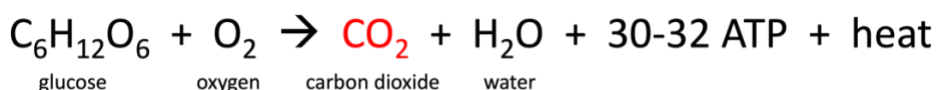


Cellular Processes and Aquatic Systems 1

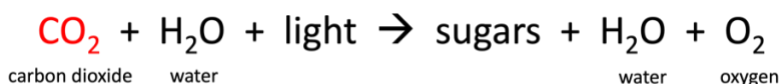
Part 1: Carbon Dioxide and pH (TA Demonstration)

Carbon dioxide (also known as CO₂) is a very important molecule in the context of biology and metabolism. Carbon dioxide is an input to photosynthesis and an output from cellular respiration.

Cellular Respiration



Photosynthesis



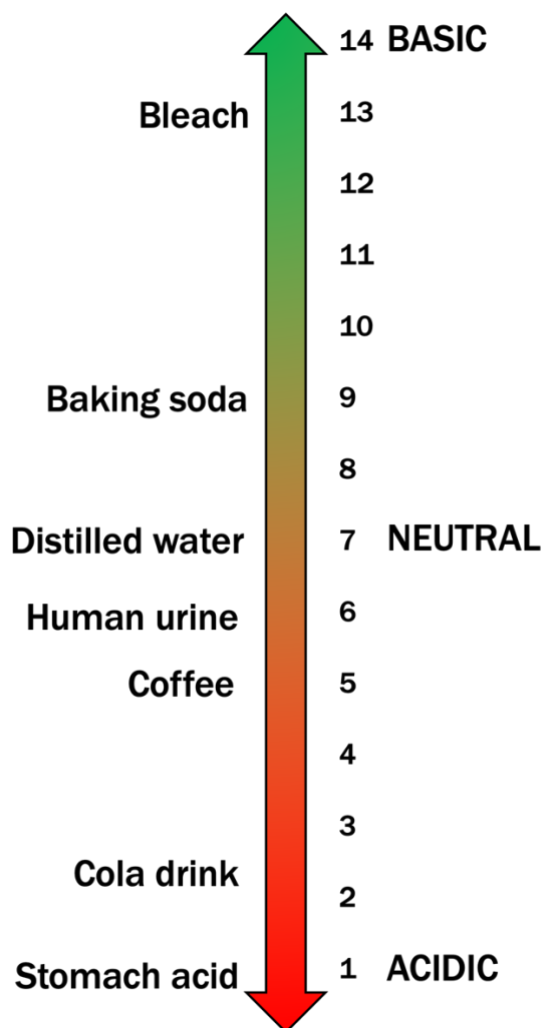
Since cellular respiration creates an output of carbon dioxide, measuring the amount of carbon dioxide present is a good way to measure how much cellular respiration is occurring.

For example, if you put a fish in a cup of water and found that the amount of carbon dioxide in the water was increasing, you would likely conclude that this was due to the fish performing cellular respiration and releasing carbon dioxide.

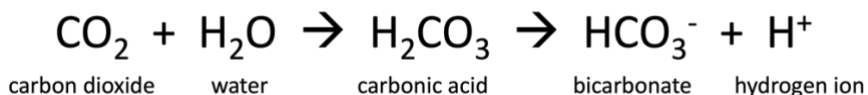
Unfortunately, we would need expensive equipment to measure the amount of carbon dioxide directly. Instead, we can measure the amount of carbon dioxide present by measuring pH.

pH stands for "potential Hydrogen" and allows us to *measure the degree to which a substance is acidic (like sulfuric acid) or basic (like bleach)*. Acidic substances have a **low pH** (less than 7) and **basic substances** have a **high pH** (greater than 7).

