

Ocean Acidification Lab

Hawai'i Institute of Marine Biology

Education Program

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Part I: Pre-activities for the classroom

Science background*

Global climate change, in the form of increasing temperatures due to rapidly elevating atmospheric levels of greenhouse gases, is one of the most significant and pressing issues of our time. Average temperatures across the planet are rising at an unprecedented rate, and most scientists agree that a major contributing factor to these drastic changes is linked to anthropogenic-generated carbon, specifically in the form of carbon dioxide (CO₂)¹. While CO₂ is a naturally occurring and biologically important gas, increased burning of fossil fuels and massive industrialization in the last century has contributed an atmospheric level of CO₂ that Earth has not experienced for at least 650,000 years^{1,2}. In fact, climate change caused by the emission of greenhouse gases is already having noticeable and measurable effects on our society today. A recent comprehensive report by the U.S. Global Change Research Program on the impact of climate change in the United States warns in part that global warming will lead to increased heat waves which will negatively affect agriculture and human health, drought especially in the Western United States, insect infestations, wildfires, sea level rise, and coastal flooding³. Elevated atmospheric CO₂ is also threatening life in the world's oceans through a process called *ocean acidification*.

To understand the process of acidification we need to understand the concept of pH, and how pH is measured. In pure water, H₂O molecules bump into one another and cause a self ionization reaction to occur. Two water molecules collide to produce a hydronium ion (H₃O⁺; note hydronium can also be represented as just H⁺), which is an *acid* because it is a source of a free proton (H⁺ ion). This reaction can be expressed in the following formula:



A simplified version of the same reaction that only shows the free proton (H⁺) is:



A hydroxide ion (OH⁻), which is called a *base* because it can readily 'accept' a proton, is also formed in the same reaction. The reaction is fully reversible and is continuously going back and forth in a state of equilibrium (symbolized by \rightleftharpoons). If you have already had a class in basic chemistry, you may recall that there are 55 moles of water molecules in one liter of water, and at room temperature only 1.0×10^{-7} moles (0.0000001 moles) of H⁺ and OH⁻ ions are formed.

* Additional information may be found on Stanford's Inquiry to Insight page:
<http://i2i.stanford.edu/AcidOcean/AcidOcean.htm>

Rather than dealing with such cumbersome numbers, a system was devised to simplify things and the term pH was developed. pH represents the concentration of hydrogen ions, and it is the 7 in the exponent of 1.0×10^{-7} moles that translates into neutral water having a pH of 7.

When an acid dissolves in water, additional H^+ ions are formed thus increasing their concentration. For example, the concentration of H^+ ions might be increased from 10^{-7} M (0.0000001 moles) up to 10^{-5} M (0.00001 moles). This change in the exponent means that the solution now contains 100 times more H^+ ions, and explains why acidic solutions have pH values lower than 7. In this example, the pH is now 5. How acidic or basic a solution is depends on the relative concentrations of H^+ and OH^- ions present. If the concentration of H^+ ions is more than the concentration of OH^- ions, the solution is considered *acidic* (e.g., pH <7). If the concentration of OH^- ions is more than the concentration of H^+ ions, then the solution is considered *basic* (e.g., pH >7). If the concentrations of H^+ and OH^- ions are equal to one another, the solution is neutral (e.g., pH=7).

Certain chemicals mixed with water result in special properties with regards to pH. These chemicals which are known as “buffering” agents, when properly mixed in a solution, cause the solution to resist changes in pH when exposed to acids or bases. This property of resisting changes in pH makes buffered solutions extremely useful in protecting sensitive equipment, dealing with chemical accidents, and even in balancing the internal processes of living things. In addition to salt, there are many dissolved chemicals that are present in seawater, for example. The complex composition of the dissolved chemicals in seawater allows it to be able to withstand changes in pH, and seawater therefore acts as a *buffer*. The buffering capacity of seawater is one of the most important reasons why there is life on this planet.

