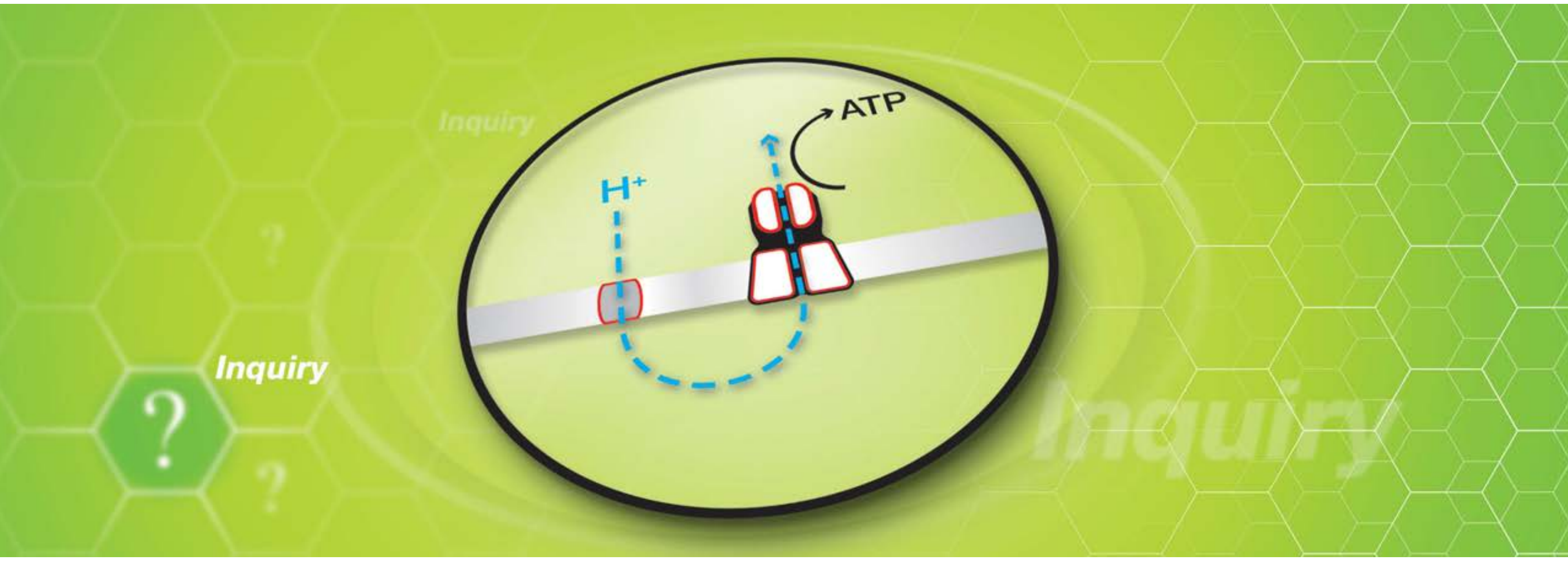
BIO-RAD Explorer™



Photosynthesis and Cellular Respiration Kit

A ThINQ!™ Investigation

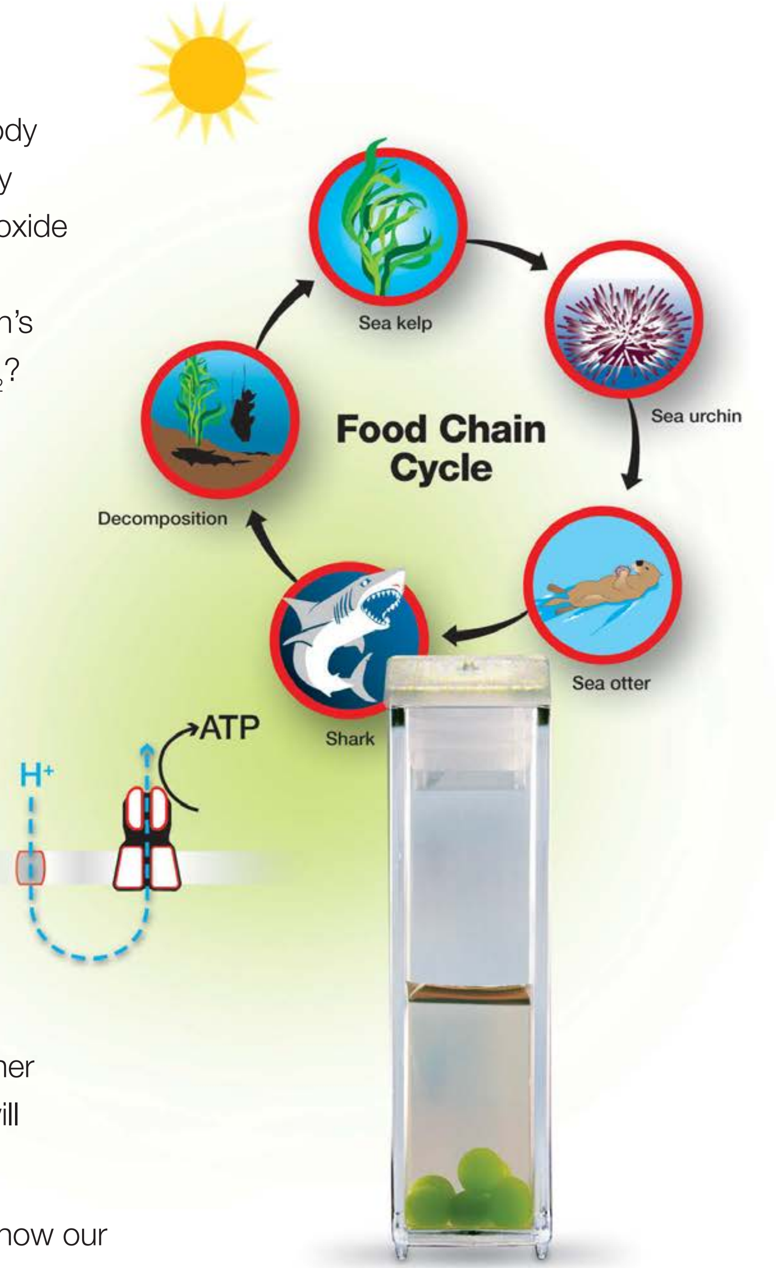
Catalog #17001238EDU

AP Biology | **Student Manual**

BIO-RAD

Dear Students

Take a deep breath in and out. What is your body doing when you inhale and exhale? Did you say absorbing oxygen (O_2) and releasing carbon dioxide (CO_2)? But where does this O_2 come from and where does the CO_2 go? Why doesn't the earth's atmosphere run out of O_2 and accumulate CO_2? Hopefully you'll be able to answer these and many other questions after investigating photosynthesis and cellular respiration through a series of experiments.



This instruction manual, and the experiments outlined within it, will help you understand the importance of photosynthesis and cellular respiration to all life on Earth: from the algae in your fish tank to the birds in the trees to the great white sharks in the ocean. While exploring these subjects you will have the opportunity to ask questions and search for answers to these questions. Sometimes you will find the answers and at other times you will discover more questions; both will contribute to your knowledge.

You may apply this knowledge by considering how our actions as human beings affect the balance of photosynthesis and cellular respiration and atmospheric O_2 and CO_2. How are greenhouse gases contributing to changes in the ocean's conditions and the species that can live there? How might replacing rainforests with farms affect the local ecosystem? How are local farming practices affecting other geographic regions? What are the benefits and detriments of our daily decisions? We hope that you will form your own opinions and perhaps suggest improvements that we as a human race can make. Most importantly, we hope you stay curious about the world around you and never stop asking questions.

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Photosynthesis and Cellular Respiration

In the following lab investigations, you will use algae beads (algae cells that are encapsulated in alginate) and a colorimetric CO_2 indicator to observe two biochemical processes that sustain most life on Earth: **photosynthesis** and **cellular respiration**. It's not an exaggeration to say that we owe our lives to these two processes. Photosynthesis converts the energy in sunlight into the sugars we eat and the oxygen we breathe; it is through cellular respiration that we are able to use the sugars and oxygen to sustain our lives.

Through a series of inquiry experiments and pre- and post-lab activities, you will explore how these two processes share mechanistic and evolutionary ties, and how all life on Earth is tied inextricably through them. This will lay a foundation for understanding how imbalances in Earth's resources can upset the balance of these processes, leading to far-reaching consequences. This introduction provides a general overview to photosynthesis and cellular respiration.

Organisms need matter and energy (food) to live

All living things need food to grow and to survive. The food we eat supplies us with the matter ("stuff") we need to make more of ourselves, and it supplies the energy we need to fuel our lives. Our lives, then, follow the **first law of thermodynamics**, which states the universe holds a finite amount of energy and, as a result, energy can be neither created nor destroyed. It can, however, be captured, transferred, and converted into other forms. We cannot simply "make" the matter and energy we need; we must get it from somewhere. In fact, all life on Earth is based on systems of energy capture, transfer, and transformation.

Life also follows the **second law of thermodynamics**, which states that during any energy transformation, a portion of the energy is lost to **entropy**. Entropy is the measure of disorder within a system. All things tend to move to a state of higher entropy, and energy input is required to maintain order and organization. To survive, living things must offset entropy by taking in more energy than they lose or expend. Otherwise, harmful or even fatal energy deficiencies can occur. This can affect population size and even cause disruptions at the ecosystem level.

Organisms need energy to survive

Photosynthesis and cellular respiration are great examples of energy-transforming systems, and they define the two ways in which organisms derive the energy they need to survive. **Autotrophs** (also called primary producers) capture free energy from the environment, including energy from sunlight (photosynthesis) or chemical sources (chemosynthesis). They transform this energy into other forms that can be used by themselves and other organisms within their environment. Some examples of autotrophs include plants, algae, and some bacteria. **Heterotrophs** (consumers), on the other hand, obtain free energy from carbon compounds produced by other organisms, including other heterotrophs and autotrophs. Within the web of life, autotrophs provide the food needed by all heterotrophs to grow, survive, and reproduce.

Energy is captured in universal units of chemical currency

The biochemical pathways that organisms use to capture energy occur in a stepwise fashion. At each stage energy is trapped in the form of energy-storing chemicals ("chemical currency"). For example, organisms can burn sugar through cellular respiration and store the energy as the following four chemicals until it is needed to perform other types of work:

ATP (adenosine triphosphate) — this molecule contains a high-energy phosphate bond. When the molecule is hydrolized, this bond is broken, and the energy released can be coupled to other reactions that require energy to proceed.

