College Biology
Instructor Guide

PASCO®

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College Biology

Instructor Guide
21st Century Science

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Introduction

PASCO scientific's probeware and laboratory investigations move students from the low-level task of memorization of science facts to higher-level tasks of data analysis, concept construction, and application. For science to be learned at a deep level, it is essential to combine the teaching of abstract science concepts with "real-world" science investigations. Hands-on, technology-based, laboratory experiences serve to bridge the gap between the theoretical and the concrete, driving students toward a greater understanding of natural phenomenon. Students also gain important science process skills that include: developing and using models, carrying out investigations, interpreting data, and using mathematics.

At the foundation of teaching science are a set of science standards that clearly define the science content and concepts, the instructional approach, and connections among the science disciplines. The Next Generation Science Standards (2012)© are a good example of a robust set of science standards.

The Next Generation Science Standards (NGSS) position student inquiry at the forefront. The standards integrate and enhance science, technology, engineering, and math (STEM) concepts and teaching practices. Three components comprise these standards: Science and Engineering Practices, Disciplinary Core Ideas, and Crosscutting Concepts. The lab activities in PASCO's 21st Century Science Guides are all correlated to the NGSS (see http://pasco.com).

- The *Science and Engineering Practices* help students to develop a systematic approach to problem solving that builds in complexity from kindergarten to their final year in high school. The practices integrate organization, mathematics and interpretive skills so that students can make data-based arguments and decisions.
- *Disciplinary Core Ideas* are for the physical sciences, life sciences, and earth and space sciences. The standards are focused on a limited set of core ideas to allow for deep exploration of important concepts. The core ideas are an organizing structure to support acquiring new knowledge over time and to help students build capacity to develop a more flexible and coherent understanding of science.
- *Crosscutting Concepts* are the themes that connect all of the sciences, mathematics and engineering. As students advance through school, rather than experiencing science as discrete, disconnected topics, they are challenged to identify and practice concepts that cut across disciplines, such as "cause and effect". Practice with these concepts that have broad application helps enrich students' understanding of discipline-specific concepts.

PASCO's lab activities are designed so that students complete guided investigations that help them learn the scientific process and explore a core topic of science, and then are able to design and conduct extended inquiry investigations. The use of electronic sensors reduces the time for data collection, and increases the accuracy of results, providing more time in the classroom for independent investigations.

In addition to supporting the scientific inquiry process, the lab activities fulfill STEM education requirements by bringing together science, technology, engineering, and math. An integration of these areas promotes student understanding of each of these fields and develops their abilities to become self-reliant researchers and innovators. When faced with an idea or problem, students learn to develop, analyze, and evaluate possible solutions. Then collaborate with others to construct and test a procedure or product.

Information and computer tools are essential to modern lab activities and meeting the challenge of rigorous science standards, such as NGSS. The use of sensors, data analysis and graphing tools, models and simulations, and work with instruments, all support the science and engineering practices as implemented in a STEM-focused curriculum, and are explicitly cited in NGSS. PASCO's lab activities provide students with hands-on and minds-on learning experiences, making it possible for them to master the scientific process and the tools to conduct extended scientific investigations.

About the College Science Lab Manual

This manual presents teacher-developed laboratory activities using current technologies to help you and your students explore topics, develop scientific inquiry skills. Using electronic-sensor data collection, display and analysis devices in your classroom fulfills STEM requirements and provides several benefits. Sensor data collection allows students to:

- observe phenomena that occur too quickly or are too small, occur over too long a time span, or are beyond the range of observation by unaided human senses
- perform measurements with equipment that can be used repeatedly over the years
- collect accurate data with time and/or location stamps
- rapidly collect, graphically display, and analyze data so classroom time is used effectively
- practice using equipment and interpreting data produced by equipment that is similar to what they might use in their college courses and adult careers

The Data Collection System

In this manual, "data collection system" refers to the system employed by students to record, visualize, and analyze sensor data during their experiments. The system consists of all components necessary to connect a sensor to a device containing the software that detects the sensor measurement and collects, records, and displays this data.

Some systems, such as the Xplorer GLX® or SPARK Science Learning System™, are stand-alone systems. These contain built-in software applications, and students simply attach a sensor and begin collecting data. Other systems use a computer or tablet with downloaded software applications. In these systems a USB or Bluetooth® interface is used to connect a sensor to the device. Software options for these include SPARKvue® 2 and PASCO Capstone™ software.

The activities are designed so that any PASCO data collection system can be used to carry out the procedures.

Getting Started with Your Data Collection System

To help you and your students become familiar with the many features of your data collection system, start with the tutorials and instructional videos that are available on PASCO's website (www.pasco.com).

Included on the storage device accompanying your manual is a Scientific Inquiry activity that acts as a tutorial for your data collection system. The activity introduces students to the process of conducting science investigations, the scientific method, and introduces teachers and students to the commonly used features of their data collection system. Start with this activity to become familiar with the data collection system.

Instructor and Student Guide Contents

All the instructor and student materials are included on the storage device accompanying the instructor's lab manual.

Lab Experiment Components

Each activity has two components: Instructor Information and Student Inquiry Worksheets.

Instructor Information is in the instructor's version of the lab manual. It contains information on selecting, planning, and implementing a lab, as well as the complete student version with answer keys. Instructor Information includes all sections of a lab activity, including objectives, procedural overview, time requirements, and materials and equipment at-a-glance.

Student Inquiry Worksheets begin with a driving question, providing students with a consistent scientific format that starts with formulating a question to be answered in the process of conducting a scientific investigation.

This table identifies the sections in each of these two activity components.

INSTRUCTOR INFORMATION	STUDENT INQUIRY WORKSHEET
Objectives	Driving Questions
Procedural Overview	Background
Time Requirement	Pre-Lab Activity
Materials and Equipment	Materials and Equipment
Concepts Students Should Already Know	
Related Labs in This Guide	
Background	
Pre-Lab Activity	
Lab Preparation	
Safety	Safety
Sequencing Challenge	Sequencing Challenge
Procedure	Procedure (+ conceptual questions)
Data Analysis	Data Analysis
Analysis Questions	Analysis Questions
Synthesis Questions	Synthesis Questions
Multiple Choice Questions	Multiple Choice Questions
Extended Inquiry Suggestions	

Electronic Materials

The storage device accompanying this manual contains the following:

- ♦ Complete Teacher Guide and Student Guide in PDF format.
- ♦ Student versions of the laboratory activities in an editable Microsoft™ Word format. PASCO provides editable files of the student lab activities so that teachers can customize activities to their needs.
- ♦ Scientific Inquiry activity: an activity that can be used to help you and your students become familiar with the data collection system.

Global Number Formats and Standard Units

Throughout this guide, the International System of Units (SI) or metric units is used unless specific measurements, such as air pressure, are conventionally expressed otherwise. In some instances, such as weather parameters, it may be necessary to alter the units used to adapt the material to conventions typically used and widely understood by the students.

Reference

NGSS Lead States. 2013. Next Generation Science Standards: For States, By States. Washington, DC: The National Academies Press.

Normal Laboratory Safety Procedures

Overview

PASCO is concerned with your safety and because of that, we are providing a few guidelines and precautions to use when exploring the labs in our Advanced Biology guide. This is a list of general guidelines only; it is by no means all-inclusive or exhaustive. Of course, common sense and standard laboratory safety practices should be followed.

Regarding chemical safety, some of the substances and chemicals referred to in this manual are regulated under various safety laws (Local, State, National, or International). Always read and comply with the safety information available for each substance/chemical to determine its proper storage, use and disposal.

Since handling and disposal procedures vary, our safety precautions and disposal comments are generic. Depending on your lab, instruct students on proper disposal methods. Each of the lab activities also has a Safety section for procedures necessary for that experiment.

General Lab Safety Procedures and Precautions

- Follow all standard laboratory procedures.
- Absolutely no food,drink or gum is allowed in the lab.
- Keep water away from electrical outlets.
- Remember to wear protective equipment (e.g., safety glasses, gloves, apron) when appropriate.
- Do not touch your face with gloved hands. If you need to sneeze or scratch, take off your gloves, wash your hands, and then take care of the situation. Do not leave the lab with gloves on.
- Wash your hands after handling samples, glassware, and equipment.
- Know the safety features of your lab such as eye-wash stations, fire extinguisher, first-aid equipment or emergency phone use.
- Insure that loose hair and clothing is secure when in the lab.
- Handle glassware with care.
- Insure you have adequate clear space around your lab equipment before starting an experiment.
- Do not wear open toe shoes, shorts or skirts in the laboratory.
- Allow heated objects and liquids to return to room temperature before moving.
- Never run or joke around in the laboratory.
- Do not perform unauthorized experiments.
- Students should use a buddy system in case of trouble.
- Keep the work area neat and free from any unnecessary objects.

Water Related Safety Precautions and Procedures

- Keep water away from electrical outlets.
- Keep water away from all electronic equipment.

Chemical Related Safety Precautions and Procedures

- Consult the manufacturer's Material Safety Data Sheets (MSDS) for instructions on handling, storage, and disposing of chemicals. Your instructor should provide the MSDS sheets of the chemicals thatyou are using. Keep these instructions available in case of accidents.
- Many chemicals are hazardous to the environment and should not be disposed of down the drain. Always follow your instructor’s instructions for disposing of chemicals.
- Sodium hydroxide, hydrochloric acid, and acetic acid are corrosive irritants. Avoid contact with the eyes and wash your hands after handling. In case of exposure, wash it off with plenty of water.
- Always add acids and bases to water, not the other way around, as the solutions may boil vigorously.
- Diluting acids and bases creates heat; be extra careful when handling freshly prepared solutions and glassware, as they may be very hot.
- Handle concentrated acids and bases in a fume hood; the fumes are caustic and toxic.
- Wear eye protection, lab apron, and protective gloves when handling acids. Splash-proof goggles are recommended. Either latex or nitrile gloves are suitable. Use nitrile gloves if you have a latex allergy.
- Read labels on all chemicals and pay particular attention to Hazard icons and safety warnings.
- When handling any bacterial species, follow aseptic techniques.
- Wash your hands before and after a laboratory session.
- If any solution comes in contact with skin or eyes, rinse immediately with a copious amount of running water for a minimum of 15 minutes.
- Follow the instructor’s instructions for disposing of chemicals, handling substances.
- Check the label to verify it is the correct substance before using it.
- Never point the open end of a test tube containing a substance at yourself or others.
- Use a wafting motion when smelling chemicals.
- Do not return unused chemicals to their original container.
- Keep flammable chemicals away from open flames.

Dangerous or Harmful Substance Related Lab Safety Precautions

- When handling any bacterial species, follow aseptic techniques.
- Always flame inoculating loops and spreaders before setting them down on the lab bench.
- Pipetting suspension cultures can create an aerosol. Keep your nose and mouth away from the tip of the pipet to avoid inhaling any aerosol.
- Use caution when working with acids.
- Use appropriate caution with the matches, burning splint and foods, and other hot materials.
- Be careful using a knife or scalpel.

Other Safety Precautions

- If water is boiled for an experiment involving heat, make sure it is never left unattended. Remember, too, that the hot plate will stay hot well after it is unplugged or turned off.

- Any injury must be reported immediately to the instructor, an accident report has to be completed by the student or a witness.
- If you are suffering from any allergy, illness, or are taking any medication, you must inform the instructor. This information could be very important in an emergency.
- Try to avoid wearing contact lenses. If a solution spills in your eye, the presence of a contact lens makes first aid difficult and can result in permanent damage. Also, organic solvents tend to dissolve in soft contact lenses, causing eye irritation.

Additional resources for Lab Safety information can be found at the websites of:

- Flinn Scientific
- The Laboratory Safety Institute (LSI)
- National Science Education Leadership Association (NSELA)/Safe Science Series

Master Materials and Equipment List

Italicized entries indicate items not available from PASCO. The quantity indicated is per student or group. NOTE: Some activities also require protective gear for each student (for example, safety goggles, gloves, apron, or lab coat).

Instructors can conduct some lab activities with sensors other than those listed here. For assistance with substituting compatible sensors and probes for a lab experiment, contact PASCO Instructor Support (800-772-8700 inside the United States or http://www.pasco.com/support).

Lab	Title	Materials and Equipment	Qty
1	**pH and Buffers** Use an advanced water quality sensor to measure the pH of buffers and the differences in pH values of various household products.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with pH Probe	1
		0.1 M Hydrochloric acid (HCl) solution	35 mL
		0.1 M Sodium hydroxide (NaOH) solution	35 mL
		Beaker, 100-mL	4
		Beaker, 500-mL	1
		Beaker, 50-mL	8
		Disposable pipet 1 mL	1
		Distilled water	1 L
		Electronic Balance	1
		Gelatin solution	150 mL
		Graduated cylinder, 10-mL	1
		Graduated cylinder, 25-mL	1
		Graduated Cylinder, 50-mL	1
		Hot Plate	1
		Labeling marker	1
		Labeling tape	1
		Meat tenderizer	7 g
		Paper towels	1
		Sodium acetate	2 g per class
		Standard buffer (pH 10)	25 mL
		Standard buffer (pH 4)	25 mL
		Test tube rack	1
		Test Tubes, 25 mm x 150 mm	15
		Various fruit juices, household ammonia, bleach and vinegar	25 mL
		Wash bottle with distilled water	1 per class
		Weighing paper	14

Lab	Title	Materials and Equipment	Qty
2	**Exploring Surface Area to Volume Ratios** Use a PASCO quad temperature sensor and ice bath to explore the relationship between the inner volume of a citrus fruit and its outer surface area.	Data Collection System	1
		PASPORT Quad Temperature Sensor	1
		Fast-Response Temperature Probe	2
		Stainless Steel Temperature Probe	1
		Citrus fruits, varying type and size	2
		Dissecting probe or pin	1
		Ice and water	2 to 3 liters
		Labeling marker	1
		Labeling tape	1
		Metric ruler	1
		Petroleum jelly	1 g
		Polystyrene cooler, plastic bucket or other vessel for ice bath	1
		Twine or string	20 to 30 cm
3	**Diffusion** Use an advanced water quality sensor and colorimeter to measure the conductivity, pH, and absorbance of two solutions separated by a semi-permeable membrane.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with pH probe and conductivity probe	1
		PASPORT Colorimeter	1
		Beaker 100-mL	2
		Beaker 400-mL	1
		Cuvettes (with colorimeter)	2
		Dialysis tubing	28 cm
		Disposable pipet	4
		Distilled water	200 mL
		Labeling marker	1
		Lint-free tissue	1
		Pickle juice	50 mL
		Plastic wrap	1 per class
		Roll of paper towels	1 per class
		Scissors	1
		Wash bottle filled with water	1 per class

Lab	Title	Materials and Equipment	Qty
4	**Diffusion and Osmosis** Use an advanced water quality sensor to measure the changes in conductivity in two solutions separated by a semi permeable membrane.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with Conductivity Probe	1
		1% starch solution	30 mL
		15% NaCl solution	2 to 3 drops
		2% IKI solution	150 mL
		2% IKI/2% NaCl solution	150 mL
		Beaker or cups, 30-mL	6
		Beaker, 250-mL	2
		Cork borer	1
		Cover slip	1
		Dental floss or string	10 to 20 cm
		Dialysis tubing	180 cm
		Disposable pipet	5
		Distilled water	1 liter
		Electronic Balance	1 per class
		Forceps	1
		Graduated cylinder, 25-mL	1
		Knife	1 per class
		Labeling marker and tape	1
		Kena™ Microscope	1
		Microscope slide	1
		Potato	3 to 4 per class
		Red Onion	1 per class
		Roll of paper towels	1 per class
		Roll of plastic wrap	1 per class
		Scissors	1
		Small funnel	1
		Sucrose solutions (0.2 M, 0.4 M, 0.6 M, 0.8 M and 1.0 M)	35 mL each
		Wash bottle	1
5	**Enzyme Catalysis** Use an oxygen gas sensor to measure oxygen gas production resulting from the decomposition of hydrogen peroxide under six conditions.	Data Collection System	1
		PASPORT Oxygen Gas Sensor with sampling bottle	1
		1.0 M Hydrochloric acid (HCl) solution	10 mL
		1.0 M Sodium hydroxide (NaOH) solution	10 mL
		Activated yeast suspension, boiled	10 mL
		Activated yeast suspension, chilled	10 mL
		Activated yeast suspension, room temperature	30 mL
		Graduated cylinder 10-mL	1
		Graduated cylinder 25-mL	1
		Hydrogen peroxide, 1.5%	120 mL
		Saltine cracker	1 per student
		Large beaker of ice	1

Lab	Title	Materials and Equipment	Qty
6	**Exploring the Effects of pH on Amylase Activity** Use an advanced water quality sensor and PASPORT colorimeter to measure the effects of pH on bacterial amylase activity.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with pH probe	1
		PASPORT Colorimeter	1
		0.1 M Hydrochloric acid (HCl) solution	30 mL
		30% Starch solution	30 mL
		Bacterial Amylase solution	4 mL
		Cuvettes (with colorimeter)	7
		Disposable graduated pipets	1
		Distilled water	200 mL
		Graduated cylinder, 10-mL	1
		Lint-free tissue	1
		Lugol's Iodine (IKI) solution	30 mL
		Saltine cracker	1
		Six buffers of varying pH (pH 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0)	5 mL each
		Small beakers or plastic cups	19
		Small funnel	1
		Standard buffer (pH 10)	25 mL
		Standard buffer (pH 4)	25 mL
		Wash bottle with distilled water	1
7	**Plant Pigments and Photosynthesis** Use a colorimeter to determine the rate of photosynthesis in a suspension of chloroplasts.	Data Collection System	1
		PASPORT Colorimeter	1
		#1 Whatman Chromatography paper	10 to 12 cm
		0.1 M phosphate buffer	4 mL
		Chloroplast suspension	2 mL
		Chromatography solvent	5 mL
		Coin	1
		Cuvettes (with colorimeter)	5
		Distilled water	13 mL
		DPIP in small amber bottle	3 mL
		Floodlight, 100 watt	1
		Glass jar, 10-12 cm tall	1
		Graduated disposable pipet 1-mL	2
		Heat sink (large beaker or flask filled with water)	1
		Ice and water	1 L
		Lint-free tissue	1
		Roll of aluminum foil	1 per class
		Spinach	1 leaf

Lab	Title	Materials and Equipment	Qty
8	**Factors that Affect Photosynthetic Activity** Use an advanced water quality sensor to measure the effects of light on the dissolved oxygen production by an aquatic plant in a PASCO Photosynthesis Tank.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with Dissolved Oxygen Probe	1
		Photosynthesis Tank	1
		Beaker, 250-mL	1
		Dark cloth to cover tank	1
		Desk lamp	1
		Distilled water	1 L
		Elodea, or other aquatic plant	2 to 3 sprigs
		Florescent or incandescent light bulb	1
		Magnetic Stirrer with Magnetic Stir Bar	1
9	**Cellular Respiration** Use the carbon dioxide and oxygen gas sensors to measure changes in gas levels in a PASCO Metabolism Chamber containing respiring peas.	Data Collection System	1
		PASPORT Oxygen Gas Sensor	1
		PASPORT Carbon Dioxide Gas Sensor	1
		PASPORT Sensor Extension Cable	1
		Dry pea seeds	25
		Germinating pea seeds, boiled	25
		Germinating pea seeds, chilled	25
		Germinating pea seeds, room temperature	25
		Glass beads	25
		Metabolism Chamber	1
		Large beaker of ice	1
10	**Measuring Aerobic Cellular Respiration in Yeast** Use an advanced water quality sensor to measure the effects of temperature on the dissolved oxygen concentration of active yeast cultures.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with Dissolved Oxygen Probe	1
		Activated yeast solution	45 mL
		Beaker, 250-mL	3
		Distilled or deionized water	1 L
		Electronic Balance	1 per class
		Graduated cylinder, 100-mL	1
		Hot Plate	1
		Ice bath, 1 L beaker filled with ice water	1
		Labeling marker	1
		Labeling tape	1
		Stirring rod	1
		Sugar	30 g
		Weighing paper	3
11	**Fermentation in Yeast** Use oxygen and ethanol gas sensors to calculate and compare the rate of fermentation in a PASCO EcoChamber containing activated yeast solution.	Data Collection System	1
		PASPORT Oxygen Gas Sensor	1
		PASPORT Ethanol Sensor	1
		Beaker, 1000-mL	1
		Beaker, 500-mL	1
		EcoChamber	1
		Magnetic Stirrer with Magnetic Stir Bar	1
		Sucrose solution, 0.5 M	500 mL
		Yeast solution	1 liter

Lab	Title	Materials and Equipment	Qty
12	**Bacterial Transformation** Use the tools of biotechnology to transform competent *E.coli* cells and then select for antibiotic resistance.	*Calcium chloride solution* *Clear labeling tape* *E.Coli starter plate* *Ice bath* *LB/Amp agar plate* *Luria Bertani (LB) agar plate* *Micropipettor with sterile tips* *pAmp plasmid* *Sterile test tubes, 15-mL* *Sterile, glass spreading rod* *Water bath (42°C)* *Wax labeling pencil* *Wire inoculating loop*	500 ul 1 1 1 2 2 1 10 ul 2 1 1 per class 1 1
13	**Mitochondrial Genetics and Biotechnology** Use restriction endonuclease digests and agarose gel electrophoresis to diagnose an inherited mitochondrial disease.	Electrophoresis buffer (1x) *Automatic micropipet (5-50 µL), with tips* Agarose gel, 0.8% *Disposable gloves* *Distilled or deionized water* *Horizontal gel electrophoresis apparatus and D.C. power supply* Edvotek QuickStrip™ DNA sample for Mitochondrial Genetics InstaStain® Blue Card *Plastic wrap* *Small plastic tray (for gel staining)* *DNA visualization system* *Waste receptacle*	300-400 mL 1 1 1 pair 1 gallon per class 1 1 1 1 roll per class 1 1 per class 1
14	**Mitosis and Meiosis** Use a microscope and prepared slides to explore the stages of mitosis and meiosis.	*Chromosome simulation kit or colored beads an magnets* Kena™ Microscope *Onion root tip prepared slide* *Sordaria ascospore (cross-over) prepared slide* *Whitefish blastula prepared slide*	1 1 1 1 1

Lab	Title	Materials and Equipment	Qty
15	**Genetics of Organisms with *Drosophila melanogaster*** Use the common fruit fly, *Drosophila melanogaster*, to create and statistically analyze genetic crosses.	*Anesthetizing material* *Culture vial* *Culture vial label or labeling tape* Kena™ Microscope *Fly morgue* *Foam plug* *Index cards* *Instant Drosophila culturing medium* *Mutant flies (autosomal monohybrid F1 cross)* *Mutant flies (autosomal recessive dihybrid F1 cross)* *Mutant flies (sex-linked F1 cross)* *Petri dish* *Screens* *Small, thin, camel hair paint brush* *Wild type flies*	1 1 1 1 1 1 2 1 to 2 g 1 vial per class 1 vial per class 1 vial per class 1 1 1 3 vials per class
16	**Evolution and Population Genetics** Use the Hardy-Weinberg equations to solve genetics problems.	*3x5 index card labeled with "A"* *3x5 index card labeled with "a"* *Calculator* *Coin*	2 2 1 1
17	**Transpiration** Use a barometer to explore the effects of environmental factors, such as air movement, on the rate of transpiration in a plant.	Data Collection System PASPORT Barometer/Low Pressure Sensor PASPORT Sensor Extension Cable *100-watt light source* *Compound light microscope* *Dicot stem prepared slide* *Disposable pipet* Electronic Balance *Fan* *Glycerin* *Heat sink (large beaker or aquarium filled with water)* *Knife or single-edge razor blade* Large Base and Support Rod *Monocot stem prepared slide* *Petroleum jelly* *Plant seedlings, 12-25 cm tall* *Scissors* *Spray bottle with water* Three-Finger Clamp *Transparent plastic bag* Utility Clamp *Wide, shallow bowl or tub filled with water*	1 1 1 1 1 1 1 1 per class 1 A few drops 1 1 1 1 2 to 3 g 1 1 1 1 1 1 1

Lab	Title	Materials and Equipment	Qty
18	**Reflex versus Reaction** Use a ruler, stopwatch, and reflex hammer to measure and compare human reflexes and reactions.	*Metric ruler* *Reflex hammer* Stop Watch	1 1 1
19	**Endotherms and Ectotherms: Temperature Regulation in Animals** Use a carbon dioxide gas sensor to explore how an endotherm and an ectotherm regulate internal temperatures by measuring the respiration rates of two different organisms exposed to varying temperatures.	Data Collection System PASPORT Carbon Dioxide Gas Sensor PASPORT Quad Temperature Sensor* PASPORT Sensor Extension Cable *Beaker, 2 L (or similarly sized container)* *Beaker, 350-mL or smaller* *Crickets* Electronic Balance *Ice and water* Large Base and Support Rod *Lint-free tissue* *Mouse, 5 to 10-g* Sampling bottle (with sensor) Three-Finger Clamp	1 1 1 1 1 1 10 1 per class 1 liter 1 1 1 1 1
20	**Physiology of the Circulatory System** Use a blood pressure sensor to measure the changes in the blood pressure and heart rate of a patient in different body positions. Use a temperature probe and the Kena™ microscope to measure the changes in heart rate of *Daphnia magna* under different temperature conditions.	Data Collection System PASPORT Fast-Response Temperature Probe PASPORT Blood Pressure Sensor *Container of room temperature water* *Container of warm water* *Daphnia Magna, large, living* *Depression slide* *Disposable pipet* Kena™ Microscope *Petri Dish* *Small container of crushed ice or an ice pack* *Small rubber band*	1 1 1 1 1 1 or 2 2 1 1 1 1 2
21	**Animal Behavior** Use a choice chamber to measure an organism's preference for different environmental factors.	*Additional stimulus agents* *Adhesive tape* *Filter paper, round* *Petri dish with lid* *Pillbugs, living* *Scissors*	1 1 3 to 4 2 10 to 15 1

Lab	Title	Materials and Equipment	Qty
22	**Air Pollution and Acid Rain** Use an advanced water quality sensor to measure the effects of CO_2, SO_2, and NO_2 on the pH of water.	Data Collection System	1
		PASPORT Advanced Water Quality Sensor with pH Probe	1
		1 M HCl solution	15 mL
		1-hole rubber stopper for flask	1
		Beaker, 40-mL	1
		Electronic Balance	1 per class
		Erlenmeyer flask, 50-mL	1
		Flexible Teflon tubing to fit glass tubing	20 cm
		Glass tubing for rubber stopper	
		Graduated cylinder, 10-mL	1
		Graduated disposable pipet	1
		Labeling marker	1
		Latex or polypropylene gloves	1
		Masking tape	1
		Planting pots, 2 inch	3
		Radish seeds	15
		Sodium bicarbonate($NaHCO_3$)	5 g
		Sodium bisulfite($NaHSO_3$)	5 g
		Sodium nitrite ($NaNO_2$)	5 g
		Vinegar	400 mL
		Wash bottle containing distilled or deionized water	1
23	**Population Ecology** Use a colorimeter to explore the effects of habitat size, nutrient availability, initial population density and temperature on the growth rate of *E.coli* bacteria.	Data Collection System	1
		PASPORT Colorimeter	1
		Culture vessels, 15-mL	3
		Culture vessels, 250-mL	3
		Culture vessels, 50-mL	3
		Cuvettes (with colorimeter)	4
		Disposable gloves	1
		Labeling marker	1
		Labeling tape	1
		Lint-free tissue	1
		Luria-Bertani (LB) broth	40 mL
		Overnight (O/N) culture of E.coli *in a 15-mL culture tube*	4 mL
		Shaking Incubator (optional)	1 per class
		Squirt bottle with 10% bleach	1
		Sterile transfer or Pasteur pipets, toothpicks, or inoculating loop	1
		Sterile water	10 mL

Lab	Title	Materials and Equipment	Qty
24	***Elodea* and the Snail** Use an advanced water quality sensor to measure pH changes in a series of simulated closed systems containing various organisms.	Data Collection System PASPORT Advanced Water Quality Sensor with pH Probe *Aquatic snails* *Bromothymol blue solution (in dropper bottle)* *De-chlorinated water* *Drinking straw* Elodea *sprigs (or other aquatic plant)* *Labeling marker* *Labeling tape* *Large test tubes* *Standard Buffer (pH 10)* *Standard Buffers (pH 4)* *Test tube rack* *Test tube stoppers*	1 1 2 500 mL 500 mL 1 2 to 4 1 1 4 25 mL 25 mL 1 4
25	**Interrelationship of Plants and Animals** Use carbon dioxide and oxygen gas sensors to measure changes in gas levels in a terrestrial ecosystem created within a PASCO EcoChamber.	Data Collection System PASPORT Oxygen Gas Sensor PASPORT Carbon Dioxide Gas Sensor PASPORT Sensor Extension Cable EcoChamber *Potting soil* *Small animal (Ex. Crickets)* *Small plant, variety*	1 1 1 1 1 2 to 3 cups 10 1
26	**AP 12 – Dissolved Oxygen and Primary Productivity** Use an optical dissolved oxygen sensor to measure the effects of temperature on the dissolved oxygen concentration of water and measure the effects of light intensity on the photosynthetic activity of algae.	Data Collection System PASPORT Advanced Water Quality Sensor with Optical Dissolved Oxygen Probe Aquatic Productivity Bottles *Beaker, 250-mL* *Dilute green algae culture* *Fluorescent light source* *Ice water* *Large vessel, 2 L (to fill bottles)* *Room temperature water* *Warm water* *Wash bottle* *Wax pencil or stickers and labeling marker*	1 1 1 3 2 L 1 200 mL 1 200 mL 200 mL 1 1

*Either the PASPORT Fast Response Temperature Probe or the Stainless Steel Temperature Probe can be used for this experiment

Calibration materials

If you want to calibrate various sensors, you will need the following:

pH Sensor

Item	Quantity	Where Used
Buffer solution, pH (4) Buffer solution, pH (10) Beaker, small Wash bottle with deionized or distilled water	25 mL 25 mL 3 1	1,6,22,24

Optical Dissolved Oxygen Sensor

Item	Quantity	Where Used
Probe storage cover (included with sensor)	1 5 mL	8,10,26

Oxygen Gas Sensor

Item	Quantity	Where Used
Sampling Bottle (included with the sensor)	1	5,11,25

Carbon Dioxide Gas Sensor

Item	Quantity	Where Used
Sampling Bottle (included with the sensor)	1	9,19,25

Ethanol Sensor

Item	Quantity	Where Used
1% ethanol solution Beaker, small	25 mL 1	11

Colorimeter

Item	Quantity	Where Used
Cuvette (included with colorimeter) Distilled water	1 7 mL	3,6,7,23

Activities by PASCO Equipment

This list shows each item needed for the activities and where the item is used.