The process of ocean acidification is the progressive decrease in the average pH of ocean waters caused by the excessive absorption of atmospheric CO_2 ². Over the past few centuries, approximately 50% of the CO_2 produced by humans has remained in the atmosphere, 20% has been taken up by terrestrial ecosystems such as tropical forests, while another 30% has been absorbed by the oceans². These numbers suggest that the effects of climate change would likely be much more severe if the oceans were not able to absorb CO_2 and act as a global buffer. Unfortunately, the ability of Earth’s oceans to absorb excess atmospheric CO_2 is finite and we are quickly approaching their buffering capacity limits⁴.

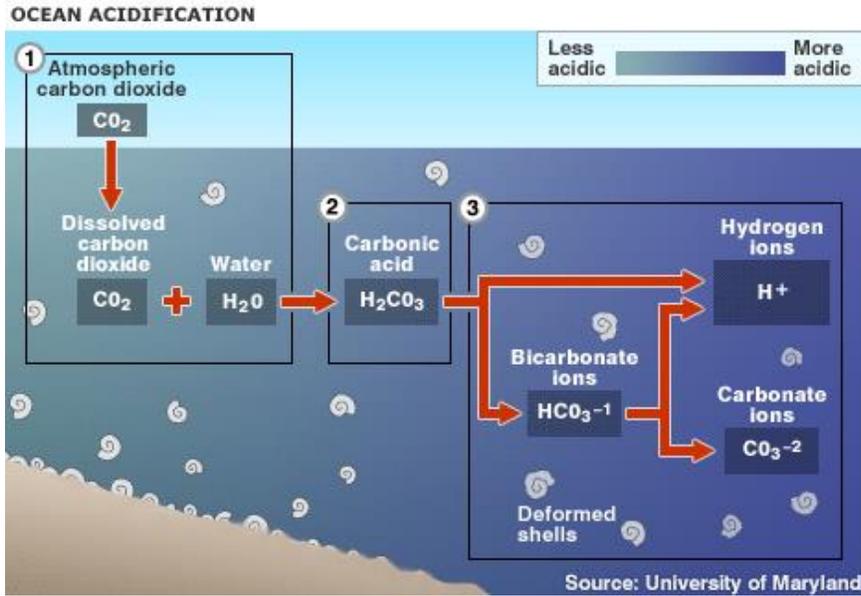


Figure 1: This figure depicts the basic carbonate chemistry involved in ocean acidification; as carbon dioxide (CO_2) is absorbed into seawater, carbonic acid (H_2CO_3) forms. Carbonic acid changes back and forth in equilibrium to bicarbonate (HCO_3^-) + H^+ and carbonate (CO_3^{2-}) + H^+ , lowering the pH due to higher concentrations of H^+ . Then additional carbon dioxide can react with the carbonate. This additional reaction consumes the carbonate availability in the oceans.

The process of acidification of the oceans is already negatively affecting ocean biogeochemistry⁵. Since the beginning of the Industrial Revolution, average surface pH readings in the oceans have decreased by about 0.1 pH units (remember that the units on a pH scale are logarithmic, so a 0.1 pH unit decrease is equivalent to about a 30% increase in H^+ ions)^{2,4,5}. Currently, Earth's atmospheric CO_2 concentration is about 380 parts per million volumes (ppmv). If current trends in anthropogenic CO_2 production continues, this value could easily double by the end of the century, resulting in a decrease in mean ocean pH of about 0.45 units from pre-industrial times^{2,4,5}.