ATP —> ADP + P_i (energy utilization reaction)

NADPH (nicotinamide adenine dinucleotide phosphate), NADH (nicotinamide adenine dinucleotide), $FADH_2$ (flavin adenine dinucleotide) — these three compounds store energy that is used in oxidation/reduction reactions.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

When did molecular oxygen (O_2) first appear in Earth's atmosphere? What organism and processes led to O_2 accumulation in our atmosphere?

Where does most of Earth's O_2 come from today (for example, oceans, rainforests, etc.)?

If photosynthesis captures only 1–2% of the energy from sunlight, what happens to the other 98–99% of energy?

Photosynthesis and chemosynthesis are two forms of autotrophy. Provide an example of a chemosynthetic organism.

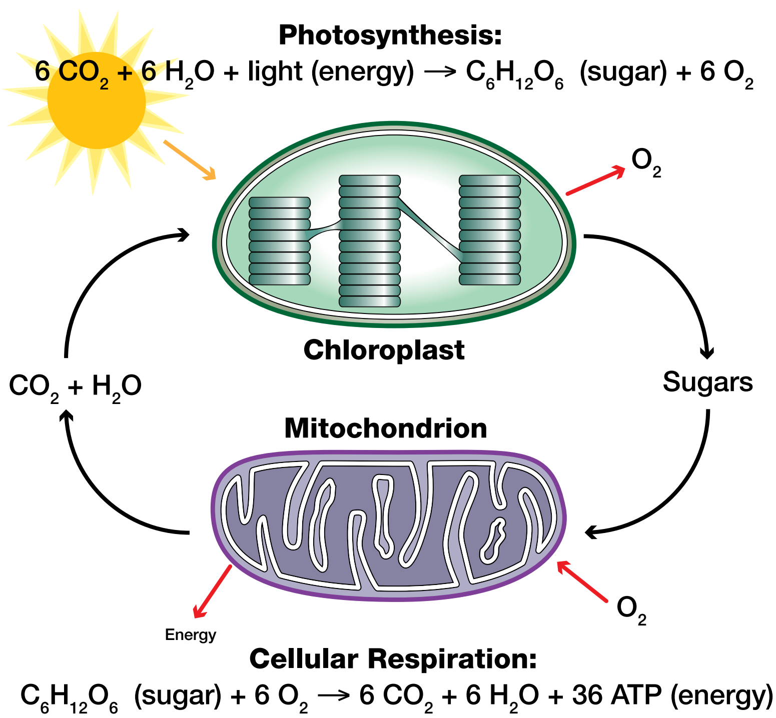
Lichens represent a symbiotic relationship between a fungus and an alga. In this relationship, which organism is the autotroph? Which is the heterotroph?

Photosynthesis and cellular respiration are also the source of the stuff we use, such as shampoo, plastic water bottles, clothes, and even medications. The materials for these items are derived from petroleum, which is a mixture of hydrocarbons formed by the compression of ancient fossilized organisms on the ocean bed. These ancient organisms were autotrophs and heterotrophs that depended on photosynthesis to convert atmospheric CO_2 to energy and organic matter. The process of converting CO_2 to organic matter, then organic matter to petroleum (hydrocarbons) can be viewed as energy storage and CO_2 capture from the atmosphere. What is happening to the captured CO_2 when we convert petroleum to energy and the things we use in our daily lives?

Photosynthesis and Cellular Respiration Are Interdependent Pathways That Are Central to Life

In one way or another, all life on Earth depends on photosynthesis and cellular respiration. Photosynthesis is the only biological process that can capture energy from sunlight and convert it into chemical compounds that all organisms — from bacteria to humans — use to power metabolism, growth, and reproduction. Cellular respiration, in turn, is the process all organisms require to derive energy from the products of photosynthesis (for example, sugars) they consume. The carbohydrates produced by photosynthesis can be used to drive multiple different metabolic processes, including cellular respiration. Cellular respiration uses the free energy from sugar, for example, to produce a variety of metabolites and to phosphorylate ADP into ATP to fuel other processes.

Although photosynthesis and cellular respiration evolved as independent processes in early prokaryotes, a look at the summary reactions (see figure below) highlights their interdependence today: The products of photosynthesis — oxygen and carbohydrates — are the reactants for cellular respiration, and vice versa.



ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

In photosynthesis, water (H_2O) is the ultimate electron donor, and it is split to yield O_2. Provide an example of an electron donor used in chemosynthesis. What is the byproduct of its electron donation?

What do synthase enzymes do? What are the two precursors that ATP synthase uses as reactants to produce ATP?

Photosynthesis Occurs in Two Phases Within chloroplasts

Photosynthesis powers 99% of Earth's ecosystems; it is through the photosynthetic activities of plants, algae, and certain bacteria that our atmosphere gains the molecular oxygen we breathe and the sugars we eat. In addition, photosynthesis helps maintain the balance between oxygen and carbon dioxide in our atmosphere. This balance is so important that scientists are trying to create artificial photosynthetic systems to capture CO_2 emissions from factories and coal-burning energy plants before they are released into the atmosphere. Photosynthesis is even responsible for fueling our cars — the energy stored within fossil fuels (natural gas, coal, petroleum, etc.) is derived from solar energy that was trapped and stored during photosynthesis long ago in the geological past. Today the global rate of energy capture through photosynthesis is approximately three times the rate of power consumption by humans. Photosynthesis occurs as two separate but coordinated sets of reactions:

- The **Hill reaction**, in which the energy from sunlight is captured and converted to chemical energy and water (H_2O) is split to form free oxygen (O_2)
- The **Calvin cycle**, in which the ATP and NADH made in the Hill reaction are used to convert CO_2 to sugar

In eukaryotes, these reactions take place in an organelle specialized to the task: the **chloroplast**. The structure of the chloroplast facilitates its function in photosynthesis. Stacked and folded inner membranes (**thylakoids**) contain the pigments (**chlorophylls** and **carotenoids**) and enzymes required for the Hill reaction; the folded and stacked structures provide a large surface area for pigments and light harvesting. In prokaryotes, these reactions occur in infoldings of the inner membrane. The carbon fixation reactions occur within the **stroma** of the chloroplast (cytosol of prokaryotes).

Directly or indirectly, nearly all ecosystems on Earth are powered by photosynthesis. For example, when a top predator, such as a coyote, preys on a rabbit, the coyote is at the end of an energy path that originated with nuclear reactions on the surface of the sun to light to photosynthesis to vegetation to the rabbits and, finally, to the coyote.

Hill Reaction Involves an Electron Transport Chain

During the Hill reaction, chlorophylls and other pigments capture the free energy from sunlight and convert it to a higher, more excited energy state. Excited chlorophylls pass electrons down an electron transport chain to successively lower energy states, creating reduced intermediates along the way. This electron transport chain ultimately yields:

- NADPH (one of the forms of universal energy currency mentioned above)
- An electrochemical gradient of protons (hydrogen ions, H^+) across the thylakoid membrane; this gradient drives the synthesis of ATP by the enzyme **ATP synthase**, which converts the energy stored in the electrochemical gradient into the energy of ATP
- A chlorophyll molecule without an electron. Chlorophyll regains this electron through the splitting of water (H_2O), which also yields O_2

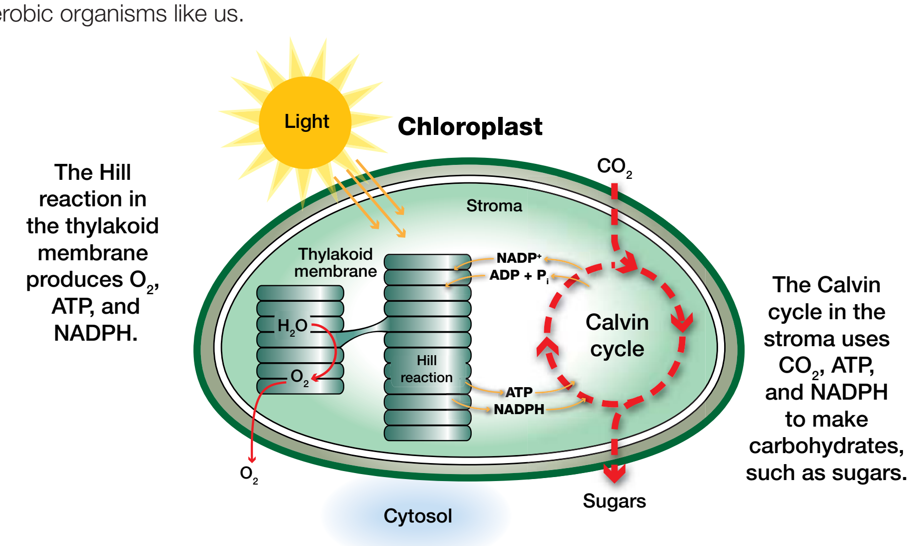
ThINQ! Exercises

Collaborate and use outside resources to answer the following question:

The carbon fixation reactions are sometimes called the dark reactions to distinguish them from the Hill reaction. Is this an accurate name? In other words, do these reactions occur only in the dark?

Hill net reaction: $2\ H_2O + 3\ ADP + 3\ P_i + 2\ NADP^+ +$ energy (light) —> $3\ ATP + 2\ NADPH + O_2$

The NADPH and ATP are energy molecules that are utilized in the Calvin cycle to make sugars; the oxygen that is generated fuels respiration by aerobic organisms like us.



The Calvin Cycle Is Powered by the Products of the Hill Reaction

The Calvin cycle (carbon-fixing) reactions of photosynthesis use the ATP and NADPH made during the Hill reaction to convert CO_2 into sugars.