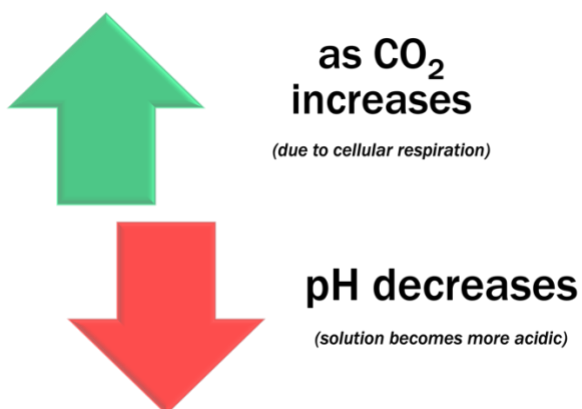
For comparison, here are the pH measurements for various familiar substances:



How can we use pH to measure the amount of carbon dioxide? Carbon dioxide (CO_2) produced during cellular respiration (or fermentation) reacts with water (H_2O) to make carbonic acid (H_2CO_3). That carbonic acid then dissociates into bicarbonate (HCO_3^-) and a hydrogen ion (H^+), as shown in the equation below. When carbon dioxide mixes with water, it dissociates into carbonic acid, which has a low pH (remember, acidic=low pH).



Remember, the more hydrogen ions a solution contains, the more acidic it will be and the lower its pH. **Therefore, the more carbon dioxide present in water the lower the pH of the liquid will be.**



Did you see that Cola drinks were at a 2.6 on the pH scale? That is partially due to the fact that sodas are carbonated by forcing carbon dioxide bubbles into the liquid (though Cola drinks also contain other substances that increase their acidity). The increase of carbon dioxide lowers the pH.

In order to measure the pH of a liquid--and therefore the amount of carbon dioxide present--we use **pH indicators**. pH indicators are *substances that change color in response to changes in pH*.

For example, in this lab we will use the pH indicator **phenol red**. In the presence of a **neutral or basic pH** (7 or higher) phenol red is a **pink-red color**. In the presence of an **acidic pH** (below 7), phenol red turns **yellow**.

Phenol Red Test	
Neutral or basic	Pink-red
Acidic	Yellow

Based on this information, discuss and answer the following questions with your lab group:

1. What color do you think phenol red would be if added to a glass of distilled water? Why?
2. What color do you think phenol red would be if you added it to a glass of sparkling (carbonated) water? Why?

3. Your TA is going to take a test tube full of phenol red and blow into it with a straw. The phenol red is diluted in distilled water, so it is starting at a neutral pH. What color would you expect the phenol red to be before your TA blows into it?

4. Write a hypothesis about what you think will happen to the phenol red when your TA blows into it. Use information from lab content to explain *why* you think this will happen.

Observe the TA demonstration. Did the results of this demonstration support your hypothesis?

Plants perform cellular respiration with their mitochondria, but they also perform photosynthesis via their chloroplasts. **While cellular respiration produces carbon dioxide, photosynthesis uses carbon dioxide as an input.**

This is the reason that planting trees can reduce the amount of carbon dioxide in the atmosphere--the chloroplasts in the tree's cells take in carbon dioxide and convert it into sugars, water, and oxygen (which animals like us breathe in to perform cellular respiration!).

Based on this information, discuss and answer the following questions with your lab group:

1. On average, do you think plants produce more carbon dioxide than they consume or vice versa? What leads you to think this?
2. Under what circumstances (if any) do you think a plant might produce more carbon dioxide than it consumes?
3. Your TA is going to place a piece of *Elodea* (a water plant) in the test tube that they just blew into (containing phenol red) and leave it under a bright light for 1-2 hours. Write a hypothesis about what you think will happen to the phenol red (if anything).

After you have completed your other lab activities, return to the test tube under the bright light and observe if there have been any changes in the phenol red. Do these changes (or lack of changes) support your hypothesis?