So what are the consequences of these changes in seawater chemistry on marine organisms? The short answer is that for the most part scientists don't exactly know for certain, but most predict that ocean acidification will have overall detrimental effects on marine organisms and ecosystems, specifically on coral reefs^{2,4}. What scientists do know is that ocean acidification leads to a decrease in the availability of carbonate ions, which are a crucial element in the formation of the shells and skeletons of a number of calcifying marine organisms such as corals, calcareous marine plankton, mollusks, and shellfish^{4,5}. Under normal oceanic conditions, carbonate is considered to be in a saturated state². In this situation, calcifying organisms such as coral are able to utilize carbonate from seawater and deposit it in their skeletons by forming calcium carbonate (CaCO_3). As the pH of the oceans becomes more acidic however, carbonate is converted in to other molecular forms of carbon (bicarbonate) which can not be used by corals, and coral carbonate skeletons begin to dissolve instead².

This scenario has severe implications for the State of Hawai'i which harbors over 70% of all of the coral reefs in the United States. If our coral reefs begin to dissolve and disappear, it will affect our way of life economically, socially, and culturally. A study in 2004 by Cesar & van Beukering estimated net benefits for Hawaii's economy at \$360 million a year, and the overall asset value of the state's 410,000 acres of potential reef area in the main Hawaiian Islands was estimated at nearly \$10 billion⁸. Many of the planktonic calcifying organisms are also very important food sources for many other marine organisms, and they make up the base of vital

Ocean acidification research at the Hawai‘i Institute of Marine Biology

There are several research laboratories working on the subject of ocean acidification and its effect on marine organisms at HIMB. Dr. Marlin Atkinson heads up the Coral Reef Biogeochemistry and Remote Sensing group who study the turnover rate of biologically-important molecules (such as carbon, oxygen, and nitrogen) through coral reefs⁶. The Atkinson Lab has been investigating the effects of ocean acidification on live coral since the late 1970s; both in controlled tanks or “flumes” at HIMB, as well as out in the environment on the patch reefs of Kāne‘ohe Bay. Dr. Atkinson and his group study the interactions of light, available food particles, and water chemistry on coral reefs to gain an understanding of the effects ocean acidification is having on coral reef ecosystems. Additionally, the Atkinson Lab is working on testing and developing remote sensing instruments which are able to more accurately monitor the changing chemical makeup of our atmosphere and oceans.



Photo credit: Atkinson Lab

Figure 3: Dr. Atkinson uses specially designed flumes like the one pictured here to study the uptake and release of nutrients in coral reef ecosystems. These flumes allow researchers to examine the effects of simulated natural cycles of light and water motion (waves) on captive populations of corals and other organisms. Performing these kinds of experiments in tanks at HIMB lets scientists alter many different environmental factors that affect reef life, including changing the atmospheric carbon dioxide levels to which the corals are exposed.

Drs. Paul Jokiel and Ku‘ulei Rodgers, who are part of the Coral Reef Monitoring and Assessment Program (CRAMP), study the long-term health of coral reefs and the direct effects of acidified seawater on captive living coral reef communities, or mesocosms⁷. Among numerous other things, Drs. Jokiel and Rodgers have found that acidified seawater significantly slows the rate at which multiple species of reef building corals and coralline algae grow. The Coral Reef Monitoring and Assessment Program (CRAMP) was founded in the late 1990s, and is still in effect today providing valuable data to marine scientists and policy makers involved in coral reef conservation efforts.



Photo credit: Ku‘ulei Rodgers

Figure 4: This is a picture of the mesocosm facility at the Point Lab at HIMB where Drs. Jokiel and Rodgers conduct the majority of their ocean acidification research. Entire coral reef communities (including reef building coral, coralline algae, associated marine bacteria, etc.) are collected and assembled into multiple fiberglass tanks. Fresh seawater is then pumped into header tanks where the water chemistry is altered before it is pumped through the experimental tanks. Various levels of carbon dioxide gas is carefully bubbled through the seawater, thus acidifying it on its way to the living coral ecosystem. This high level of control allows the researchers to mimic predicted atmospheric concentrations of CO₂ and observe the effects of this altered environment on the health of the reefs.