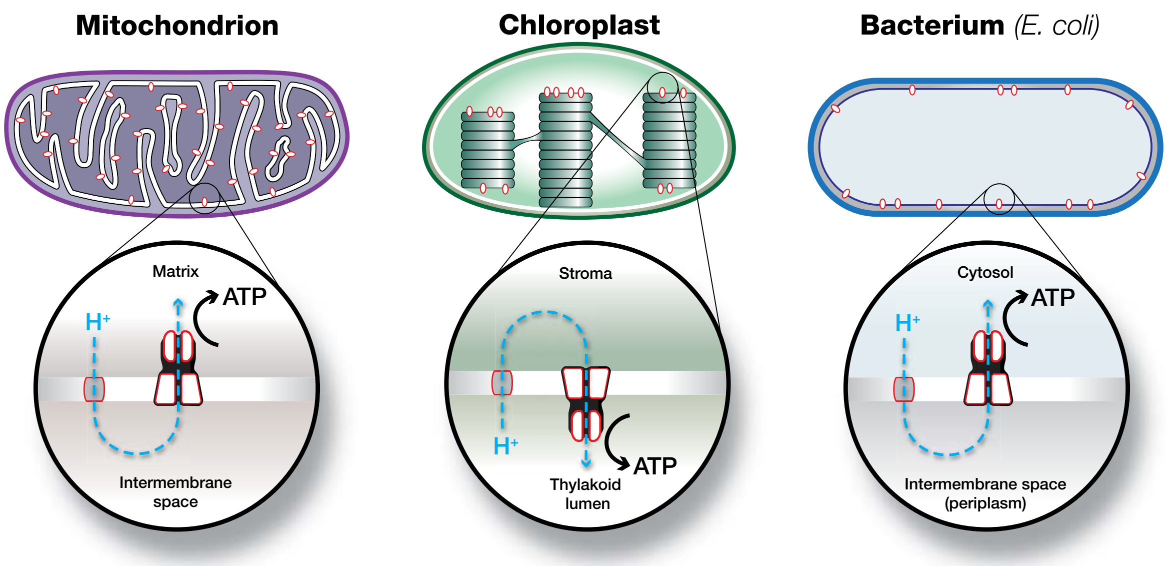
Calvin net reaction: 6 CO_2 + 18 ATP + 12 NADPH —> $C_6H_{12}O_6$ (glucose) + 12 $NADP^+$ + 18 ADP + 18 P_i

The enzyme **RuBisCO** (**R**ib**u**lose **Bis**phosphate **C**arboxylase **O**xygenase) catalyzes the CO_2 fixation step of the Calvin cycle. Highlighting its critical role in sustaining life, RuBisCO is the most abundant protein on Earth — ~20–50% of the protein in every leaf is RuBisCO.

The sugar and other intermediates produced by photosynthesis are the branch points for a number of different metabolic pathways, including those that synthesize fats, amino acids, and other critical building blocks of life. Glucose also serves as a vital food source and as a primary entry point to glycolysis and cellular respiration.

Reactions of Glycolysis and Cellular Respiration Release Energy from Food

Where photosynthesis uses sunlight, water, CO_2, ADP, and $NADP^+$ as input and generates the sugars we eat and O_2 we breathe, glycolysis and cellular respiration essentially reverse these processes: aerobic organisms eat compounds like glucose and, in the presence of oxygen that is breathed, convert the glucose to CO_2 and water and extract a huge amount of energy in the process.



And as with photosynthesis, the reactions of glycolysis and cellular respiration are compartmentalized in eukaryotes: In eukaryotes, glycolysis (the initial breakdown of sugar) occurs in the cytosol, and cellular respiration — which also involves an electron transport chain and an ATP synthase — occurs within mitochondria.

In fact, as we sleep each night, we lose weight just from respiration. It is estimated that, for every gram of air we breathe in as we sleep, we lose about 0.013 gram of carbon (in CO_2) and 0.019 gram of water vapor. This can add up to a pound of weight loss overnight!

Aerobic Respiration of Glucose

$C_6H_{12}O_6$ (glucose) + 6 O_2 —> 6 CO_2 + H_2O + energy

In the presence of oxygen, the stepwise combustion of glucose yields energy in the forms of ATP, NADH, and $FADH_2$. Ultimately, since the latter two can be converted to units of ATP, one molecule of glucose yields approximately 36 molecules of ATP! Remember, ATP is used for many different processes, including muscle action, nerve impulses, and other metabolic processes . . . a lot of them!

Photosynthesis and Cellular Respiration Occur within the Same Cell

It is important to understand that, although only autotrophs perform photosynthesis, **ALL organisms** (you, your teacher, the neighbor's cat, and the tree at the end of the street) **perform glycolysis and cellular respiration**. In fact, the reactions that break down glucose in the presence of oxygen are universal. Even autotrophs, who produce their own food, use glycolysis and cellular respiration to break down the sugars they synthesize in order to extract energy and metabolites along the way. Where photosynthesis is the capture and transformation of light energy to chemical energy (**photosynthates**), respiration is the burning of those photosynthates for energy to grow and to do the work of living. Both plants and animals (including microorganisms) need oxygen for aerobic respiration. This is why overly wet or saturated soils are detrimental to root growth and function, as well as to the decomposition processes carried out by microorganisms in the soil.

In autotrophs such as algae, these pathways occur within the same cells! In fact, if you could look inside one of the algal cells you will be using in the lab investigations, you'd see a large central chloroplast as well as smaller mitochondria — all within the same cell. Though photosynthesis and cellular respiration are connected through common intermediate metabolites in the cytosol, elegant regulatory pathways and differences in resource availability ensure the algal cells balance the rates of photosynthesis and cellular respiration as needed to survive environmental changes.

The algae beads used in the following investigations allow you to observe both pathways simultaneously. You will incubate the algae beads in a CO_2 indicator solution that is sensitive to changes in pH caused by gaseous CO_2 dissolving in water to form carbonic acid:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+$$

When the CO_2 levels are high, the CO_2 indicator will turn yellow, and when CO_2 levels decrease, it turns purple.

ThINQ! Exercises

Collaborate and use outside resources to answer the following question:

In the absence of oxygen, organisms may undergo anaerobic fermentation to extract chemical energy and metabolic intermediates from glucose. What are some of the products of fermentation that occur in nature?

Summary

The following table summarizes some of the hallmarks of photosynthesis and cellular respiration.

Table 1. Hallmarks of photosynthesis and respiration.

	Photosynthesis	*Cellular Respiration*
Input	CO_2, H_2O, light energy	Sugars, O_2
Output	Sugars, O_2	CO_2, H_2O, chemical energy
Organism type	Autotrophs (producers) only	Autotrophs and heterotrophs
Organelle	Chloroplast	Mitochondrion
Electron transport chain?	In thylakoid membranes	In cristae (inner membrane of mitochondria)
Terminal electron acceptor	$NADP^+$ to generate $NADPH_2$ for CO_2 fixation	Oxygen (O_2) to generate water (H_2O)
Requires light?	Yes	No

Investigations #1 and 2: *Scenedesmus obliquus* and Examining the Rates of Photosynthesis and Cellular Respiration

Scenedesmus obliquus is a eukaryotic microalgae. In these investigations, you will observe its physiology and monitor the overall rates of photosynthesis and cellular respiration under light and dark conditions.

Focus Questions

Scientific investigations begin with an observation about the natural world and the formulation of questions about those observations. Below are a few questions to ponder as you observe photosynthesis and cellular respiration.

Question 1: How can the rates of both photosynthesis and cellular respiration be monitored using the same system?

1.1 Scientists measure the rates of biochemical processes by monitoring either substrate depletion or product generation. Considering this, what substrates or products might you monitor to determine the rate of photosynthesis? Of cellular respiration?

ThINQ! Exercises

Collaborate and use outside resources to answer the following question:

In your own words (or using chemical reactions), describe how photosynthesis and cellular respiration are interdependent:

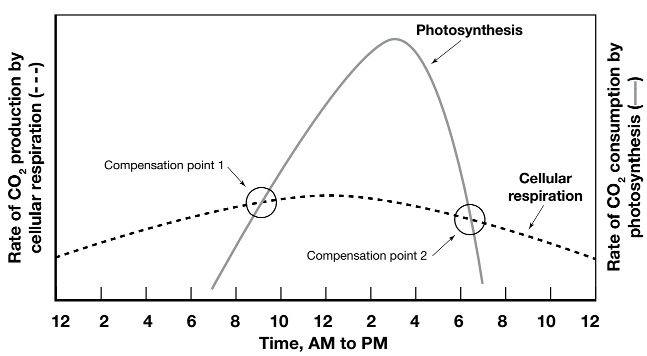
1.2 What type of organism would you need to use to be able to monitor both photosynthesis and cellular respiration? Why are the eukaryotic algal cells in the Photosynthesis and Cellular Respiration lab a good choice?

Question 2: How can one process be investigated over the other?

1.3 Which process (photosynthesis, cellular respiration, or both) do the algae perform when incubated in the light? In the dark?

1.4 Photosynthesis uses CO_2 and cellular respiration produces CO_2. We call the point when the two processes are in balance — when there is no net production of CO_2 — the compensation point. How might you limit one of the processes in order to achieve a compensation point?

1.5 Examining the data below, how do you expect the rate of cellular respiration to impact the rate of photosynthesis that you can measure in the light and the dark?



1.6 What would happen to life on Earth if the rates of photosynthesis and cellular respiration in all phototrophs were equal?

Investigation #1: Algae Microscopy

Overview

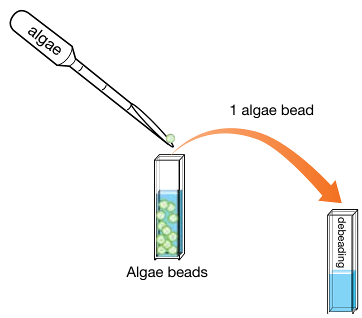
In this exercise, you will depolymerize the algae bead to free the algae (*Scenedesmus obliquus*) and observe the algae under a microscope.

The *Scenedesmus* genus is one of the most common unicellular freshwater algae. Though it can exist in a single cell (unicell) stage, it is also often seen in **coenobia** of four to eight cells. The coenobia you observe may have end cells with two long spines protruding from the outer corners. Each cell contains a single, plate-like chloroplast.

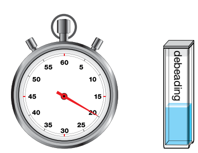
Scenedesmus is used as an experimental system in research on pollution, photosynthesis, and biofuels. In another practical application, *Scenedesmus* provides oxygen for the bacterial decomposition of organic matter in sewage purification processes.

Protocol

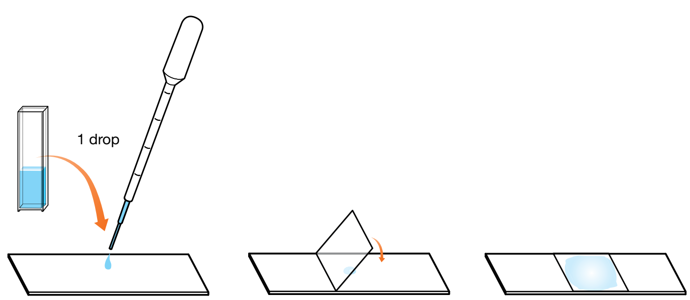
1. Use the **algae** transfer pipet that has been cut into a scoop to transfer one algae bead into the cuvette labeled **debeading** and cap tightly.