Part 2: pH and Marine Ecosystems

You just observed how adding CO₂ to a small volume of water could change the pH. Now we will expand our scope--how could a worldwide increase in CO₂ impact large volumes of water, like our oceans?

Discuss and answer the following questions in your lab group:

1. How do you think an increase in atmospheric CO₂ might impact the CO₂ concentration and pH of the world's oceans?

Take a look at [this graph](#). Does the data support your hypothesis?

2. Hard corals and many shelled sea organisms (ex. snails, oysters, clams, etc.) build their support structures out of calcium carbonate, which is a very basic material (i.e. high on the pH scale). How do you think these organisms might be impacted by increased atmospheric CO₂ concentrations?

During the first lab session of the week, students weighed three seashells, then placed one in regular water, one in sparkling water, and one in vinegar. Over the course of this week, each subsequent lab section will weigh the shells and add their data to the table.

Before your lab section looks at the shells, take a moment to discuss the following question with your lab group:

1. What do you think will happen to the weight of the shells? Why?
2. Do you think all three shells will be impacted equally? Why?

Use the following procedure to collect seashell data.

Procedure

1. Carefully remove Shell 1 from the cup. Use a paper towel to gently wipe off as much liquid as possible.
2. Record any qualitative observations of the shell in your lab notebook. What is the surface texture like? Are there any holes? How thick do the walls of the shell appear?

3. Place the shell on the triple beam balance and record the weight.

4. Repeat this procedure for Shell 2 and Shell 3.

Once you have entered your data in the table, you can take a look at the data that has been collected by lab sessions before you. Next week, you can look at the final results.

Part 3: Culturing Algae

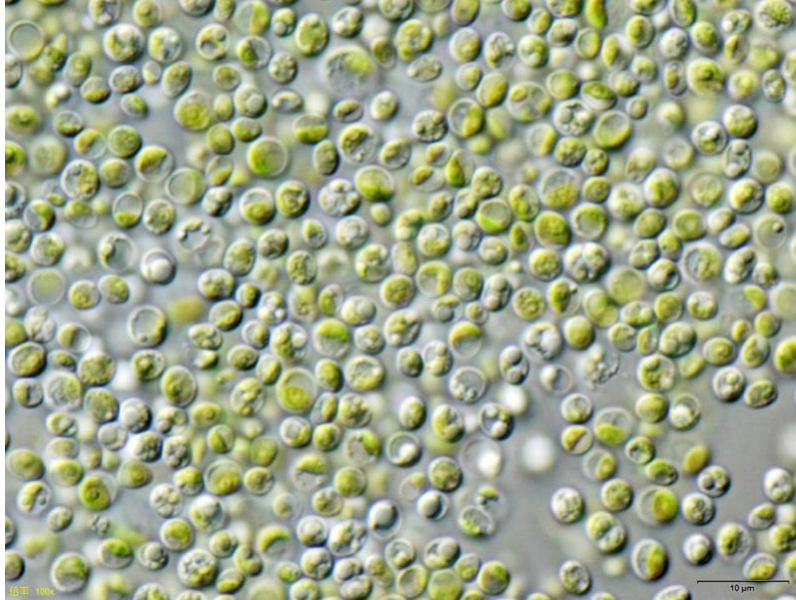
The general term "algae" includes a wide variety of organisms including diatoms (eukaryotic), green algae (eukaryotic and related to plants), and cyanobacteria (prokaryotic)--all types of algae perform both cellular respiration and photosynthesis.

Algae is a major source of photosynthesis in freshwater and marine ecosystems--photosynthetic algae produces around 70% of the oxygen in Earth's atmosphere. Algae can reproduce much faster than most plants and some species of microalgae absorb carbon dioxide through photosynthesis many times faster than plants.

Humans use various species of algae for many different purposes:

- dietary supplement
- food for livestock
- [biofuel](#) (including research being done by the Peers Lab at CSU!)
- wastewater treatment
- ingredients in sustainable plastic
- aquarium and pond filtration
- removing carbon dioxide from the atmosphere

In your lab, you will be conducting an experiment to determine the optimal conditions under which to grow freshwater green algae in the genus *Chlorella*.