Classroom Laboratory: The Buffering Capacity of Seawater

In all living systems on Earth, organisms have evolved mechanisms to maintain their internal pH. The maintenance the pH inside an organism within a very small and optimal range is crucial for basic biological processes such as breathing, heartbeats, brain activity, and photosynthesis to occur. Using some very simple tools, we will measure the pH of different solutions and assess how acidic (pH <7) or basic (ph>7) they are. Equally important is that we will assess how easy or difficult it is to change the pH of a solution, and introduce the concept of a ‘buffer’.

Laboratory at a Glance:

Values of pH range between 1 – 14, with a pH of 7 being neutral. Very low (strongly acidic) pH values also indicate that a solution can be highly reactive, as it contains a high concentration of free protons (H⁺ ions) which are available to quickly and energetically react with other molecules. Alternatively, solutions that have very high (strongly basic) pH values are also highly reactive, as they have high concentrations of hydroxyl ions (OH⁻) which are also readily available to react with other molecules. For these reasons, solutions at both ends of the pH spectrum are hazardous to living systems. By obtaining the pH values of a variety of solutions that can be commonly found in households, you will 1) determine which of them fall into pH ranges that can support living things. Using the same tools to quantify pH, you will 2) also examine the ability of certain solutions to resist changes in pH.

1) What is the pH of my solution?

Have students bring one solution from home (e.g., drinking water, soft drinks, juices, mouth wash, shoyu, bleach, vinegar, kim chee sauce, cleaning solutions, etc) which they would like to determine the pH of. Using one of the simplest tools to measure pH (e.g., test strips or test kits) they will measure various solutions that are common to the household. However, both the test kits and test strips have a limited range of pH units (4.5 – 10) that they can measure. To measure the entire range of pH (e.g., 1 – 14) a pH meter is required. Using the pH test kit or test strips (generally purchased from your local aquarium store, or pH meter if available at your school), have students measure their respective solutions, and enter the name of the solution across from its respective pH on the data table provided below. Based on the results of the data collected, have students conclude what range in pH values are supportive of living systems, and which of the pH values are hazardous.

Item	pH
	1
	2
	3
	4
	5
	6
	7
	8
	9
	10
	11
	12
	13
	14

2) What is the buffering capacity of saltwater?

Items Needed

- Hawaiian salt
- One 1 Liter beaker
- Distilled or de-ionized water (if available rainwater is a natural substitute)
- Tap water
- 1 Liter bottle of soda (e.g., Coke, Sprite) to generate CO₂
- Airline tubing
- Hot glue gun or duct tape
- Three 100mL beakers
- Total hardness test kit**
- pH Test kit or test strips (pH 4.5-10)**
- Stopwatch or timer

** Hardness and pH kits or strips are available in local aquarium stores. To measure the entire pH range of 1 – 14 a pH meter is required.

Procedure

1) Dissolve 35 grams of Hawaiian salt in one Liter of distilled or de-ionized water. This stock solution is to be distributed to students and can be made in advance and stored indefinitely.

2) Set up 3 separate 100 mL beakers and place approximately 60 mL of saltwater, tap water, or distilled/de-ionized water in each beaker, respectively.

3) Follow the directions for the use of the pH test strips or pH meter, and obtain the pH of each of the respective solutions before exposing them to CO₂. Record the data in the appropriate box in Table 1 below, and discard the strip immediately after recording the pH.

4) Follow the directions for the test kit for water hardness, and obtain the total hardness for each of the water samples. Please note that the saltwater sample might be off the scale and should be recorded as greater than the highest value of the kit if it is undetectable. Record the data into the appropriate column in the table.

5) Remove cap from soda bottle and poke a hole in the cap, push airline tubing through hole, and seal tubing in the cap with a glue gun or duct tape.

6) While gently shaking the soda bottle, you can pinch the tubing to control the flow of CO₂ gas out of the soda bottle.