2. Incubate the solution at room temperature for 20 min, rigorously shaking the cuvette every 5 min. After 20 min, enough of the bead will have depolymerized to release enough algae cells to proceed with the microscopy activity.

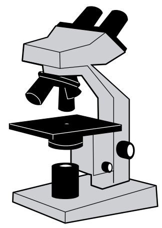


3. Gently invert the cuvette to mix, and then use a fresh transfer pipet to transfer 1 drop of dissolved algae bead solution to the center of a microscope slide.



4. Place a coverslip over the microscope slide.

5. Observe under a microscope, taking notes and making sketches.



Data Collection and Analysis

1.7 Draw some of the cells you see. Do you see coenobia? If so, how many cells do you typically see per coenobium?

1.8 Do the *S. obliquus* vary in color? What does the intensity of the color tell you about the algae?

1.9 Draw a diagram that represents the interdependence of photosynthesis and cellular respiration within the algae cells. This diagram should include the connections between the products and reactants for each process, the location/organelle in which each process occurs, and a short description of what is occurring during photosynthesis and cellular respiration. If you have drawn this diagram in Pre-Lab #3, then copy it here for future reference for Investigations #3–6.

Investigation #2: Photosynthesis and Cellular Respiration Core Lab

Overview

All life on Earth ultimately relies on two biochemical processes: photosynthesis and cellular respiration. In this exercise, you will use algae beads to measure rates of photosynthesis and cellular respiration. The beads contain eukaryotic microalgae (*Scenedesmus obliquus*) encapsulated in alginate. You will incubate them in a CO_2 indicator solution that is sensitive to changes in pH caused by gaseous CO_2 dissolving in water to form carbonic acid:

$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

When the CO_2 indicator is at equilibrium with the atmosphere, it is dark orange. When the CO_2 levels increase, it changes to yellow, and when CO_2 levels decrease, it changes to purple (see Indicator Color Guide). The CO_2 indicator spans the range of pH change that will be seen in the algae beads (pH 6.9–9.1), making it a convenient way to measure photosynthesis and cellular respiration.

In this exercise, you will compare the rates of color change of the CO_2 indicator caused by algae beads incubated under bright light and in complete darkness. The color/pH change of the CO_2 indicator can be determined using the Indicator Color Guide or a spectrophotometer set to measure absorbance at 550 nm.

pH	6.9	7.1	7.3	7.5	7.7	7.9	8.1	8.3	8.5	8.7	8.9	9.1
Absorbance at 550 nm	−0.485	−0.434	−0.402	−0.353	−0.223	−0.126	0.000	0.178	0.400	0.616	0.849	1.009

Indicator Color Guide
Compare the CO_2 indicator in your sample to the colors in this Indicator Color Guide to help you determine its pH. Each color corresponds to values for specific pH and absorbance at 550 nm.

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0 mm 10 20 30 40 50 60 70 80 90 100 110 120 130 140

Indicator Color Guide and corresponding pH values.

Focus Questions

2.1 As the algae photosynthesize, how will the pH of the CO_2 indicator change? Why? How will the pH change if the cells begin to respire?

2.2 Imagine that the algae are experiencing the light conditions that would result in the graph from page 7. Predict what color changes will happen in the CO_2 indicator between compensation points 1 and 2, and explain why. What about after compensation point 2?

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

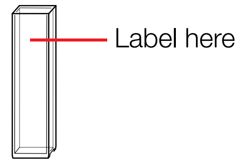
The algae beads provide a convenient experimental system because they are uniform in size and contain roughly the same number of algal cells per bead. Why are these advantages for the experiments you will perform?

Under the light, which process(es) will be taking place: photosynthesis, cellular respiration, or both?

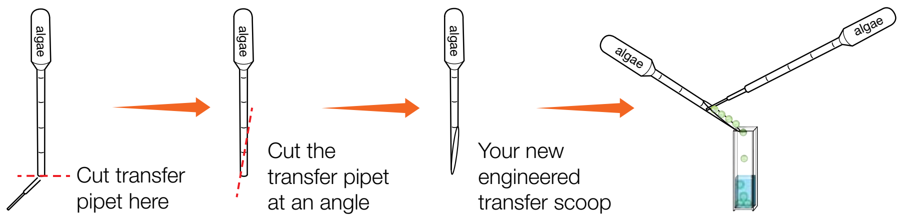
Why are you measuring the absorbance of the solution at 550 nm?

Protocol

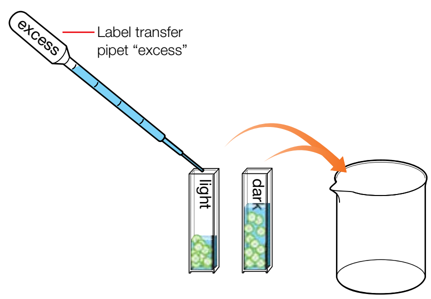
1. Label one empty cuvette **light**, and the other cuvette **dark**. Label each cuvette so that it does not obstruct light reaching the algae beads.



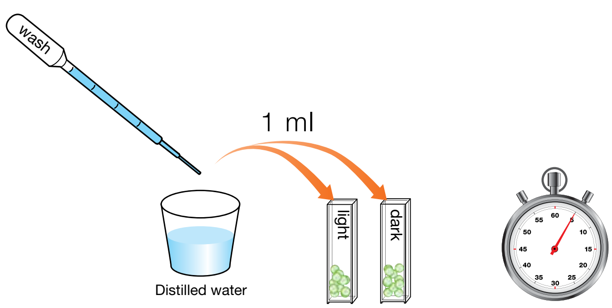
2. Label a transfer pipet **algae** and convert it into a scoop by cutting the transfer pipet at the 100 µl mark diagonally. Use the **algae** transfer pipet to transfer 10 algae beads into each of the **light** and **dark** cuvettes.



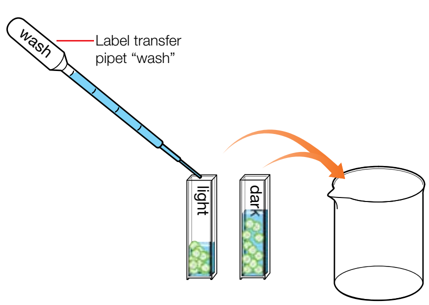
3. Label a new transfer pipet **excess** and use it to remove and discard the liquid that transferred along with the beads.



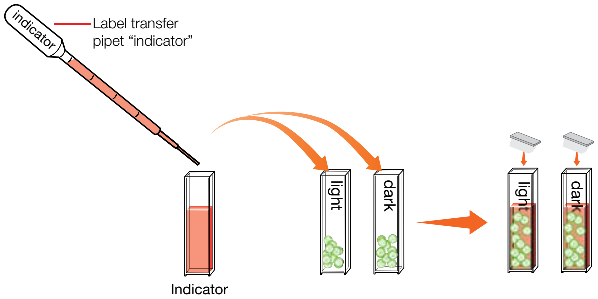
4. Label a new transfer pipet **wash** and use it to add 1 ml of distilled water to each of the cuvettes. Let the algae beads incubate in the water for 5 min to allow indicator within the bead to wash out.



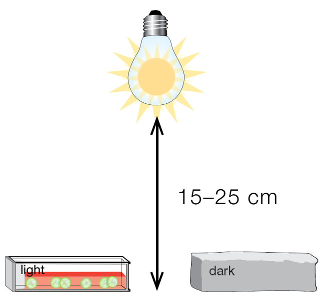
5. Use the **wash** transfer pipet to remove the water from the cuvette. Discard the water into the waste container.



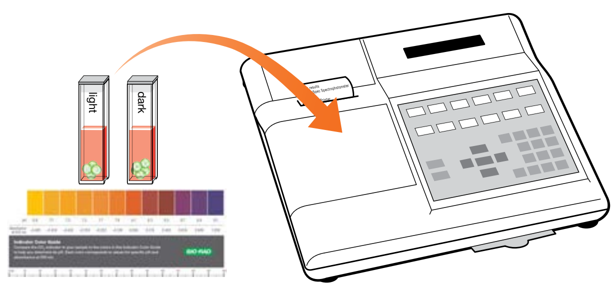
6. Label a new transfer pipet **indicator** and use it to transfer 1 ml of CO_2 indicator to each cuvette. Cap cuvettes tightly.



7. Wrap the cuvette labeled **dark** in aluminum foil. Place both the cuvettes labeled **light** and **dark** on their sides 15–25 cm from the lamp. Ensure that the beads are distributed evenly throughout the cuvette and the clear side of the cuvette faces the light.



8. Collect data starting at time = 0 min. Every 5 min, thoroughly mix the CO_2 indicator in the cuvettes and determine the color. This can be done by comparing the color of the CO_2 indicator in your cuvette to the provided Indicator Color Guide, or by reading the absorbance at 550 nm (A_{550}) in a spectrophotometer (make sure your teacher has zeroed the machine). Be quick about taking this reading and immediately return the cuvettes to the experimental conditions.



9. If enough time remains after the last time point, switch the light and dark cuvettes. Place the cuvette labeled **light** in the dark and the cuvette labeled **dark** in the light. Continue to record pH or A_{550} every 5 min.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Why is it important to keep the cuvettes at a consistent distance from the lamp as you perform this activity?

What other variables must you keep constant as you examine the relative rates of photosynthesis and respiration?