Chlorella algae cells

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<https://commons.wikimedia.org/w/index.php?curid=35450226>

What factors influence the rate of algae growth? Like plants, algae need the following basic components to live and grow:

- water
- light
- carbon dioxide
- nutrients

Although there are many nutrients that photosynthetic organisms need to grow and thrive, in this lab we will be focusing on two major nutrients:

- Nitrogen:** an important ingredient in **chlorophyll** (*the green pigment in chloroplasts that allows plants and algae to generate energy from light*) and amino acids (the building blocks of protein)
- Phosphorous:** needed to make ATP (remember, the P stands for phosphate) and nucleic acids (the building blocks of DNA)

These nutrients are not found abundantly in *forms that living organisms can use* (also known as **bioavailable**)--so the amount of nitrogen and phosphorous available to algae can be a major limiting factor to its growth.

Discuss and answer the following questions with your lab group:

1. How are the growth requirements of algae related to the process of photosynthesis?
2. Would changes in the availability of these growth requirements also impact the process of cellular respiration? Why?
3. Based on your background knowledge and what you have learned so far in this course, what specific impacts do you think low levels of each of the following growth requirements would have on algae? (Hint: Think about their role in the algae's cellular processes)

- water
- carbon dioxide
- light
- phosphorous
- nitrogen

4. Do you think it would be possible to add too much of any of the growth requirements? What do you think would happen to your algae if it received too much of each of the growth requirements?
5. Which of these factors do you think places a limit on the rate at which algae can grow? Do you think it is always the same factor?
6. Which of the growth requirements (water, carbon dioxide, light, nutrients) are you most interested in manipulating for your experiment? Why?

Note: Remember that Chlorella is freshwater algae, so you cannot change the water requirements without killing the algae.

7. In conducting an experiment on algae growth, how many growth requirements (carbon dioxide, light, nutrients) should you change for a single experiment? Why?

For this activity, each lab group will be responsible for setting up and measuring a single algae culture. As a lab section, you should decide on a single variable to manipulate, so that you can compile and compare your data at the end of the experiment.

For example, if your lab section decided to manipulate the color of light that the algae received, you might have 2 groups place their cultures under red light, 2 under blue light, and 2 under yellow light (unfortunately, we don't have different colored light in lab to do this experiment).

Start with the general procedure below to grow algae, then work with your lab section to alter the procedure for your experiment.

Algae Culture Setup

1. Fill an 8 oz bottle 3/4 full with culture media. *Chlorella* requires specific water quality to grow, so you cannot use tap water for this step.
2. Add 5 drops of nutrient solution to your bottle with a transfer pipette.
3. Add 1 mL of algae to your bottle from the starter culture using a different transfer pipette.
4. Replace the cap on your bottle, but only rest it loosely on top to allow for gas exchange.
5. Write your lab date and time + the initials of your group member on a piece of tape. Place the piece of tape on the bottom of your bottle.
6. Place your algae culture under grow lights or on a well-lit windowsill.

Work with your lab section to decide how you will conduct your experiment. Remember to record your procedure--that's good science!