Items Available from PASCO	Qty	Where Used
PASPORT Carbon Dioxide Gas Sensor	1	9,19,25
PASPORT Barometer/Low Pressure Sensor	1	17
PASPORT Colorimeter	1	3,6,7,23
PASPORT Oxygen Gas Sensor	1	5,9,11,25
PASPORT Quad Temperature Sensor with Fast-Response and Stainless Steel Temperature Probes	1	2,19,20
PASPORT Advanced Water Quality Sensor with pH probe	1	6,18,22,24
PASPORT Advanced Water Quality Sensor with Optical Dissolved Oxygen Probe		8,10,26
PASPORT Advanced Water Quality Sensor with Conductivity Probe		3,4
PASPORT Blood Pressure Sensor	1	20
PASPORT Ethanol Sensor	1	11
PASPORT Sensor Extension Cable	1	9,17,19,25
Photosynthesis Tank	1	8
EcoChamber	1	11,25
Metabolism Chamber	1	9
Aquatic Productivity Bottles	1	26
Kena™ Microscope	1	4,14,15
Mitochondrial Genetics Kit	1	13
Large Base and Support Rod	1	17,19
Magnetic Stirrer with Magnetic Stir Bar	1	8,11
Stop Watch	1	18
Electronic Balance	1	1,4,11,13,17,19,22
Hot Plate	1	1,10
Three-Finger Clamp	1	19,17
Utility Clamp	1	17

The Cell

1. pH and Buffers

Objectives

Biological systems are incredibly sensitive to the pH of their environments. In this lab, students discover the pH of some common household products and then learn how biological systems buffer large-scale changes in pH. In this experiment, students:

- Explore the effects of pH on the experiment of an enzyme
- Understand that buffers minimize the effects of acids and bases
- Understand how acids and bases affect the functions of enzymes and the functions of proteins

Procedural Overview

Students gain experience conducting the following procedures:

- Creating a buffer, and measuring the difference in the change of the pH in buffered and non-buffered solutions
- Measuring and understanding pH values for household products.

Time Requirement

♦ Preparation time	45 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	Day one: 60 minutes Day two: 15 minutes

Materials and Equipment

For each student or group:

- Data collection system
- pH sensor
- 500-mL beaker
- 100-mL beaker (4)
- 50-mL beaker (8)
- 10-mL graduated cylinder
- 25-mL graduated cylinder
- 50-mL graduated cylinder
- Disposable pipets, 1-mL
- Test tubes, 25 mm x 150 mm, (15)
- Test tube rack
- Weighing papers (14)
- Standard buffers pH 4 and pH 10
- Gelatin solution, 150 mL
- Meat tenderizer, 7 g
- Various fruit juices (100%), household ammonia, household bleach, vinegar[1]
- 0.1 M Hydrochloric Acid(HCl), 35 mL
- 0.1 M Sodium Hydroxide (NaOH), 35 mL
- Sodium acetate ($NaCH_3 CO_2$), 2 g
- Hot plate
- Electronic balance, 1 per class
- Distilled water, 1 L
- Distilled water in a rinse bottle
- Labeling tape and marker
- Paper towels

[1] You can choose household items or juices other than those described in this lab. Refer to the Data Analysis section for a list of the sample items tested and described in this lab.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Basic principles of ions and solutions
- Properties of water
- Structure and function of enzymes
- Factors that affect enzyme experiment

Related Labs in This Guide

Labs conceptually related to this one include:

- Exploring the Effects of pH on Amylase Experiment
- *Elodea* and the Snail
- Air Pollution and Acid Rain

Background

Using the simplest definition of acids and bases, we can say that:

- an acid is a molecule that when dissolved in water releases hydrogen ion (H^+) or the hydronium ion (H_3O^+);
- a base is a molecule that when dissolved in water releases a hydroxide ion (OH^-).

This is the way Arrhenius defined acids and bases in 1886. These definitions work well today for biological systems because most are aqueous. When you mix an acid and a base together, they will neutralize each other (if there are equal numbers of H^+ and OH^-).

In biological systems, the pH level is a measure of the concentration of H^+ ions. pH impacts the effectiveness of enzymes and proteins. Amino acids, which contain carboxyl functional groups (acidic) and amine functional group (basic), make up enzymes and proteins. The amino acids in the chains and the sequence of the amino acids determine a protein's particular shape, with specific exposed side chains. Alterations of the shape or functional side groups can cause the protein to become denatured or non-functional. Enzymes are particularly sensitive to pH changes, which can change their three-dimensional structure. A change in the shape of the active site renders the enzyme ineffective because the molecule can no longer attach to the substrate molecule.

A buffer works this way: If you add 100 ions of H^+ to a non-buffered solution, all 100 H^+ ions can interact with the other ions or molecules in the solution. However, if you add 100 ions of H^+ to a solution with a buffer, then the buffer picks up 90 of the ions, leaving only 10 free to interact with the enzymes in the solution. With fewer acid ions in the solution, the chances of them interacting with all the enzymes in the solution (making them inactive) are much less.

Pre-lab Discussion and Experiment

Discuss the basic concepts of pH, the properties of water, and buffers. Ask students to list several liquids they use in their everyday life. Ask them to predict the pH of these substances.

Ask students where they have heard the term "buffer" or "pH-balanced." Discuss the idea of neutral pH, and whether all living things require a neutral environment.

Discuss the basics of proteins and their structure. Talk about how the structure of a protein intimately relates to its function.

Discuss factors that cause a protein to denature, like pH and temperature.

Discuss the topic of enzymes. Help students understand that enzymes are catalytic proteins, and they will therefore denature like proteins.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Purchase gelatin, meat tenderizer, and juices at the grocery store. The powdered meat tenderizer can be found in the spice aisle and the gelatin in the baking aisle. Be sure to purchase unflavored gelatin in packets. Each packet usually contains 7 g of gelatin.
2. Prepare the gelatin solution before class. To prepare solution for 8 groups:

   a. Add 400 mL of room-temperature water to a very large beaker.

   b. Sprinkle four packets of gelatin on top of the water.

   c. Stir, and let stand for 1 to 5 minutes.

   d. Add 1200 mL of boiling water to the mixture, and stir until it all dissolves.

   This will last all day, but it might start to gel if it cools. To reliquify it, heat it gently on a hot plate, and stir constantly.
3. Each group will need eight 50-mL beakers and four 100-mL beakers. If you do not have enough beakers, use paper or plastic cups of similar size.

Safety

Follow all standard laboratory procedures.

Procedure

Day 1

Part 1 – pH of common household substances

Set Up

1. Start a new experiment on the data collection system.

2. Connect the pH Sensor to the data collection system.

3. Use the pH 4 and pH 10 buffer solutions to calibrate the pH sensor.

4. Label a 50-mL beaker for each juice and household substance.

5. Add 25 mL of each juice and household substance to a different beaker.

6. Record a predicted pH value for each of your samples in the Table 1.

Collect Data

7. Place the pH probe in the first sample and start recording pH data.

8. Allow the pH value to stabilize, and then record the value in the Table 1.

9. Remove the pH probe from the sample.

10. Rinse the probe with distilled water.

11. Repeat the Collect Data steps until the pH has been recorded for each sample.

Part 2 – pH and enzyme experiment

Set Up

12. Place 15 test tubes in the test tube rack.

13. Label each test tube #1 through #15.

14. Add 10 mL of 0.1 mL HCl to test tube #1.

15. Add 9 mL of distilled water to test tubes #2 through #7.

16. Using a disposable, graduated pipet, remove 1.0 mL of fluid from test tube #1, and add it to test tube #2.

17. Swirl to mix tube #2.

18. Rinse your pipet or obtain a new pipet, and remove 1.0 mL of fluid from test tube #2.

19. Add it to test tube #3.

20. Swirl to mix tube #3.

21. Continue this process of adding 1.0 mL of fluid to the next test tube, stopping at test tube #7.

22. How will the concentration of acid differ in each of the first 7 test tubes?

Because this is a 1 to 10 dilution, each tube should have one-tenth the amount of acid. The pH should increase by 1 pH unit (less acidic). In reality, this is a good approximation. (Students may need to update their answers after they have collected their data.)

23. Add 10 mL of 0.1 M NaOH to test tube #14.

24. Add 9 mL of distilled water to test tubes #8 through #13.

25. Using a disposable, graduated pipet, remove 1.0 mL from test tube #14 and adding it to test tube #13.

26. Stir or gently shake the tube to mix test tube #13.

27. Rinse your pipet or obtain a new pipet, and remove 1.0 mL from test tube #13 and add it to test tube #12.

28. Swirl to mix test tube #12.

29. Continue this process adding fluid to the next test tube, stopping at test tube #8.

30. Add 10 mL of distilled water to test tube #15.

31. How will the concentrations of base differ in test tubes #8 through #14?

This is a 1:10 dilution, so the concentration of base will decrease and the pH will drop towards 7 in test tube #8.

32. Using an electronic balance and weighing papers, create 14 individual samples, 0.5 g each, of meat tenderizer.

33. Add 0.5 g of meat tenderizer to each of the test tubes labeled #1 through #14.

34. Swirl to mix each test tube.

35. What is the function of a meat tenderizer?

Meat tenderizers are used to make poor cuts of meat, which are usually tough to chew, more tender. They usually contain an enzyme that helps break down the protein structure of the meat, making it easier to chew.

36. Add 10 mL of the warm gelatin solution to test tubes #1 through #15.

37. Swirl to mix each test tube.

38. What will happen to the gelatin as it cools?

The gelatin is made from collagen, a protein found in the bones and connective tissue of mammals. Gelatin is usually derived from domestic animals, such as cattle and horses. It is used as a thickening agent so solutions will gel when they cool. It is a reversible process; heat returns the solution to a liquid state.

39. What is the purpose of test tube #15?

This is the control. It has all the variables except the tenderizer, and the pH is assumed to be neutral. Water that has been standing and exposed to carbon dioxide in the air will absorb the carbon dioxide and become acidic. However, since the amount of carbon dioxide is small, the solution will have very few H+ ions to affect the tenderizer.

40. Place the test tube rack in the refrigerator overnight.

Part 3 – Buffers

Set Up

41. Place 50 mL of vinegar into a 100-mL beaker.

42. Add 1.0 g of sodium acetate to the vinegar.

43. Swirl the beaker until the solid dissolves.

Collect Data

44. Place the pH probe into the solution.

45. Start recording the pH.

46. What do you predict will happen to the pH of the vinegar when you add 0.1 M hydrochloric acid?

The pH will remain fairly constant because adding the sodium acetate creates a buffer using the acetate ions. When H^+ ions are added, they react with the acetate ion and form a weak acid, acetic acid. Weak acids do not ionize easily, so the H^+ ions are removed from the solution.

47. Add a drop of 0.1 M HCl to the solution. Be certain to add the acid to the solution, not directly on the probe.

48. Continue to add a drop of 0.1 M HCl every 10 seconds for 3 minutes, swirling the test tube as you add the HCl. Be certain to add the acid to the solution, not directly on the probe.

49. Stop recording data after 3 minutes. Remove and rinse the probe.

50. Name this data run "Unbuffered Acid".

51. Add 50 mL of distilled water to a new 100-mL beaker.

52. Place the pH probe into the solution, and begin recording the pH.

53. What do you predict will happen to the pH of the water when you add hydrochloric acid?

The pH will drop rapidly as there are no ions to remove the extra H^+ ions that were added. After a while the pH will start to level off.

54. Add a drop of 0.1 M HCl to the distilled water. Be certain to add the acid to the solution, not directly on the probe.

55. Continue to add a drop of 0.1 M HCl every 10 seconds for 3 minutes, swirling the test tube as you add the HCl. Be certain to add the acid to the solution, not directly on the probe.

56. Stop recording data after 3 minutes. Remove and rinse the probe.

57. Name this data run "Buffered Acid".

58. Place 50 mL of vinegar into a new 100-mL beaker.

59. Add 1.0 g of sodium acetate.

60. Swirl the beaker until the solid dissolves.

61. Place the pH probe into the solution, and begin recording the pH.

62. How will adding a base instead of an acid affect the pH of the solution?

The pH of the solution will stay fairly constant due to the presence of the acetic acid (vinegar). As you add the OH^- from the base, the acetic acid dissociates to release H^+ ions, which neutralize the added OH^- ions.

63. Add a drop of 0.1 M NaOH to the solution and stir. Be certain to add the base to the solution, not directly on the probe.

64. Continue adding a drop of 0.1 M NaOH every 10 seconds for 3 minutes, swirling the solution as you add the NaOH.

65. Stop the data recording after 3 minutes. Remove and rinse the probe.

66. Name this data run "Unbuffered Base".

67. Place 50 mL of distilled water into a new 100-mL beaker.

68. Place the pH probe into the solution, and begin recording the pH.

69. How will adding a base instead of an acid affect the pH of the distilled water?

The pH will rise, as the number of OH^- ions will increase and not react with any ions in the distilled water.

70. Add a drop of 0.1 M NaOH to the solution. Be certain to add the base to the solution, not directly on the probe.

71. Continue adding a drop of 0.1 M NaOH every 10 seconds for 3 minutes, swirling the solution as you add the NaOH.

72. Stop the data recording after 3 minutes. Remove and rinse the probe.

73. Name this data run "Buffered Base".

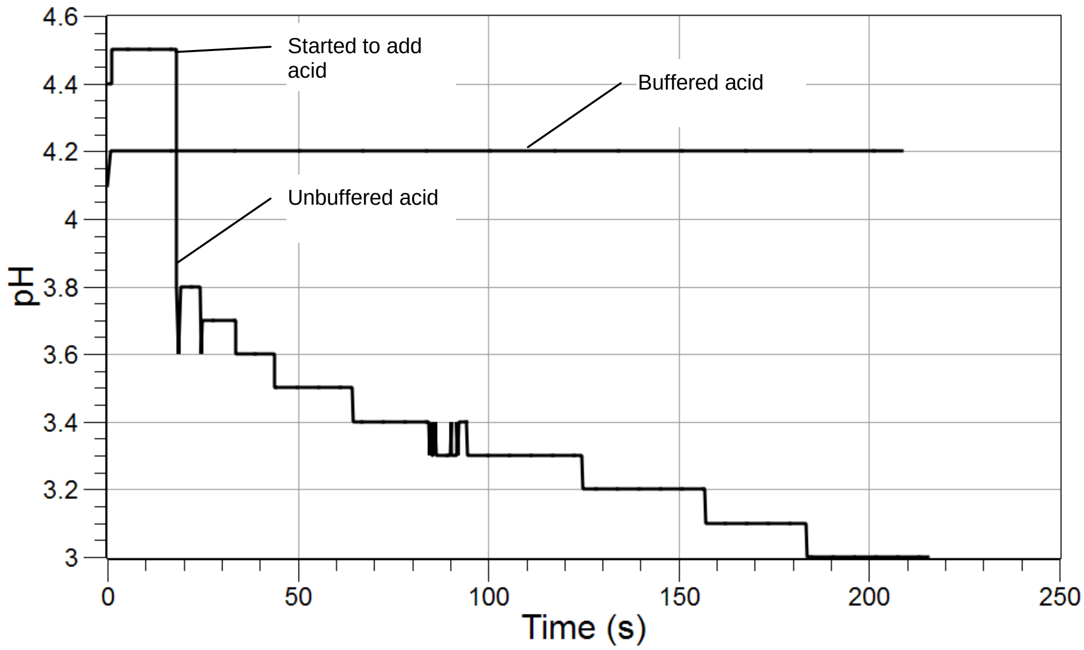
Day Two

Collect Data

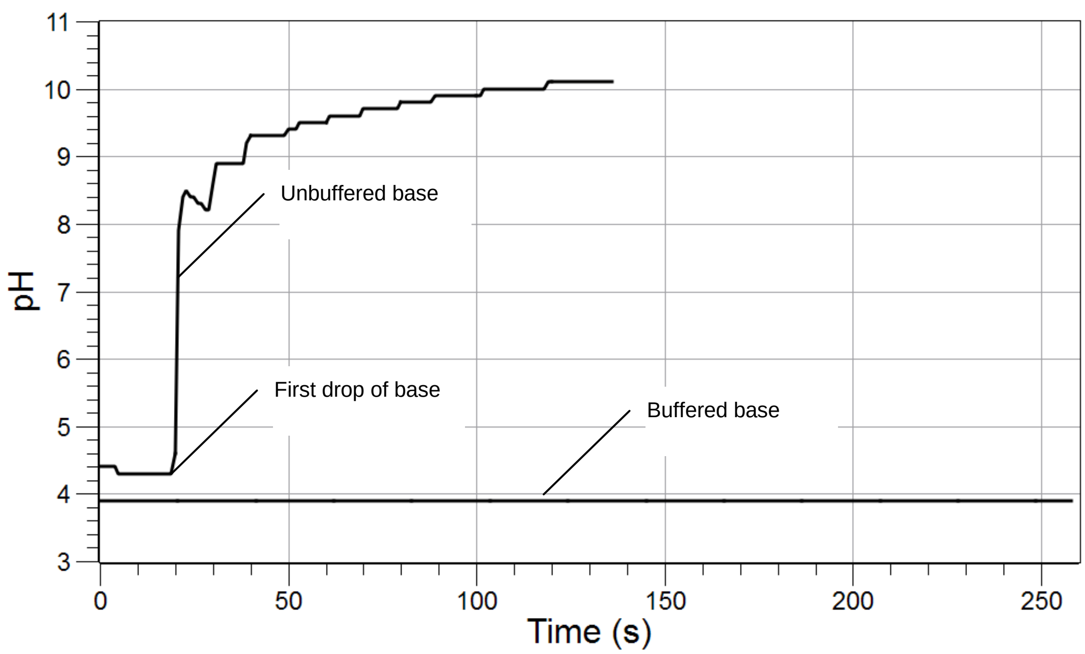
74. Remove the test tubes from the refrigerator, and observe the consistency of the gelatin in the test tubes. Record your observations in the Table 2.

Sample Data

Unbuffered and Buffered Acid



Unbuffered and Buffered Base



Data Analysis

Part 1

Table 1: pH of Common Household Substances

Sample	Predicted pH	Measured pH
Orange Juice	Will vary	4.30
Apple Juice	Will vary	4.10
Milk	Will vary	6.80
Pineapple Juice	Will vary	4.10
Vinegar	Will vary	2.80
Coke	Will vary	2.90
Bleach (chlorine)	Will vary	9.50
Household ammonia	Will vary	10.90

Part 2

Table 2: pH and Enzyme Experiment

Test tube number	Observation	Measured pH (Instructor reference only)
1	Gel	1.10
2	Gel	1.90
3	Liquid	2.90
4	Liquid	5.10
5	Liquid	6.20
6	Liquid	6.80
7	Liquid	6.80
8	Liquid	7.50
9	Liquid	7.90
10	Liquid	8.40
11	Liquid	9.50
12	Liquid	11.1
13	Gel	12.1
14	Gel	12.8
15	Gel (Control, no meat tenderizer)	6.90

Note: The papain in the meat tenderizer functions best in the pH range of 2 through 11, which is a large range for an enzyme. The pH levels were measured before the gelatin and meat tenderizer were added.

Part 3

1. Use the statistics tool to find the minimum and maximum pH values for all four data runs. Record the values in Table 3.

2. Use the data in Table 3 to calculate the change in pH for all four data runs.

Change in pH (acid) = maximum pH - minimum pH

Change in pH (base) = minimum pH - maximum pH

Table 3: pH Changes

Run	**Minimum pH**	**Maximum pH**	**Change in pH**
#1 – Buffered Acid	4.1	4.2	0.1
#2 – Unbuffered Acid	3.0	4.5	1.5
#3 – Buffered Base	3.9	3.9	0.0
#4- Unbuffered Base	4.3	10.1	5.8

Analysis Questions

1. Were your predicted values in Table 1 similar to the results? Can you make any generalizations about acids and bases that will help you to predict the pH value of other household products?

Fruit juices tend to be acidic especially from citrus fruits. Cleaning products tend to be basic.

2. Did you observe a pattern in the results from Part 2? Explain what factor appeared to influence the pH of the solution.

Extreme pH (very high or very low) caused the enzyme to be ineffective. This is apparent because the gelatin remains liquid when the enzyme is effective. Since the gelatin remained liquid at pH of 3 to 12, we know that the enzyme was effective in this range. In environments more acidic or basic than this, range, the enzyme was ineffective and the gelatin solidified.

3. In the buffered solutions in Part 3, will the pH remain a constant no matter how much acid or base is added? Why or why not?

No, the solutions will not resist a change in pH no matter how much acid or base is added. At some point the solution will reach the buffer capacity, and the solution will no longer resist a change in pH. The buffer capacity depends on the ratio of the weak acid to the salt (sodium acetate), which we added.

Synthesis Questions

1. Papain is one of the most important enzymes found in meat tenderizer. How sensitive is papain to denaturation by temperature? Explain your answer.

The enzyme is not very sensitive because the gelatin solution was very hot when we added it to the test tube with the meat tenderizer. The enzyme still inhibited the gelatin from jelling.

2. People often shake meat tenderizer on a bee sting to reduce the pain and swelling Why do you think this is effective?

The venom from bee and some other insects is a protein. So adding a meat tenderizer, which breaks down proteins, will break down the venom, reducing the pain and swelling.

3. Can you think of any important uses for buffers in industry?

Shampoo and other beauty products are "pH-balanced" to protect the skin and hair. Since our hair and skin are slightly acidic, beauty products contain buffers that help the product function without being affected by the pH of our bodies.

Multiple Choice Questions

Questions 1 through 3 refer to the table below.

	Sample	**pH**
A	Milk	6.8
B	Ammonia solution	10.9
C	Apple juice	4.1
D	Orange juice	4.3
E	Coke	2.9

1. Which sample listed in the table is the most acidic?

- **A.** Milk
- **B.** Ammonia solution
- **C.** Apple juice
- **D.** Orange juice
- **E. Coke**

2. Which sample listed in the table has the highest H^+ concentration?

- **A.** Milk
- B. Ammonia solution
- **C.** Apple juice
- **D.** Orange juice
- **E. Coke**

3. When a protein is denatured, which of the following is most likely to occur?

- **A.** It changes color
- **B.** It changes pH
- **C.** The sequence of amino acids changes
- **D. The three-dimensional shape is altered**
- **E.** The organism dies

Extended Inquiry Suggestions

Have students investigate the optimum pH of the enzymes that breakdown the major food groups and where in the digestive tract these enzymes are located.

Have students investigate hair and the ways in which hair care manufacturers alter the pH of their products to achieve the desired effects.

Have students investigate competitive inhibitors, which block the active site of enzymes and make them ineffective without denaturing the enzyme.

Have the students design a controlled experiment to investigate the relationship of an enzyme's experiment to the temperature of the solution.

Design a controlled study to investigate buffer capacity, the point at which the solution no longer absorb H^+ or OH^- ions from a solution.

Investigate where gelatin and papain come from. Why do they behave the way they do in this experiment.

2. Exploring Surface Area to Volume Ratios

Objectives

In this experiment, students investigate how a cell's surface area to volume ratio affects the way the cell responds to the environment. To learn this, students:

- Use citrus fruits as a model to investigate how the surface area to volume ratio influences the way a cell interacts with its environment
- Calculate the mathematical relationship between surface area and volume
- Apply the concept of surface area to volume ratios to biological systems

Procedural Overview

In this lab, students will gain experience in the following procedures:

- Measure the circumference of two or three different citrus fruits.
- Calculate the radius, surface area, volume, and the ratio of surface area to volume of each fruit.
- Place the fruits in an ice-water bath, and record how quickly each fruit releases heat into its environment.

Time requirement

♦ Preparation time	20 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	45 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Quad Temperature Sensor
- Fast response temperature probe (2)
- Stainless steel temperature probe
- Citrus fruit, varying type and size (2) [1]
- Dissecting probe or pin
- Metric ruler
- Petroleum jelly
- Ice and water
- Sharpie or other marker
- Tape, labeling or masking
- Twine or string
- Polystyrene cooler, plastic bucket, or other vessel for ice bath

[1] This Instructor Information provides data for three different fruits: grapefruit, orange, and lime. However, students will use only two pieces of fruit.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Free energy
- Solute versus solvent
- Basic principles of diffusion and osmosis
- Basic principles of cell structure (specifically organelles, cell membranes, and cell walls)
- Basic principles of heat, thermal energy, and specific heat

Related Labs in This Guide

Labs conceptually related to this one include:

- Diffusion
- Diffusion and Osmosis

Background

The ratio of surface area to volume is a fundamental concept that governs the function of nearly all biological systems. The relationship between the surface area and the volume of a cell, organ, or organism changes as the size of the cell, organ, or organism changes. The relationship between surface area and volume drives adaptations as diverse as:

- cell size and shape
- the endomembrane system of eukaryotic cells
- the organization and function of the human circulatory system
- the size and shape of mammals living in different biomes
- Surface area to volume ratios even influence nutrient cycling in aquatic ecosystems.

Pre-lab Discussion and Experiment

1. How big are cells? What limits a cells size?

Typical prokaryotic cells are approximately 5 to 10 microns (µm) at their longest point. Most plant and animal cells are between 10 and 100 microns (µm) in diameter. Cells must be small enough to maintain an adequate surface area in order to transport nutrients in and waste products out. This also allows for the exchange of respiratory gasses (O_2 and CO_2). A cell must be large enough to contain genetic material as well as the molecular machinery to replicate and express the genetic material.

2. What shapes do cells assume?

Cells are three-dimensional. Typical prokaryotic cell shapes are rod, spiral, or spherical. Eukaryotic cells come in a variety of shapes and sizes. Plant cells are typically rectangular or cylindrical. We often visualize cells as circles or squares because we're looking at them in cross section. Animal cells can assume any shape that nature selects. Human cheek cells are flat (or squamous), while the small intestinal cells are columnar with finger-like appendages (called microvilli). Nerve cells and some lymphoid cells (white blood cells) are highly branched and amoeba-shaped.

3. How does cellular shape allow cells to interact with their environment?

Shape, whether rod, spherical, or cylindrical, allows cells to increase their interactions with the environment. For example, absorptive cells (such as, in the small intestine) need increased surface area for absorption. An increased surface area to volume ratio also allows for an increased rate of cellular respiration as well as an increased reception of chemical signals.

4. How does the surface area to volume ratio change as cells or organisms get larger?

As cells get bigger, the surface area to volume ratio decreases. As organisms get bigger, the surface area to volume may or may not change. As they grow, organisms generally add cells that may or may not have different surface area to volume ratios..

5. How does the relationship between surface area and volume influence the way a cell interacts with its environment?

A cell with a larger surface area relative to volume will have more contact with the surrounding environment, speeding up or facilitating any interaction with the environment. This would result in faster importing or exporting material into or out of a cell as well as increased cell-to-cell contact for communication and cooperation among cells. A cell with a smaller surface area relative to volume would be more insulated from the surrounding environment, resulting in slower cell transport and slower rates of heat loss or gain.

6. What adaptations do eukaryotic cells have to deal with a smaller surface area to volume ratio and increasing diffusion distances?

The adaptations that eukaryotic cells have developed to compensate for their smaller cell surface area to volume ratio compared with prokaryotic cells include the endo-membrane system and the membrane-bound organelles.

7. In terms of cell size versus cell number, how do multicellular organisms grow and develop?

Multicellular organisms grow and develop by adding more cells, not bigger cells.

8. Why do we use models in science?

Models help us visualize a difficult concept by creating simple versions of a natural system. These models allow us to analyze one change to that system at a time.

9. How do we calculate surface area to volume ratio?

We use geometry. See the formulas in Table 1.

10. Predict what will happen when you submerge fruits in ice water. Encourage students to talk specifically about this experiment and to make comparisons between the fruits used in this experiment.

The larger fruit will cool more slowly than the smaller fruit because heat diffuses faster out of the fruit with a higher surface area to volume ratio.

11. Ask students to think about the changes to the internal temperature of an organism that is placed in a cold aqueous, environment.

The organism will lose heat to the environment at a rapid rate. Several adaptations have evolved to deal with this problem. Adaptations include ectothermy (being a thermoconformer), synthesis of lipids, and production of hair. Another adaptation is increasing size and decreasing the surface area to volume ratio. Other organisms (homeotherms) have increased metabolic rates and have evolved countercurrent exchange systems to conserve heat.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Oranges, limes, and key limes work well. Grapefruits also work well, but can be expensive and less dense than key limes. The thicker grapefruit peel can also act as an insulator, decreasing the cooling rate. This lab provides sample data for grapefruit, orange, and lime.

2. Keep a large cooler of ice in a central location. Let the students set up their own ice bath. Tell students that a slurry (half ice, half water) will work best, but allow them to experiment with their set up. It is important that students lower all their fruit into the slurry simultaneously.
3. This lab requires careful set up. The experiment itself runs for only 10 minutes. However, allow students to run the lab longer if there is time or if temperature equilibrium has not been reached (if the temperature continues to drop noticeably).
4. Make sure the fruit is at room temperature before beginning the experiment. If the fruit have been refrigerated, leave them out overnight. You can also place them under an incandescent bulb. A larger temperature gradient between fruit and the ice bath will yield more dramatic results. Ideally, all fruit should have the same initial temperature.