Data Collection

1. Enter your data in the table below.

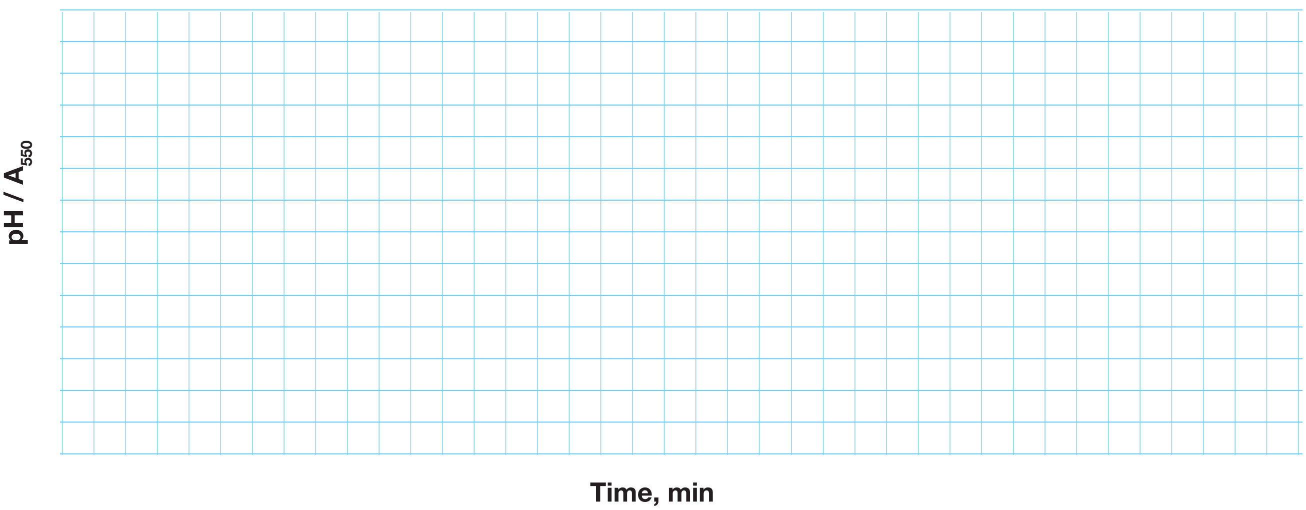
Time, min	***Light*** ***Indicator color, pH, or A_{550}***	***Dark*** ***Indicator color, pH, or A_{550}***
0		
5		
10		
15		
20		
25		
30		
35		
40		
45		

2. Make some general observations about your experimental setup (type of light bulb, light bulb color, brightness of the light bulb, distance of your cuvettes from the light, temperature of the room, location of your experimental setup relative to other light sources, etc.). It might be useful to sketch your experimental setup. Why might these general observations be important?

Analysis of Results

The goal of this analysis is to determine the rates of photosynthesis and cellular respiration in the light and in the dark.

1. Graph your results. Label the y-axis with pH or A_{550} value intervals that are appropriate to your data. Plot the color change versus time for both your light and dark samples on the same graph, as below. Use a ruler to draw a best fit line for the linear region of your **light** and **dark** datasets. Hint: use a different color to plot your **light** and **dark** results, or use solid and dashed lines.



2. Calculate the slope. Mark two points along your **light** best fit line. Try to choose points that are far apart but are still in the linear range of the graph. Label the point on the left $\mathbf{L_i}$ and the point on the right $\mathbf{L_f}$. Do the same with the **dark** best fit line but label the points $\mathbf{D_i}$ and $\mathbf{D_f}$.

   Fill out the chart below with the coordinates of the points you marked.

	Time, min	***pH or A_{550}***
L_i		
L_f		
D_i		
D_f		

The slope of the graph indicates the change in CO_2 over time. Calculate the slope from your **light** and **dark** best fit lines using the following equation (replace A_{550} with pH if you visually assessed color in your experiment):

$$\text{Slope}_{\text{light}} = \frac{(\text{A550}_{\text{Lf}} - \text{A550}_{\text{Li}})}{(\text{time}_{\text{Lf}} - \text{time}_{\text{Li}})} =$$

$$\text{Slope}_{\text{dark}} = \frac{(\text{A550}_{\text{Df}} - \text{A550}_{\text{Di}})}{(\text{time}_{\text{Df}} - \text{time}_{\text{Di}})} =$$

2.3. Are your slopes positive or negative for light and dark conditions? What does this mean about the change in CO_2?

2.4. Under which condition did the CO_2 indicator turn more alkaline? Why?

2.5 Under which condition did the solution start to change color more quickly (light or dark)? (Hint: look at the absolute value of the slopes you calculated.)

2.6 How does cellular respiration impact the observed rate of photosynthesis? Is your calculated rate of photosynthesis accurate? Why or why not?

2.7 Revisit your diagram of photosynthesis and cellular respiration from either Pre-Lab #3 or Investigation #1. Revise your diagram to demonstrate how the experimental design of your current investigation affects these processes. Indicate where the rates of these processes may increase or decrease and describe why. Also indicate how the organism will be affected if the conditions of your investigation were to continue.

2.8 Look up current ocean pH values. How do the current values compare to those from previous years? Consider what you've just learned about algae and how the chemistry of the indicator used in the experiments you just performed works. Hypothesize why oceans are at their current pH. How is the pH of the ocean changing and why? How might this affect the organisms that live in the ocean?

Investigations #3–6: Examining Rates of Photosynthesis and Cellular Respiration under Various Conditions

Overview

Plants cannot move from place to place like animals can when their environment changes or becomes inhospitable. When temperatures or light availability change, or even when predators attack, plants have no choice but to stay where they are and survive as best they can. Plants have adapted to a wide range of habitats, from the very cold and shaded to the very bright, hot, and humid. Plants have adopted a variety of strategies to accommodate changing environmental conditions, but how do environmental fluctuations affect rates of photosynthesis and cellular respiration?

As biochemical processes, photosynthesis and cellular respiration are affected by an array of biotic and abiotic factors, including the availability of substrates, availability of light, and the surrounding temperature. In the activities that follow, you will use the algae beads, CO_2 indicator, and other supplies to design and perform your own experiments to examine some of the factors that may influence rates of photosynthesis and cellular respiration.

Effects of Light Quantity and Quality

Sunlight is the starting point for photosynthesis, and the quantity and quality of light available to a plant — or alga — can fluctuate widely over both time and space. How do you think the quantity and quality of light might affect plant growth?

Light Intensity

When we talk about light quantity, we are talking about light intensity, the amount of light hitting a plant leaf. Light intensity also refers to the degree of brightness the leaf might be exposed to, with high light intensity being brighter than low light intensity.

Light intensity varies significantly in nature. When you plant a garden, you select plants that can thrive in the various conditions in your yard. Plants are often classified as sun plants or shade plants based on the light intensity to which they are best adapted. In addition, individual leaves of a tree often show developmental adaptations to different conditions: Leaves growing on the exterior of the tree canopy, for example, may exhibit differences in anatomy, shape, and metabolism (such as photosynthesis) from leaves growing within the crown of the tree, where they are shaded by surrounding leaves.

Light intensity also changes with the time of day, the season, geographic location, distance from the equator, and weather. For example, light intensity increases from sunrise to the middle of the day and then decreases; it is high during summer, moderate in spring and fall, and low in winter. Geographically, maximum light intensity occurs at the equator and decreases with increasing proximity to the poles. Light intensity is also affected by dust and water particles in the air.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

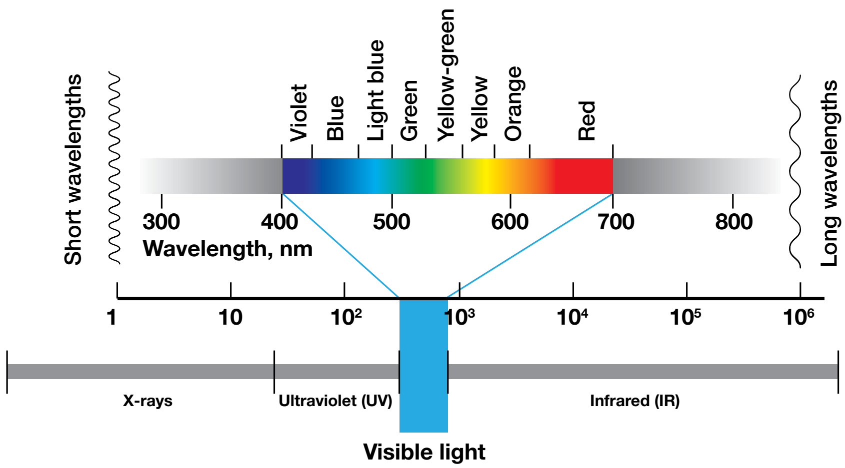
How does light intensity affect the rate of photosynthesis?

How does excessive light intensity reduce chlorophyll content?

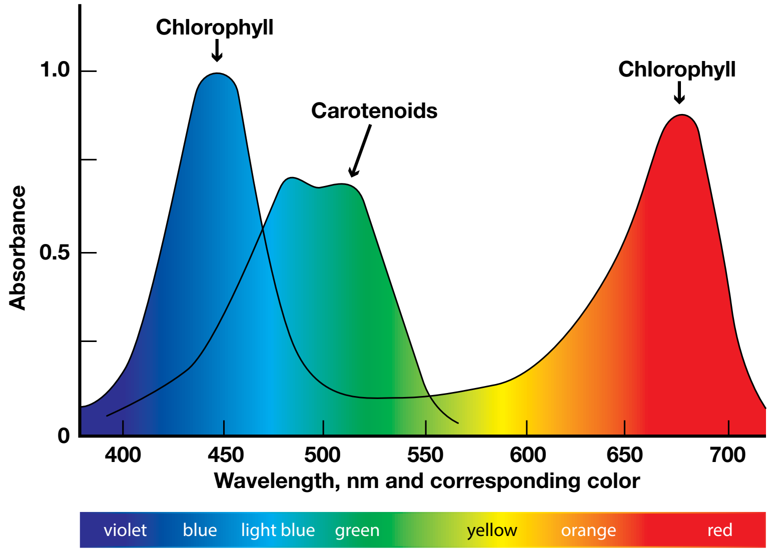
Light quality (color)

Where light intensity refers to the amount of light available, light quality refers to the wavelength or color of light available.

As a source of energy, visible light occupies only a small portion of the electromagnetic spectrum (see figure below), which extends from high-energy gamma rays at one end to lower-energy radio waves at the other. Life on Earth is made possible by our atmosphere, which filters out most of the higher-energy, ionizing radiation (<295 nm) that would be hazardous to living organisms.



Light energy must be absorbed by a pigment in order to have a biological effect. In the plants, algae, and cyanobacteria that are capable of oxygen-producing photosynthesis, the primary pigment molecule used for capturing sunlight is chlorophyll *a*. Six other forms of chlorophylls also exist in nature, along with other accessory pigments that help increase the range of light that can be harvested. Each of these pigments has a characteristic absorption spectra (see figure below). For example, chlorophyll and carotenoid pigments are optimized for absorption of blue and red wavelengths of light.