7) While continuing to shake the bottle and pinching the air tubing to control the flow of CO₂ gas out of the soda bottle, put the end of the tubing in the bottom of each of the beakers one at a time. The length of time each sample is bubbled (30 seconds), and the rate of bubbling (number of bubbles per second) should be as close to identical as possible between the beakers.

8) Using the stop watch, bubble the solution for a set amount of time (30 seconds) and again measure the pH by dipping the pH strip or pH meter directly into the water in the beaker. If there has been a measurable pH change of at least one pH unit the treatment is completed. However, if there has been no change in at least 1 pH unit repeat bubbling and measuring pH as necessary until a change in pH occurs. Record the time in took for a change in pH to occur in the table above. It is very important to be consistent with the timing of reading of the pH strips, and the strips should be discarded immediately after the pH value has been recorded.

Table 1

Solution	Total Hardness	pH at Beginning	Time for change in pH to occur
Distilled or De-ionized water			
Tap Water			
Seawater			

Expected results

Values for total water hardness should increase from distilled/de-ionized water, to tap water and then to saltwater. Note that the total hardness of saltwater might be off the scale for the test kit. Record the value as greater than the highest value the kit can measure. If the rate of bubbling remained relatively constant between the different samples, the pH of de-ionized water should change very quickly, followed by tap water, and finally by saltwater. The differences in the time taken to change the pH directly reflect the differences in total water hardness, and demonstrate the impact of dissolved minerals in acting as buffers in solution. Clearly, saltwater will possess the greatest buffering capacity, and because a similar solution (seawater) covers 75% of the Earth's surface, these results underscore the importance of buffered water to the planet's ecosystem.

What to expect during the field trip day

During your field trip day to HIMB you will examine the effects of changing the pH of seawater, and how this change affects the release of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions from the skeletons of five species of corals.

Part II: Field trip day at HIMB

Introduction

In this activity, you will simulate the process of ocean acidification by lowering the pH of seawater in glass jars via bubbling 100% CO₂ gas through it. The skeletons of various species of coral and sand will be placed in the jars and incubated with either the acidified seawater or fresh seawater for a period of time. After the incubation period, you will quantify the dissolution of the calcium carbonate (the primary chemical which makes up coral skeletons), by estimating the amount of calcium and carbonate ions released into the water. You will estimate the concentration of these ions using the aquarium test kits provided in the lab.

Below is a series of questions which will help to give you ideas about different hypotheses and tests you can perform during your time at HIMB. Read them carefully, and start to think about what question you'd like to address (hypothesis) and the experiment you'd like to conduct (method).

Guiding Questions

- What factors may influence the amount of calcium or carbonate released from the coral skeletons?
- Would the length of time a coral was exposed to acidified water affect the results? How long is long enough to see an effect?
- Does the species of coral matter (i.e., is one species more likely to dissolve or will it dissolve more quickly in acidified seawater than another)?
- Does the morphology of the coral matter?
- What about the pH of the water; if the pH were lowered more than one pH unit, how would that have affected the results?
- Was it important to maintain a constant flow of CO₂ gas during the experiment?
- Is this experiment biologically relevant; meaning is a 1 pH unit drop in seawater even likely in the next 100 years? If not, do you think you would observe similar results with a smaller change in pH? How small?

Tools available

In the lab we can conduct experiments by manipulating pH of sea water through bubbling CO₂ into glass jars with non-living coral skeletons or live plankton samples in them. Using 60 mL jars and small pieces of coral skeletons, we can measure changes in pH, calcium ions, and calcium carbonate using test strips and test kits, relative to untreated samples (controls). We will demonstrate how to use these tools in the lab, after which you will have all the necessary information to create and test your own hypothesis (see example in the **Appendix**).