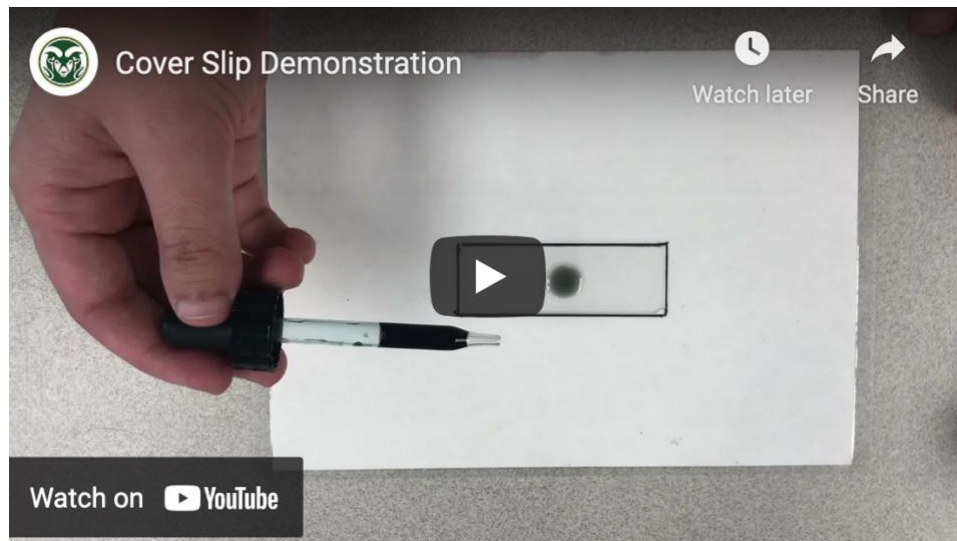
You will measure the starting values of your algae cultures in two different ways:

1. Observing algae cells under the microscope
2. Measuring the chlorophyll concentration in your culture

First, each member of your group should observe a sample of your group's algae culture under the compound microscope.

Algae Sample Microscopy

1. Obtain a blank slide and cover slips.
2. Shake or otherwise agitate your algae culture to make sure algae is suspended in the water.
3. Place 1 drop of water from your culture on your slide and create a wet mount. You can review how to make a wet mount using the video below:



4. Examine the slide under a compound microscope at 400x. If you need to review how to use the compound microscope, check out this video:



5. Find the area on your slide where algae cells are densest. Count and record the number of algae cells that you can see in your field of vision (i.e. how many algae cells you can count in a single spot without moving the microscope slide).
6. Record qualitative (i.e. non-numerical) observations of your algae sample. What color and shape are the cells? Do they look alive? Do they look healthy? Are they clumped or arranged in a particular way? You may want to make a quick sketch or take a picture of your sample.
7. Record the number of algae cells you were able to count on your slide. You may want to make a data table to keep track of the number of cells counted by each member of your group.
8. Calculate the average of the cell counts from your lab group, then report that value to your TA. (Were there any outliers you felt you should exclude from this number?)

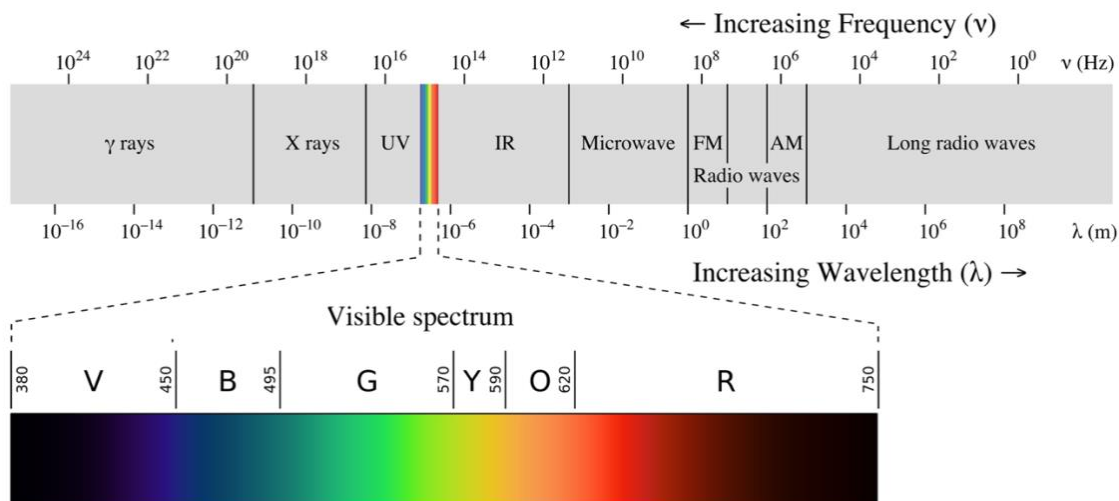
Next, you will measure the concentration of chlorophyll A in your algae culture. You may remember from earlier that **chlorophyll** is the *pigment found in chloroplasts which allows photosynthetic organisms to capture energy from light*--chlorophyll A is the most prevalent photosynthetic pigment. The concentration of chlorophyll in your sample is a good measurement of the concentration of algae and potential for photosynthesis.