As with light intensity, the growth of plants is strongly correlated to the wavelengths of light available to them. A number of developmental changes result from changes in light quality, including flowering and senescence. In the laboratory or classroom, wavelengths of light from tungsten or fluorescent bulbs are different from those of natural sunlight. As a result, the rates of photosynthesis measured under artificial light sources will be different from those observed under the sun.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Why do kelp leaves appear green to us?

What can cause light quality changes and fluctuations in the environment?

Effects of Other Environmental Factors

Plants in your garden are sensitive to other environmental factors as well, including water availability, soil conditions, nutrient availability, presence of pests and disease, and temperatures. The ability of algae to perform photosynthesis and cellular respiration is similarly sensitive to environmental factors, including substrate availability (CO_2, O_2, water) and temperature.

In the laboratory investigations that follow, you will design and conduct experiments to test the effects of different conditions on photosynthesis and cellular respiration. The following sections will help you ask a question about photosynthesis and cellular respiration, formulate a hypothesis, develop an experiment to test this hypothesis, and predict results.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Why might having accessory pigments be useful for photosynthetic organisms?

Millions of years ago, many plants used carotenoids — red/purple colored pigments — as their primary pigment. Today, most plants use chlorophyll as their primary pigment instead. What could explain this change?

Examine the absorption spectra (graph on the previous page), and explain why chlorophyll appears green. How does your reasoning apply to carotenoids?

Investigation #3: Effect of Light Intensity

In nature, light intensity can vary significantly, depending on the time of day or year, location, climate, and a host of other factors. In the laboratory, **neutral density filters**, which reduce transmittance of all wavelengths of light, can be used to manipulate light intensity in a controlled manner. In this investigation, you will design and conduct an experiment using neutral density filters to understand the impact of light intensity on the rate of photosynthesis by the algae beads.

For this investigation, all the materials and equipment from Investigation #2 will be made available to you as well as the following:

- Neutral density filters that block 0%, 50%, 85%, and 100% of light
- Ruler or yard/meter stick or measuring tape (optional)

Observation and Hypothesis

3.1 Observe the world for a phenomena involving light intensity and photosynthetic organisms. Describe this phenomenon and ask a question about it.

3.2 Formulate a hypothesis to explain your observation.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

What are some ways you can change light intensity in the laboratory?

Why do you think light intensity affects photosynthesis?

Writing the Procedure

When you develop a protocol for your experiment, it can be helpful to draw a picture of your experimental setup. Think about the materials you have and the question you are trying to answer. Use this page to illustrate your setup and articulate your procedure. Ask your teacher to review the experiment before you begin.

Results

Use this page to restate your hypothesis and record and analyze your results.

Hypothesis

Results

Record any observations relevant to your experiment.

Data Analysis and Interpretation

Graph your results

Make any calculations that may be relevant to your data:

What do these calculations/results mean?

How do the data support or contradict your hypothesis?

3.3 Revisit your diagram of photosynthesis and cellular respiration from either Pre-Lab #3 or Investigation #1. Revise your diagram to demonstrate how the experimental design of your current investigation affects these processes. Indicate where the rates of these processes may increase or decrease and describe why. Also indicate how the organism will be affected if the conditions of your investigation were to continue.

List any ideas you have for further refining your hypothesis and testing your experimental design.

Investigation #4: Effect of Light Color

In nature, light quality, or the color or wavelength of light available to an organism for photosynthesis, can fluctuate depending on a variety of conditions, including location and weather. In the laboratory or classroom, **color filters**, which reduce transmittance of specific wavelengths of light, can be used to manipulate the quality of light available to your samples. In this investigation, you will design and conduct an experiment using color filters to understand the impact of light quality (color) on the rate of photosynthesis by the algae beads.

For this investigation, all the materials and equipment from Investigation #2 will be made available to you as well as the following:

- Color filters that allow only specific colors (wavelengths) of light to pass through

Observation and Hypothesis

4.1 Observe the world for phenomena involving light color and photosynthetic organisms. Describe one phenomenon and ask a question about it.

4.2 Formulate a hypothesis to explain your observation.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Based on what you know about the light spectrum, photosynthesis, and chlorophyll pigments, which color(s) of filters do you expect will yield the greatest rates of photosynthesis?

If your absorbance data were to demonstrate that photosynthesis was taking place in the algae beads when exposed only to green wavelengths of light, what might this suggest about the algae beads?

Writing the Procedure

When you develop a protocol for your experiment, it can be helpful to draw a picture of your experimental setup. Think about the materials you have and the question you are trying to answer. Use this page to illustrate your setup and articulate your procedure. Ask your teacher to review the experiment before you begin.

Results

Use this page to restate your hypothesis and record and analyze your results.

Hypothesis

Results

Record any observations relevant to your experiment.

Data Analysis and Interpretation

Graph your results

Make any calculations that may be relevant to your data:

What do these calculations/results mean?

How do the data support or contradict your hypothesis?

4.3 Revisit your diagram of photosynthesis and cellular respiration from either Pre-Lab #3 or Investigation #1. Revise your diagram to demonstrate how the experimental design of your current investigation affects these processes. Indicate where the rates of these processes may increase or decrease and describe why. Also indicate how the organism will be affected if the conditions of your investigation were to continue.

List any ideas you have for further refining your hypothesis and testing your experimental design.

Investigation #5: Effect of Temperature

All living organisms have a wide variety of adaptations that allow them to survive temperature extremes. For example, some plants have adapted to extreme conditions like the Arctic tundra.

Temperature is another environmental factor that can affect the rate of photosynthesis, as well as respiration, in the algae beads. In this investigation, you will design and conduct an experiment to understand the impact of temperature on the rate(s) of one or both of these processes.

For this investigation, all the materials and equipment from Investigation #2 will be made available to you as well as the following:

- Water bath
- Ice
- Thermometer

Observation and Hypothesis

5.1 Observe the world for phenomena involving temperature and photosynthetic organisms. Describe one phenomenon and ask a question about it.

5.2 Formulate a hypothesis to explain your observation.

ThINQ! Exercises

Collaborate and use outside resources to answer the following question:

In Investigation #2, why was it important to keep your algae beads at a controlled distance from the light source?

Writing the Procedure

When you develop a protocol for your experiment, it can be helpful to draw a picture of your experimental setup. Think about the materials you have and the question you are trying to answer. Use this page to illustrate your setup and articulate your procedure. Ask your teacher to review the experiment before you begin.

Results

Use this page to restate your hypothesis and record and analyze your results.

Hypothesis

Results

Record any observations relevant to your experiment.

Data Analysis and Interpretation

Graph your results

Make any calculations that may be relevant to your data:

What do these calculations/results mean?

How do the data support or contradict your hypothesis?

5.3 Revisit your diagram of photosynthesis and cellular respiration from either Pre-Lab #3 or Investigation #1. Revise your diagram to demonstrate how the experimental design of your current investigation affects these processes. Indicate where the rates of these processes may increase or decrease and describe why. Also indicate how the organism will be affected if the conditions of your investigation were to continue.

List any ideas you have for further refining your hypothesis and testing your experimental design.

Investigation #6: Mini-Ecosystem

In nature, organisms are generally not found in isolation. They are part of a complex and dynamic ecosystem where organisms interact to exchange energy and matter. In Investigation #2 you studied the balance between photosynthesis and cellular respiration in a single photosynthetic organism.

How might you investigate photosynthesis and cellular respiration in an ecosystem?

For this investigation, all the materials and equipment from Investigation #2 will be made available to you as well as the following:

- Aquatic snails or another heterotroph

Observation and Hypothesis

6.1 Observe the world for phenomena involving matter exchanges between heterotrophs and photosynthetic organisms. Describe one phenomenon and ask a question about it.

6.2 Formulate a hypothesis to explain your observation.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

For an enclosed ecosystem such as Earth to be sustainable, does the overall rate of photosynthesis need to be greater or less than that of cellular respiration? Why?

Which reactants and products of photosynthesis and cellular respiration are easily diffusible in and out of the cell and which are not? Why is this important?

Writing the Procedure

When you develop a protocol for your experiment, it can be helpful to draw a picture of your experimental setup. Think about the materials you have and the question you are trying to answer. Use this page to illustrate your setup and articulate your procedure. Ask your teacher to review the experiment before you begin.

Results

Use this page to restate your hypothesis and record and analyze your results.

Hypothesis

Results

Record any observations relevant to your experiment.

Data Analysis and Interpretation

Graph your results

Make any calculations that may be relevant to your data:

What do these calculations/results mean?

How do the data support or contradict your hypothesis?

6.3 Revisit your diagram of photosynthesis and cellular respiration from either Pre-Lab #3 or Investigation #1. Revise your diagram to demonstrate how the experimental design of your current investigation affects these processes. Indicate where the rates of these processes may increase or decrease and describe why. Also indicate how the organism will be affected if the conditions of your investigation were to continue.

List any ideas you have for further refining your hypothesis and testing your experimental design.

Part 1

Where Have All the Brown Shrimp Gone?

Hank Despaux leaned over to inspect his net. It was his eighth try of the day, and he was beginning to lose hope. As with the seven tries before, this one pulled up a water haul — a virtually empty net, containing only eight lethargic crabs, a number of tiny silver fish flapping about, and a solitary brown shrimp, the real goal of his trip. Hank is a shrimper whose family has made a living in the waters off the Louisiana coast for three generations. The last ten years, however, have been increasingly difficult, and this season, he fears, may be his last.

A number of economic factors affect the shrimp industry in the Gulf of Mexico. For one, the price of shrimp has dropped dramatically over the last few decades. As demand has increased, cheaper imports have come in to fill the void. Because they are less expensive, these imports have forced Gulf shrimpers to lower their price per pound by as much as 50%. Add to that increasing costs of fueling, running, and maintaining a boat, and it becomes clear the profession has become very difficult to sustain.

In the last few decades, an ecological phenomenon has also contributed to the hardships of shrimpers like Hank: the annual Dead Zone. The Dead Zone is a phenomenon in which the waters at the bottom of the Gulf become hypoxic (have low concentrations of dissolved oxygen). This forces the shrimp, fish, and other creatures that live there to flee to fresher waters or die. In the case of the brown shrimp Hank is after, the Dead Zone blocks juvenile brown shrimp from reaching their offshore spawning grounds, where they reproduce.