Materials to bring:

Pen/pencil and paper/notebook

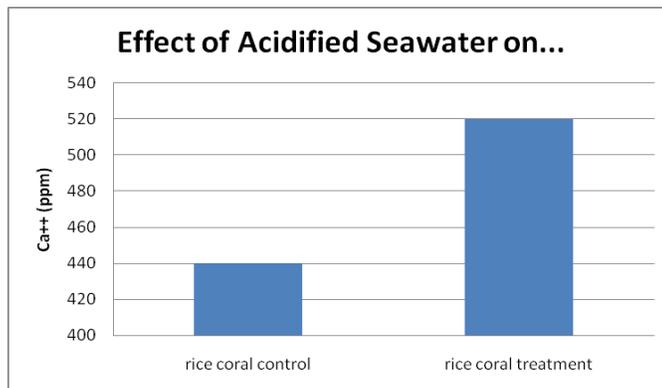
Materials available:

- 100 mL graduated cylinder
- 200 gram electronic balance (accurate to 1 gram)
- 60 mL glass jars
- CO₂ bulk tank with regulator
- Airline tubing
- Small fragments of: rice coral (*Montipora capitata*), mushroom coral (*Fungia scutaria*), finger coral (*Porites compressa*), lobe coral (*Porites lobata*), cauliflower coral (*Pocillopora meandrina*), or urchin tests (*Tripneustes gratilla*)
- Sand or various shells
- Seawater
- KH (Carbonate Hardness) Test Kits
- Ca²⁺ (Calcium Hardness) Test Kits
- pH Test Strips (4.5-10) or pH meter (0-14)
- Stopwatch or timer
- 10 mL glass pipette with pipetting ball
- 1L glass beakers
- Gloves

Exploring the data (data processing and organization)

For each species of coral/ urchin/ sand/ shells tested, depending on your experiment, you should be able to generate up to three separate graphs; pH, calcium, and carbonate. The treatment (control first, then treatment) can be plotted on the X-axis, while the parameter which was measured (i.e., pH, calcium, or carbonate) can be plotted on the Y-axis.

EXAMPLE:



What will you do with the data back in the classroom?

When you return to your home classroom after your visit to HIMB, you will need to begin the process of analyzing your data. If you were unable to finish graphing your data at HIMB, complete the graphs before you go to your home classroom so you are ready to begin discussing your results. You will compare results, discuss your hypotheses, make conclusions, and reflect on the importance of your findings.

Part III: Post-activities back in the classroom

Step by step analysis

Begin class with a general discussion of your experience at HIMB. Were you prepared for the lab exercise and able to complete the activity? Did you enjoy your time there? Now begin to discuss the subject matter:

A) Were your hypotheses supported by the data? Did CO₂ gas lower the pH of seawater? Was this change in pH enough to cause the dissolution (release) of calcium and carbonate from coral skeletons into the surrounding water? Did the species of coral matter? Did the length of time the coral was exposed to acidified seawater affect how much calcium or carbonate you detected?

B) Do your findings influence the way you view the ocean? Will it change your behavior in terms of reducing your carbon footprint?

Lab report

For your laboratory exercise at HIMB, you will be expected to eventually produce an in-depth laboratory report including the following independent sections:

- **Title:** summarize the entire experiment in several words.
- **Introduction:** in one half to three-quarters of a page describe the subject of ocean acidification, what its effects on coral reefs might be, and why we should care.
- **Hypothesis:** based on your background knowledge of ocean acidification and the information about what tools are available in the lab at HIMB, describe the hypothesis you tested.
- **Materials and Methods:** develop and describe, in detail, the experiment you conducted to test your hypothesis; include all of the materials you used to complete it as well.
- **Results:** compile your data and express them visually and where appropriate in graphs, tables, or figures.
- **Discussion:** analyze your data in essay form; discuss the results, emphasize what did and didn't work, and propose a new experiment or changes to your original experiment which may help explain the results.
- **Conclusion:** in a paragraph or so, summarize your results and make concise conclusions about them; also include a sentence or two conveying your general conclusions about your results in the context of global warming and ocean acidification.