To measure the chlorophyll concentration, we will use a machine called a **spectrophotometer**. Spectrophotometers *measure the light absorbance of a sample* (how much light it absorbs).

To do this, the spectrophotometer shines a beam of light through your sample towards a light sensor on the other side. By measuring the difference between the starting intensity of the light beam and the light intensity detected by the sensor, the spectrophotometer can calculate how much light was being absorbed by your sample.

First, you will need to calibrate the spectrophotometer using the culture media that the algae is suspended in. This is to cancel out the absorbance of the culture media, so you are just measuring the algae. You can think of this like placing a liquid in a cup on a scale to be weighed--you need to cancel out the weight of the cup first, so you are only measuring the weight of the liquid.

You will be setting the spectrophotometer to 450 nm (nanometers) which is the wavelength of light that chlorophyll A absorbs best. Remember that wavelength corresponds to color.



Wavelengths (in nm) of the visible light spectrum

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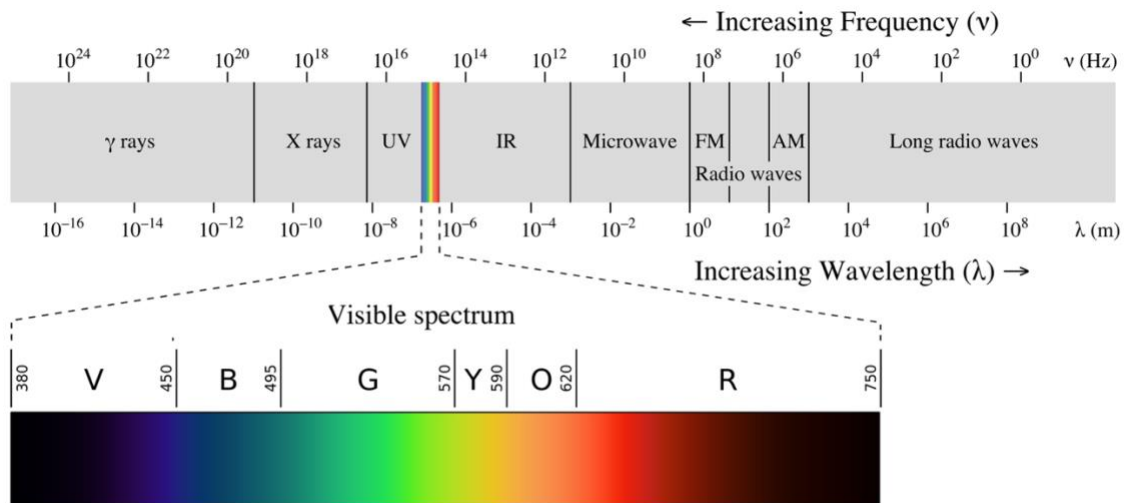
Spectrophotometer Procedure

1. Check that the sample compartment in the spectrophotometer is empty, then turn on the spectrophotometer using the toggle switch near the power cord. Allow 20 minutes for the spectrophotometer to warm up--do not press any buttons until the initialization sequence is complete.
2. Obtain 2 cuvettes and clean them with a KimWipe. Once the cuvettes are clean, try to only hold them by the top and bottom. Any fingerprints or other smudges on the sides of the cuvettes may influence the spectrophotometer's measurements.
3. Use a pipette to fill 1 cuvette with pure culture media. Fill the second cuvette with a sample from your algae culture. Be sure to shake or otherwise agitate the culture to suspend the algae before taking your sample.
4. Press the blue "A/T/C" button until "A" appears on the right side of the display window.