Instructor Tip: Students may experience difficulty keeping water out of the hole where they insert the temperature probe. Solutions to this problem include: 1) make as small of a hole as possible by boring the hole with a stainless steel temperature probe; 2) seal the space around the temperature probe with petroleum jelly; 3) during data collection, hold the fruit in the ice bath such that the hole is just above the surface of the water.

Safety

Add these important safety precautions to your normal laboratory procedures:

♦ Use caution if you use dissecting probes to poke holes in the fruit.

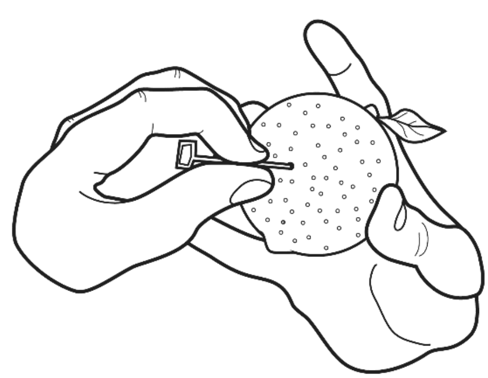
Procedure

Set Up

1. Obtain two different citrus fruits. Make sure you have different types of fruit of similar shape, but that differ in size.
2. Use the tape measure, twine, marker, and a ruler to measure the circumference of each piece of fruit.
3. Record the data in the Table 1.
4. Complete the rest of Table 1 by calculating the radius, surface area, volume, and surface area to volume ratio for each piece of fruit using your circumference measurements and the formulas in Table 1. Show your work in the space below.

Work space to calculate radius, surface area, volume, and surface area to volume ration provided here.

5. Use a dissecting pin or a dissecting probe to bore a small hole through the peel and pulp of the fruit. The pin should reach to the center of the fruit, but no further. The hole depth should precisely equal the radius you calculated for the fruit. When you insert the fast response temperature probe into the fruit later in the experiment, its tip will be at the center of the fruit.



6. Why are you using citrus fruit to simulate a cell?

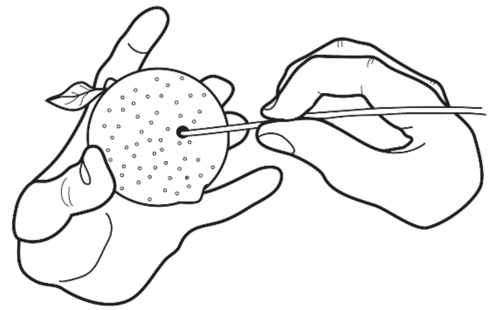
Citrus fruits are inexpensive models of cells. They have a spherical shape, which helps us visualize a eukaryotic cell.

7. What might be the adaptive significance of cells being small and spherical?

Small, spherical cells have high surface area to volume ratios resulting in increased capacity for interaction with other cells and increased ability transport of nutrients, gasses, and waste products across the cell membrane.

8. Measure from the tip of the temperature probe to a point equal to the radius of the fruit, and then mark that measurement on the probe with tape.

9. Thread a fast response temperature probe carefully into the hole you bored in a fruit up to the tape mark. The tip of the probe should be in the center of the fruit.



10. Repeat the steps above to measure and thread the probe into the second piece of fruit.

11. Inspect for gaps between the peel on each fruit and the temperature probe. Seal any openings you find around the hole with petroleum jelly.

12. Why wouldn't you want water flowing into this hole?

If cold water flows directly onto the temperature probe, the data will indicate rapid (perhaps instantaneous) cooling of the fruit that is not representative of the actual temperature of the fruit cells.

13. Start a new experiment on the data collection system.

14. Plug the Quad Temperature Sensor into your data collection system after you insert the temperature probes into both pieces of fruit. Connect two fast response temperature probes and one stainless steel temperature probe to the sensor.

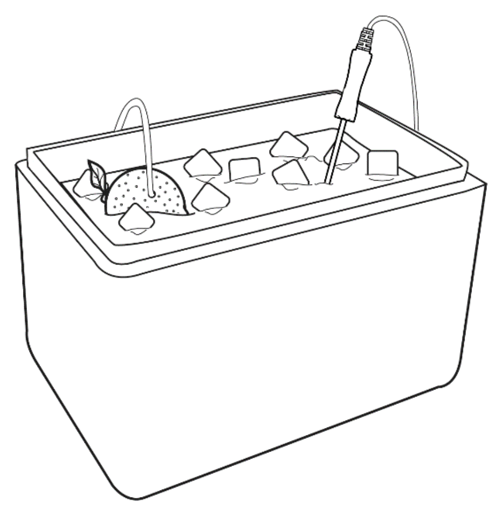
15. Set up the digits display so that all 3 temperatures are displayed simultaneously.

16. Which fruit do you predict will lose heat most quickly? Why?

The smaller fruit should cool more quickly because it has a greater surface area relative to its volume.

17. Fill a tub or cooler with an ice and water mix.

The amount of ice is up to you. Make sure there's enough water to actually float the fruit, but not so much that the fruit sink to the bottom of the tub.



18. Place the stainless steel temperature probe into the ice and water mix.

The temperature should read between 0 and 4 °C. This temperature probe serves to monitor the constant temperature of the water bath.

19. Why do you need to place a temperature probe in the cooler?

This monitors a constant in the experiment: the temperature of the environment surrounding the test subjects. If the ice-bath temperature changes, the fruit temperature measurements might be considered inaccurate and would need to be re-collected.

20. Do you expect the ice bath temperature to change significantly during the course of the experiment? If it did, what would that tell you about your experiment set up?

The temperature of the ice bath should not change much. If it does, it indicates that the fruits are warm enough to heat up the ice bath. It could also mean that the experimental set up was not correct.

Collect Data

21. Place both of your fruits in the cooler simultaneously, and immediately begin recording temperature data.

Be sure to hold the temperature probes upright throughout the course of the experiment with the hole containing the probe just above the ice bath surface. This helps keep water from flowing into the hole.

22. What other types of cell shapes exist? What is the adaptive significance of these other cell shapes?

Examples include: spiral, rod (bacillus), rectangular (or brick shaped), cylindrical, cuboidal, squamous (flat), columnar, and amoeboid. Cell shapes are almost limitless. Cylindrical and rectangular shapes confer strength and rigidity; flat cells allow for diffusion; columnar cells allow for increased packing of individual cells for secretion and absorption. Amoeboid cells, and branched cells (such as neurons) allow for increased connections between cells.

23. What other characteristics of the fruit besides size might affect the rate of cooling in your fruits. Be as specific as possible.

Characteristics include: thickness of the peel; the density of the fruit; the shape of the fruit; the amount of pulp within the fruit (the pulp might increase the transfer of heat through the fruit); the initial temperature of the fruit.

24. Record the temperatures for each fruit into Table 2 at 1-minute intervals for 10 minutes.

25. What do you notice about the temperature change in each fruit? Be specific.

Answers will vary.

26. Stop recoding data after 10 minutes.

27. Remove the fruit from the cooler.

28. Remove the temperature probes from the fruit and the cooler.

29. Clean the temperature probes with a damp paper towel.

30. Save your experiment and clean up according to your instructor's instructions.

Data Analysis

Table 1: Measurements and calculations

Fruit	Circum-ference (cm)	Radius (cm) (C/2pi)	Surface Area (cm) (4pi*r^2)	Volume (cm^3) ((4/3)*pi*r^3)	SA/V Ratio	Cooling Rate (°C/min)
Grape-fruit	37.5	5.97	445.551	890.823	0.500	0.076
Orange	23	3.66	168.334	205.263	0.820	0.189
Lime	17.5	2.78	97.11	89.95	1.0795	0.729

1. Calculate the cooling rate of each fruit over the entire 10-minute period, and record in Table 1. Use the initial and final temps (0 minutes and 10 minutes) to calculate the rate as follows:

Rate of cooling (°C/min) = Temperature Initial – Temperature Final ÷ 10 min.

Rate of cooling (°C/min) = Temperature Initial – Temperature Final/10 minutes

Grapefruit = (17.3 – 16.54)/10 = 0.076 °C/min

Orange = (24.20 – 22.31)/10 = 0.189 °C/min

Lime = (25.61 – 18.32)/10 – 0.729 °C/min

Table 2: Loss of heat during incubation in water bath

Time (Min)	Temp (°C) Fruit 1 (Grapefruit)	Temp (°C) Fruit 2 (Orange)	Temp (°C) Fruit 3 (Lime)	Temp (°C) Ice Bath
0	17.3	24.20	25.61	2.4
1	17.25	24.14	25.34	1.8
2	17.19	24.10	25.11	4.0
3	17.12	24.06	24.85	3.9
4	17.05	24	24.32	3.2
5	16.98	23.89	23.59	1.6
6	16.91	23.71	22.69	1.3
7	16.83	23.46	21.69	1.2
8	16.74	23.14	20.60	1.1
9	16.65	22.74	19.42	1.1
10	16.54	22.31	18.32	1.0

Analysis Questions

1. Describe the trends that you observe in your table. Make a claim as to which fruit cooled the fastest. Be sure to include the evidence you used to make your claim.

Answers will vary based on the students' run. Ideally, the students will say that the fruits cooled continuously as the experiment progressed. They might also say that the smaller fruit seemed to cool faster than the larger fruit. In the pilot experiment, the lime cooled the fastest. The rate was 0.79 degrees C/minute.

2. Calculate and compare the rate of cooling for each fruit, where:
Rate of cooling (°C/min) = Temperature Initial – Temperature Final ÷ 10 minutes

Grapefruit = (17.3 – 16.54)/10 = 0.076 °C/min

Orange = (24.20 – 22.31)/10 = 0.189 °C/min

Lime = (25.61 – 18.32)/10 = 0.729 °C/min

The lime experienced the fastest rate of cooling, followed by the orange. The grapefruit experienced the slowest rate of cooling.

3. Explain the difference, if any, in the cooling rates for each fruit. If there was not a difference between cooling rates, explain why.

The smaller fruits have the highest cooling rates and so cooled the fastest. The small fruit cooled faster because of its higher surface area to volume ratio.

4. Are there any characteristics of these fruits other than the surface area to volume ratio that might alter the results? How would you control for these differences if you ran the experiment a second time?

The most obvious characteristic that could alter results is the presence of the peel. The peel might serve as an insulator between the ice bath and the core of the fruit. As another approach, you could peel all the fruit as you set up the experiment. Alternatively, you could peel all the fruit and then wrap them in plastic bag to keep ice water from directly seeping into the fruit and altering the results.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Describe a real situation where a high surface area to volume ratio would increase an organism's chance of survival.

Answers will vary. One example is: Cells of a developing embryo have a high surface area to volume ratio which allows the cells to take up nutrients and get rid of waste products.

2. How does a low surface area to volume ratio limit a cell's ability to survive?

A low surface area to volume ratio results in slower transport of nutrients into a cell and wastes out of the cell. It also limits the contact the cell has to other cells for the purpose of communication and coordination between cells.

3. If you needed to cool a beverage quickly, why might it be better to place it in cold water, or an ice-water bath, instead of a refrigerator?

Water has a higher specific heat than air. Therefore, a beverage should lose more heat to the water, thus cooling faster and remaining cool longer than it would to refrigerated air.

4. One method of lowering a fever is to administer a medication, such as aspirin, to the patient. Using the information from this lab, what is another method you could

use to reduce a high fever in a human? Why is this method so effective at bringing down a fever?

You could place the patient in a cool bath, and pack ice around the patient. If you maximize the surface area of the patient with the cool water, you draw heat away from the body and into the water. Also, water has a high specific heat, making water better at absorbing heat from another object than air.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following cellular processes would be limited by low surface area of plasma membrane relative to volume of the cytosol?

- **A.** Synthesis of ribosomal subunits
- **B.** Diffusion of oxygen into the cell
- **C.** Diffusion of carbon dioxide out of the cell
- **D.** All of the above
- **E. B and C only**

2. Which of the following organelles have highly folded inner membranes that increase their internal surface area?

- **A. Mitochondria**
- **B.** Prokaryotes
- **C.** Lysosomes
- **D.** Vacuoles
- **E.** Peroxismes

3. The lining of the small intestine and the lining of the lungs are similar in that they both

- **A.** Allow for the absorption of glucose from food.
- **B.** Allow for the diffusion of atmospheric oxygen.
- **C. Have a folded interior to increase internal surface area relative to their volume.**
- **D.** Secrete digestive enzymes.
- **E.** Release carbon dioxide into the atmosphere.

Extended Inquiry Suggestions

The concept of surface area to volume ratio and its implications is pervasive at every level of biological organization. The opportunities for lab extensions are abundant.

If students find on the idea of a rind or pulp altering the cooling rates of fruit distracting, students could use different sized scoopers or melon ballers to make different sized spheres of the same fruit and then perform the experiment again.

Prokaryotic and eukaryotic cells come in all shapes and sizes. If time permits, students could cut out different shapes (for example, rod shaped, cuboidal, columnar, “star,” or amoeboid shaped) from a fleshy fruit like an apple, pear, or banana; place a temperature probes in the center of the shapes; and then perform the experiment again.

The morphology and bathymetry of lakes and streams influences how they interact with their watersheds. Deeper, round lakes tend to be oligotrophic, while small, shallow and curvy lakes (such as reservoirs or oxbow lakes) tend to be more mesotropic or eutrophic. Simulate this phenomenon by creating plaster (or clay) molds with different shaped basins. Heat the molds in an incubator or under an incandescent bulb. Then, fill the basins with a known volume of cold water, and use probes to measure the change in water temperature over time.

3. Diffusion

Objectives

Students will investigate the following concepts related to diffusion and membrane permeability:

- Determining concentration gradients by evaluating movement of molecules across a semipermeable membrane
- Determining which molecules are small enough to cross the membrane
- Determining the rate of diffusion across the membrane

Procedural Overview

Students will gain experience conducting the following procedures:

- Setting up an experiment to measure rate of diffusion out of a dialysis bag containing pickle juice
- Collecting data from three different probes to determine which molecules or ions are diffusing across the dialysis membrane
- Using evidence from their experiments to make claims about which molecules or ions are diffusing across the dialysis membrane.

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	45 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Advanced ater quality sensor
- Conductivity probe
- pH probe
- Colorimeter
- Cuvettes (2)
- 400-mL beaker or similar-sized cup
- 50-mL beaker or similar-sized cup (2)
- Pickle juice containing FD&C yellow dye # 5, 50 mL
- Disposable pipet (4)
- Soaked dialysis tubing 28 cm
- Squirt bottle of water and cup or beaker
- Paper towels
- Plastic wrap
- Labeling marker
- Distilled water
- Scissors
- Lint-free tissue

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ Cell membrane structure and function
- ♦ Basic chemistry: pH, properties of water, molecular and ionic bonding

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Exploring Surface Area to Volume Ratios
- ♦ Diffusion and Osmosis

Background

Many aspects of cellular experiment depend on the kinetic energy of molecules and atoms. This kinetic energy causes molecules to bump into each other and move in new directions. One result of this molecular motion is the process of diffusion. Diffusion is the random movement of molecules from an area of higher concentration of those molecules to an area of lower concentration until a dynamic equilibrium is reached. Dynamic equilibrium is when the concentration of a substance is approximately equal within an area or on both sides of a membrane and there is no net movement of the molecule from one area to another.

Diffusion is affected by several factors. The most notable is the concentration gradient across the membrane. The size of the concentration gradient and the distance the molecules must travel also affect the rate of diffusion. The closer a solute is to a membrane, the more likely it is to cross the membrane and move along its concentration gradient. Cells maximize surface area to volume ratios to decrease diffusion distances and increase the rate of diffusion. The rate of diffusion is also affected by the size of the solute and the temperature of the solution.

Consider what is actually diffusing during this experiment. Pickle juice contains, among other things, NaCl (salt), acetic acid (vinegar), and yellow dye #5. Acetic acid in solution will dissociate (or separate) into protons (H^+) and acetate ions. Students will learn that the membrane is permeable to protons by measuring changes in pH with a pH probe. In solution, NaCl will dissociate into Na^+ and Cl^- ions. Students will learn that the membrane is permeable to these ions by measuring changes in conductivity with a conductivity probe. The yellow dye in the pickle juice is a pigment that will absorb various colors of light. Students will learn that the membrane is impermeable to the dye by measuring changes in the absorbance of blue light with a colorimeter.

The membrane in this lab is dialysis tubing, a cellulose sheet with uniform distribution of similar-sized pores. Dialysis tubing is used to purify a solution consisting of several solutes. When the solution is placed in a dialysis bag and the bag is placed in a hypotonic solution, like pure water, the small solutes travel out of the bag, while the larger solutes stay in the bag.

Cell membranes are more complex than dialysis tubes. The current model of cell membranes is known as the fluid mosaic model. The major component of cell membranes is a phospholipid bilayer. This layer is amphipathic, meaning it has a hydrophilic exterior and a hydrophobic core. This structure sets up spontaneously in aqueous solutions (for example, cytosol and extracellular fluid). Floating within the membrane are various proteins. Peripheral proteins are found only on the inside or outside of the cell membrane, while transverse proteins, integral proteins, and channel proteins span the entire membrane. Phospholipids can be associated with short carbohydrates (glycolipids), while proteins can be associated with short carbohydrates (glycoproteins) and proteins of the extracellular matrix and the cytoskeleton. These molecules allow for cell-to-cell recognition, cell-to-cell communication, and signal transduction.

Cell membranes are semipermeable. Large molecules do not easily pass through the phospholipid bilayer. The “heads” of phospholipids repel nonpolar molecules, while many polar molecules cannot penetrate the hydrophobic core of cell membranes. Oxygen and carbon dioxide gas and small polar molecules (such as salts and water) can diffuse through the membranes.

Pre-Lab Discussion and Experiment

Use any of these activities individually, or all of them in sequence.

Observe diffusion without membranes by filling petri dishes with water and placing one drop of food coloring into the dish with a disposable pipet. Allow students to observe the diffusion of the dye through the water over time. Next, place drops of two different colors of dye into a dish. Allow students to observe what happens. Ask students if the concentration of one dye affects the diffusion of the other dye.

Show students the dialysis tubing, demonstrate how to fill it with pickle juice, and let students know that this is a semipermeable membrane.

Ask students to name the compounds and molecules that are found in pickle juice. Ask a few brave student volunteers to taste the straight pickle juice and describe the taste to the class. Ask other students to explain what the volunteers are tasting. Do not focus on taste buds or how nerves conduct signals to the brain, but rather on the salts and vinegar in the pickle juice.

Test the pH of water and any other common household products or beverages.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Purchase dill pickles that are pickled in a solution containing NaCl, acetic acid solution (vinegar), and yellow dye #5. The juice might also contain $CaCl_2$ and EDTA, but these molecules will not affect the results of the experiment.

2. Cut enough dialysis tubing so that each student group has two 28-cm lengths of tubing. They will need one piece of dialysis tubing, but it's good to have extra in case they rip or tear a bag. Place the two sections of tubing in a plastic cup and soak in distilled water for at least half an hour. Soaking the dialysis tubing is a necessary step because it removes chemicals that preserve and protect the membrane during shipping and storage.

Safety

Add these important safety precautions to your normal laboratory procedures:

- ♦ These exercises are "cable intensive". Make sure cords are wrapped or taped down and out of the way of hands and feet.
- ♦ None of the solutions should be tasted unless you are instructed to do so by your instructor..

Procedure

Part 1 – Pre-soak

Set Up

1. Start a new experiment on the data collection system.)

2. Connect a pH probe, conductivity probe, and colorimeter to your data collection system.

3. Create a digits display of pH.

4. Set the conductivity probe to a measurement range of 0 to 100,000 (the wave button), and connect a 10X probe to the sensor. Create a digits display of conductivity.

5. Set the colorimeter to measure absorbance. Create a digits display of Blue Absorbance.

6. Why measure absorbance of blue light?

Pickle juice is yellow. Therefore, it transmits yellow light and absorbs other colors. Pickle juice absorbs blue light more than any other wavelength available on the colorimeter.

7. Pour approximately 50 mL of pickle juice into one of your 50-mL beakers. Label this beaker "pickle juice".

8. Fill a clean cuvette with pickle juice using a clean pipet, and screw the cap on the cuvette. Do not dispose of the pickle juice.

9. Fill the other 50-mL beaker with distilled water. Label this beaker "distilled water".

10. Using a clean pipet, fill the second cuvette with distilled water and screw the cap on the cuvette. Do not dispose of the distilled water.

Collect Data

Note: Be sure to rinse the conductivity probes and the pH probes before and after each measurement in the following steps.

11. Start data recording.

12. Use the pH probe to measure the pH of the pickle juice and record the data in Table 1.

13. Use the conductivity probe to measure the conductivity of the pickle juice and record the data in Table 1.

Note: You may need to tip the cup to completely soak the conductivity sensor.

14. Use the pH probe to measure the pH of the distilled water and record the data in Table 1.

15. Use the conductivity probe to measure the conductivity of the distilled water, and record the data in Table 1.

16. Do not dispose of the pickle juice or the distilled water.

17. Clean the outside of the cuvette with lint free tissue.

18. Calibrate the colorimeter with the cuvette of distilled water.

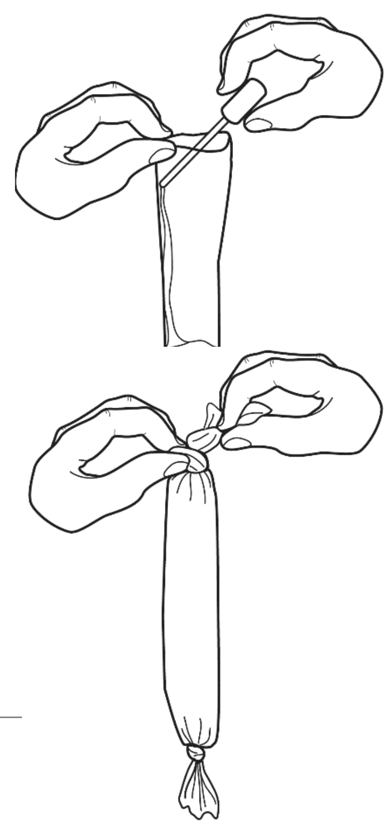
19. Use a lint-free tissue to clean the outside of the pickle juice cuvette.

20. Insert the cuvette into the colorimeter, close the top, measure the absorbance of blue light, and record the data in Table 1.

21. Stop data recording.

Part 2 – Post-soak

Set Up



22. Wash your hands, and then obtain a piece of dialysis tubing soaking in water.

23. Tie a half-knot in one end.

24. Open the other end of the dialysis tubing by rubbing it back and forth between your wet fingers. Be patient. Once you get the tube open, hold it open.

25. Using a disposable pipet, fill the dialysis bag with 15 to 20 mL of pickle juice. Be sure to leave enough room in the bag for expansion.

26. Why should room be left in the bag?

Water will diffuse into the bag by osmosis and the bag will fill. This should dilute the yellow dye # 5.

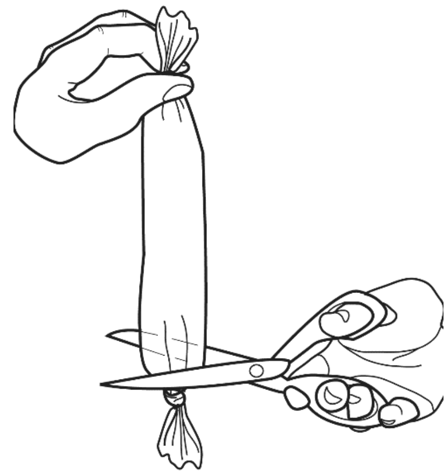
27. Tie off the open end of the bag with another half-knot.

If any excess dialysis tubing is exposed above or below your knots, cut it off with scissors. You should have 0.5 cm of dialysis tubing above and below each knot.

28. Thoroughly rinse the bag with distilled water, and set it on a piece of plastic wrap until you are ready to begin the experiment.

29. Why is it important that the bag be thoroughly rinsed?

If pickle juice is on the outside of the bag, the juice will easily diffuse into the cup and alter the results. We would not have any way of knowing if the change in the cup is caused by diffusion through the dialysis membrane or by “contamination”.



30. Place the bag containing pickle juice into the beaker or cup of water, and allow it to soak for 30 to 45 minutes (depending upon your instructor's instructions).

31. What do you think will happen to the pH of the solution in the dialysis bag and the solution in the beaker?

Answers will vary based on what students know about diffusion. Most will predict the pH will decrease in the cup and increase in the beaker.

32. Allow the incubation to finish and then remove the dialysis bag from the water. (Do not dispose of the water in the cup.)

33. Hold the dialysis bag over the "pickle juice" beaker, and carefully cut the bag open so the contents empty into the beaker.

34. Using a clean pipet, fill a cuvette with soaking fluid from the cup. Screw on the cap and clean the outside of the cuvette with lint free tissue.

35. Using a clean pipet, fill another cuvette with fluid from the dialysis bag. Screw on the cap and clean the outside of the cuvette with lint free tissue.

Collect Data

Note: Be sure to rinse the conductivity probes and the pH probes before and after each measurement.

36. Start data recording.

37. Insert the cuvette filled with soaking fluid into the colorimeter, close the top, measure the absorbance of blue light. Record the data in Table 1.

38. Remove the cuvette and replace with the cuvette filled with fluid from the dialysis bag. Close the top, measure the absorbance of blue light and record the data in Table 1.

39. Use the pH probe to measure the pH of the fluid in the beaker labeled "distilled water", and record the data in Table 2.

40. Use the conductivity probe to measure the conductivity of the same solution, and record the data in Table 2.

41. Use the pH probe to measure the pH of the fluid from the dialysis bag, and record the data in Table 2.

42. Use the conductivity probe to measure the conductivity of the fluid from the dialysis bag, and record data in Table 2.

43. Stop data recording.

44. Clean up your lab station, rinse all probes and cuvettes, and store the probes according to your instructor's instructions.

Data Analysis

Table 1: Pre-soak conditions

	pH	**Conductivity (µS/cm)**	**Abs Blue Light (480 nm)**
Distilled water	6.6	12	0
Pickle juice	4.1	30,566	0.769

Table 2: Post-soak conditions (30 minutes)

	pH	**Conductivity (µS/cm)**	**Abs Blue Light (480 nm)**
Contents of beaker	4.1	478	0
Contents of dialysis bag	4.2	18,214	0.682

1. Fill in Table 3 with the appropriate data from Tables 1 and 2.

2. Calculate the change in pH for the distilled water and the pickle juice. Show your work below and record your results in Table 3.

Change = Final – Initial

For example, the change in pH of distilled water = 4.1 – 6.6 = –2.5

3. Calculate the percent change in pH for the distilled water and the pickle juice. Show your work below and record your results in Table 3.

Percent change = Change in pH * 100 / Initial pH

For example, the percent change in pH for distilled water is 2.5 * 100 / 6.6 = 37.9%

Table 3: Change in pH

	Initial pH	**Final pH**	**Change in pH**	**Change in pH (%)**

Distilled water	6.6	4.1	–2.5	–37.9
Pickle juice	4.1	4.2	0.1	2.4

4. Fill in Table 4 with the appropriate data from Tables 1 and 2.

5. Calculate the change in conductivity for the distilled water and the pickle juice. Show your work below and record your results in Table 4.

Change = Final – Initial

For example, the change in conductivity of distilled water = 478 – 12 = 456 µS/cm

6. Calculate the percent change in conductivity for the distilled water and the pickle juice. Show your work below and record your results in Table 4.

Percent change = Change in conductivity * 100 / Initial conductivity

For example, the percent change in conductivity for distilled water is 466 * 100 / 12 = 3,883%

Table 4: Change in conductivity

	Initial Conductivity (µS/cm)	Final Conductivity (µS/cm)	Change in Conductivity (µS/cm)	Change in Conductivity (%)
Distilled Water	12	478	466	3,883
Pickle juice	30,556	18,214	–12,342	–40.4

7. Fill in Table 5 with the appropriate data from Tables 1 and 2.

8. Calculate the change in absorbance of blue light for the distilled water and the pickle juice. Show your work below and record your results in Table 5.

Change = Final – Initial

For example, the change in absorbance of pickle juice = 0.682 – 0.769 = –0.087

9. Calculate the percent change in absorbance of blue light for the distilled water and the pickle juice. Show your work below and record your results in Table 5.

Percent change = Change in absorbance * 100 ÷ Initial absorbance

For example, the percent change in absorbance for pickle juice is –0.087 * 100/0.769 = –11.3%

Table 5: Change in absorbance of blue light

	Initial Absorbance	Final Absorbance	Change in Absorbance	Change in Absorbance (%)
Distilled Water	0	0	0	0
Pickle juice	0.769	0.682	–0.087	–11.3

Analysis Questions

1. Which molecules was the dialysis membrane permeable to? Which way did these molecules diffuse: from the bag into the beaker, or from the beaker into the bag? What evidence do you have to support your claims?

Na^+ and Cl^- ions (from the salt) diffused through the membrane out of the bag and into the beaker. We know this because the conductivity in the bag decreased and the conductivity in the water increased.

The protons from dissociation of the acetic acid (vinegar) moved from the bag into the cup. We know this because the pH of the bag decreased and the pH of the water increased.

Also, water moved from the beaker into the bag. We know this because the dye seems to have been diluted during the experiment.

2. Was the membrane impermeable to any of the molecules? What evidence do you have to support your claims?

The membrane was impermeable to yellow dye #5, so it was not able to diffuse into the dialysis bag. The contents of the beaker did not absorb blue light at the end of the experiment, meaning there was no yellow dye in the beaker.