References

Science background information condensed and/or compiled from the following sources:

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Appendix A. Relevant Next Generation Science Standards

Science and Engineering Practices

1. Scientific Knowledge is Open to Revision in Light of New Evidence
 - a. Most scientific knowledge is quite durable, but is, in principle, subject to change based on new evidence and/or reinterpretation of existing evidence.
2. Engaging in Argument from Evidence
 - a. Evaluate the claims, evidence, and reasoning behind currently accepted explanations or solutions to determine the merits of arguments.
3. Planning and Carrying Out Investigations
 - a. Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly.

Connections to Nature of Science

1. Scientific Investigations Use a Variety of Methods

- a. Scientific inquiry is characterized by a common set of values that include: logical thinking, precision, open-mindedness, objectivity, skepticism, replicability of results, and honest and ethical reporting of findings.
- 2. Analyzing and Interpreting Data
 - a. Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and problems, using digital tools when feasible.
- 3. Obtaining, Evaluating, and Communicating Information
 - a. Communicate scientific information (e.g., about phenomena and/or the process of development and the design and performance of a proposed process or system) in multiple formats (including orally, graphically, textually, and mathematically).

Disciplinary Core Ideas

- 1. LS2.C: Ecosystem Dynamics, Functioning, and Resilience
 - a. A complex set of interactions within an ecosystem can keep its numbers and types of organisms relatively constant over long periods of time under stable conditions. If a modest biological or physical disturbance to an ecosystem occurs, it may return to its more or less original status (i.e., the ecosystem is resilient), as opposed to becoming a very different ecosystem. Extreme fluctuations in conditions or the size of any population, however, can challenge the functioning of ecosystems in terms of resources and habitat availability.
- 2. LS4.D: Biodiversity and Humans
 - a. Biodiversity is increased by the formation of new species (speciation) and decreased by the loss of species (extinction). (secondary)
 - b. Humans depend on the living world for the resources and other benefits provided by biodiversity. But human activity is also having adverse impacts on biodiversity through overpopulation, overexploitation, habitat destruction, pollution, introduction of invasive species, and climate change. Thus sustaining biodiversity so that ecosystem functioning and productivity are maintained is essential to supporting and enhancing life on Earth. Sustaining biodiversity also aids humanity by preserving landscapes of recreational or inspirational value. (secondary) (Note: This Disciplinary Core Idea is also addressed by HS-LS4-6.)
- 3. LS4.C: Adaptation
 - a. Changes in the physical environment, whether naturally occurring or human induced, have thus contributed to the expansion of some species, the emergence of new distinct species as populations diverge under different conditions, and the decline—and sometimes the extinction—of some species.
 - b. Species become extinct because they can no longer survive and reproduce in their altered environment. If members cannot adjust to change that is too fast or drastic, the opportunity for the species' evolution is lost.

Crosscutting Concepts

- 1. Cause and Effect
 - a. Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.

Common Core State Standards Connections:

ELA/Literacy -

2. SL.11-12.5
 - a. Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest. (HS-LS1-4)
3. RST.11-12.1
 - a. Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. (HS-LS2-2)
4. WHST.9-12.2
 - a. Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes. (HS-LS2-2)
5. RST.11-12.7
 - a. Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem. (HS-LS2-6)
6. WHST.9-12.7
 - a. Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation. (HS-LS2-7)
7. WHST.9-12.9
 - a. Draw evidence from informational texts to support analysis, reflection, and research. (HS-LS4-4)

Mathematics -

1. MP.2
 - a. Reason abstractly and quantitatively. (HS-LS2-2)
2. HSN.Q.A.3
 - a. Choose a level of accuracy appropriate to limitations on measurement when reporting quantities. (HS-LS2-2)

Appendix B: An example of how to phrase a hypothesis and materials and methods section

The following section describes an EXAMPLE of a possible hypothesis and experiment you could conduct at HIMB. By reading through this example, you will also get a better sense of how to use the tools available in the lab. Your experimental design should be DIFFERENT from this activity, and must directly address your hypothesis. The techniques and tools (i.e., the glass jars, bubbling CO₂ into seawater, measuring pH with strips, and measuring calcium and carbonate with test kits) described in this experiment are the same ones you will be using, but you must come up with your own question and your own way to answer it.