During these summer months, shrimpers and fishermen like Hank can spend weeks, if not months, not catching much of anything, and this puts an even tighter squeeze on their bottom line. They must fish in waters much farther off the coast, which costs more and which is not even possible for some of the smaller boats. Some years, the fishing industries curtail the official shrimping seasons in order to ensure the long-term health of the fishing and shrimping industries. “If you’re already struggling to provide for your family, if your profit margin is so small that you rely on every month’s catch to make a living, it’s going to affect you,” Hank says. “You can be in real trouble.”

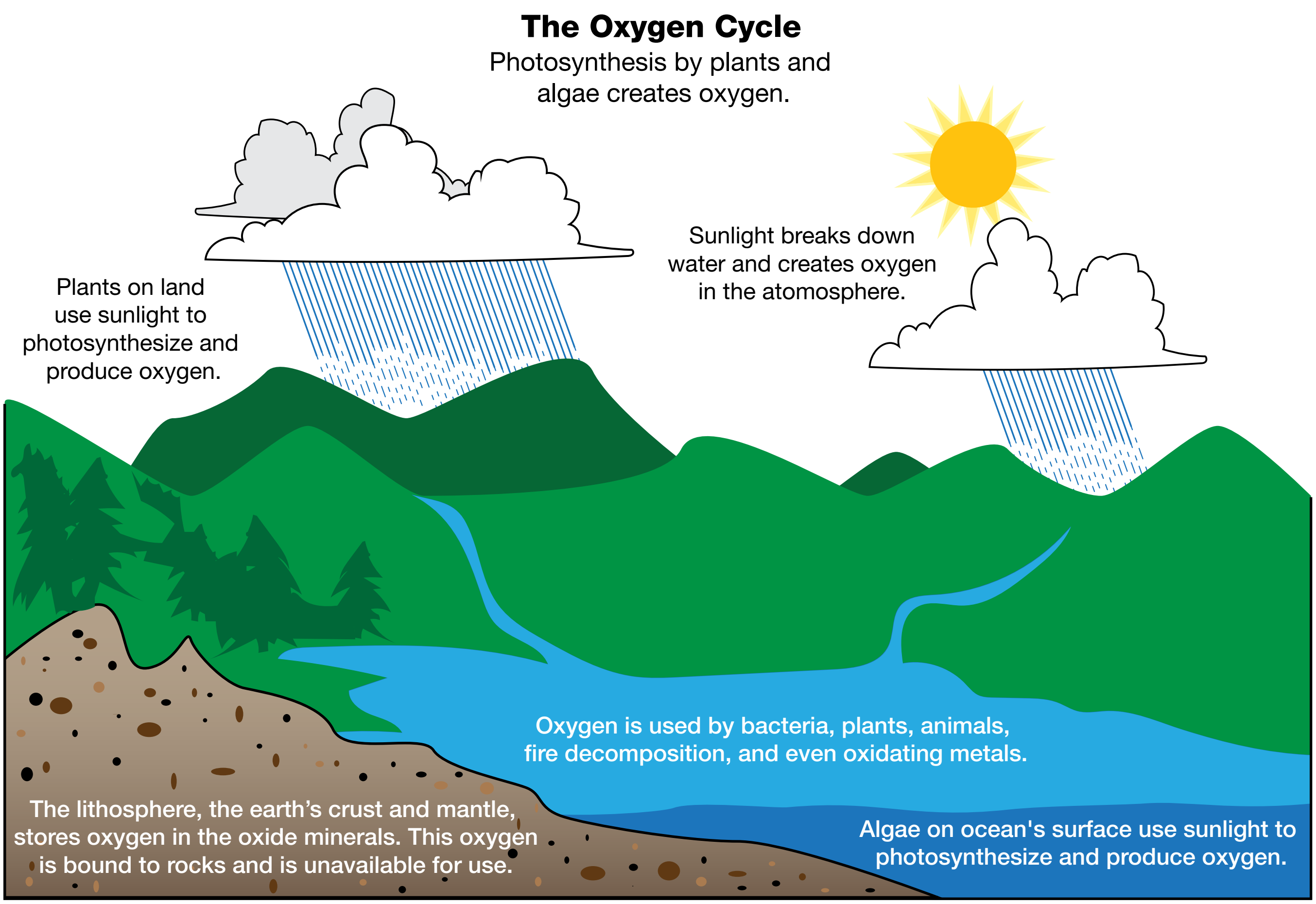


Fig. 1. The oxygen cycle.

1. Water is considered hypoxic (lacking oxygen) if it contains 2 mg/l or less dissolved oxygen. Think of the food chain that exists in the Gulf of Mexico: What are the types of organisms that likely live at the bottom of the Gulf, where the water is most hypoxic? How can a lack of oxygen affect them? Which cellular processes are affected by a lack of oxygen?

2. Oxygen is much less abundant in water than in air. Air contains 21% oxygen, but the oxygen content in water is only 0.001%. What factors (physical and biological) can affect the levels of dissolved oxygen in the Gulf waters? Think of the oxygen cycle (Figure 1).

Part 2

Too much photosynthesis?

The Dead Zone is a huge ecological and economic problem that is caused by some of the Gulf's smallest residents: microalgae and aerobic bacteria.

Microalgae (microscopic algae) live and grow in the sundrenched top layers of the Gulf waters where, under normal conditions, they perform their photosynthetic duties and, as its primary producers of oxygen, make life in the Gulf possible. In fact, microalgae around the world make all our lives possible, as they produce about half of the oxygen we breathe. Ironically, these tiny microscopic sustainers of life are also the ultimate cause of the Dead Zone. When favorable conditions prevail (abundant sunlight, nutrients, and the right water conditions), their growth can spiral out of control, and a vast algal bloom (overgrowth) appears. These blooms can stretch for hundreds of miles and suffocate life in the waters below them. This is because as the microalgae grow and ultimately die, they sink into the waters below, where they are digested by aerobic bacteria in a process that consumes oxygen (cellular respiration).

Under normal conditions, wind-driven ocean churning (due to storm activity and natural upwelling) helps stir the waters enough to bring oxygenated surface water down to the lower depths to alleviate any temporary hypoxia. In the Gulf of Mexico, however, the warm surface waters and cooler bottom waters — and diminished storm activity in spring and summer — create a stable water column that discourages this churning. In addition, fresh water pouring into the Gulf from the Mississippi River also traps oxygen-depleted saltwater below (Figure 2). As a result, organisms living at greater depths, including most marine animals, cannot acquire necessary oxygen. This timing is especially bad, as the summer months are a time of active reproduction by fish and benthic (bottom-dwelling) invertebrates.

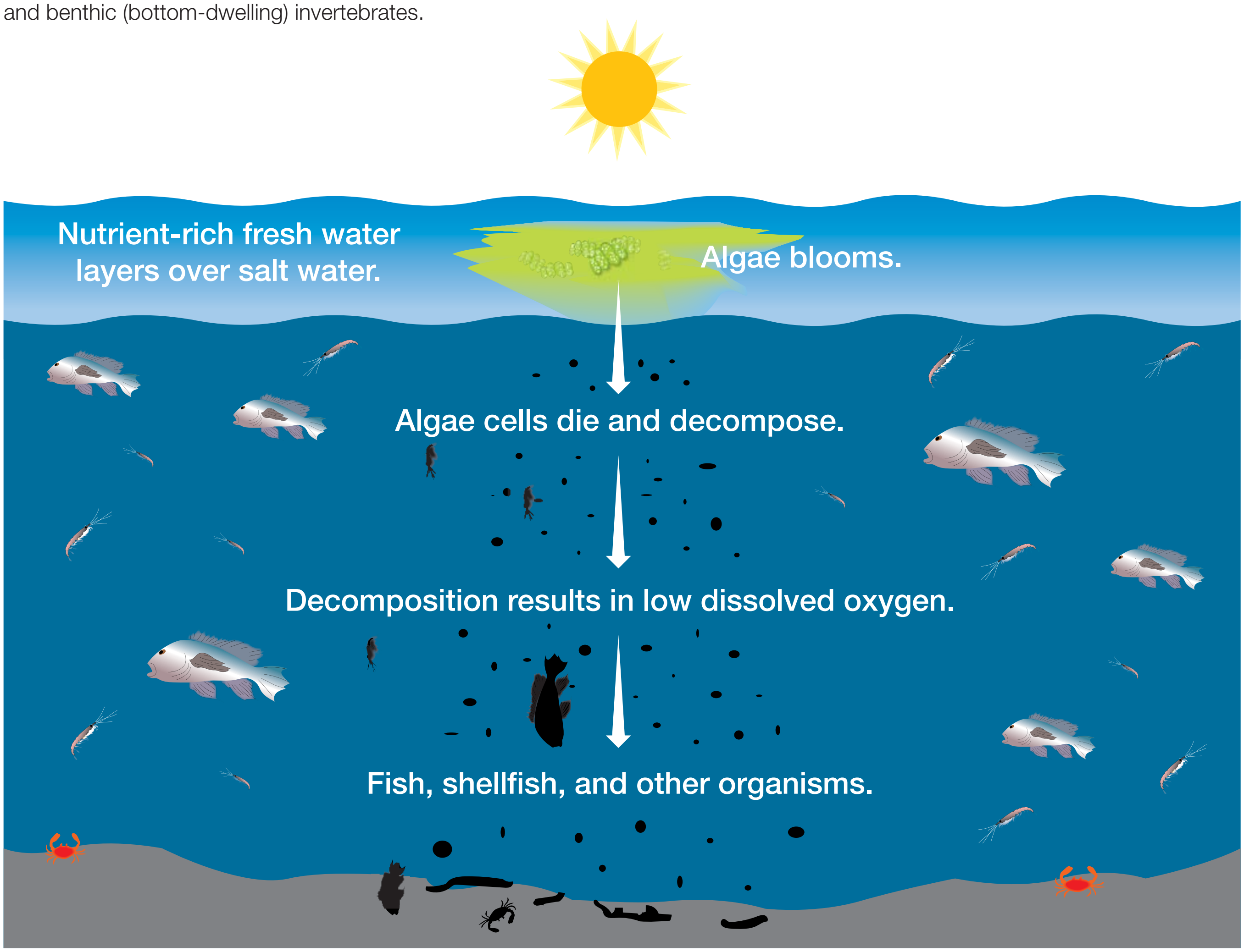


Fig. 2. Factors that lead to Dead Zone formation in the Gulf of Mexico.