3. Did osmosis occur during the experiment? What evidence do you have to support your claim?

The absorbance of the contents of the dialysis bag decreased during the experiment. However, we know that the dye is too large to cross the membrane and we didn't see any yellow color in the distilled water, so the decrease in absorbance is not the result of less dye inside the bag. It must be due to dilution of the dye by water moving into the bag from the beaker.

4. What does it mean for a molecule to be in equilibrium? Does it mean that there are equal concentrations of a solute on both sides of the membrane?

Not necessarily. To be in equilibrium means that the molecules are moving from one side of the membrane to the other at equal rates. In a state of equilibrium, here is no net movement of molecules from one side to the other.

Synthesis Questions

Use available resources to help you answer the following questions.

1. How does the structure of biological membranes affect the rate of diffusion into or out of a cell?

The polar, hydrophilic nature of the outside of the cell membrane repels many nonpolar molecules. Therefore, these molecules do not diffuse through the membrane. Conversely, the hydrophobic core of the cell membrane slows or stops many small, charged molecules. Large molecules and many nonpolar molecules must pass through special channel proteins in order to diffuse into or out of a cell.

2. How would you change this experiment if you wanted to measure the rate of diffusion across the dialysis tubing?

Set up three dialysis bags of pickle juice and place them in three separate beakers of distilled water. Remove one bag after 10 minutes, one after 20 minutes and one after 30 minutes. Measure the pH and conductivity of the solutions in each beaker and determine which one reached equilibrium with the pickle juice first.

3. How do molecules that cannot diffuse easily across the phospholipid bilayer of a cell membrane get into or out of cells?

These molecules usually move (along their concentration gradient) through transmembrane proteins called channel or carrier proteins. When molecules move through these proteins, it is called facilitated diffusion. Some molecules must be actively transported across the membrane against their concentration gradients, a process that requires the use of the cell's energy currency, ATP.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following types of molecules are the major structural components of the cell membrane?

- **A.** Phospholipids and cellulose
- **B.** Nucleic acids and proteins
- **C. Phospholipids and proteins**
- **D.** Proteins and cellulose

2. The surface of an integral membrane protein would be best described as?

- **A. Hydrophilic**
- **B.** Hydrophobic
- **C.** Amphipathic (both hydrophobic and hydrophilic)
- **D.** Completely covered with phospholipids

3. Which of the following statements is correct about diffusion?

- **A.** It occurs very rapidly over long distances.
- **B.** It requires an expenditure of energy by the cell.
- **C. It is a passive process in which molecules move from a region of higher concentration to a region of lower concentration.**
- **D.** It is an active process in which molecules move from a region of lower concentration to one of higher concentration.

4. Glucose molecules diffuse slowly through artificial membranes. However, the cells lining the small intestine rapidly move large quantities of glucose from the glucose-rich food into their glucose-poor cytoplasm. Using this information, which transport mechanism is most probably functioning in the intestinal cells?

- **A.** Simple diffusion
- **B.** Phagocytosis
- **C.** Exocytosis
- **D. Facilitated diffusion**

Extended Inquiry Suggestions

Work with your students to perform serial dilutions of a 10% NaCl solution to make (1%, 0.1% and 0.001% NaCl solutions. Complete this experiment again, but use the NaCl solutions instead of pickle juice. Explore the relationship between the salt concentration and rate of diffusion out of the dialysis bag.

These experiments were performed at room temperature. Students can raise or lower the temperature of the experimental set up to observe how temperature affects diffusion.

4. Diffusion and Osmosis

Objectives

The concept of transport across membranes is fundamental to understanding cell structure and function. In this lab, students investigate diffusion, osmosis, and facilitated diffusion using artificial membranes, tissue cores, and individual cells. Students:

- Investigate the processes of diffusion and osmosis in model membrane systems
- Investigate the processes of osmosis in living plant tissue, and relate solute concentration to water potential
- Use light microscopy to observe osmosis in living cells
- Understand the concepts of diffusion and osmosis, and why they are important for understanding cell structure and function
- Observe the effects of solute size and concentration gradients on diffusion across a semipermeable membrane

Procedural Overview

By performing this laboratory, students should be able to:

- Use a conductivity sensor to determine the relative size of water molecules, solutes, and membrane pores
- Conceptualize molarity/solute concentration, and how it relates to osmotic potential of a solution
- Use experimental evidence to determine the sucrose concentration of unknown solutions
- Set up a controlled experiment to calculate the water potential of cells
- Understand the importance of water potential, and how it relates to solute concentration and pressure potential within a cell
- Use basic light microscopy skills to observe the effects of the movement of water into and out of plant cells
- Understand the following concepts: tonicity, plasmolysis, crenation, tugor, and cell lysis

Time Requirement

♦ Preparation time	45 minutes
♦ Pre-lab discussion and experiment	45 minutes
♦ Lab experiment - Part 1	30 minutes
♦ Lab experiment - Part 2	40 minutes
♦ Lab experiment - Part 3	20 minutes
♦ Lab experiment - Part 4	20 minutes

Materials and Equipment

For each student or group:

Part 1 – Diffusion

- Data collection system
- Advanced water quality sensor
- Conductivity probe
- Soaked dialysis tubing (2), 30 cm long
- Graduated cylinder, 25-mL
- Beaker (2), 50-mL
- Beaker (2), 250-mL
- Disposable pipets
- 2% IKI solution[1], 150 mL
- 2% salt, 2% IKI solution[2], 150 mL
- 1% starch solution[3], 30 mL
- Wash bottle for sensor
- Small funnel
- Dental floss or string
- Scissors

[1] To prepare 2% IKI solution, refer to the Lab Preparation section.
[2] To prepare 2% salt, 2% IKI solution, refer to the Lab Preparation section
[3] To prepare 1% starch solution, refer to the Lab Preparation section.

Part 2 – Osmosis

- Beakers or plastic cups (6), 250-mL
- Graduated cylinder, 25-mL
- Soaked dialysis tubing (6), 30 cm long
- Unknown sucrose solutions[4] (6)
- Distilled water
- Disposable pipets
- Electronic balance (one per class)
- Small funnel
- Plastic wrap
- Labeling marker and tape
- Scissors
- Dental floss or string

[4] To prepare sucrose solutions, refer to the Lab Preparation section.

Part 3 – Water potential

- ♦ Beakers or cups (6), 30-mL (for potatoes)
- ♦ Several medium-sized potatoes
- ♦ Unknown sucrose solutions
- ♦ Potato corers (0.5 cm is preferred)
- ♦ Electronic balance (one per class)
- ♦ Plastic wrap
- ♦ Knife
- ♦ Forceps
- ♦ Paper towels

Part 4 – Onion cell plasmolysis

- ♦ Microscope
- ♦ Red onion
- ♦ 15% NaCl solution[5]
- ♦ Disposable pipets
- ♦ Microscope slides and cover slips
- ♦ Distilled water
- ♦ Lint-free tissue

[5] To prepare NaCl solution, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

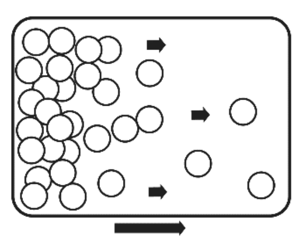
- ♦ Basic principles of free energy
- ♦ Solute versus solvent
- ♦ Solutions and molarity
- ♦ Basic principles of water potential
- ♦ Basics principles of diffusion and osmosis
- ♦ Basic cell structure (specifically organelles, cell membranes and cell walls)

Related Labs in This Guide

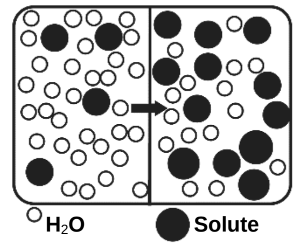
Labs conceptually related to this one include:

- ♦ Diffusion
- ♦ Exploring Surface Area to Volume Ratios

Background



Diffusion is the random movement of molecules from an area of higher concentration to an area of lower concentration. For example, if one were to open a covered dish containing a rotten fish in the front of the lab, students on the front row would quickly recognize the molecule dimethylamine with their nose. Students in the back of the lab would succumb to the smell a few seconds later. The diemethylamine gas is highly concentrated in the rotten fish carcass, and diffuses into the less concentrated corners of the laboratory until a dynamic equilibrium is reached. What is a dynamic equilibrium? It is when the concentration of dimethylamine is approximately equal throughout the lab, and there is no net movement of the molecule from one area to another.



Osmosis is a special case of diffusion. It is the diffusion of water through a selectively permeable membrane (a membrane that allows for diffusion of certain solutes and water) from a region of higher water potential to an area of lower water potential. Water potential is the measure of free energy of water in a solution. Recent research indicates that in many organisms (including plants) osmosis is actually a special case of facilitated diffusion. Water does move through the plant cell membrane, but water also moves more rapidly through membrane-bound channel proteins called aquaporins.

Diffusion and osmosis do not entirely explain the movement of ions or molecules into and out of cells, or across intercellular membranes. Some of that movement is attributed to active transport. This process uses the energy from ATP to move substances across a membrane against the concentration gradient, from an area of low concentration to area of higher concentration.

Pre-Lab Discussion and Experiment

1. How does the pore size of a semipermeable membrane affect solute transport?

In this experiment, your students will observe the movement (or lack of movement) of starch and glucose across dialysis tubing (a membrane with tiny pores). Small solutes (like IKI and glucose) and water should pass freely through the membrane, while larger molecules (like starch) will move more slowly (or not at all) through the membrane.

2. How can we tell if certain molecules have moved across the dialysis tubing?

Your students will use a conductivity sensor and the Lugol's iodine test to determine which molecules diffused across the dialysis tubing and which molecules did not diffuse across the dialysis tubing.

3. What is osmosis?

Osmosis is a special case of diffusion. Osmosis is the diffusion of water through a selectively permeable membrane (a membrane that allows for diffusion of certain solutes and water) from a region of higher water potential to an area of lower water potential. Water potential is the measure of free energy of water in a solution. The symbol for water potential is the Greek letter psi (ψ).

4. What do the terms hypertonic, hypotonic, and isotonic mean?

When two solutions have the same concentration of solutes, they are said to be isotonic to each other. If a selectively permeable membrane separates two solutions, water will move between the two solutions, but there will be no net change in the amount of water in either solution.

If two solutions differ in the concentration of solutes that each has, the one with more solute (less water) is hypertonic to the one with less solute. The solution with less solute is said to be hypotonic to the one with more solute. These terms are used to compare solutions, and can be applied to cells and the extracellular environment.

5. If solutions with different solute concentrations are separated by a selectively permeable membrane, which way will water flow?

Consider a hypertonic solution and a hypotonic solution separated by a selectively permeable membrane in which water can pass through the membrane, but the solute cannot. Water moves along a water potential gradient, and the hypotonic solution has a higher water potential than the hypertonic solution. Think of it this way: the hypertonic solution has more solute, and therefore less water; the hypotonic solution has less solute, and therefore more water. Teach students the following phrase: “water chases the solutes…until equilibrium is reached.” It is important to stress that we are predicting net movement of water. Some water molecules will move randomly across the membrane, against their concentration gradient, but most water molecules will diffuse along their concentration gradient.

6. What are some other ways to determine the sucrose concentrations of these solutions?

Ask students for ideas. Some might suggest tasting the solutions. Other ideas include, but are not limited to: finding the mass of the solutions, placing something in solutions to see which is the most dense, or looking at the opacity. All the solutions are colored, but some might look more “syrupy” than others.

7. Does water potential help us predict which way water will flow?

Water will always move from an area of higher water potential (higher free energy, more water molecules) to an area of lower water potential (lower free energy, less water molecules). Therefore, water potential measures the tendency of water to leave one place in favor of another place. Picture the water diffusing “down” a water potential gradient.

8. What two factors determine water potential?

Water potential is affected by two physical factors, pressure and solute concentration. Therefore, water potential has two components: a physical pressure component (Ψ_P –"pressure potential") and a solute concentration component (Ψ_S – "solute potential").

$\Psi = \Psi_P + \Psi_S$

Water potential = Pressure potential + Solute potential

Pressure potential is directly related to water potential, while solute potential is inversely related to pressure water potential. In other words, increasing the pressure within a cell increases the water potential of a cell. Conversely increasing the solute potential of a cell lowers the water potential of a cell.

By convention, the water potential of pure water at room temperature and sea level is defined as being zero (Ψ = 0). A 0.1 M sucrose solution at atmospheric pressure is –2.3 bars* because the pressure potential of this solution is 0 ($\Psi_P = 0$) while the solute potential is –2.3 ($\Psi_S = -2.3$)

*bars are a metric measure of pressure, measured with a barometer, which is about the same as 1 atmosphere. Another measure of pressure is a kilopascal (100 kPa = 1 bar).

9. How does water move across a cell membrane into an extracellular environment?

Water moves into and out of cells based on solute potential (relative solute concentration) on either side of the cell membrane. If water moves out of a cell, the cell can shrink—plasmolyze or crenate. If water moves into a cell, the cell can swell, become turgid, and, in the case of animal cells, potentially burst or lyse. In plant cells, the presence of a cell wall prevents cells from lysing as water enters the cell, but pressure builds up within the cell and affects the net movement of water. As water enters a dialysis bag or a cell with a cell wall, pressure builds up within the bag or cell, raising water potential, and slowing the net flow of water into the system. Adding solutes to this system will lower the water potential and increase the net flow of water into the system.

10. Does water potential have a value?

It is important to be clear about the numerical relationships between the water potential (Ψ), pressure potential (Ψ_P), and solute potential (Ψ_S). The water potential value can be positive, zero, or negative. Remember that water will move across a membrane in the direction of lower potential. An increase in pressure potential results in a more positive water potential value. A decrease in pressure potential (pulling or tension) results in a more negative water potential value. In contrast to pressure potential, solute potential is always negative. An increase in solute concentration of a system decreases water potential system.

11. Can you calculate the water potential of potatoes and of other plants?

Yes, once you determine the solute potential (ψ_S) of a sucrose solution using the formula below, you can then calculate the water potential of the potato (or any other plant cell). We will assume the pressure potential of the plant cell is zero ($\psi_P = 0$).

$\psi_S = -iCRT$

where i = ionization constant (for sucrose this is 1.0 because sucrose does not ionize in water)

C = molar concentration

R = pressure constant (R = 0.0831 liter bars/mole K)

T = temperature K (273 + °C of solution)

The units of measure will cancel as in the following example:

A 1.0 M sugar solution at 22 °C under standard atmospheric condition

$\psi_S = -i \times C \times R \times T$

$\psi_S = -$ (1) (1.0 mole/liter) (0.0831 liter·bars/mole·K) (295 K)

12. What is plasmolysis?

Plasmolysis is when plant cell membranes shrink away from the cell wall when placed in a hypertonic solution (high solute concentration). Crenation is the analogous process in animal cells. Turgor in plant cells, and cell swelling (and even cell lysis) are the reverse processes in plant and animal cells, respectively. Cells shrink and swell based on the tonicity of their extra cellular environment.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

Part 1 – Diffusion

1. 2% IKI solution:

   IKI (iodine/potassium iodide) solution is also known as Lugol's iodine solution. You can purchase it from any science supply company.

   To prepare 400 mL of 100% IKI solution from scratch, dissolve 3.0 g of potassium iodide (KI) in 400 mL of distilled water, add 0.6 g of iodine (I_2), and slowly stir. Store the solution in dark amber bottles. Pour the solutions into small amber dropper bottles, and you will always have IKI solution on hand!

   To make 1 L of 2% IKI solution, pour 20 mL of 100% IKI solution into a beaker, and add distilled or deionized water to make 1L. Store the solution in an amber colored bottle.

2. To make 1 L of 2% salt, 2% IKI solution, add 20 g of table salt to 20 mL of 100% IKI solution, and add distilled or deionized water to make 1 L. Store the solution in an amber colored bottle.

3. To prepare a 1% starch solution, add 2.0 g soluble starch to 200 mL of distilled or deionized water.

   Starch is not very soluble in water. If you have a hotplate/magnetic stirrer, it will speed up the process significantly. If not, bring the solution to a boil and stir often until all of the starch has dissolved. If you plan to keep the solution for longer than a week, store it in the refrigerator to prevent mold growth.

4. Cut the dialysis tubing into approximately 30-cm strips. Cut two for each group, plus a few extra, and soak the tubing in a cup or beaker of water. The tubing should soak for at least 30 to 60 minutes; it is easy to cut the tubing the day before the lab and let it soak overnight.

5. During the lab, your students might have trouble tying off the ends of the dialysis tubing. Show them how to tie a knot in the tubing. Don't stretch or pull the tubing too hard, as it may tear, which opens up unwanted, large pores in the membrane. If you still have trouble, try tying a knot with dental floss.

6. Soak the ends of the conductivity probes in distilled water for 5 to 10 minutes before the students begin collecting data.

Part 2 – Osmosis

1. Prepare "unknown" sucrose solutions: Make 1 L of each unknown sucrose solution (0.0 M; 0.2 M; 0.4 M; 0.6 M; 0.8 M; 1.0 M). If you don't purchase the pre-weighed bags of sugar, make 3 L of 1.0 M sucrose, and then dilute as follows. Remember to set aside 1 L of the 1.0 M solution.

   To make 1 L of 1.0M sucrose solution, dissolve 342.3 g of sucrose (cane (table) sugar) into 600 mL distilled water. Getting the sugar in the 1.0 M solution will be difficult. You may need to put it on a hot plate and stir until dissolved. After the sugar is dissolved, add distilled water to bring the final volume up to 1 L.

   To make the 0.8 M solution: add 200 mL of distilled water to 800 mL of the 1.0 M solution.

   To make the 0.6 M solution: add 400 mL of distilled water to 600 mL of the 1.0 M solution.

   To make the 0.4 M solution: add 600 mL of distilled water to 400 mL of the 1.0 M solution.

   To make the 0.2 M solution: add 800 mL of distilled water to 200 mL of the 1.0 M solution.

Instructor Tip: Don't label the bottles! Add a few drops of food coloring to each of the solutions to make each a different color. Make a note of the colors that correspond to each molarity. If you don't have food coloring, label the six bottles with a different color of tape, or label them with a different letter (A through F).

2. Cut the dialysis tubing into 20- to 30-cm strips. Cut 6 strips per group, plus a few extra. Soak the tubing in a cup or beaker of water. The tubing should soak for at least 30 to 60 minutes; it is easy to cut the tubing the day before the lab and let them soak overnight.

Part 3 – Water potential

Buy a 5-lb bag of potatoes. Refrigerate them until the day of the lab. Buying the potatoes in a 5-lb bag is a good way to make sure that the initial water potential of all of the potatoes is fairly consistent.

Part 4 – Onion cell plasmolysis

1. Purchase a red onion at the grocery store. You only need one onion for the whole class.
2. Prepare a 15% NaCl solution: add 30 g of NaCl to 200 mL of distilled water, and stir until dissolved.

Instructor Tip: Have enough red onion available for students to make several slide preps. It is unlikely they will get it right the first one or two times. It's easiest to take one layer of onion, break it against its natural curve, and then peel one side of the broken layer back. This should create a big enough tear in the red skin to hold it with forceps. Pull as much skin off the onion as possible, mount the skin on a microscope slide, and cover with a cover slip. Show your students once, and then let them try it. The layer of skin should look translucent to the naked eye, and it might even look translucent under the microscope.

Safety

Add these important safety precautions to your normal laboratory procedures:

- ♦ These exercises are "cable intensive." Make sure cords are wrapped or taped down and out of the way of hands and feet.
- ♦ None of the solutions should be tasted.
- ♦ Wear safety glasses and lab coats or aprons because iodine can stain your hands and clothing.
- ♦ Use caution when using cork borers and knives. Always bore or cut away from your hands, and onto a plate. Don't bore directly into your lab bench.

Procedure

Part 1 – Diffusion

1. Soak the end of the conductivity probe in distilled or deionized water for 5 to 10 minutes before you start collecting data.
2. Obtain two 250-mL beakers.
3. Label one "IKI" and the other "IKI/Salt."
4. Pour 150 mL of 2% IKI solution into the beaker labeled "IKI."
5. Pour 150 mL of 2% IKI/2% salt solution into the beaker labeled "IKI/Salt."
6. Record the color of the solutions in Table 1.1 and 1.2.
7. Obtain a 50-mL beaker and label it "starch."
8. Pour approximately 30 mL of 1% starch solution into the beaker.
9. Record the color of this solution in Table 1.1 and 1.2.
10. Start a new experiment on the data collection system.
11. Connect the conductivity sensor to the data collection system. Create a digits display of 10X Conductivity.

12. Use the conductivity probe to measure the conductivity of the three solutions (IKI, IKI/Salt, and Starch):

a. Select the appropriate conductivity range by pressing one of the three green buttons on the sensor prior to the start of data collection. For the IKI solution, choose the "flask" setting. For the salt/IKI solution, choose the "wave" setting. For the starch solution, choose the "cup" setting.

b. Begin data recording.

c. Place the end of the probe into the IKI solution and wait for the reading to stabilize. Record the reading in Table 1.1.

d. Rinse the probe with water before measuring another solution.

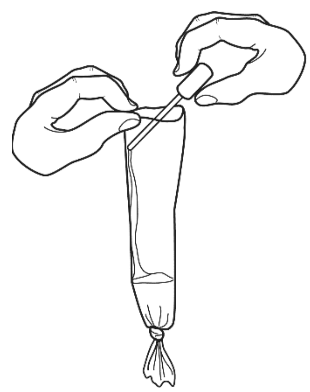
e. Repeat the data collecting and rinsing steps above for the IKI/Salt solutions (making sure to rinse the sensor in between measurements), and record the reading in Table 1.2.

f. Repeat the data collecting and rinsing steps above for the starch solution, and record the reading in Tables 1.1 and 1.2.

g. Stop data recording.

13. Obtain two pieces of dialysis tubing that have soaked in water.

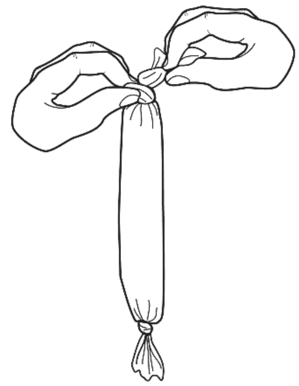
14. Tie a knot in one end of each piece of tubing to make two dialysis bags. If you cannot tie a knot in the bag, try using dental floss or string.



15. Pour approximately 15 mL of the 1% starch solution into one bag.

You do not have to pour exactly 15 mL into the bag. It is more important that you leave room in the bag for expansion and that you can tie the open end into a knot. If you cannot pour all of the solution into the bag, then you need a longer piece of tubing or slightly less starch solution.

16. Tie off the open end of the dialysis bag.



17. Repeat the steps above to fill and tie the second dialysis bag.

Again, it is not necessary to pour exactly 15 mL into the bag. However, it is important that the bag contains the same volume as the first bag. So, if you poured 13 mL into the first bag, pour 13 mL into the second bag.

18. Rinse the outside of the dialysis bags with water, and place one in the beaker of IKI solution and the other in the beaker of IKI/salt solution.

19. Let the bags soak in the water for approximately 15 minutes.

20. Why is it necessary to rinse the bag before placing it in the cup?

So that any starch or glucose solution that dripped on the bag doesn't react with the indicators in the beaker. The only color changes are caused by diffusion across the dialysis tubing.

21. Predict the movement of salt, starch, and IKI during this experiment.

Answers will vary. Students will be able to confirm their prediction in the data analysis section. Salt will move out of the bag into the beaker. Starch will not move. Iodine (IKI) will move into the dialysis bag.

22. Do you think the bag will change shape during the course of the experiment? Why or why not?

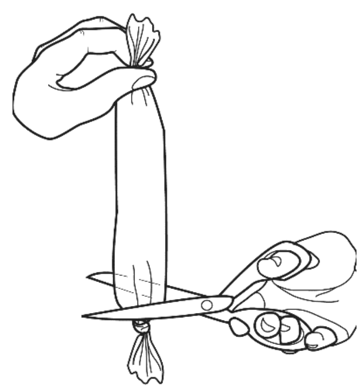
Answers will vary. If students think water will diffuse into the bag because of the higher solute concentration in the bag, then they might predict that the bag will swell.

23. After 15 minutes, observe the color in the beakers and the bags, and record your observations in Tables 1.1 and 1.2.

24. Remove the dialysis bags from the beakers, and rinse the outside of the bags with water.

25. Obtain two clean 50-mL beakers.

26. Label one "starch/IKI" and the other "Starch/IKI/Salt".



27. Cut carefully to open the dialysis bag that was soaking in IKI solution, and pour the contents into the cup you labeled 'starch/IKI".

28. Cut carefully to open the dialysis bag that was soaking in Salt/IKI solution, and pour the contents into the beaker you labeled "Starch/IKI/Salt".

29. Use the conductivity probe to measure the conductivity of the contents of the bag and the solutions in the beakers.

30. Record the conductivity of all four solutions in Tables 1.1 and 1.2.

31. Dispose of the contents of the cups and beakers as instructed by your instructor.

Part 2 – Osmosis

32. Obtain six strips of soaked dialysis tubing.

33. Tie a knot in one end of each strip to create a bag. If you cannot tie a knot in the bag, try using dental floss or string.

34. For each of the six unknown sucrose solutions, perform the following steps:

a. Use the graduated cylinder to obtain 15 mL of an unknown sucrose solution.

b. Fill one of the dialysis bags with the solution. You may want to use a funnel to help you fill the bag.

c. Tie off the open end of the dialysis tubing.

d. Rinse the outside of the bag with water, blot the outside of the bag with a paper towel, and place the bag on plastic wrap.

35. Why should you place the bags on plastic wrap as opposed to a paper towel?

The dialysis bag is placed on plastic wrap because it will not absorb water and create a situation in which water diffuses out of the bag and makes the sucrose more concentrated in the bag.

36. Find the mass of each of the six dialysis bags, and record the data in Table 1.3.

37. Obtain six 250-mL beakers or cups.

38. Fill each of them two-thirds full with distilled water.

39. Place each of the dialysis bags into a separate beaker or cup, making sure the bags are completely submerged. Label the beaker or cup in such a way that you know which unknown solution was placed in each beaker or cup.

40. Can you determine the sucrose concentrations of the unknown solutions if you don't label the cups?

No, because you would not remember which unknown went with which color. It is imperative to label the beakers or cups so you can identify the different unknown solutions.

41. Let the dialysis bags soak for 30 minutes.

42. Remove the bags after 30 minutes, and blot them quickly with a paper towel.

43. Find the mass of each of the bags again, and record data in Table 1.3.

44. Why would you use the same balance that you used for the initial mass?

Each balance is calibrated differently. If you used a different balance, it may indicate an incorrect mass relative to the original balance. This value could make the percent change in mass calculation inaccurate, and if the percent change in mass calculations are inaccurate, you may not be able to properly identify the unknowns.

Part 3 – Water potential

45. Your instructor will assign your group 2 of the 6 sucrose solutions.

46. Use a graduated cylinder to measure 20 mL of each of your assigned sucrose solutions.

47. Pour each sucrose solution into separate beakers or cups. Be sure to label the beaker or cup.

48. Obtain a medium-sized potato.

49. Using a knife, peel the potato.

50. Cut the peeled potato into smaller, equal-sized chunks.

51. Use a cork borer and a metric ruler to cut 4 potato cores for each cup. The potato cores should be 5 mm in diameter and 3 cm long.

52. Cover the potato cores with plastic wrap or a damp paper towel.

53. Why should you use long and skinny potatoes instead of cubes or spheres?

The cylinder has a higher surface area to volume ratio than spheres or cubes. Therefore, there is greater surface area for water to diffuse into and out of the cells.

54. Why should you keep the potatoes covered? What would happen to the water potential if the potatoes were allowed to dehydrate?

Covering the potatoes keeps the system “closed.” If potatoes dehydrate, then their water potential will decrease.

55. Find the mass of 4 potato cores at a time, and record the values in Table 1.5.

56. Place 4 cores into each of your cups of sucrose solution.

57. Cover the cups with plastic wrap, and let the cores soak overnight. Do not let the cores soak for more than 24 hours.

58. Remove the cores from the solution within 24 hours, blot them quickly on a paper towel, and find the mass of all four cores again.

59. Why is it necessary to blot the potatoes?

Some of the solutions are isotonic (or close to isotonic) to the potatoes. Any water clinging to the outside of the potato cores will be weighed along with the potato cores. This will yield inaccurate results and make it difficult to accurately determine the sucrose concentration of the potatoes.

60. Record the final masses in Table 1.5.

61. Talk to other groups, and record class data in Table 1.6.

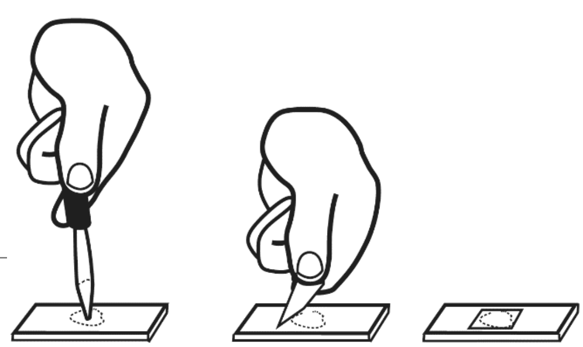
62. Why do you need class averages?

Some groups might have inaccurate data or “outliers.” Averaging all the trials for each group will yield more accurate data, or allow for easier determination of the sucrose concentration of the potatoes.