Hypothesis (EXAMPLE):

If coral skeletons are exposed to acidified seawater (1 pH unit lower than normal) for 30 minutes, they will release calcium and carbonate through the process of dissolution.

Methods (EXAMPLE):

1. I placed two pieces of same species of coral, weighing within a gram of each other, in separate 60 mL glass jars. Then I filled the jars to the internal rim with seawater.
2. I labeled one jar “Control”; this jar contained seawater, a piece of coral or sand, but was NOT exposed to CO₂ gas. I labeled the other jar “Treatment”; this jar contained seawater, a piece of coral identical in size and species to the control, and this was exposed to CO₂ gas.
3. I positioned both control and treatment jars on the bench in front of me with both of the lids removed. I took a pH reading of the water in both jars using the pH strips or pH meter and recorded (they should be identical).
4. I began a constant flow of 100% CO₂ gas from the regulator through a piece of airline tubing. (IMPORTANT: the flow rate of the gas must remain constant throughout the experiment).
5. I placed the end of the airline tubing in the bottom of the treatment jar such that bubbles of gas rose from the bottom to the top of the jar through the seawater. I allowed the CO₂ gas to bubble for 30 seconds and then removed the airline tubing from the jar.
6. I took another pH measurement from the treatment water. If the pH had decreased by 1 pH unit (example: from 8.3-7.3), I screwed the lids on both the control and treatment jars and began my incubation period. If the pH had not changed 1 pH unit, I placed the airline tubing back in the treatment jar and continued bubbling with CO₂ gas for another 30 seconds. After this 30 second bubbling, I repeated the measurement again and, if necessary, repeated this procedure until I had achieved a 1 pH unit decrease in my treatment jar water.
7. I allowed both the control and treatment jars to incubate at room temperature for 30 minutes with their lids screwed on.
8. Following the incubation period, I removed the lids and again record the pH of the seawater in both jars. Next, I removed 5 mL of seawater from the control jar using the glass pipette and

pipette ball (NOTE: if you are using sand be careful not to suck up sand into the pipette, and try to take water from the top of the jar instead).

9. I pipetted the 5 mL of seawater into the glass vial provided in the calcium test kit. I followed the instructions in the test kit, and recorded my data. Briefly, the calcium test kit's procedure went as follows:

-Fill the provided test tube with 5 mL of water

-Add 10 drops of Calcium Test Solution (Bottle #1), cap the test tube, and invert for 10 seconds

-Invert Calcium Test Solution (Bottle #2) for 10 seconds

-Add Calcium Test Solution (Bottle #2), drop by drop to the test tube until the solution changes from pink to the blue endpoint color

-Based on the number of drops of Calcium Test Solution (Bottle #2) added, use the chart on the back of the instructions to determine the calcium level

10. I then pipetted an additional 5mL of seawater into the glass vial provided in the carbonate test kit. I then followed the instructions in the test kit and recorded my data. Briefly, the carbonate test kit's procedure went as follows:

-Fill the provided test tube with 5 mL of water

-Add KH Test Solution, drop by drop to the test tube (count the number of drops you add!)

-Cap the test tube and invert after every drop you add

-When the water in the test tube turns from blue to yellow, the measurement is complete

-Based on the number of drops of KH Test Solution added to turn the solution from blue to yellow, use the chart on the back of the instructions to determine the carbonate level

-Pour all used chemicals into the plastic containers labeled WASTE; DO NOT pour experimental water down the drain! Make sure all other seawater (without chemicals in it) is discarded outside and not down the drain!