidification: biological constraints on geological dilemmas. *Coral Reefs*, 27, 459–472.

Wang, X., Wang, M., Jia, Z., Song, X., Wang, L. & Song, L. (2017). A shell-formation related carbonic anhydrase in *Crassostrea gigas* modulates intracellular calcium against co2 exposure: implication for impacts of ocean acidification on mollusk calcification. *Aquatic Toxicology*, 189, 216–228.

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