Part 4 – Onion cell plasmolysis

63. Prepare a wet mount of a small piece of the epidermis of an onion. To do this:

a. Obtain a thin slice of onion skin from your instructor, and use forceps to

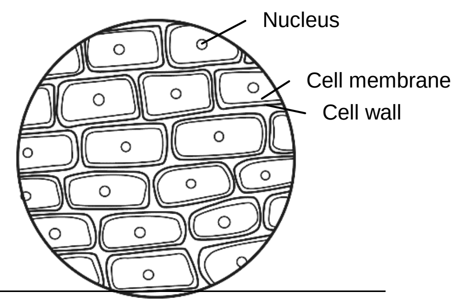


carefully place the onion skin on a clean microscope slide. Flatten the onion skin so that it is laying flat on the slide.

b. Place one drop of water over the onion skin, and put the cover slip over the drop of water.

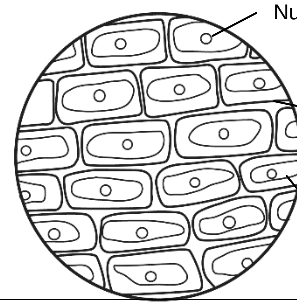
c. Use a piece of paper towel to blot any excess water from the slide.

64. With a compound light microscope, observe the cells under 100 x magnification. Look for the cell wall, the cell membrane, and the nucleus. Label them in your drawing. Use the circle for drawing the onion cells and the lines for describing what you see.

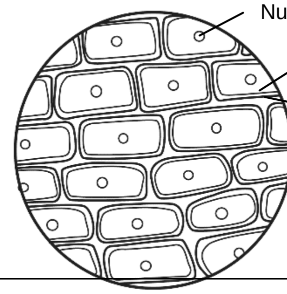


65. Add 2 or 3 drops of 15% NaCl to the edge of the cover slip. Draw the salt solution across the slide by touching a piece of paper towel to the opposite side of the cover slip.

66. Sketch the onions cells as they appear now. Describe what you see, and explain in the space below what happened.



67. Can you return the cells to a turgid state? What did you do to increase the water potential inside the cells? Describe what you did, and why you think it worked in the space provided. Draw the cells now that you have performed your procedure.



Data Analysis

Table 1.1: IKI solution

	Color of solution		Conductivity (µS/cm)		Change in conductivity (µS/cm)	Change in conductivity (%)
	Initial	Final	Initial	Final		
Starch solution in bag	Colorless and cloudy	Dark blue	68	123	+55	+80.8
IKI solution in beaker	Brownish-yellow	Brownish-yellow	480	380	–100	–20.8

Table 1.2: IKI/salt solution

	Color of solution		Conductivity (µS/cm)		Change in conductivity (µS/cm)	Change in conductivity (%)
	Initial	Final	Initial	Final		
Bag	Colorless and cloudy	Blue	68	427	+359	+527.9
Beaker	Brownish-yellow	Brownish - yellow	30,000	28,800	–1,200	–4.00

Table 1.3: Individual group determination of sucrose solutions

Contents in dialysis bag (color)	Initial mass (g)	Final mass (g)	Mass difference (g)	Change in mass (%)*	Concentration of sucrose (M)
Solution 1 (green)	15.8	15.7	–0.1	–0.63	0.0
Solution 2 (red/green)	15.5	15.6	0.1	+0.64	0.2
Solution 3 (red/blue)	17.5	18.4	0.9	+5.1	0.4
Solution 4 (red)	16.3	17.8	1.5	+9.2	0.6
Solution 5 (blue)	15.7	17.3	1.6	+10.2	0.8
Solution 6 (white)	16.1	19.1	3.0	+18.6	1.0

Table 1.4: Class determinations of sucrose solutions

Contents in dialysis bag (color)	Change in mass of dialysis bags (%)						[Sucrose] (M)
	Group 1	Group 2	Group 3	Group 4	TOTAL	Class average	
Solution 1 (green)	+1.61	–1.30	-0.79	–0.91	–1.39	–0.35	0.0
Solution 2 (red/green)	+1.12	+1.81	+0.92	+1.47	+5.32	+1.33	0.2
Solution 3 (red/blue)	+6.42	+8.50	+4.90	+8.72	+28.5	+7.14	0.4
Solution 4 (red)	+10.3	+8.85	+8.90	+13.2	+41.2	+10.3	0.6
Solution 5 (blue)	+11.8	+17.5	+10.2	+8.53	+48.0	+12.10	0.8
Solution 6 (white)	+16.4	+14.4	+18.6	+21.8	+71.2	+17.8	1.0

Table 1.5: Potato core: individual data

Contents in cup (color) and [sucrose] (M)*	Initial mass (g)	Final mass (g)	Mass difference (g)	Change in mass (%)
Solution 1 (green) (0.0)	5.92	6.26	+0.34	+5.74
Solution 2 (red/green) (0.2)	6.07	6.66	+0.59	+9.87
Solution 3 (red/blue) (0.4)	6.61	6.87	+0.26	+3.93
Solution 4 (red) (0.6)	5.78	5.06	–0.72	–12.5
Solution 5 (blue) (0.8)	6.12	4.60	–1.52	–24.8
Solution 6 (white) (1.0)	5.99	4.16	–1.83	–30.6

Table 1.6: Potato core results: class averages

Contents in cup (color) and [sucrose] (M)*	Change in mass of potato cores (%)**				
	Group 1	Group 2	Group 3	Group 4	Class average
Solution 1 (green) (0.0)	–2.92	+12.5	+5.90	+5.78	+5.32
Solution 2 (red/green) (0.2)	+16.0	+14.8	+22.7	+9.70	+15.8
Solution 3 (red/blue) (0.4)	+2.92	+2.97	+10.0	+3.91	+4.95
Solution 4 (red) (0.6)	–12.9	–13.9	–3.53	–12.5	–10.7
Solution 5 (blue) (0.8)	–24.2	–23.5	–12.3	–24.9	–21.2
Solution 6 (white) (1.0)	–27.9	–32.4	–31.7	–30.1	–30.5

*These sucrose concentrations should have been determined in Part 2, and these data should be entered from lowest sucrose concentration (distilled water) to highest sucrose concentration (1.0 M).

**Notice how variable the percent change in mass is, especially between 0.0 and 0.2, and 0.2, and 0.4 M sucrose. This occurs for three reasons: 1) the potatoes have a different sucrose concentration, especially if they have dried out, 2) students might “over-dry” / “over-blot” their potato cores before obtaining final mass, showing a greater loss in mass than might be expected, 3) 0.2 and 0.4 M are almost isotonic to the potatoes, therefore the net flow of water varies from one group to another. The dialysis bag data is usually much more definitive. The types of variability and error that occur in this experiment present opportunities for you to teach your students about experimental design, evaluating evidence, and averaging data.

1. Use the data in Table 1.1 and 1.2 to calculate the change in conductivity and the percent change in conductivity for both solutions. Record your results in Tables 1.1 and 1.2.

Change in conductivity = final conductivity – initial conductivity

% change in conductivity = [(change in conductivity) / initial conductivity] X 100

Change in conductivity in bag (IKI): 123-68 = +55
Change in conductivity in beaker (IKI): 380-480 = -100
Change in conductivity in the bag (IKI/salt): 427-88 = +359
Change in conductivity in the beaker (IKI/salt): 28,800 – 30,000 = -1,200

% change in conductivity in bag (IKI): (55 / 68) x 100 = +80.8%
% change in conductivity in beaker (IKI): (-100 / 480) x 100 = -20.8%
% change in conductivity in bag (IKI/salt): (359/ 68) x 100 = +527.9%
% change in conductivity in beaker (IKI/salt): (-1,200 / 30,000) x 100 = -4.00%

2. Use the data in Table 1.3 to calculate mass difference and percent change in mass for each of the six unknown solutions. Record your results in Table 1.3.

Change in mass = final mass – initial mass

% change in mass = [(change in mass) / initial mass] X 100

Change in mass Solution 1: 15.7 – 15.8 = -0.1g
Change in mass Solution 2: 15.6 – 15.5 = 0.1g
Change in mass Solution 3: 18.4 – 17.5 = 0.9g
Change in mass Solution 4: 17.8 – 16.3 = 1.5g
Change in mass Solution 5: 17.3 – 15.7 = 1.6g
Change in mass Solution 6: 19.1 – 16.1 = 3.0g

% Change in mass Solution 1: (-0.1 / 15.8) x 100 = -0.63%
% Change in mass Solution 2: (0.1 / 15.5) x 100 = +0.64%
% Change in mass Solution 3: (0.9 / 17.5) x 100 = +5.1%
% Change in mass Solution 4: (1.5 / 16.3) x 100 = +9.2%
% Change in mass Solution 5: (1.6 / 15.7) x 100 = +10.2%
% Change in mass Solution 6: (3.0 / 16.1) x 100 = +18.6%

3. Use the percent change in mass to determine the sucrose concentration of each unknown solution. Record your claims in Table 1.3.

Increased % change in mass is correlated with an increase in the solution's molarity, so the solution with the greatest % change in mass is the solution with highest molarity. The solution with the lowest % change in mass is distilled water

Solution 6 had a +18.6% change, so it is 1.0 M sucrose.

Solution 5 had a +10.2% change, so it is 0.8 M sucrose.

Solution 4 had a +9.2% change, so it is 0.6 M sucrose.

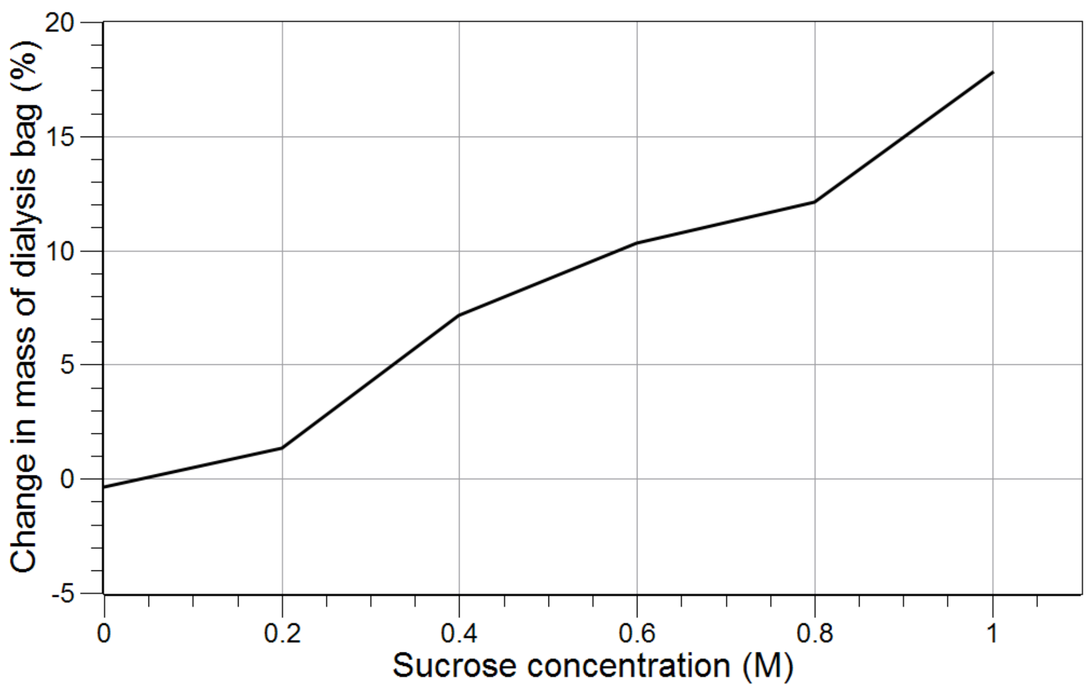
Solution 3 had a +5.1% change, so it is 0.4 M sucrose.

Solution 2 had a +0.64% change, so it is 0.2 M sucrose.

Solution 1 had a -0.63% change, so it is distilled water.

4. Gather data from other groups, and record it in Table 1.4. Get a class average % change in mass for each solution.

5. Draw Graph 1.1. Plot the data from Table 1.4 on a graph showing "% change in mass of the dialysis bag (class average)" on the y-axis versus "sucrose concentration (M)" on the x-axis. Label the overall graph, the x- and y-axes, and include units on the axes.



6. Calculate the mass difference and the percent change in mass of the potato cores. Complete this calculation using your own data from Table 1.5 as well as the class data in Table 1.6.

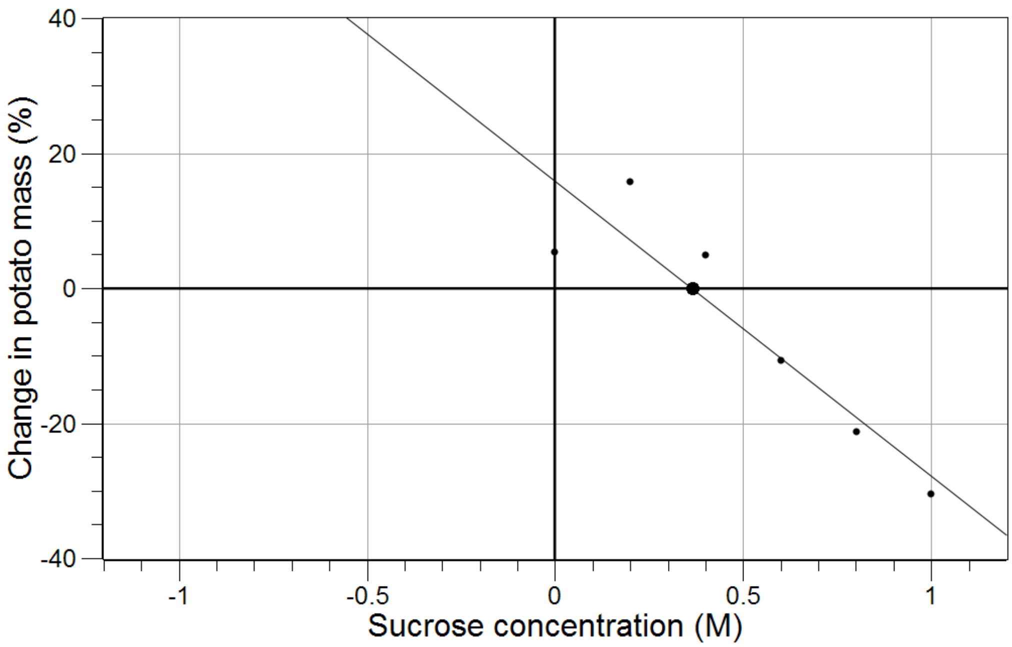
Change in mass = final mass – initial mass

% change in mass = [(change in mass) / initial mass] X 100

Change in mass Solution 1: 6.26– 5.92= +0.34g
Change in mass Solution 2: 6.66 – 6.07 = +0.59g
Change in mass Solution 3: 6.87 – 6.61 = +0.26g
Change in mass Solution 4: 5.06 – 5.78 = -0.72
Change in mass Solution 5: 4.60 – 6.12 = -1.52
Change in mass Solution 6: 4.16 – 5.99 = -1.83

% Change in mass Solution 1: (0.34/ 5.92) x 100 = 5.74%
% Change in mass Solution 2: (0.59/ 6.07) x 100 = +9.87%
% Change in mass Solution 3: (0.26 / 6.61) x 100 = +3.93%
% Change in mass Solution 4: (-0.72/ 5.78) x 100 = -12.5%
% Change in mass Solution 5: (-1.52 / 6.12) x 100 = -24.8%
% Change in mass Solution 6: (-1.83/ 5.99) x 100 = -30.6%

7. Draw Graph 1.2. Plot the data from Table 1.6 on a graph of "% change in mass of the potato cores (class average)" on the y-axis versus "sucrose concentration (M)" on the x-axis. Label the overall graph, the x- and y-axes, and include units on the axes. Use a key to identify the sucrose solutions.



8. Use Graph 1.2 to determine the sucrose concentration (molarity) of the potato cores.

This is the sucrose molarity in which the mass of the potato cores does not change. To find this, have your students draw a line of best fit for the data they generated. The point at which the line crosses the x-axis represents the molar concentration of sucrose with a water potential that is equal to the potato tissue water potential. At this concentration there is no net gain or net loss of water from the tissue.

Analysis Questions

1. Using the data that you collected in the diffusion experiment, explain why there was a difference in the percent change in conductivity of the dialysis bag in the IKI solution compared with the bag in the IKI/Salt solution.

There are significantly more ions in the IKI/salt solution than in the IKI solution. Because there are more ions in the IKI/salt solution than in the IKI solution, there is a larger concentration gradient. The larger concentration difference drives more ions across the membrane, leading to a greater conductivity change in the solution.

2. What effect did increased sucrose concentration have on the mass of the dialysis bags in Part 2?

As the sucrose concentration in the bag increases, the change in mass increases. Bags containing lower sucrose concentration had little change in mass, while bags containing more sucrose had a larger change in mass. Students should highlight specific values from their runs, or class averages in their answer.

3. Why did you calculate the percent change in mass rather than simply using the change in mass?

All the bags used in this experiment had a different initial mass. Smaller bags, or bags with less fluid, are likely to gain more mass over time. By calculating percent change in mass, it is possible to compare results within each group and compare results across trials regardless of the initial mass of any of the bags.

4. How are plasmolysis and water potential related? Describe an example where plasmolysis would occur in a plant.

As water potential decreases in the environment surrounding plant cells, both in the soil or in the atmosphere, plant cells will lose water to the environment and the plant cells will plasmolyze. Plasmolysis will occur in a potted plant that has been left on a sunny porch without water for a week.

5. What do you think would happen if you tried this same procedure with red blood cells (RBC) or human cheek cells? Be sure to explain your response in terms of tonicity and use the proper terms for cell shrinking or swelling or bursting.

Red blood cells or human cheek cells are hypotonic to 15% saline solution and will shrivel, or crenate in the high salt solution. These cells are hypertonic compared with distilled water, therefore they will swell and potentially lyse when flooded with distilled water.

Synthesis Questions

Use available resources to help you answer the following questions.

1. What if your instructor presented you with results of an experiment similar to the Diffusion experiment, but the bag was filled with dark purple liquid and the beaker contained a pale yellow fluid. Can you explain what the initial fluids in both the cup and the bag were? Explain what happened during the experiment.

The cup contained starch, and the bag contained water and IKI. The IKI diffused out of the bag along its concentration gradient, turning the starch in the cup blue. The starch did not diffuse into the bag because it was too large to fit through the pores in the bag.

2. How would your diffusion results have been different if you didn't leave enough room in the bag for expansion during the experiment?

Determining the sucrose concentration of the 0.8M and 1.0 M would have been difficult because there might not be enough room for water to osmose into the bags before equilibrium was reached between the bag and the beaker.

3. If a potato core were allowed to dehydrate by sitting in the open air, would the water potential of the potato cells increase or decrease? Why?

The water potential would decrease because the solutes would become concentrated within the cells, and there would be less water in the cells.

4. What adaptations do plants have to deal with hypertonic soils?

Answers will vary, but one good example would be: root hairs can actively transport solutes into the cells to decrease water potential in those cells so that water will diffuse out of the soil and into the roots by osmosis.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following types of molecules are the major structural components of the cell membrane?

- **A.** Phospholipids and cellulose
- **B.** Nucleic acids and proteins
- **C. Phospholipids and proteins**
- **D.** Proteins and cellulose
- **E.** Glycoproteins and cholesterol

2. Which of the following statement is correct about diffusion?

- **A.** It is very rapid over long distances.
- **B.** It requires an expenditure of energy by the cell.
- **C. It is a passive process in which molecules move from a region of higher concentration to a region of lower concentration.**
- **D.** It is an active process in which molecules move from a region of lower concentration to one of higher concentration.
- **E.** It requires integral proteins in the cell membrane.

3. Water passes quickly through cell membranes because

- **A.** The bilayer is hydrophilic.
- **B.** It moves through hydrophobic channels.
- **C.** Water movement is tied to ATP hydrolysis.
- **D.** It is a small, polar, charged molecule.
- **E. It moves through aquaporins in the membrane.**

4. A patient has had a serious accident and has lost a significant amount of blood. In an attempt to replenish body fluids, distilled water, equal to the volume of blood lost, is transferred directly into one of his veins. What will be the most probable result of this transfusion?

- **A.** It will have no unfavorable effect as long as the water is free of viruses and bacteria.
- **B.** The patient's red blood cells will shrivel up because the blood fluid is hypotonic compared to the cells.
- **C. The patient's red blood cells will swell because the blood fluid is hypotonic compared to the cells.**
- **D.** The patient's red blood cells will shrivel up because the blood fluid is hypertonic compared to the cells.
- **E.** The patient's red blood cells will burst because the blood fluid is hypertonic compared to the cells.

5. Celery stalks that are immersed in fresh water for several hours become stiff and hard. Similar stalks left in a salt solution become limp and soft. From this we can deduce that the cells of the celery stalks are

- **A.** Hypotonic to both fresh water and the salt solution.

B. Hypertonic to both fresh water and the salt solution.

C. **Hypertonic to fresh water but hypotonic to the salt solution.**

D. Hypotonic to fresh water but hypertonic to the salt solution.

E. Isotonic with fresh water but hypotonic to the salt solution.

Extended Inquiry Suggestions

Decalcify two dozen eggs (12 eggs/1 gallon of vinegar soaking for 48 hours), and have lab groups use the decalcified eggs to determine the sucrose concentration of four unknown solutions ranging from 0.0 M to 2.0 M in 0.5 M increments. The methods can vary, but the experiment must be quantitative. Students need to identify their independent and dependent variables and their controls (if they have one). They should generate a hypothesis, predict their results, and report their data in table and graphic form. A one-page write up should suffice.

Try this same experiment with different foods or different solutions. You can also experiment with different shapes of potato cores. This will result in discussions about surface area and volume, and how that relationship affects diffusion rates.

Use the microscopes to observe the dialysis tubing. Can you see the pores? Are they uniform in size?

Use the microscopes to observe a thin section of potato tissue. Record your observations. Next, place a drop of iodine next to the potato tissue, and draw the fluid across the slide. Can you explain the results? How do potatoes store excess carbohydrate?

5. Enzyme Catalysis

Objectives

In this lab, students learn about the enzyme catalase and how factors like temperature and pH affect its activity. Students:

- ♦ Observe the conversion of hydrogen peroxide to water and oxygen gas by the enzyme catalase
- ♦ Measure the amount of oxygen generated and calculate the rate of the enzyme-catalyzed reaction
- ♦ Explore the effects of pH and temperature on the rate of the enzyme-catalyzed reaction

Procedural Overview

Students will gain experience conducting the following procedures:

- ♦ Measuring and analyzing oxygen gas production as the result of the decomposition of hydrogen peroxide under six conditions: spontaneously, in the presence of the enzyme catalase, increased environmental pH, decreased environmental pH, increased environmental temperature, and decreased environmental temperature
- ♦ Calculating the rate of oxygen production for each data run

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	60 minutes [1]

[1] You should attempt to complete this lab in one lab period. To shorten the required time, you can reduce the data collection time by half, or have each student group complete a different part and then share their data with the class.

Materials and Equipment

For each student or group

- ♦ Data collection system
- ♦ Oxygen gas sensor
- ♦ Graduated cylinders (2), 10-mL and 25-mL
- ♦ 1.5% hydrogen peroxide (H_2O_2), 100 mL
- ♦ 1.5% hydrogen peroxide (H_2O_2), chilled, 20 mL
- ♦ Activated yeast suspension, 30 mL[1]
- ♦ Activated yeast suspension, boiled, 10 mL[1]
- ♦ Activated yeast suspension, chilled, 10 mL[1]
- ♦ 1.0 M sodium hydroxide (NaOH), 10 mL
- ♦ 1.0 M hydrochloric acid (HCl), 10 mL
- ♦ Sampling bottle
- ♦ Saltine crackers, 1 per student
- ♦ Large beaker of ice

[1] To formulate room temperature, boiled, and chilled activated yeast suspensions, refer to the Lab Preparation Section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ The general functions and activities of enzymes
- ♦ The relationship between the structure and function of enzymes
- ♦ The concept of initial reaction rates of enzymes
- ♦ How the concept of free energy relates to enzyme activity
- ♦ That changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Exploring the Effects of pH on Amylase Activity

Background

Enzymes are naturally occurring, complex, proteins, found in living cells. Enzymes function as organic catalysts, helping to speed up reactions. Like catalysts in other chemical reactions, enzymes are not consumed during the reaction, but rather help turn the substrate into the final product. Once the enzyme releases the final product, the enzyme can pick up additional substrate and catalyze another reaction. Enzymes are substrate-specific. Only one type of molecule can fit into the active site of the enzyme and have the reaction proceed.

Many factors affect how well enzymes function. All enzymes have an optimal temperature and pH at which they have maximum efficiency. Enzymes found in most human cells typically work best at 37 °C and pH 7. Enzymes in the acidic environment of the stomach adapted to work best at pH 2. You will find some enzymes in virtually all living organisms. These enzymes have a wide range of optimal temperatures and pH. When an enzyme's environment strays too far away from its optimal pH and temperature, the enzyme's protein structure unravels, and it no longer functions. This process is called denaturation. Once an enzyme becomes denatured, it never returns to its original confirmation and never functions properly again.

Enzymes have varied functions within human cells. For example, the enzyme lactase catalyzes the breakdown of the sugar lactose into its component sugars, galactose and glucose. In order for digestion of lactose (milk sugar) to occur, lactase must be present. People with a lactase deficiency were born without the gene to produce the enzyme lactase and cannot digest lactose. Another important example of enzyme function is the enzyme catalase, an enzyme produced by most living cells. It protects the cell from the damaging effects of hydrogen peroxide by splitting it into water and oxygen gas.

$$H_2O_2 \rightarrow H_2O + O_2(g)$$

Pre-Lab Discussion and Experiment

Give each student a saltine cracker. Ask them to place the saltine cracker in their mouth and chew exactly three times. After chewing three times, instruct them not to chew anymore. They should simply let the saltine sit in their mouth. After several minutes, the students will notice that the saltine is liquefying in their mouth.

1. Why is the saltine being digested even though it is not being chewed?

Guide the students to the conclusion that there must be a chemical within their saliva digesting the saltine.

Saliva contains an enzyme called salivary amylase, which begins the chemical digestion of starch within the mouth. The process of chewing breaks the food down into smaller pieces to increase the surface area of the food. This maximizes the contact between the food and the enzyme.

You can optionally demonstrate what occurs when you place a few drops of hydrogen peroxide on a small piece of liver or potato. Let the students observe, and ask them to formulate an explanation about what they see.

2. Why does it bubble and fizz when you pour hydrogen peroxide on a cut?

Guide students to the conclusion that most living cells contain the enzyme catalase.

When catalase comes into contact with hydrogen peroxide, it breaks the H_2O_2 molecule down into oxygen gas and water. The bubbles that you observe on the cut are the oxygen gas. Human cells supply some of the catalase. Bacteria and yeast that live naturally on the skin also supply some catalase. Two very important groups of bacteria live naturally on the human skin, staph and strep. Staph bacteria can produce catalase to protect themselves from hydrogen peroxide, but strep bacteria cannot. So, using hydrogen peroxide to clean a cut will kill most, but not all bacteria.

3. Describe the primary, secondary, tertiary, and quaternary structure of proteins.

The primary structure of a protein is its unique sequence of amino acids. The DNA sequence on a particular gene determines the primary structure of a protein. Even a slight change in primary structure can affect a protein's conformation and ability to function.

The secondary structure consists of coils and folds that result from hydrogen bonds at regular intervals along the polypeptide backbone. Typical shapes that develop from secondary structure are coils (an alpha helix) or folds (beta pleated sheets). The alpha helix is a coil held together by an hydrogen bond at every fourth amino acid. The beta pleated sheet is 2 or more regions of the polypeptide chain that lie parallel to each other, held together by hydrogen bonds.

Irregular contortions of the secondary structure resulting from interactions between the amino acid form the tertiary structure. Tertiary structure is determined by interactions among R groups (side chains) and between R groups and the polypeptide backbone. These interactions include hydrogen bonds among polar and/or charged areas, ionic bonds between charged R groups, hydrophobic interactions among R groups, van der Waals interactions among hydrophobic R groups, and disulfide bridges. Disulfide bridges are strong covalent bonds that form between the sulfhydryl groups (SH) of cysteine monomers. These bonds stabilize the tertiary structure.

Quaternary structure results from the aggregation of two or more polypeptide subunits. Quaternary structures may be fibrous and rope-like, such as collagen or globular-like hemoglobin.

4. Why is the structure of an enzyme (its shape) essential to its function?

When a substrate binds to an enzyme, the enzyme catalyzes the conversion of the substrate to the product. The active site of an enzyme is a pocket or groove on the surface of the protein into which the substrate fits. The specific fit between the active site and that of the substrate allows the enzyme and substrate to conform and react. We call this enzyme specificity. Changes in shape influence the reaction rate.

5. Predict how pH and temperature will affect enzyme activity.

Some conditions lead to the most active conformation and lead to optimal rate of reaction. Other conditions change the shape of the active site, rendering it dysfunctional. Temperature greatly influences reaction rate. As temperature increases, collisions between substrates and active sites occur more frequently because molecules move faster. However, at some point, thermal agitation begins to disrupt the weak bonds that stabilize the protein's active conformation, and the protein denatures. Each enzyme has an optimal temperature. Because pH also influences shape (and therefore reaction rate), each enzyme also has an optimal pH. The optimal range of pH falls between pH 6 and 8 for most enzymes. However, digestive enzymes in the stomach work best at pH 2 while those in the intestine prefer pH 8. Both optimal pH ranges match their working environments.

6. Discuss denaturation.

An enzyme in an environment where temperature, pH, or salinity is significantly above or below its optimum will begin to lose its confirmation. The quaternary structure will fall apart, followed by the tertiary and secondary structure. Depending upon the environment, the protein could revert back to its primary structure. Most enzymes cannot return to their active confirmation after denaturation.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare a room-temperature activated yeast suspension by starting a culture of active yeast approximately 30 minutes before class begins. To do this for a class of eight groups:

a. Add approximately 600 mL of warm water (30 to 35 °C) to 4 packages of dry yeast.

b. Add 4 g of sucrose (table sugar), and mix well to assure that the yeast is thoroughly wetted. The yeast will become active in 5 to 10 minutes and will begin to metabolize the sugar you have added. The suspension will be suitable for use for the remainder of the day, but you should not store it overnight.