5. Use the blue "nm ▼" (nanometers down) or "nm ▲" (nanometers up) keys to set desired wavelength (450 nm).
6. Press the blue "0 ABS/100%T" button. Make sure sample compartment is empty and the cover is closed.
7. Insert your pure culture media cuvette into the sample compartment. Make sure the guide mark on the cuvette faces toward the front of the machine and the cuvette is seated firmly in the compartment. Gently lower the lid on the sample compartment.
8. Press the blue "0 ABS/100%T" button. The display should read "0.000 A". You have now calibrated or "zeroed" the spectrophotometer.
9. Remove the pure culture cuvette from the sample compartment and replace it with the cuvette containing a sample from your algae culture. Make sure the guide mark on the cuvette faces toward the front of the machine and the cuvette is firmly seated in the compartment. Gently lower the lid on the sample compartment.
10. Read and record the percent absorbance value from the digital readout. Remove the algae culture cuvette and close the compartment lid. Report the percent absorbance value to your TA.

Discuss and answer the following questions with your lab group:

1. What do you predict the results of your group experiment will be?
2. Why did you need to take measurements of your algae culture at the start of the experiment instead of just comparing measurements at the end of the experiment?
3. You were instructed to set your spectrophotometer to 450 nm because that is the wavelength of light that chlorophyll absorbs best. Chlorophyll also absorbs light well around 635 nm. What do you think this indicates about which types of light would be best for growing plants?



Wavelengths (in nm) of the visible light spectrum

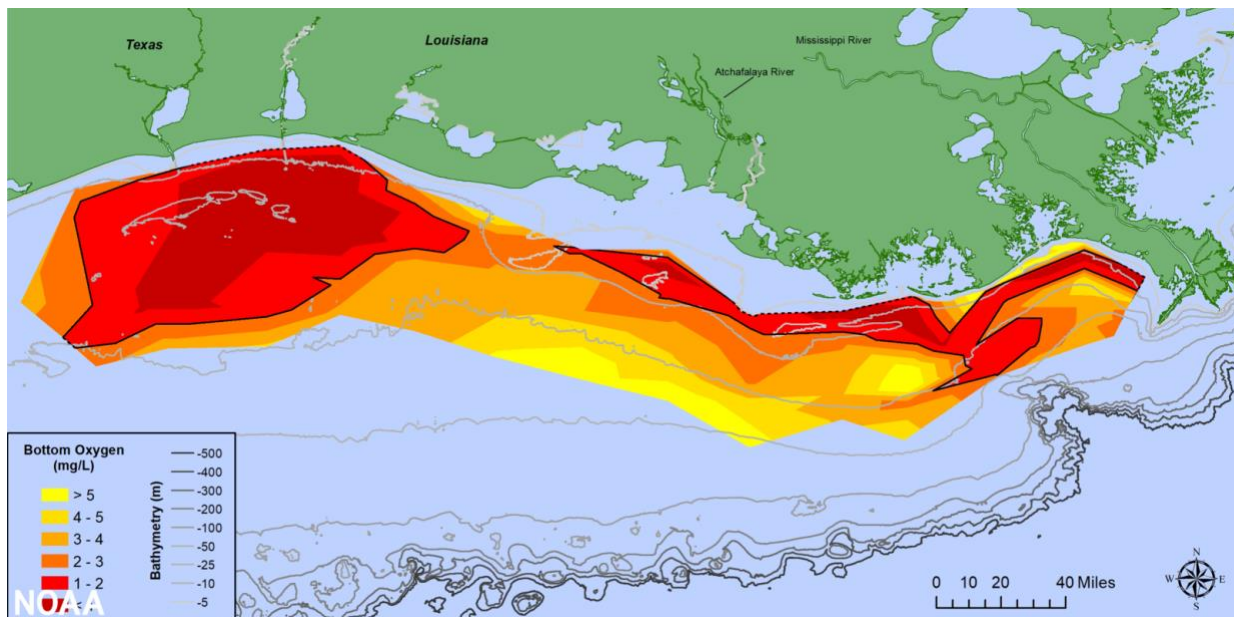
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Part 4: Hypoxic Zones