Although they are often noted for their harmful effects, algal blooms (red tides, for example) occur as a part of natural upwelling cycles and sustain the beginning of the marine food chain. In healthy marine ecosystems, algae grow and reproduce as conditions permit, producing food, energy, and oxygen that support the rest of the food chain. As they and the organisms that feed on them die, they become part of the sediment at the bottom. Decomposers such as bacteria then effectively recycle these organisms back into the food chain. Then, as offshore winds blow across the ocean surface, surface water is displaced, allowing cooler water to rise up from below. This water that rises to the surface as a result of upwelling is also rich in nutrients that "fertilize" surface waters, triggering new algal blooms and supporting the growth and reproduction of the organisms that depend on them. It is not surprising that good fishing grounds typically are found where upwelling is common.

Fig. 3. Size and locations of the Mississippi River basin and Dead Zone.

3. Microalgae are primary producers (see Introduction for the definition of producer) in the Gulf of Mexico. What environmental factors are necessary to sustain their growth? What do they produce in return?

4. Draw a diagram that connects substrates and products of photosynthesis and cellular respiration (CO_2, O_2, H_2O, etc.). Which of these processes is (are) performed by microalgae? By aerobic bacteria?

5. Compare the algae in the Gulf of Mexico with terrestrial plants (for example, crop plants): Where and how do they each obtain the nutrients they need to grow and reproduce?

6. Scientists mapping the size of the algal blooms and Dead Zone have noticed a correlation between the amount of annual rainfall and the size of the bloom. In 1988, a year of drought, the Dead Zone was relatively small, but in 1993, a year of flooding on the Mississippi River, the Dead Zone was quite large. In 2014, the Dead Zone was larger than average: at 5,840 square miles, it was about the size of Connecticut. As described above, the freshwater boundary caused by Mississippi freshwater runoff is one factor that contributes to Dead Zone formation. Refer to Figure 3. Can you provide another hypothesis as to how and why the size of algal bloom might be affected by annual rainfall totals? Consider your answers to the previous two questions in the context of the major economic activity in the Mississippi River basin: agriculture.

7. What types of measurements or experiments could you perform to explore your hypothesis?

8. The Atlantic hurricane season runs from June through November. What environmental occurrences/factors might contribute to reoxygenation of the hypoxic layer and removal of the Dead Zone at the end of the summer?

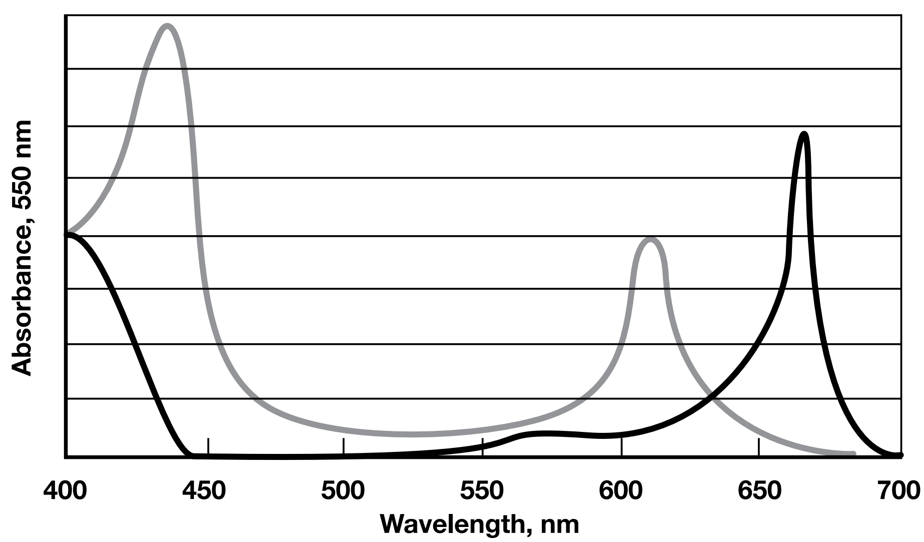
Post-Lab Assessment

On a recent exploratory mission, marine biologists discovered a large field of brown kelp in the Indian Ocean. Brown kelp typically grows in cool oceans and has never been seen before living in warm ocean waters. Aside from its location, the marine biologists also noticed that the green kelp indigenous to the area where they were conducting their research seemed to be growing less densely than normal. In fact, they observed that the green kelp population had decreased by 50% since the biologists were there two years before. The marine biologists took samples of the brown and green kelps back to the lab for analysis.

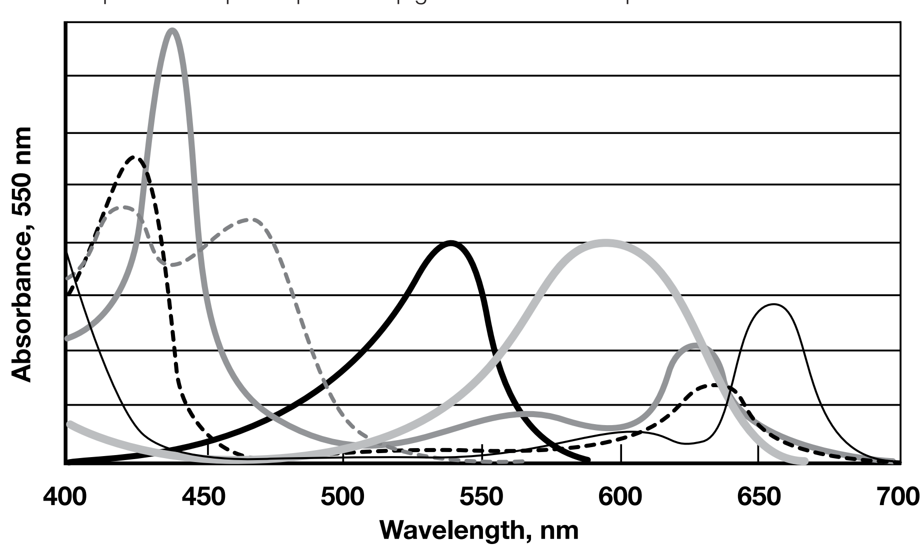
Claim: The brown kelp has developed unique characteristics that allow it to undergo photosynthesis in a variety of conditions and it is outcompeting green kelp in the Indian Ocean.

Evidence: Back at the lab, the biologists performed tests to compare the brown and green kelps. They determined what pigments each kelp uses for photosynthesis (graphs 1 and 2) and performed analyses to determine how well the kelps can photosynthesize under different conditions such as temperature, light intensity, and light color (graphs 3, 4, and 5). The graphs below show their results:

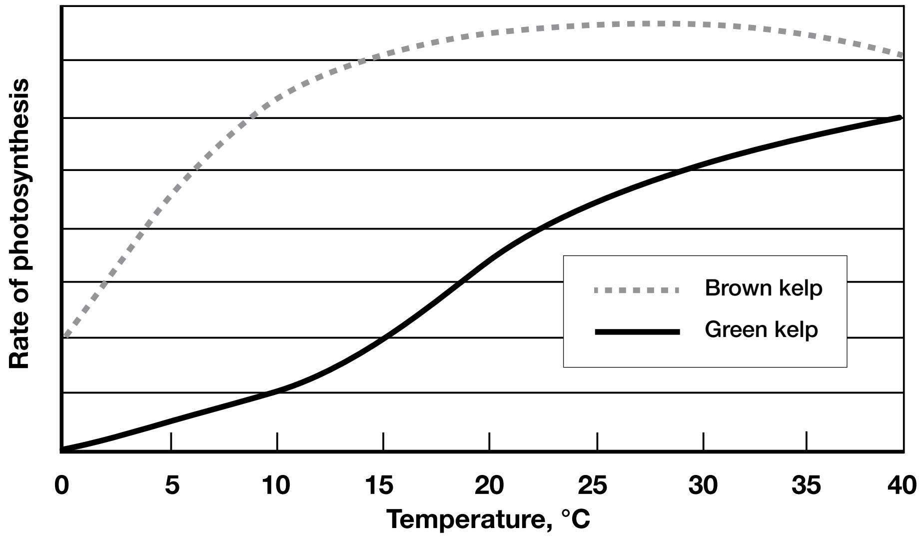
Graph 1. Absorption spectra of pigments in green kelp



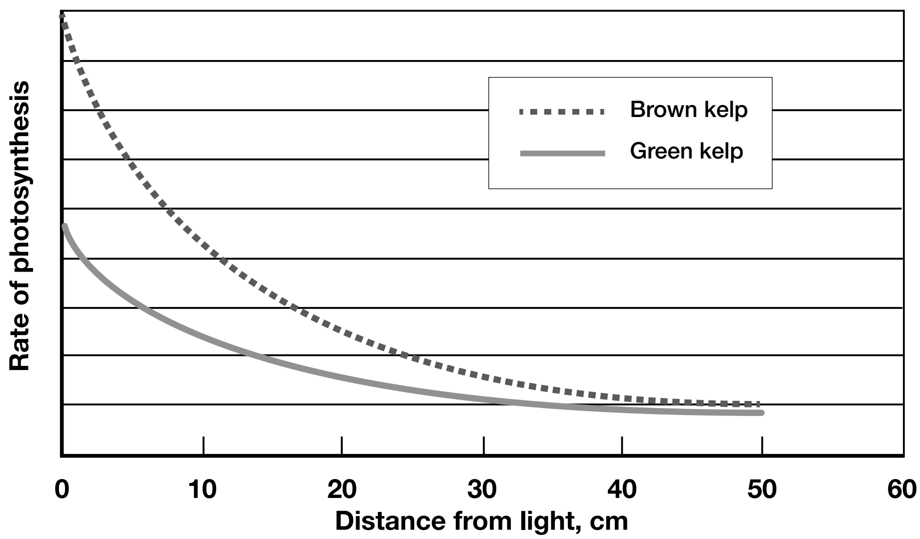
Graph 2. Absorption spectra of pigments in brown kelp



Graph 3. Rate of photosynthesis at different temperatures



Graph 4. Rate of photosynthesis at different distances from light source



Explain: Do you agree with the claim that the marine biologists made regarding the brown kelp? Justify your answer using the evidence in the graphs.

Predict: Given what you know about each type of kelp, what would you expect the photosynthetic activity of each kelp to look like under conditions when only red, blue, or green wavelengths of light are available?

Predict: How do you think the presence of brown kelp in the Indian Ocean may affect the ecosystem in that location? What evidence do you currently have to support your claim?

Design: Describe what evidence you would need to collect in order to prove your claim. What would your next research question be?

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