If you need to make yeast solutions for more than one class, make a larger batch by adjusting the recipe accordingly. To ensure maximum yeast activity for each class, mix the yeast and water at the start of the day, but initially only add 1 gram of sugar. Add 1 more gram of sugar 10 to 15 minutes before each class. This will ensure that each class uses yeast with the same level of activity. Once you have made the initial solution, there is no need to maintain the yeast at 30 to 35 °C. However, make sure that the temperature of the yeast does not fall below 20 °C or rise above 45 °C.

Note: Activated yeast solution is an easy and inexpensive alternative to catalase. If you do not want to use a yeast solution, you can order catalase from a biological supply company.

2. Prepare the boiled yeast suspension. For a class of eight groups:

a. Place 100 mL of the yeast suspension into a test tube after the yeast has become active.

b. Place the test tube into a beaker of boiling water, and allow it to heat for 10 minutes.

c. Remove the test tube from the hot water, and place it in a test tube rack to cool.

d. Label the test tube, "boiled yeast". It may sit out at room temperature until ready for use.

3. Prepare cold yeast suspension. For a class of eight groups:

a. Place 100 mL of the yeast suspension into a small beaker after the yeast has become active.

b. Label the beaker, "chilled yeast".

c. Place the small beaker into a larger beaker full of ice. Keep the beaker of ice and yeast in the refrigerator until ready for use.

d. Make sure that the yeast stays chilled during the lab by adding ice cubes to the water around the yeast.

4. Your hydrogen peroxide will most likely be a 3% solution. If so, dilute the hydrogen peroxide to 1.5% by using a 1:1 ratio of distilled water to hydrogen peroxide.

5. After diluting the hydrogen peroxide, pour 20 mL per group into a beaker and place on ice (or in the refrigerator) to chill.

6. To reduce the amount of glassware used, do not give each group their own graduated cylinders. Set up one station where students can get their yeast suspension, and place 1 graduated cylinder at that station. Set up one station where students can get their hydrogen peroxide, and place 1 graduated cylinder at that station. Set up a third station where students can get their acid and base, and place 2 graduated cylinders at that station. Make sure that they know not to cross-contaminate the glassware.

7. If you do not have hydrochloric acid or sodium hydroxide, you may use other strong acids and strong bases instead. Any 1 M strong acid or strong base will have similar effects on the enzyme. A good substitute for HCl is H_2SO_4 (sulfuric acid), HNO_3 (nitric acid). Good substitutes for NaOH are KOH (potassium hydroxide) and $Ca(OH)_2$ (calcium hydroxide.

8. To save time in the lab, assign only one part of the lab to each group, and ask them to present their data to the class at the end of the lab.

Safety

Add these important safety precautions to your normal laboratory procedures:

- Use caution when working with acids, bases, and hot plates.
- Keep water away from all electronic equipment.

Procedure

Part 1 – Spontaneous decomposition of hydrogen peroxide

Set Up

1. Start a new experiment on the data collection system.

2. Connect the oxygen gas sensor to the data collection device.

3. Calibrate the oxygen gas sensor.

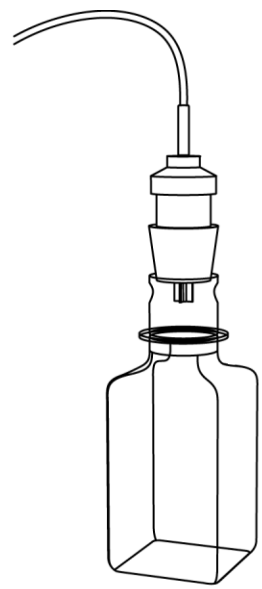
4. Display O_2 concentration (%) on the y-axis of a graph with Time on the x-axis.

5. Pour 20 mL of 1.5% hydrogen peroxide into the sampling bottle.

6. Gently swirl the bottle to mix the solutions.

7. Insert the oxygen-sensing element into the top of the sampling bottle.

Note: Inserting the element too tightly into the bottle may cause it to pop off during data collection.



Collect Data

8. Start data recording #1.

9. Record data for 300 seconds (5 minutes), and then stop recording data.

10. Name the data run "Spontaneous".

11. What purpose does measuring the amount of oxygen released by the natural breakdown of hydrogen peroxide serve?

Answers will vary. Finding the rate of uncatalyzed decomposition of catalase allows us to compare other rates calculated to this rate. It serves as our control group, the group not exposed to the independent variable.

12. Remove the oxygen-sensing element from the sampling bottle.

13. Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Part 2 – Enzyme-catalyzed breakdown of hydrogen peroxide

Set Up

14. Pour 20 mL of 1.5% hydrogen peroxide into the sampling bottle.

15. Add 10 mL of yeast suspension to the peroxide solution in the sampling bottle. Do not add the yeast until you are ready to start collecting data.

16. Gently swirl the bottle.

17. Insert the oxygen-sensing element into the sampling bottle. Keep your hand on the bottle to ensure the stopper does not pop off as pressure builds in the bottle.

Collect Data

18. Start data recording #2.

19. Record data for 300 seconds (5 minutes), and then stop recording data.

20. Name the data run, "Room Temp".

21. Did you observe any physical changes when you added the yeast to the hydrogen peroxide?

Answers will vary. Students should observe bubbling or fizzing when yeast solution is added to hydrogen peroxide. When live yeast comes into contact with hydrogen peroxide, the catalase present in the yeast cells breaks hydrogen peroxide down into water and oxygen gas. The bubbles that the students observed are composed of this oxygen gas.

22. Remove the oxygen-sensing element from the sampling bottle.

23. Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Part 3 – Effects of increased pH on catalase activity

Set Up

24. Pour 20 mL of 1.5% hydrogen peroxide into the sampling bottle.

25. Add 10 mL of sodium hydroxide to the sampling bottle.

26. Make a prediction about the difference between oxygen production in the original yeast suspension and oxygen production in the yeast suspension in a basic environment. Explain your answer.

Answers will vary. Students should predict that oxygen production will decrease as pH increases. Catalase is an enzyme and therefore a protein. An increase in pH can cause the enzyme to denature.

27. Add 10 mL of the room-temperature yeast suspension to the sampling bottle. Do not add the yeast until you are ready to collect data.

28. Gently swirl the bottle.

29. Insert the oxygen-sensing element into the sampling bottle.

Collect Data

30. Start data recording #3.

31. Record data for 300 seconds (5 minutes), and then stop recording data.

32. Name the data run, "Increased pH".

33. Remove the oxygen-sensing element from the sampling bottle.

34. Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Part 4 - Effects of decreased pH on catalase activity

Set Up

35. Pour 20 mL of 1.5% hydrogen peroxide into the sampling bottle.

36. Add 10 mL of hydrochloric acid to the sampling bottle.

37. Do you expect that the acid will have the same effect on enzymatic activity as the base? Explain your answer.

Yes. Students should predict that oxygen production will decrease as pH decreases. Catalase is an enzyme, and therefore a protein. A decrease in pH can cause the enzyme to denature.

38. Add 10 mL of the room-temperature yeast suspension to the sampling bottle. Do not add the yeast until you are ready to begin collecting data.

39. Gently swirl the bottle.

40. Insert the oxygen-sensing element into the sampling bottle.

Collect Data

41. Start data recording #4.

42. Record data for 300 seconds (5 minutes), and then stop recording data.

43. Name the data run, "Decreased pH".

44. Remove the oxygen-sensing element from the sampling bottle.

45. Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Part 5 – Effects of increased temperature on catalase activity

Set Up

46. Pour 20 mL of 1.5% hydrogen peroxide into the sampling bottle.

47. Add 10 mL of boiled yeast suspension to the sampling bottle. Do not add the yeast until you are ready to collect data.

48. Gently swirl the bottle.

49. Insert the oxygen-sensing element into the sampling bottle.

Collect Data

50. Start data recording #5.

51. Record data for 300 seconds (5 minutes), and then stop recording data.

52. Name the data run, "Increased Temp".

53. Imagine that you placed the boiled yeast suspension in a beaker of ice water to cool. Do you think the catalase would regain its enzymatic activity? Explain your answer.

Answers will vary. No, the catalase would not regain activity if placed on ice after being boiled. The enzyme was probably denatured when boiled, and most enzymes cannot regain functionality after denaturation.

54. Remove the oxygen-sensing element from the sampling bottle.

55. Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Part 6 – Effects of decreased temperature on catalase activity

Set Up

56. Pour 20 mL of chilled 1.5% hydrogen peroxide into the sampling bottle. Place the sampling bottle into the beaker of ice to ensure the solution remains cold.

57. Make a prediction about the difference between oxygen production by a room-temperature yeast suspension and oxygen production by a cold yeast suspension. Explain your answer.

Answers will vary. Students should understand that the cold yeast suspension will have decreased oxygen production compared to the room-temperature yeast suspension. However, these temperatures are not cold enough to denature the enzyme, so there will be some enzyme activity, with oxygen gas production as evidence.

58. Add 10 mL of chilled yeast suspension to the sampling bottle. Do not add the yeast until you are ready to collect data.

59. Gently swirl the bottle.

60. Insert the oxygen-sensing element into the sampling bottle.

Collect Data

61. Start data recording #6.

62. Record data for 300 seconds (5 minutes), and then stop recording data.

63. Name the data run, "Decreased Temp".

64. Save your experiment and clean up according to your instructor's instructions.

- **a.** Remove the oxygen-sensing element from the sampling bottle.
- **b.** Discard the contents of the bottle as directed, and rinse the bottle thoroughly.

Data Analysis

1. Find the minimum and maximum oxygen concentrations for each of the six data runs. Record your results in Data Table 2.1.

2. Calculate the change in oxygen concentration, and record your result in Data Table 2.1. Show your work in the space below.

Maximum $[O_2]$ – Minimum $[O_2]$

Run #1 21.0 – 20.6 = 0.4%

Run #2 42.4 – 20.8 = 21.6%

Run #3 24.3 – 20.5 = 4.2%

Run #4 21.9 – 20.7 = 1.2%

Run #5 22.6 – 20.9 = 1.7%

Run #6 29.5 – 20.9 = 9.4%

3. Calculate the rate of oxygen production for each part of the lab. Record your results in Data Table 2.1. Show your work in the space below.

Change in $[O_2]$/ time (s)

Run #1 0.4%/300 s = 0.001 %/sec

Run #2 21.6%/ 300 s = 0.072 %/sec

Run #3 4.2%/ 300 s = 0.014 %/sec

Run #4 1.2%/ 300 s = 0.004 %/sec

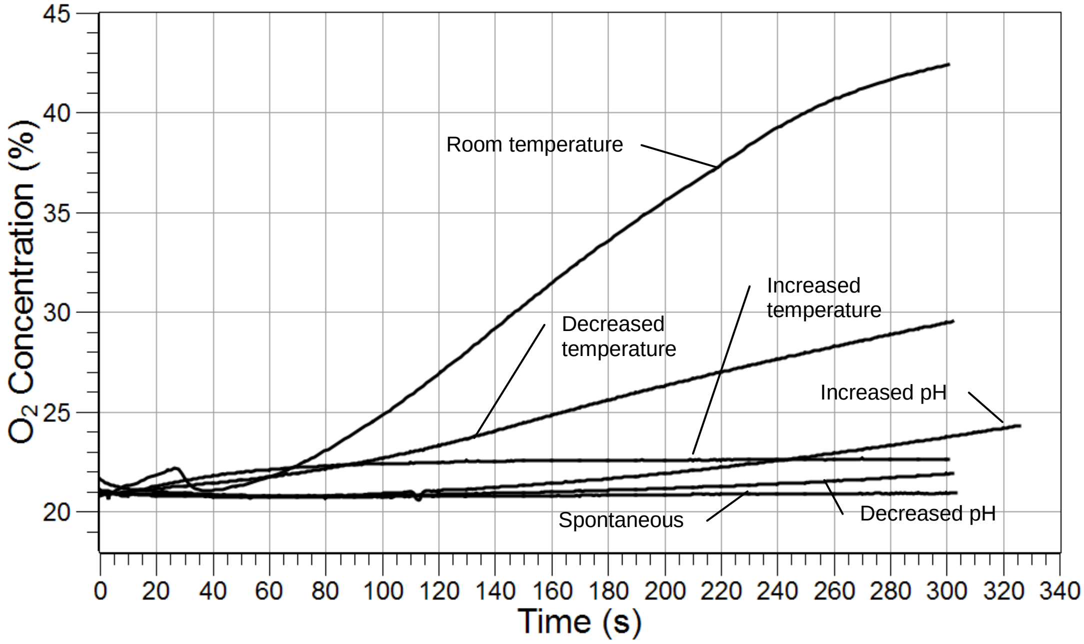
Run #5 1.7%/ 300 s = 0.006 %/sec

Run #6 9.4%/ 300 s = 0.031 %/sec

Table 2.1: Catalase activity

Data Run #	Setup	Minimum % O_2	Maximum % O_2	Change in $[O_2]$	Rate of Reaction (%/s)
1	Spontaneous decomposition	20.6	21.0	0.4	0.001
2	Enzyme-catalyzed	20.8	42.4	21.6	0.072
3	Increased pH	20.5	24.3	4.2	0.014
4	Decreased pH	20.7	21.9	1.2	0.004
5	Increased temperature	20.9	22.6	1.7	0.006
6	Decreased temperature	20.9	29.5	9.4	0.031

4. Sketch a graph of all six data runs. Use a different shape or color for each of the 6 data runs, and record the shape or color in the Key. Label the overall graph, the x-axis, the y-axis, and include units on your axes.



Graph 1: Oxygen gas concentration (%) versus Time (seconds)

Analysis Questions

1. We measured oxygen production as a way to determine the activity of the enzyme catalase. What is the function of catalase, and why is oxygen production used to measure its activity?

Catalase protects cells from the damaging effects of hydrogen peroxide by breaking it down into oxygen gas and water. Oxygen levels can be used as way to measure catalase activity because it is a product of the catalytic breakdown of hydrogen peroxide by catalase.

2. Why is it important to get a baseline reading of the amount of oxygen that is released from the spontaneous decomposition of hydrogen peroxide?

The baseline measurement is very important to the experiment because it represents the control group. The control group setup is not exposed to the independent variable. Because we are testing catalase activity, the control group must be void of catalase. By showing how slowly hydrogen peroxide would spontaneously breakdown, it is possible to then show how catalase would affect the reaction.

3. Identify the independent and dependant variable in each trial.

	Independent	Dependent
Part 1	Control Group	Oxygen gas production
Part 2	Presence of catalase	Oxygen gas production
Parts 3 and 4	pH	Oxygen gas production
Parts 5 and 6	Temperature	Oxygen gas production

4. Which data run had the smallest increase in oxygen concentration? What does this tell you about the activity of catalase in this environment? Which data run had the greatest increase in oxygen concentration? Explain why.

Answers will vary. Students should see the smallest increase in oxygen concentration in data run #1, the spontaneous decomposition of hydrogen peroxide. The data run with the greatest increase should be data run #2, the enzyme-catalyzed reaction. This is evidence that a significant amount of activation energy is required to cause the spontaneous decomposition of hydrogen peroxide. Catalase decreases the activation energy and catalyzes the reaction.

5. Which factor had a greater effect on catalase activity: increased or decreased temperature? Explain why.

Increasing the temperature should have had a greater effect on catalase activity than decreasing the temperature. When the yeast was boiled, the protein structure of catalase was denatured and should no longer function properly. The cold catalase should have shown a decreased activity compared to the room temperature catalase. However, this temperature should not have been cold enough to completely inactivate the enzyme.

6. Which group represented the control group for Part 2? For Part 3? For Part 5? Explain why you chose your answers.

The control group for Part 2 is Part 1. In order to understand the effects of catalase on the breakdown of hydrogen peroxide, you have to compare it to the effects of the uncatalyzed breakdown of hydrogen peroxide, which occurred in Part 1.

The control group for Part 3 is Part 2. In Part 3, we explored the effects of pH on catalase activity. In order to understand how changing the pH affects catalase, we have to see how catalase functions under its optimal pH. This is what occurred in Part 2.

The control group for Part 5 is Part 2. In Part 5, we explored the effects of temperature on catalase activity. In order to understand how changing the temperature affects catalase, we have to see how catalase functions under its optimal temperature. This is what occurred in Part 2.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Most living cells contain catalase or a very similar enzyme called peroxidase. These enzymes help protect the cell by breaking down hydrogen peroxide before it can damage the cell. How does this information help explain the results you obtained in the chilled sample?

Enzymes are catalytic proteins that function best at specific temperature ranges referred to as "optimal temperature" Catalase and peroxidase are enzymes with an optimal temperature similar to that of human body temperature. When the temperature decreases below the optimal range, enzyme function decreases. In this experiment, the chilled yeast suspension did exhibit some catalase activity, as evidenced by the oxygen gas production. However, less oxygen gas was produced by the chilled yeast than the room temperature yeast. This supports the hypothesis that the chilled conditions were colder than optimal for catalase activity.

2. Design a controlled experiment to test the effects of salinity on enzyme function.

Answers will vary, but students should identify the problem or question, create a hypothesis, set up a controlled experiment, identify controls, identify independent and dependent variables, indicate how they will measure the independent variable, indicate how they will organize their data, indicate how they will analyze their data, make predictions about the results of the experiment, and indicate that the experiment must be repeatable and validated by the scientific community.

3. Lipases and proteases are groups of enzymes commonly found in mammalian cells. They are sequestered by the cell because they would otherwise severely damage the cell. Why can these enzymes cause so much damage to cells?

Lipases are enzymes that break down fats. Biological membranes, like the cell membranes, are phospholipid bilayers. Some lipases attack this double layer of phospholipids and could cause the cell to lyse and die. Also, many of the cells' organelles are membrane-bound (surrounded by a membrane). These organelles could be permanently damaged or destroyed by lipases.

Proteases are enzymes that break down or denature proteins. Many parts of the cell are composed of proteins, most importantly the cytoskeleton. Cells are also constantly creating protein products at ribosomes. All of these proteins could be permanently damaged or destroyed by proteases.

Multiple Choice Questions

$$\begin{array}{ccccccc} & E1 & & E2 & & E3 & \\ A & \rightarrow & B & \rightarrow & C & \rightarrow & D \end{array}$$

1. In the series of enzymatic reactions shown above, product D is able to occupy the active site of enzyme E2. Product D can therefore first inhibit the production of

- **A.** Reactant A
- **B.** Reactant B
- **C. Reactant C**
- **D.** Enzyme 1
- **E.** Enzyme 3

2. What of the following best describes "enzyme specificity"?

- **A.** Enzymes are made in a specific part of the cell.
- **B. One enzyme binds to a specific substrate and only catalyzes one reaction.**
- **C.** One enzyme can catalyze many different reactions.
- **D.** Substrates are transported to enzymes along the electron transport chain.
- **E.** Enzymes specifically bind to as many substrates as possible.

3. Enzymes belong to which class of organic compounds?

- **A.** Lipids
- **B.** Carbohydrates
- **C.** Nucleic acids
- **D.** Glycoproteins
- **E. Proteins**

4. An enzyme's function is to increase the rate of a reaction by

- **A.** Increasing the concentration of substrate
- **B. Decreasing the activation energy**
- **C.** Competing with the substrate
- **D.** Breaking down ATP
- **E.** **H**ydrolyzing the substrate

Extended Inquiry Suggestions

Explore the effects of other variables on catalase activity. Allow students to design an experiment to test the effects of salinity, humidity, or pressure on catalase activity.

Use living sources of catalase other than yeast, such as liver or potatoes.

6. Exploring the Effects of pH on Amylase Activity

Objectives

In this experiment, students investigate the concept that enzymes function within a range of pH. During the lab, students find the pH at which the enzyme bacterial amylase functions optimally. Students:

- Observe the effects of bacterial amylase on a starch solution
- Explore the effects of pH on the rate of enzyme activity using six buffers of unknown pH
- Determine the optimal pH for bacterial amylase activity using qualitative and quantitative data

Procedural Overview

Students will gain experience conducting the following procedures:

- Use the colorimeter to test the effects of pH on bacterial amylase activity.
- Use qualitative and quantitative data to find the optimal pH for bacterial amylase activity. pH will be varied using six buffers of unknown pH, varying from approximately 3.0 to 8.0.

Time Requirement

♦ Preparation time	60 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	45 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Advanced water quality sensor
- pH sensor
- Colorimeter
- Small beakers or plastic cups (19)
- Graduated cylinder, 10-mL
- Disposable graduated pipets
- Cuvettes (7)
- Small plastic or glass funnel
- Starch solution[1] (30%), 35 mL
- Standard buffers pH 4 and pH 10
- 6 Buffers of varying pH[1] (pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0), 5 mL each
- Bacterial amylase, 4 mL
- HCl solution (0.1 M), 10 mL
- IKI solution (Lugol's iodine solution), 15 mL[2]
- Distilled water, 200 mL[3]
- Wash bottle or cup of distilled water for pH sensor
- Saltine crackers, 1 per student
- Kimwipes® or other lint-free tissue

[1] To make starch solution, refer to the Lab Preparation section.

[2] You can mix your own IKI (iodine/potassium iodide) solution from common chemicals, or you can purchase it pre-mixed from a chemical supply company. Refer to the Lab Preparation section for instructions that include additional materials you might need.

[3] You may need additional distilled water, depending on the solutions you choose to prepare. Refer to the Lab Preparation section for instructions that include additional materials you might need.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Protein structure: primary, secondary, quaternary, and tertiary
- Basics principles of enzyme structure and function
- Basic differences between monosaccharides, disaccharides, and polysaccharides

Related Labs in This Guide

Labs conceptually related to this one include:

- Measuring Aerobic Cellular Respiration in Yeast
- Enzyme Catalysis
- Cellular Respiration

Background

Enzymes are catalytic proteins that help to speed up specific chemical reactions without being consumed by the reaction. Enzymes do this by lowering the amount of energy that it takes for the uncatalyzed reaction to take place. In other words, enzymes lower the activation energy of a reaction. Enzymes are substrate-specific. Only one type of molecule can fit into the active site of the enzyme to have the reaction proceed.

Many factors affect how well enzymes function. All enzymes have an optimal temperature and pH at which they have maximum efficiency. Enzymes found in most human cells typically work best at 37 °C and pH 7. Enzymes in the acidic environment of the stomach are adapted to work best at pH 2. You will find some enzymes in virtually all living organisms. These enzymes have a wide range of optimal temperatures and pH. When an enzyme's environment becomes more acidic or basic than its optimal pH, the enzyme's protein structure unravels, and it no longer functions. Temperature has a similar effect on enzyme structure. This process is called *denaturation*. Once an enzyme becomes denatured, it never returns to its original confirmation and never functions properly again.

We can determine the optimal pH of the enzyme amylase. Because many organisms rely on carbohydrates as a major source of energy, organisms must have a method of carbohydrate digestion. Amylase breaks specific bonds in a starch molecule, releasing smaller carbohydrates, or sugars. Amylase exists in the saliva and pancreatic juices of many animals. Amylase is essential in the digestion of high starch plants like corn, potato, rice and wheat. The mouth and the small intestines provide strikingly different environments for their respective amylases, so you might expect that pancreatic and salivary amylases have a different optimal pH. Amylases produced by other organisms, like bacteria and fungi, differ as well.

Pre-lab Discussion and Experiment

Give each student a saltine cracker. Ask them to place the saltine cracker in their mouth and chew exactly three times. After chewing three times, instruct them not to chew anymore. They should simply let the saltine sit in their mouth. After several minutes, the students will notice that the saltine is liquefying in their mouth.

1. Why is the saltine being digested even though it is not being chewed?

Guide the students to the conclusion that there must be a chemical within their saliva digesting the saltine.

Saliva contains an enzyme called salivary amylase, which begins the chemical digestion of starch within the mouth. The process of chewing breaks the food down into smaller pieces to increase the surface area of the food. This maximizes the contact between the food and the enzyme.

2. Are enzymes catalytic proteins? Explain your answer.

Proteins are organic molecules that have specific three-dimensional configurations. The specificity of an enzyme depends on the shape of its active site (the location on the protein where the enzyme will bind to its substrate). Enzymes have an optimal pH and temperature at which they work most efficiently. When pH and temperature deviate too far past optimal, the enzyme will denature. This means shape to the active site will change, no longer specifically fitting the substrate.

3. Describe the primary, secondary, tertiary, and quaternary structure of proteins.

The primary structure of a protein is its unique sequence of amino acids. The DNA sequence on a particular gene determines the primary structure of a protein. Even a slight change in primary structure can affect a protein's conformation and ability to function.

The secondary structure consists of coils and folds that result from hydrogen bonds at regular intervals along the polypeptide backbone. Typical shapes that develop from secondary structure are coils (an alpha helix) or folds (beta pleated sheets). The alpha helix is a coil held together by a hydrogen bond at every fourth amino acid. The beta pleated sheet is 2 or more regions of the polypeptide chain that lie parallel to each other, held together by hydrogen bonds.

Irregular contortions of the secondary structure resulting from interactions between the amino acid form the tertiary structure. Tertiary structure is determined by interactions among R groups (side chains) and between R groups and the polypeptide backbone. These interactions include hydrogen bonds among polar and/or charged areas; ionic bonds between charged R groups; hydrophobic interactions among R groups; van der Waals interactions among hydrophobic R groups; and disulfide bridges, strong covalent bonds that form between the sulfhydryl groups (SH) of cysteine monomers. Disulfide bridges stabilize the tertiary structure.

Quaternary structure results from the aggregation of two or more polypeptide subunits. Quaternary structures may be fibrous and rope-like, like collagen, or globular like hemoglobin.

4. Why is the structure of an enzyme essential to its function?

When a substrate binds to an enzyme, the enzyme catalyzes the conversion of the substrate to the product. The active site of an enzyme is a pocket or groove on the surface of the protein into which the substrate fits. The specific fit between the active site and that of the substrate allows the enzyme and substrate to react. We refer to this as enzyme specificity. If the shape of the enzyme changes, then the substrate no longer fits into the active site, and the enzyme will not function.

5. Predict how pH and temperature will affect enzyme activity.

Changes in shape influence reaction rate. Some conditions lead to the most active conformation and optimal rate of reaction. Other conditions change the shape of the active site, rendering it nonfunctional. Temperature greatly influences reaction rate. As temperature increases, collisions between substrates and active sites occur more frequently as molecules move faster. However, at some point, thermal agitation begins to disrupt the weak bonds that stabilize the protein's active conformation, and the protein denatures. Each enzyme has an optimal temperature. Because pH also influences shape and therefore reaction rate, each enzyme has an optimal pH too. This falls between pH 6 to 8 for most enzymes. However, digestive enzymes in the stomach work best at pH 2, while those in the intestine prefer pH 8, both matching their working environments.

6. Describe denaturation.

An enzyme in an environment whose temperature, pH, or salinity is significantly above or below its optimum will begin to lose its confirmation. The quaternary structure will fall apart, followed by the tertiary and secondary structure. We call this denaturation. Most enzymes cannot return to their active confirmation after denaturation.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

You can purchase pre-made buffer solutions for this experiment from a chemical supply company, or you can mix them yourself. To create the buffers, follow the steps in the "Creating Buffers" section below. If you purchase buffers individually from a chemical supply company, be sure to order colorless buffers (Flinn offers all six of these buffers), and skip the A through F labeling steps.

Creating Buffers

To create six different buffer solutions (pH 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0) ahead of time, follow these steps:

1. Prepare a 0.2 M sodium phosphate solution:

a. Add 28.3 g of Na_2HPO_4 to 1 L of distilled water.

b. Stir to mix.

c. Adjust the mass and volume of the water to make the amount of sodium phosphate buffer you need.

2. Prepare a 0.1 M citric acid solution.

a. Add 19.2 g of citric acid to 1 L of distilled water.

b. Stir to mix.

c. Adjust the mass and volume of the water to make the amount of citric acid buffer you need.

3. Add the following volumes of sodium phosphate buffer and citric acid buffer together to make 100 mL of each.

Label	pH of buffer	Sodium phosphate solution (mL)	Citric acid solution (mL)
E	3.0	20.55	79.45
B	4.0	38.55	61.45
F	5.0	51.50	48.50
A	6.0	63.15	36.85
D	7.0	82.35	17.65
C	8.0	97.25	2.75

Note: These buffers will not likely match the desired pH exactly. This is OK because the students will measure the exact pH of each buffer at the end of the lab.

4. Label each of the six buffer storage bottles with the pH of each.

5. If necessary, prepare the 0.1 M hydrochloric acid solution (30 mL/class).

Caution: Do not pour water into the concentrated acid.

Note: 0.1 M HCl is the same as 0.1 N HCl.

Preparing and Setting Up the Lab

1. On the day of the lab, remove the labels that identify the pH of each buffer, and replace them with labels A through F to correspond with the pH values in the table above.

2. Prepare 500 mL of 30% starch solution. If you do not purchase soluble potato starch from a biological supply company, you can make your own from potatoes with one of the two following procedures:

♦ To make the starch solution using soluble potato starch:

a. Bring 450 mL of water to a gentle boil in a large beaker.

b. Mix 15 g of soluble potato starch in 25 mL of cold water.

c. Add the starch slurry to the boiling water while stirring.

d. Add more water to bring the volume up to one liter.

e. Mix well, and stir until the starch completely dissolves.

f. Cool the starch solution to room temperature. If you have a hot plate/magnetic stirrer combination, use it to dissolve the starch.

♦ To make the starch solution with potatoes:

a. Peel and dice a potato.

b. Weigh 500 g of potato chunks.

c. Blend them in a blender with 500 mL of distilled water.

d. Pour the potato solution into several centrifuge tubes. Be sure to pour equal volumes of liquid into each tube.

e. Spin at 3000 RPM for 4 minutes.