Algae growth can have tremendous impacts, both positive and negative, on aquatic ecosystems.

Today, you will look at several figures related to the phenomenon of **hypoxic zones**, or **areas of a body of water with extremely low dissolved oxygen** (remember, "hypo"=low, "hypo" + "oxic"=low oxygen). Hypoxic zones are also sometimes called **"dead zones"** because extremely low dissolved oxygen can kill aquatic life.

Below is a figure showing a large **hypoxic zone in the Gulf of Mexico**.



Hypoxic zone in the Gulf of Mexico

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Take a look at this figure with your group, then discuss and answer the following questions:

1. What is this figure displaying? What do the different colors mean?
2. Just from looking at this figure with no other context, what information can you learn?
3. What further questions do you have from looking at this figure?

With your lab group, read the first two paragraphs from the paper *Nutrients and the "dead zone": the link between nutrient ratios and dissolved oxygen in the northern Gulf of Mexico*.

Remember, that you don't have to understand every word or concept in a scientific paper to derive meaning from it. You may want to read the passage multiple times to increase your understanding.

After reading the passage, discuss and answer the following questions with your lab group:

1. What are the molecular inputs and outputs of the algae growing in the hypoxic zone? (Hint: Think back to the equations for different energetic processes from the pre-lab).
2. What are the molecular inputs and outputs of the microorganism community that break down the dead algae? (Hint: Think back to the equations for different energetic processes from the pre-lab).
3. Make a sketch or diagram showing how hypoxic conditions occur in the Gulf of Mexico. Be sure to include which molecules are being produced and consumed by different organisms (your answers to the previous two questions may be a good guide!).
4. How do you think the dissolved oxygen levels in the hypoxic zone change at night? Justify your answer.
5. Besides consuming oxygen, what other impacts might the microorganism breakdown of dead algae be having on this ecosystem? (Hint: You may want to think back on previous activities in this lab or take a look at [this paper](#)).

Next, you'll look at real data from satellites to see this phenomenon in action.

[Click here](#) to view an interactive map of the world showing aquatic chlorophyll concentrations in the world's oceans. This data was collected by NOAA using satellites, which are also used to collect important data about ocean surface temperature and salinity.

Set the time settings to *Monthly* and zoom in on the Gulf of Mexico. By moving the slider, you can scroll through the chlorophyll concentrations of different months and years.

Discuss and answer the following questions with your lab group:

1. What patterns or trends do you notice about the location of intense chlorophyll concentrations? Why do you think this trend exists?
2. What patterns or trends do you notice in how the concentration of chlorophyll changes month to month? Based on this data, when do you think the hypoxic conditions in the Gulf of Mexico are worst? Why do you think this trend exists?
3. What patterns or trends do you notice in how the concentration of chlorophyll changes year to year? Do you think the hypoxic conditions in the Gulf of Mexico are improving? Why do you think this trend exists?
4. Zoom out from the Gulf of Mexico. Do any of the trends you identified apply to chlorophyll concentrations in other parts of the Earth?
5. How can you connect this new information to your algae experiment? Does this change what you expect your outcomes to be? Why?

Algae growth activity adapted from [Algae-in-a-Bottle Experiment: A High-Impact Learning Activity](#) by W. Sean Chamberlin. NOAA View Global Data Explorer activity inspired by [Nitrates, Phosphates, and Algae, Oh My!](#) by Erin Bardar and Sarah L. Hill.