3. Prepare a 4% bacterial amylase solution: Add 4 mL of bacterial amylase to a beaker, fill the beaker with distilled water to make 100 mL of diluted amylase solution, and label the bottle.

4. Prepare a diluted IKI (iodine/potassium iodide) solution. Mix 50 mL of IKI with 100 mL of distilled water. Use an amber bottle for long-term storage. (If you do not have IKI solution, dissolve 6.5 g of potassium iodide in 30 mL of distilled water. Add 3.18 g of iodine and slowly add stir. Slowly add distilled water to bring the volume of the solution up to 500 mL.)

5. Test the starch and iodine solutions. Put a few drops of the starch solution on a glass plate or bowl. Add 1 drop of the iodine reagent and see observe the color change. If a deep blue color develops, then both your starch and iodine solutions are functional.

Safety

Add these important safety precautions to your normal laboratory procedures:

♦ Always use your protective eyewear when working with acids.

♦ Keep water away from electrical outlets.

♦ Use caution when working with iodine. It can stain skin and clothing.

♦ Use caution when working with acids.

Procedure

Set Up

1. Label 6 clean beakers, A through F. Also label a 7th beaker "blank".

2. Use a graduated cylinder to add 5.0 mL of the starch solution into each of the labeled beakers.

3. Rinse the graduated cylinder.

4. Use the graduated cylinder to add 5.0 mL of the unknown buffer solution A into the beaker labeled "A".

5. Rinse the graduated cylinder.

6. Repeat the steps above for adding 5 mL of unknown buffer solutions for the beakers labeled B through F, making sure the label of the unknown solution matches the label of the other beaker. (Do not add buffer to the beaker labeled "blank".)

7. Swirl the beakers gently to mix the solutions.

8. Use a graduated, disposable pipet to add 0.5 mL of the bacterial amylase solution to each of the beakers labeled A through F.

9. Swirl the beakers to gently mix the solutions.

10. Add 6.0 mL of distilled water to beaker labeled "blank". Nothing else will be added to this beaker.

11. Let the reaction proceed for exactly 5 minutes at room temperature (25 °C). While waiting, conduct the following steps to create beakers with HCL and IKI and to prepare your data collection system.

12. Label 6 clean beakers with both "HCl" and a letter A through F.

13. Add 10 mL of 0.1 M HCl to a graduated cylinder and use a plastic pipette to quickly add 1 mL of HCl solution into each of the beakers you just labeled, and set them aside.

14. Label 6 clean beakers with "IKI" along with a letter A through F.

15. Use a clean graduated cylinder and pipette to add 2.0 mL of IKI into each of the beakers you just labeled, and set them aside.

16. Start a new experiment on the data collection system.

17. Connect the colorimeter to the data collection system.

18. Create a digits display of "Orange 610 nm absorbance."

19. After the 5-minute waiting period, add the reacted starch solutions to the HCl beaker with the corresponding letter. For example, add the reacted starch solution in beaker A to beaker labeled "HCl A".

20. Swirl the beakers gently to mix the solutions.

Collect Data

21. Observe the color of each of the six solutions, and record the color in Table 1 as "Initial color."

22. What effect does adding the hydrochloric acid have on the enzymatic reaction occurring between bacterial amylase and starch?

When you added bacterial amylase to the starch solution, it began the enzymatic breakdown of starch into smaller sugars. The HCl acts as a "stopping solution" and stops enzymatic breakdown of starch. Once you add the HCl, no more starch breaks down.

23. Add the reacted starch/HCl solutions to the IKI beakers with corresponding letters. For example, add the reacted starch solution in beaker “HCl A” to beaker "IKI A."

24. Swirl the beakers gently to mix the solutions.

25. Observe the color of the solutions in each beaker, and record as "Final Color" in Table 1.

26. Based on your observations of the color of the solutions, can you determine which solution had the highest enzyme activity? How do you know?

Yes, the lightest color solution had the highest enzyme activity. The lightest solution might be the optimal pH for enzyme activity. The solution should turn deep purple or blue if there is any unconverted starch present. The solution is brown-red colored for partially degraded starch, while it is clear for totally degraded starch.

27. Use a funnel or clean pipette to add some ofthe reacted starch/HCl/IKI solution A into a clean, glass cuvette.

28. Place a new sticker on the cap, and label the cuvette "A."

29. Repeat the above steps for creating and labeling cuvettes for the remaining five beakers of reacted starch/HCl/IKI solution, rinsing the funnel in between each cuvette and marking each cuvette B through F.

30. Use the funnel to fill the 7th cuvette with "blank" solution.

31. Clean any fingerprints or smudges from all of the cuvettes with the lint-free tissue.

32. Open the top of the colorimeter, and insert the blank, cuvette #7 into the cuvette holder. Snap the lid closed.

33. Press the green button on the top of the colorimeter and release.

You will see a green light indicating that the colorimeter is calibrating. When the green light turns off, calibration is complete.

34. Open the top of the colorimeter, and remove cuvette #7.

35. What is the purpose of cuvette #7?

Cuvette #7 is the blank. You calibrate colorimeters and spectrophotometers with solutions called blanks. Calibrating the device with dilute iodine tells the colorimeter that the amount of light transmitted through cuvette

#7 is the amount that the device should always read as 100% transmittance. By doing this, we take care of any background absorbance that would have occurred as a result of the iodine.

36. Open the top of the colorimeter, and place cuvette A in the cuvette holder.

37. Press "Start" to measure the absorbance.

38. Record the measurement in Table 1.

39. Remove cuvette A from the colorimeter. You do not have to stop collecting data between each cuvette. Pull one cuvette out, and put the next one into the colorimeter. Repeat the steps above to measure and record absorbance for cuvettes B through F.

40. Use the color change data and the absorbance data in Table 1 to predict the pH of each buffer, and record in Table 1.

41. Connect the pH sensor to the data collection system.

42. Use the pH4 and pH 10 buffer solutions to calibrate the pH sensor.

43. Create a digits display of pH.

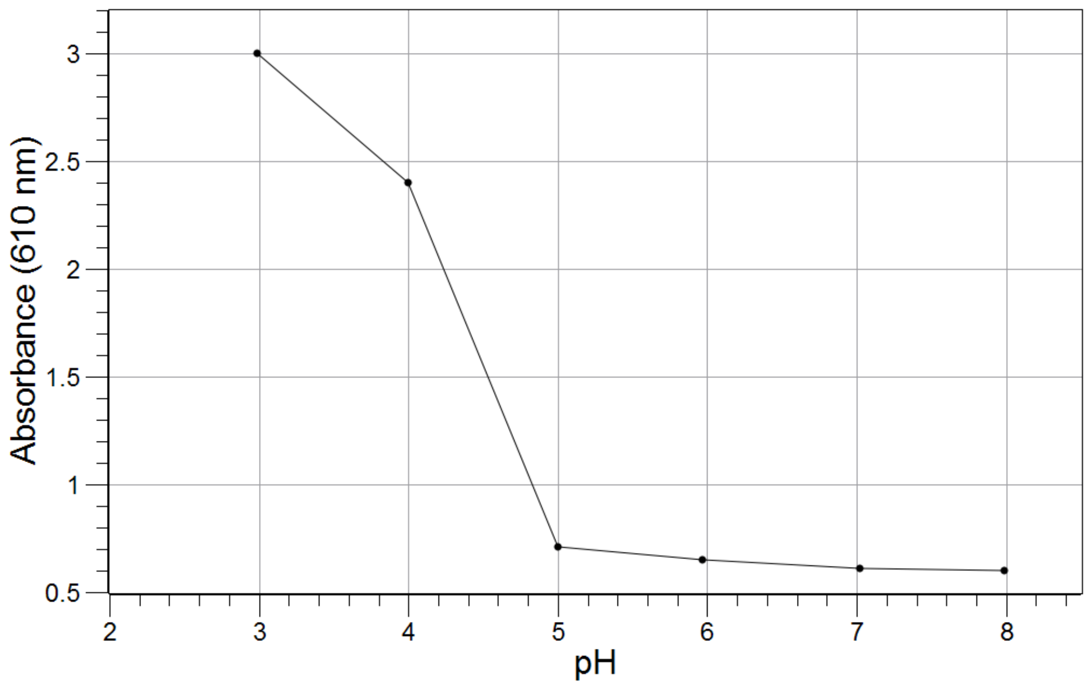
44. Use the pH sensor to measure the actual pH of each of the six buffers and record each measured pH value in Table 1.

Data Analysis

Table 1: Absorbance and pH

Unknown Buffer	Initial Color	Final Color	Absorbance (610 nm)	Predicted pH	Measured pH
A	Colorless	Dark orange/red	0.647	Answers will vary	5.97
B	Colorless	Very dark reddish/blue	2.403	Answers will vary	4.00
C	Colorless	Light orange/red	0.598	Answers will vary	7.99
D	Colorless	Dark orange/red	0.610	Answers will vary	7.12
E	Colorless	Dark blue	3.00	Answers will vary	2.99
F	Colorless	Dark orange/red	0.713	Answers will vary	5.00

1. Create a graph of the absorbance (610 nm) versus pH. Label the overall graph, the x-axis, the y-axis, and include units on the axes.



Analysis Questions

1. Now that you know the pH of each of the buffer solutions, which of the buffer solutions provided the optimal pH for bacterial amylase activity at 25 °C?

Answers will vary based on data, but the optimal pH of bacterial amylase at 25 °C is 8.0. Students should indicate that buffer C had the optimal pH.

2. What data or previous knowledge did you use to determine the optimal pH of the enzyme?

When starch comes into contact with IKI, the solution turns bluish or purple. Therefore, the darker the solution, the more starch is present. Bacterial amylase breaks starch down into smaller sugars that do not react with IKI in the same way that starch does. Therefore, the higher the activity of the enzyme, the less starch there will be in the solution, and the lighter the color will be. The colorimeter measures the amount of light absorbed by the solution, so darker solutions absorb more light. The lightest solution will contain the least amount of starch and will transmit the most light. This solution must be at the optimal pH since the enzyme activity is the highest.

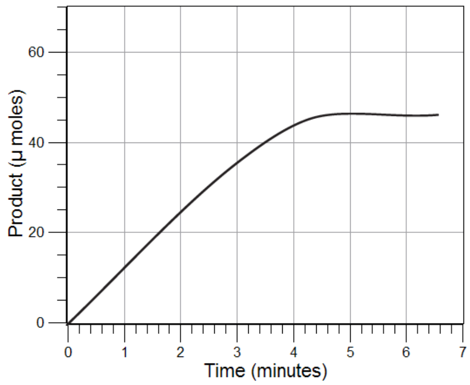
3. What happened to the amylase activity when the pH dropped below 4.0? Why did this occur?

Enzyme activity significantly decreased at pH more acidic than 4.0. The "effective range" of bacterial amylase is between 4.0 and 9.0. When the pH drops below 4.0 (or goes above 9.0), the enzyme becomes unstable and begins to denature.

4. Can you identify any steps in your procedure that may have caused erroneous results?

Answers will vary. Examples include contamination by not rinsing your glassware in between solutions, measuring solutions incorrectly, or not labeling properly.

5. Look at the graph below.



A scientist determined the rate of this enzyme-catalyzed reaction by measuring the amount of product formed over time. The curve was generated from the collected data. Make a statement about trends that you observe in the graph. Can you propose an explanation for the trend observed after the 4-minute mark?

Answers will vary. Students should indicate that the rate of production, or enzyme activity, remained constant for the first 3 to 4 minutes. After 3 minutes, the rate of increase slowed. At 4 minutes, the rate of enzyme activity was zero. At this point, the amount of product did not change. One possible explanation for this plateau is that the substrate had all been converted to the product.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Design a controlled experiment to test the effects of temperature on enzyme function.

Answers will vary. Students should identify the problem or question, create a hypothesis, set up a controlled experiment, identify controls, independent and dependent variables, indicate how they will measure the independent variable, indicate how they will organize their data, indicate how they will analyze their data, make predictions about the results of the experiment, indicate that the experiment must be repeatable and validated by the scientific community.

2. What is the purpose of the hydrochloric acid in this experiment? How would your results be affected if you left this step out?

The HCL acts as a "stopping solution." When you added HCl to the reacted starch solution, it immediately stops the reaction of amylase and starch. HCl has a very low pH, and it denatures amylase. This step is important because it ensures that all buffered reacted starch solutions are exposed to amylase for only 5 minutes. If you forgot to add HCl, the reaction would go to completion in all of the buffered solutions, and you would see no differences in the absorbance of the solutions.

3. Knowing that amylase breaks starch down into smaller sugars, can you think of any reason that food industry might use amylase?

Answers will vary. Amylase is used in brewing, baking, and the production of cereals, confections, and sugar syrups. In all of these cases, amylase increases the sugar content of the food by breaking starch down into smaller sugars.

4. In the experiment described below, water was added to each of six test tubes. Sucrose (a disaccharide) and living yeast were added to some, but not all of the test tubes. One of the test tubes was boiled after the yeast was added, one was chilled, and the others were kept at room temperature. After 10 minutes, the presence of glucose (a monosaccharide) was determined.

Test tube	Water added	Yeast added	Sucrose added	Temperature treatment	Presence of glucose after 10 minutes
1	yes	no	no	room temperature	none present
2	yes	yes	no	room temperature	none present
3	yes	no	yes	room temperature	none present
4	yes	yes	yes	room temperature	glucose present
5	yes	yes	yes	boiled	none present
6	yes	yes	yes	chilled	trace of glucose present

Which two test tubes would you use as evidence to support the hypothesis that yeast are utilizing an enzyme to convert sucrose to glucose? Explain why the other pairs do not provide this evidence.

Test tubes 2 and 4 both contain living yeast at room temperature, but only test tube 4 has sucrose. These tubes provide evidence that sucrose is required for the presence of glucose in the final solution.

Test tubes 1 and 3 do not contain yeast. Test tube 1 does not contain sucrose, but tube 3 does. After 10 minutes, glucose is present in neither of these tubes. This is evidence that the starch hydrolysis is associated with the yeast.

Test tubes 5 and 6 both contain yeast and sucrose, but tube 5 was boiled, and tube 6 was chilled. Glucose is present in trace amounts in the chilled tube, but is absent in the boiled tube. This provides additional evidence that the sucrose hydrolysis is associated with the yeast. Test tubes 4 and 5 have the same components, sucrose and yeast, but tube 4 is kept at room temperature and tube 5 is boiled. Tube 4 contains glucose after 10 minutes, but tube 5 does not. This is evidence that yeast is associated with sucrose hydrolysis and that temperature changes affects sucrose hydrolysis. Tubes 5 and 6 indicate that temperatures associated with boiling stop sucrose hydrolysis, but lowering the temperature only slows the process. This combination of evidence from tubes 4, 5, and 6 suggests that the sucrose hydrolysis is enzyme catalyzed.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Because enzymes are biological catalysts, they:

- **A.** Prevent reactions from occurring spontaneously.
- **B. Lower the activation energy needed for a reaction to occur.**
- **C.** Permanently bind to reactants, allowing a reaction to occur.
- **D.** Raise the activation energy until a reaction begins.
- **E.** Break down all molecules at the same rate.

2. In the enzyme-catalyzed reaction represented below, which molecules are considered to be the substrate(s)?

monosaccharide + monosaccharide → disaccharide + water

- **A. Monosaccharide and monosaccharide**
- **B.** Disaccharide and water
- **C.** Monosaccharide and water

- **D.** Monosaccharide and disaccharide
- **E.** Starch and monosaccharide

3. A cell contains

- **A. Thousands of different kinds of enzymes, each promoting different chemical reactions.**
- **B.** One kind of enzyme that promotes thousands of different chemical reactions.
- **C.** Approximately 100 kinds of enzymes, each promoting a different chemical reaction.
- **D.** One enzyme that promotes photosynthesis, and one enzyme that promotes cellular respiration.
- **E.** Only the types of enzymes that are normal for the environment.

4. Which of the following best describes the effect of temperature on enzyme activity?

- **A.** For enzyme activity to be high, the temperature must be as high as possible.
- **B.** For enzyme activity to be high, the temperature must be as low as possible.
- **C. Optimal enzyme activity occurs within a narrow range of temperatures.**
- **D.** Temperature is not important because the role of enzymes is to overcome the need for activation energy.
- **E.** Temperature is very important, but only when the enzyme is in an environment with optimal pH.

Extended Inquiry Suggestions

Test the effects of temperature on bacterial amylase activity. Use the same procedure that is listed in this experiment, but instead of varying the pH with buffers and an acid, vary the temperature.

Complete the experiment again, but vary the concentration of the substrate, the concentration of the enzyme or the incubation time.

Conduct this same experiment with different enzymes to discover whether all enzymes have the same optimal pH.

7. Plant Pigments and Photosynthesis

Objectives

Students learn how to separate plant pigments using paper chromatography and how to measure the rate of photosynthesis in chloroplast suspensions.

Procedural Overview

Students gain experience conducting the following procedures:

- Separating plant pigments based on their solubility using paper chromatography.
- Determining the rate of photosynthesis in a suspension of chloroplasts using a colorimeter and the reduction of 2,6-dichlorophenolindophenol (DPIP).

Time Requirement

♦ Preparation time	60 minutes
♦ Pre-lab discussion and experiment	15 to 20 minutes
♦ Lab experiment : Part 1	20 minutes
♦ Lab experiment: Part 2	55 minutes

Materials and Equipment

For each group:

- Data collection system
- Colorimeter
- Cuvettes (5)
- Glass jar, 10 to 12 cm tall
- Disposable graduated pipet (2), 1-mL
- Chloroplast suspension[1], 2 mL
- 0.1 M phosphate buffer[2], 4 mL
- DPIP in small amber bottle[3], 3 mL
- #1 Whatman® chromatography paper
- Chromatography solvent[4], 5 mL
- Spinach
- Quarter or other coin
- Kimwipes® or other lint-free tissue
- Distilled water, 13 mL
- Floodlight, 100 watt
- Heat sink (large beaker or flask filled with water)
- Aluminum foil
- Cheesecloth
- Ice

[1] To formulate using spinach leaves and sucrose solution, refer to the Lab Preparation section.

[2] To formulate using dibasic and monobasic potassium phosphate and distilled water, refer to the Lab Preparation section.

[3] To formulate using DPIP and distilled water, refer to the Lab Preparation section.

[4] To formulate using petroleum ether and acetone, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- How chromatography separates two pigments in a solution
- The basic process of photosynthesis
- The function of plant pigments
- The relationship between light wavelength and photosynthetic rate
- The relationship between light intensity and photosynthetic rate

Related Labs in This Guide

Labs conceptually related to this one include:

- Factors that Limit Photosynthetic Activity
- Elodea and the Snail
- Dissolved Oxygen and Primary Productivity

Background

Plants are photosynthetic organisms, able to harness light energy from the sun to convert carbon dioxide gas from the atmosphere into sugar through photosynthesis summarized as follows:

$$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2.$$

Chloroplasts, the organelles where photosynthesis occurs, contain pigments that absorb varying wavelengths of light. In the leaves of the plant, the pigments are indistinguishable; however, they may be separated using chromatography.

Paper chromatography separates a mixture into its various components. The mixture is placed onto a piece of chromatography paper, and then a solvent is allowed to migrate (through diffusion) up the paper. As the solvent migrates, it carries the components of the mixture along with it. Each of the components will migrate at varying rates based on their solubility, size, and hydrogen bonding with the paper.

The photosynthetic pigments absorb light energy from the sun. As light hits the chloroplasts, electrons are excited and passed along an elaborate electron transport chain within the thylakoid membrane of the chloroplasts. The electrons eventually reduce the molecule nicotinamide adenine dinucleotide phosphate ($NADP^+$) to form NADPH.

Preparing the chlorophyll suspension physically breaks apart the thylakoid membrane so that the natural electron transport chain is nonfunctional. DPIP is added to the suspension to accept the excited electrons. When DPIP is reduced, it changes color from dark blue to colorless. The colorimeter will measure these color changes by determining the % Transmittance of light in each sample.

Pre-Lab Discussion and Experiment

Begin the pre-lab discussion with a brief review of the role of plant pigments in photosynthesis.

1. Go over the procedures in the chromatography portion of the lab and discuss the technique of paper chromatography.

Paper chromatography separates a mixture into its various components. The mixture is placed onto a piece of chromatography paper, and then a solvent is allowed to migrate (through diffusion) up the paper. As the solvent migrates, it carries the components of the mixture along with it. Each of the components will migrate at varying rates based on their solubility, size, and hydrogen bonding with the paper.

Perform a quick chromatography demonstration. Place a small dot of water-soluble ink on a piece of chromatography paper. Secure the top of the paper with a paper clip. Place the paper in a 100-mL beaker filled with just enough water to touch the tip of the paper, using the paper clip to hold the paper in place. In just a few minutes the water will move up the paper and separate the black ink into its various pigments.

2. Discuss the second portion of the lab and explain the role of 2,6 dichlorophenolindophenol (DPIP) in the experiment.

DPIP is a blue compound that is easily reduced. In this experiment, it will take the place of the electron acceptor NADP. When DPIP becomes reduced (accepts an electron), it turns from blue to colorless. So, if photosynthesis is occurring in a solution of chloroplasts and DPIP, the solution should turn colorless. The colorimeter will help us quantify how much color change occurs in each cuvette.

3. Make sure students know what a colorimeter is and how it will be used in this experiment.

The colorimeter is a device that measures light absorbance or light transmittance through a solution. The colorimeter will allow you to choose the % Absorbance or % Transmittance of several different wavelengths of visible light.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

One or two days before the lab:

1. Prepare chromatography solvent: Mix 9 parts petroleum ether (PET) with 1 part acetone. The solvent should be kept tightly sealed when not in use.
2. Prepare 0.5 M sucrose solution (for chloroplast suspension): Dissolve 342 g of sucrose in 800 mL of distilled water. Adjust the final volume to 2 L with distilled water. Refrigerate until use.
3. Prepare DPIP solution: Dissolve 0.07 g of DPIP in 500 mL of distilled water. Adjust the final volume to 1 L with distilled water. Transfer the DPIP solution to amber bottles and store in the refrigerator.

Note: DPIP will quickly become reduced (and useless) if exposed to light. Always store it away from light in an amber bottle.

4. Prepare 0.1 M phosphate buffer: Dissolve 174 g of K_2HPO_4 (dibasic phosphate) in 800 mL of distilled water. Adjust the final volume to 1 L using distilled water. In a separate container, dissolve 136 g of KH_2PO_4 (monobasic phosphate) in 800 mL of distilled water. Adjust the final volume to 1 L using distilled water. In a new container, mix together 685 mL of monobasic buffer and 315 mL of dibasic buffer. This is a 1 M phosphate buffer solution. To dilute it to 0.1 M, add 100 mL of the phosphate buffer to 900 mL of distilled water.
5. Buy a bag of spinach. Pre-washed spinach works well, but for best results, buy the loose spinach that you have to bag yourself.

The night before the lab:

6. Incubate the spinach: Put a few paper towels on the floor or lab bench. Spread your spinach over the towels. Place a light source far enough away from the spinach that you do not feel the heat of the lamp when you place your hand in front of the spinach. Leave the spinach in the light overnight.

The morning of the lab:

7. Blend spinach: Add 0.5 M sucrose solution to a blender. Add enough to just cover the blades (100 to 200 mL). Then add spinach leaves to about 2 cm above the blades. Blend the spinach and sucrose solution in three 10-second pulses. Repeat as necessary.
8. Fill a large beaker halfway with water. Place it on a hot plate set to high so the water will be boiling for step 12.

9. Prepare chloroplast suspension: Cut a piece of cheesecloth to cover the top of a large beaker. Make sure the cloth is no more than two layers thick. Cover the top of the beaker with the cheesecloth. Wrap a rubber band around the cloth and the beaker to keep the cheesecloth in place. Place the beaker in a bowl of ice. Make sure that the beaker is stable in the ice and will not tip over. Filtering the spinach solution through the cheesecloth provides the chloroplast suspension.

10. For each group, put at least 2 mL of the chloroplast suspension into a test tube for the preparation of the boiled chloroplast suspension.

11. For each group, pour 2 mL of the chloroplast suspension into a small amber vial and label. Store on ice until ready to use. Alternately, pour the suspension into a large amber bottle, label, and store on ice.

Note: This suspension is only good for 24 hours.

12. Prepare boiled chloroplast suspension: Place the test tubes of chloroplast suspension in the boiling water for 5 minutes. Transfer the boiled chloroplast suspension to amber vials or bottles, label them, and place on ice.

Lab Safety

Add this important safety precaution to your normal laboratory procedures:

- Due to the volatility of the chromatography solvent, ensure all containers remain tightly sealed.

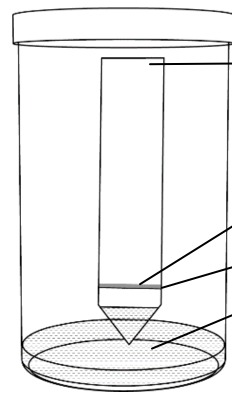
Procedure

Part 1 – Plant pigments

Set Up

1. Obtain a small glass jar and fill with chromatography solvent to a level of 1 cm.

2. Obtain a piece of chromatography paper about as long as the height of the glass jar. Cut one end of the paper into a point and draw a line in pencil across the width of the paper 1.5 cm above the point.



3. Lay a spinach leaf on the paper above the pencil line. Deposit the plant pigments onto the paper by firmly rolling the edge of a quarter over the leaf about 15 times until a heavy green line appears on the paper.

4. Lower the paper into the jar ensuring that only the tip of the paper touches the solvent. The green line must be above the solvent. Tightly close the lid of the jar.

5. What do you notice happening to the solvent in the jar?

Students should note that the solvent will quickly wick up the paper, dissolving and separating the chlorophyll into its various pigments.

Collect Data

6. When the solvent has traveled to about 1 cm below the top of the paper, remove the paper from the jar.

7. Using a pencil, quickly mark the location of the solvent's furthest point of travel before the solvent evaporates.

8. Measure the distance the solvent traveled (the distance between the two lines) and record this value in Table 4.1.

9. On the paper, mark the location of the top of each of the pigments.

10. Measure the distance each pigment traveled from the origin to the top of each band. Record these measurements in Table 4.2.

11. How many different pigments bands do you see? Why do some of the bands migrate further than others?

Depending on the species of plant used, students will observe three to four pigment bands. The yellow or yellow-orange band is carotene. The yellow pigment is xanthophyll. The bright green-to-blue green pigment is chlorophyll a and the yellow green to olive-green pigment is chlorophyll b.

The distance each pigment travels is dependent upon its solubility, molecular weight, and attraction to the chromatography paper.

12. How do your measurements compare with other groups?

Students may notice variations in the distances traveled for similar pigments.

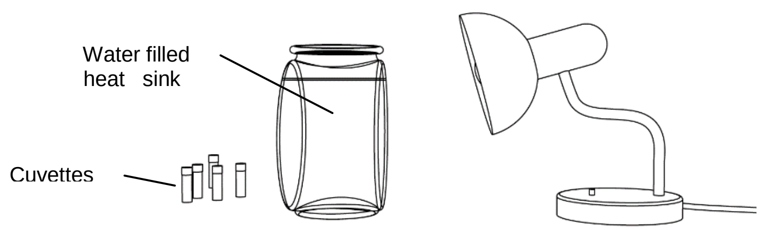
Part 2 – Photosynthesis

13. Start a new experiment on the data collection system.

Set Up

14. Connect a colorimeter to the data collection system.

15. Prepare the incubation area. You will need a flood light and a heat sink (a large beaker or flask filled with water). Place the flood light directly in front of the heat sink. The heat sink will absorb the heat from the flood light while still allowing light to pass through to the cuvettes that are placed a few inches behind the flask.



16. Obtain five cuvettes and label the tops from "1"to "5".

17. Cover the sides and top of cuvette 2 with aluminum foil.

18. Fill each of the cuvettes according to Table 4.1 below, but do not add either the unboiled or boiled chloroplasts yet.

Table 4.1: Setup for photosynthesis experiment

Contents	Cuvette 1 Blank (no DPIP)	Cuvette 2 Unboiled chloroplasts (Dark)	Cuvette 3 Unboiled chloroplasts (Light)	Cuvette 4 Boiled chloroplasts (Light)	Cuvette 5 No chloroplasts (Light)
Phosphate buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Distilled water	4 mL	3 mL	3 mL	3 mL	3 mL + 3 drops
DPIP	None	1 mL	1 mL	1 mL	1 mL
Unboiled chloroplasts	3 drops	3 drops	3 drops	None	None
Boiled chloroplasts	None	None	None	3 drops	None

Collect Data

19. Add 3 drops of the unboiled chloroplast suspension to cuvette 1.

20. Screw the lid onto the cuvette and mix by inverting the cuvette several times.

21. Wipe the sides of the cuvette gently with a Kimwipe® or other lint-free tissue

22. Insert the cuvette into the cuvette holder on the colorimeter and close the colorimeter lid tightly.

23. Calibrate the colorimeter

24. Remove the cuvette.

25. What is the purpose of cuvette 1?

Cuvette 1 is the blank. The % Transmittance of each of the experimental samples will be compared to the % Transmittance in this tube, which only contains unboiled chloroplasts without DPIP.

26. Add 3 drops of the unboiled chloroplast suspension to cuvette 2.

27. Screw on the lid and invert to mix.

28. Remove the aluminum foil from the cuvette, and insert into the cuvette holder on the colorimeter. Close the colorimeter lid tightly.

29. Display % Transmittance in a digits display.

30. Start data recording data.

31. Record the % Transmittance value in Table 4.3.

32. Stop data recording data.

33. Remove the cuvette from the colorimeter. Recover the cuvette with aluminum foil.

Note: Be sure to mix the contents of the cuvettes before each reading.

34. Predict how the rate of photosynthesis will change for the sample in cuvette 2 (chloroplasts, dark).

Because this cuvette is denied light, students should predict that photosynthesis does not occur and therefore the % Transmittance does not change.

35. Add 3 drops of the unboiled chloroplast suspension to cuvette 3.

36. Screw on the lid and invert to mix.

37. Insert into the cuvette holder on the colorimeter and tightly close the lid.

38. Start data recording data.

39. Record the % Transmittance value in Table 4.3.

40. Stop data recording data.

41. Remove the cuvette from the colorimeter.

42. Predict how the rate of photosynthesis will change for the sample in cuvette 3 (chloroplasts, light).

This cuvette shows increasing rates of photosynthesis over time.

43. How will the change in photosynthetic rate change the % Transmittance?

The % Transmittance increases over time as the DPIP becomes colorless.

44. Add 3 drops of the boiled chloroplast suspension to cuvette 4.

45. Screw on the lid and invert to mix.

46. Insert into the cuvette holder on the colorimeter and tightly close the lid.

47. Start data recording data.

48. Record the % Transmittance value in Table 4.3.

49. Stop data recording data.

50. Remove the cuvette from the colorimeter.

51. Will this cuvette show signs of photosynthesis? Why or why not?

Because this sample contains the boiled chloroplast suspension, photosynthesis will not occur and the % Transmittance should not change.

Note: The boiled suspension usually contains small fragments that can cause % Transmittance readings to fluctuate slightly.

52. Screw on the lid to cuvette 5 and invert to mix. (This cuvette does not receive any chloroplasts.)

53. Insert into the cuvette holder on the colorimeter and tightly close the lid.

54. Start data recording data.

55. Record the % Transmittance value in Table 4.3.

56. Stop data recording data.

57. Remove the cuvette from the colorimeter.

58. Place all cuvettes in the incubation area.

59. Note the time that the cuvettes are put in the incubation area. ____________

60. Measure the % Transmittance of all five cuvettes again at 5 minutes and record the data in Table 4.3.

61. Measure the % Transmittance of all five cuvettes again at 10 minutes and record the data in Table 4.3.

62. Measure the % Transmittance of all five cuvettes again at 15 minutes and record the data in Table 4.3.

63. Save your experiment and clean up according to your instructor's instructions.

64. What is the purpose of cuvette 5?

Cuvette 5 is a control. Because this sample contains no chlorophyll, the DPIP will not be reduced and will not change color (the % Transmittance remains constant).

Data Analysis

Table 4.2: Distance moved by pigment bands (millimeters)

Band Number	Distance (mm)	Band color	R*f*
1	23	Light-green	0.26
2	34	Blue-green	0.38
3	85	Yellow	0.94
4			
5			

Note: The number of bands that your students observe will vary between three and five.

Table 4.3: % Transmittance

Cuvette	Conditions	% Transmittance (Initial)	% Transmittance (5 min)	% Transmittance (10 min)	% Transmittance (15 min)
#2	Unboiled, Dark	7.1	8.0	7.6	7.5
#3	Unboiled, Light	11.3	18.7	26.5	35.3
#4	Boiled, Light	10.0	12.3	13.6	14.4
#5	No Chloroplasts, Light	16.4	16.2	16.4	16.4

1. Within your class results you may notice some variation in the distance traveled by the same pigments. While the migration distance of each pigment may vary, the distance relative to the migration of the solvent does not. The migration of the pigment relative to the solvent is expressed as the constant R*f*.

2. In the space below, calculate the R*f* of each of the pigments you observed and record these values in Table 4.2.

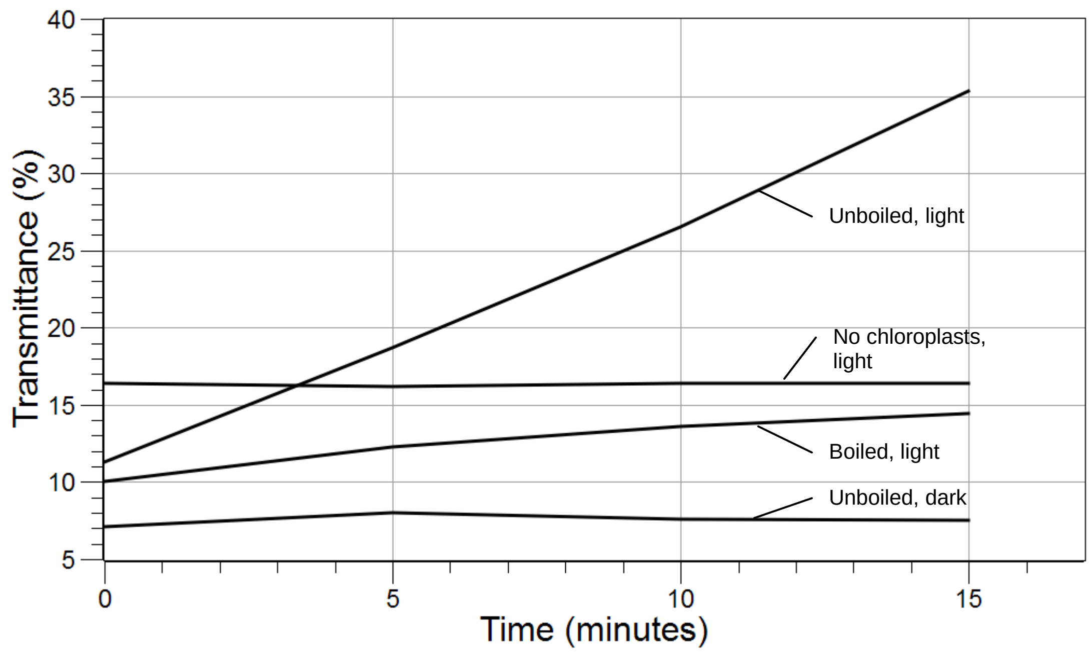
R*f* = distance traveled by the pigment / distance traveled by the solvent

Band 1: 23/90 = 0.26 mm

Band 2: 34/90 = 0.38 mm

Band 3: 85/90 = 0.94 mm

3. Plot a graph of % Transmittance versus Time. Use a key to differentiate your four cuvettes.



Analysis Questions

1. Which of the pigments that you observed in the chromatography experiment is most easily dissolved by the solvent? How do you know?

The pigment that moved the farthest, xanthophyll, is dissolved most easily and is the lightest of the pigments.

2. How would the *Rf* value change if a different solvent were used?

Separation of the pigments is dependent upon the solvent's ability to dissolve the pigments. Using a solvent in which the pigments are insoluble would cause the experiment to fail.

Note: One way to demonstrate this is to repeat the black ink demo using insoluble ink and water as the solvent and then repeat using acetone as the solvent.

3. What is the role of DPIP in this experiment? What would happen to your results if you forgot to add the DPIP to cuvette #3?

DPIP is the electron acceptor and is reduced by electrons from chlorophyll.

If you forgot to add DPIP to cuvette #3, the % Transmittance would mimic that of the boiled chloroplast. Without DPIP, it would appear that photosynthesis was not occurring and the solution would not change color.

4. Compare the % Transmittance in cuvettes #3 and #4.

The unboiled chloroplasts should show a fairly constant rate of increase in % Transmittance during the experiment. The boiled chloroplasts should show little to no change in % Transmittance.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Explain in your own words why the colorimeter was used in this lab. What data did it help you collect?

The colorimeter quantifies how much color change occurred in each cuvette. The colorimeter is a device that measures light absorbance or light transmittance through a solution. The colorimeter allows you to measure the % absorbance or the % Transmittance of several different wavelengths of visible light.

2. Relate leaves changing color in the fall to the accessory pigments.

During the fall the decreasing amounts of sunlight trigger the storage of chlorophyll in the stem of the plant. This allows us to see the normally masked accessory pigments. The accessory pigments are also able to absorb the other wavelengths of light that chlorophyll is not able to.

3. How would your results change if you added sodium bicarbonate (baking soda) to the solutions in the cuvettes? Explain your answer.

When baking soda ($NaHCO_3$) is added to a solution, it dissociates into protons, water, and carbon dioxide. So, adding baking soda to the cuvettes would increase the carbon dioxide concentration of the solutions. Since the Calvin cycle of photosynthesis is dependent upon carbon dioxide, the rate of photosynthesis in cuvette #3 would probably increase.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. The light reactions of photosynthesis use _____ and produce _____.

A. NADPH, $NADP^+$
B. **Water, NADPH**
C. Carbon dioxide, oxygen
D. Carbon dioxide, sugar
E. NADPH, oxygen

2. Where in a plant cell are chlorophyll molecules found?

A. **Thylakoid**
B. Stroma
C. Stomata
D. Plasma membrane
E. Golgi apparatus

3. What is the role of $NADP^+$ in photosynthesis?

A. It assists chlorophyll in capturing light.
B. It acts as the primary electron acceptor for the photosystems.
C. As part of the electron transport chain, it manufactures ATP.
D. It assists photosystem II in the splitting of water.
E. **It is reduced and then carries electrons to the Calvin cycle.**

Extended Inquiry Suggestions

The same technique can be used to measure changes in the rates of photosynthesis under other conditions such as temperature, light intensity, or wavelength (by using a colored light bulb).

8. Factors that Affect Photosynthetic Activity

Objectives

Students explore factors that limit oxygen and carbohydrate production during photosynthesis.

- Students quantify the reactions of photosynthesis.

Procedural Overview

Students gain experience conducting the following procedures:

- Measuring photosynthetic activity using the dissolved oxygen sensor and photosynthesis tank
- Manipulating the amount of light in the system to determine the effects on the rate of dissolved oxygen production

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	10 minutes
♦ Lab experiment	55 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Advanced water quality sensor with optical dissolved oxygen probe
- Photosynthesis tank
- Beaker, 250-mL, filled with water
- Distilled water
- *Elodea, Cabomba* or other aquatic plant, 2 to 3 sprigs[1]
- Desk lamp and incandescent or fluorescent light bulb
- Dark cloth to cover the tank
- Magnetic stirrer with stir bar

[1]See the Lab Preparation section for aquatic plant options.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Basic principles of photosynthesis
- Basic principles of cellular respiration

Related Labs in This Guide

Labs conceptually related to this one include:

- *Elodea* and the Snail
- Plant Pigments and Photosynthesis

♦ Dissolved Oxygen and Primary Productivity

Background

The sun provides the energy for most living organisms, but few organisms can capture the energy and transfer it to other organisms. Animals can warm themselves in the sun, but the energy is not incorporated into their bodies as chemical potential energy. Only plants, through their chloroplasts, can absorb the energy from the sun, incorporate it into molecules, and store it for other organisms. This process is known as photosynthesis.

Photosynthesis is a complex process involving numerous enzymatic reactions. But it can be simply represented by the chemical equation: $6CO_2 + 6H_2O + \text{sunlight} \rightarrow 6O_2 + C_6H_{12}O_6$.

Glucose ($C_6H_{12}O_6$) is the fundamental energy source for living organisms. Animals that eat plants are able to convert the glucose into energy through the process of respiration, which is the reverse chemical reaction of photosynthesis. In order for plants to produce glucose, three factors must be present, sunlight, carbon dioxide, and water. If any of these three factors is absent or limited, glucose production in the plant will be effected.

Photosynthesis consists of two major phases, one that requires light and one that does not. In the light-dependent reactions, the energy from the sun is absorbed by chlorophyll molecules in the chloroplast producing adenosine triphosphate (ATP) molecules. During the light-independent reactions, or the Calvin cycle, the energy from the ATP molecules is released to drive the creation of carbohydrates.

This energy is used to drive several synthesis reactions that fix carbon dioxide entering the leaf through the stomata and make simple carbohydrates. Some of these carbohydrates are used to make glucose, which is used by the mitochondria in cell respiration. Other carbohydrates are immediately used by the plant in its daily activities like growth and reproduction.

Pre-Lab Discussion and Experiment

Engage students in a discussion about photosynthesis, how it is measured, and what factors might affect it. Ask the following series of questions.

1. How could you measure photosynthesis?

Lead students to determine that you could measure the amount of sugar produced, the amount of carbon dioxide consumed, or the amount of oxygen produced. Tell the students that they will be using oxygen production to measure photosynthetic activity in this lab.

2. Ask students to name factors they think could limit the production of oxygen and glucose in photosynthesis.

Factors include: light, temperature, carbon dioxide levels, water availability and nutrient availability.

3. Focus on the idea that carbon dioxide levels can affect photosynthetic activity. Ask students to think of a way to control the amount of carbon dioxide in a room. Then ask students if they think it is possible to change carbon dioxide levels in water.

Tell students how you prepared the "carbon dioxide–free" water. Also, tell students what happens when you dissolve sodium bicarbonate in water. The ions dissociate, or separate from each other, and carbon dioxide is released into the solution.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Buy aquatic plants from any pet store that carries aquarium supplies. Many people use Elodea for photosynthesis experiments. Unfortunately, it is illegal in some states to purchase Elodea. Cabomba is a good alternative. You will need two or three sprigs per group. After you purchase the plant, remove the rubber band and place the plant in de-chlorinated water. De-chlorinate the water by leaving it out on the counter for several hours.

If you cannot find an aquatic plant, or if the plants look unhealthy, algae solution is a great alternative. You can purchase green algae, like chlorella from most biological supply companies. When the algae arrive, immediately remove it from the box. Place the test tube in a test tube rack and remove the cap. Use a disposable pipette to squirt air bubbles into the test tube and then place the algae in a cool place with indirect lighting until ready to culture.

When you are ready to do the lab, obtain a very large, sterile container. Depending upon the volume of solution you wish to create, add 20 ml of Alga-Gro® per liter of water. Also add the test tube of algae. Stir to mix. Place the algae in a cool place with indirect lighting until ready to use.

Safety

Add this important safety precaution to your normal laboratory procedures:

♦ Be careful when handling the light bulb after it has been on. It will be hot and could cause burns.

Procedure

Part 1 – Monitoring dissolved O_2 in ambient light

Set Up

1. Obtain a photosynthesis tank, a magnetic stirrer, a stir bar and a 250-mL beaker of de-chlorinated water containing 2 to 3 sprigs of aquatic plant.

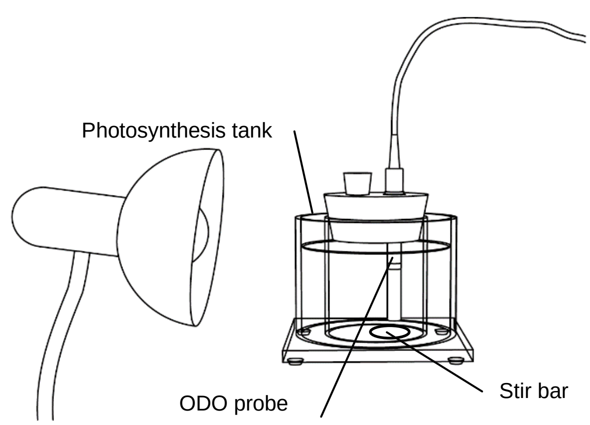
2. Fill the outer ring of the photosynthesis tank with cold water.

3. Fill the inner ring of the photosynthesis tank with approximately 500 mL distilled or deionized water. Pour as much water into the inner tank as possible (it is ok if it overflows into the outer tank).

4. Drop a stir bar into the inner chamber and place the tank on top of the magnetic stirrer.

5. Put 2-3 sprigs of the aquatic plant into the inner chamber.

6. Start a new experiment on the data collection system.



7. Connect the optical dissolved O_2 sensor to the data collection system.

8. Calibrate the optical dissolved O_2 sensor.

9. Carefully insert the dissolved oxygen probe into the larger hole in the rubber stopper. With the probe in the stopper, insert the probe into the water and press the stopper firmly into the inner ring of the tank. Make sure that the smaller hole in the tank is plugged with the small rubber stopper.

Note: Ensure proper placement of the DO probe. The silver ring near the top of the probe should be under the water. The end of the probe should be about 1 cm above the stir bar, and no plant material should be touching the end of the probe.

10. Turn the magnetic stirrer on to a high speed.

Collect Data

11. Monitor the dissolved O_2 level in the tank.

12. When the level stabilizes, display Dissolved O_2 (%) on the y-axis of a graph with Time on the x-axis

13. Start data recording.

14. Adjust the scale of the graph to show all data.

15. Record data for 5 minutes. While you are waiting, obtain a desk lamp and place it close to the tank.

16. After 5 minutes, turn the light on and shine it directly on the plant. Do not stop recording data.

17. Create a note indicating the time at which the light was turned on.

18. How will the dissolved O_2 levels change due to the increased light intensity?

Students should understand that the dissolved O_2 levels in this part of the experiment should be higher than those measured in the first five minutes. The light should stimulate photosynthetic activity and increase dissolved oxygen levels in the tank.

19. Record data for 15 minutes with the light on. While you are waiting, obtain a dark cloth.

20. After 15 minutes, turn the light off and cover the tank with a dark cloth. **Continue to record data**.

21. Create a note indicating the time at which the light was turned off.

22. How will the dissolved O_2 levels change during the dark period? Explain your answer.

Students should understand that the dissolved O_2 levels in this part of the experiment should decrease slightly. The lack of light will cause photosynthetic activity to decrease and the dissolved oxygen levels in the tank will decrease. Even though the plant is not photosynthesizing, it will continue to respire and use oxygen in the process. This is what causes the decrease in dissolved levels in the tank.

23. After 15 minutes in the dark, stop data recording and remove the dark cover.

24. Remove the top of the tank, take the dissolved O_2 sensor out of the stopper and put it back into its bottle.

25. Remove the plants from the tank and place them back in the beaker of water.

26. Save your experiment and clean up according to your instructor's instructions.

Data Analysis

1. Select the data in the first 5 minutes, before the light was turned on.

2. Apply a linear fit to this data to find the slope of the line.

3. Record the slope as the rate in Table 1.

4. Select the data points during the period of time when the plant was exposed to light.

5. Apply a linear fit to this data to find the slope of the line.

6. Record the slope as the rate in Table 1.

7. Select the data points during the time period when the plant was in the dark.

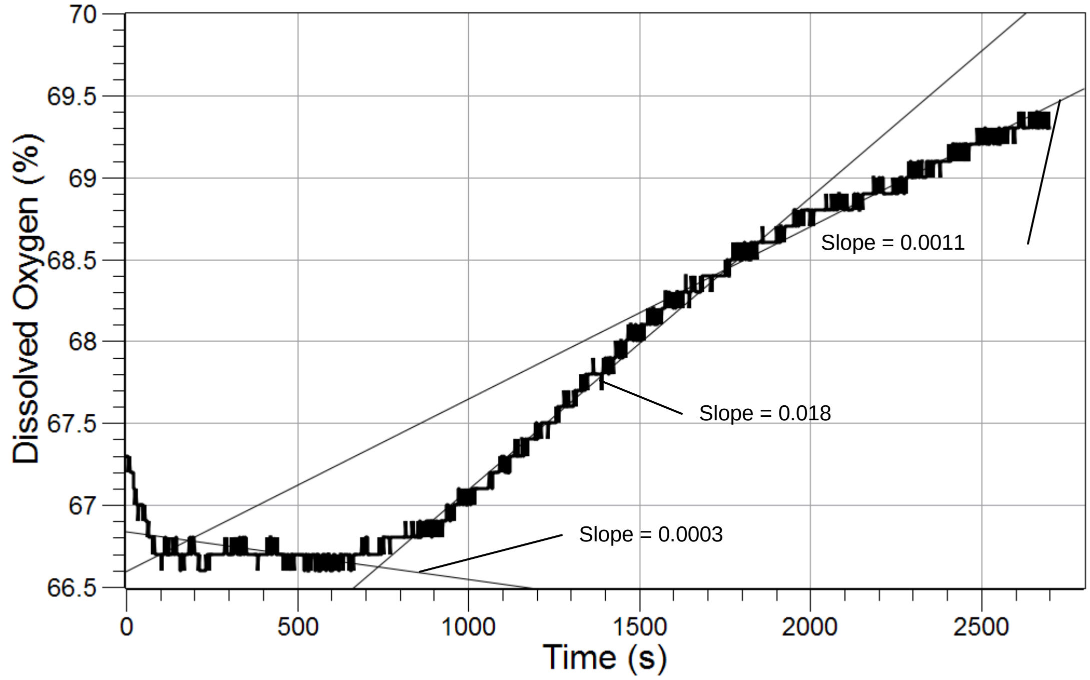
8. Apply a linear fit to this data to find the slope of the line.

9. Record the slope as the rate in Table 1.

Table 1: Rate of dissolved oxygen production

Light Intensity	Rate of Change in Dissolved Oxygen Concentration (%/s)
Ambient Light	–1.94 x 10–4
Full Light	+0.002
No Light	+0.001

10. Sketch a graph of the data run. Label the overall graph, the x-axis, the y-axis, and include units on the axes. Indicate the periods of time when the plant was exposed to ambient light, full light and no light.



Dissolved Oxygen (%)
70
69.5
69
68.5
68
67.5
67
66.5
0
500
1000
1500
2000
2500
Time (s)
Slope = 0.0011
Slope = 0.018
Slope = 0.0003

Analysis Questions

1. Compare the rates of dissolved oxygen production during these three periods of time: ambient light, full light and no light.

Dissolved oxygen production was very low during the ambient light period, increased during the full light period and then slightly decreased during the dark period.

2. Relate the rate of oxygen production during the three time periods to photosynthetic activity.

The rates of dissolved oxygen production in this experiment provide evidence that photosynthetic activity was highest when the light intensity was the greatest.

3. Predict what would happen if this experiment was continued for two more hours with the plant in the dark. Explain your answer.

Students should predict that the dissolved oxygen levels would slowly begin to decrease as photosynthetic activity decreased due to the lack of light, and respiration rates remained the same, but continued to consume oxygen.

Synthesis Questions

Use available resources to help you answer the following questions.

1. The oceans are an important carbon sink in the cycling of carbon in nature. Explain how aquatic plants and algae could help to reduce carbon dioxide levels in the atmosphere.

Through photosynthesis, algae, phytoplankton, and other organisms capable of photosynthesis remove CO_2 from the ocean water. When they die, much of the material falls into the depths of the ocean where it is sequestered for a long time.

2. What do you think would happen to the dissolved oxygen levels in the tank if you added glucose solution to the inner chamber containing an aquatic plant? Explain your answer.

Students should indicate that the dissolved oxygen levels would decrease. Glucose will decrease dissolved oxygen levels in two ways. First, it will increase the rate of respiration within the mitochondria of the plant cells. The reactions of respiration use oxygen as a reactant. Second, sugars like glucose are end products of the photosynthetic reactions. Therefore, the increased concentration of glucose will cause a negative feedback mechanism that will decrease the rate of photosynthesis.

3. Water is one of the inputs to photosynthesis. If there is not enough water vapor in the air, a plant will not open the stomata on its leaves and photosynthesis will not occur. What are some of the ways plants have adapted to conserve water in their leaves?

Adaptations include thick waxy leaves, stomata on the underside of the leaves, and long narrow leaves, to name a few.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. In order for photosynthesis to occur in green plants which of the following inputs are required?

- **A.** Light, oxygen, carbon dioxide
- **B.** Light, water, oxygen
- **C. Light, water, carbon dioxide**
- **D.** Darkness, glucose, carbon dioxide
- **E.** Darkness, oxygen, glucose

2. Which reaction is matched with the correct products?

- **A. Light-dependent reactions — ATP and NADPH**
- **B.** Light-dependent reactions — water and ATP
- **C.** Light-dependent reactions — glucose and ADP
- **D.** Light-independent reactions — ATP and glucose
- **E.** Light-independent reactions — carbon dioxide and water

3. Which of the following statements is true?

- **A.** Photosynthesis occurs at night and during the day.
- **B. Respiration occurs at night and during the day in green plants.**
- **C.** Respiration only occurs when the plant is photosynthesizing.
- **D.** Respiration only occurs in the dark.
- **E.** Photosynthesis and respiration cannot occur at the same time in green plants.

Extended Inquiry Suggestions

Have the students design and carry out an investigation to determine if temperature or light intensity has an effect on the production of oxygen in aquatic plants.

A further extension could be to find the optimum levels for all three factors (light, carbon dioxide level, and temperature) combined. For this, the students would have to plan an experiment with three variables.

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9. Cellular Respiration

Objectives

Students use oxygen gas consumption and carbon dioxide gas production as measures of seed respiration rates.

- Students compare the rate of cell respiration in germinating versus dormant pea seeds.
- Students determine the effects of temperature on the rate of cell respiration in germinating pea seeds.

Procedural Overview

Students gain experience conducting the following procedures:

- Using the carbon dioxide gas sensor to measure changes in CO_2 levels in a Metabolism Chamber containing respiring pea seeds
- Using the oxygen gas sensor to measure changes in O_2 levels in a Metabolism Chamber containing respiring pea seeds
- Calculating the respiration rate of germinating versus non-germinating pea seeds
- Calculating the respiration rate of germinating pea seeds exposed to differing temperatures

Time Requirement

♦ Preparation time	30 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	70 minutes

Materials and Equipment

For each group:

- Data collection system
- Oxygen gas sensor
- Carbon dioxide gas sensor
- Sensor extension cable
- Metabolism Chamber
- Glass beads (25)
- Dry pea seeds (25)
- Germinating pea seeds (25), room temperature
- Germinating pea seeds (25), chilled
- Germinating pea seeds (25), boiled
- Large beaker of ice

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- The basic principles of cellular respiration
- Seed dormancy and seed germination

♦ The relationship between cell respiration rates and cellular activity

Related Labs in This Guide

Labs conceptually related to this one include:

♦ Measuring Aerobic Cellular Respiration in Yeast

♦ Fermentation in Yeast

♦ Interrelationships of Plants and Animals

♦ Elodea and the Snail

♦ Physiology of the Circulatory System

♦ Dissolved Oxygen and Primary Productivity

Background

The term "respiration" refers to the exchange of gases between an organism and its environment. This intake of oxygen gas and exhalation of carbon dioxide gas is closely linked to the production of ATP at the cellular level, a process called cellular respiration. ATP is generated by mitochondria within the cell. During cellular respiration, the energy stored within macromolecules such as glucose is released and harnessed to phosphorylate ADP, producing ATP.

In the presence of oxygen, glucose can be fully oxidized releasing large amounts of energy. The process of cellular respiration also produces water and carbon dioxide gas as waste products.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{energy}$$

Organisms that utilize oxygen for the breakdown of glucose are called aerobic organisms. Plants and animals are both examples of aerobic organisms. Even dormant plant seeds undergo respiration, although at a much lower level than after germination starts.

Pre-Lab Discussion and Experiment

Engage students in a discussion about aerobic respiration by asking the following series of questions.

1. Review the major points of aerobic respiration with the class. What is the basic formula for aerobic cellular respiration? What is the basic formula for anaerobic respiration?

Ask students to come up with these basic equations and to note that the number of ATP molecules produced in anaerobic respiration is significantly less than the amount produced by aerobic respiration.

Aerobic respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36 \text{ or } 38 \text{ ATP}$

Anaerobic respiration is technically glycolysis + fermentation. When glycolysis occurs in an anaerobic environment, glucose is still converted to pyruvate. However, the fate of pyruvate differs if oxygen is not present at the end of glycolysis. Instead of entering the Krebs cycle, as it does in aerobic respiration, pyruvate enters one of two fermentation pathways, lactic acid fermentation or ethyl alcohol fermentation.

Ethyl alcohol fermentation: pyruvate + ADP + NADH → ethyl alcohol (C_2H_6O) + CO_2 + ATP + NAD^+

Lactic acid fermentation: pyruvate + ADP + NADH → lactic acid ($C_3H_5O_2$) + ATP + NAD^+

2. How can the rate of aerobic cellular respiration be measured in pea seeds?

Students should understand that they can measure the amount of carbon dioxide produced as a byproduct of cell respiration, or they could measure the amount of oxygen consumed by the organism during respiration.

3. What part of aerobic cell respiration produces carbon dioxide? What part of aerobic cell respiration uses oxygen?

There are three major parts of aerobic cellular respiration, glycolysis, the Krebs cycle, and the electron transport chain and oxidative phosphorylation. Carbon dioxide is released during the Krebs cycle. Oxygen is used in the electron transport chain.

4. What is glycolysis?

Glycolysis is series of enzyme-catalyzed reactions that occur in the cytosol. In glycolysis, one molecule of glucose is oxidized to two molecules of pyruvate. Electrons and protons that are stripped from glucose are stored in the high energy carrier, NAD^+. Two NADH and two net ATP molecules are produced in glycolysis.

5. What is the fate of pyruvate?

If oxygen is present, pyruvate is transported across the outer mitochondrial membrane and is converted to acetyl-CoA. Acetyl-coA then becomes a reactant in the Krebs cycle.

6. What happens in the Krebs cycle?

Within the mitochondrial matrix, another series of enzymatic reactions that oxidizes acetyl-CoA and stores the electrons and protons in NAD^+ and FADH, reducing them to NADH and $FADH_2$. Two net ATP are produced by the Krebs cycle. NADH and $FADH_2$ carry those stored electrons and protons to the inner mitochondrial membrane where the electrons are donated to the first protein in the electron transport chain.

7. Where is the ATP made?

Most of the ATP produced by aerobic cell respiration is produced by the electron transport chain, a series of proteins in the inner mitochondrial membrane that are easily oxidized and reduced. As electrons are passed from protein to protein in the chain, the energy of the electrons is used to pump protons from the inner membrane space into the mitochondrial matrix. The large proton gradient this creates drives the production of ATP through the membrane-bound enzyme, ATP synthetase. When the electron reaches the end of the electron transport chain, it reduces the terminal electron acceptor, oxygen, and with the addition of protons, water is produced. This process, known as oxidative phosphorylation, produces 32 ATP. This is the only step of aerobic cellular respiration where oxygen is required.

8. Does temperature affect respiration rates in pea seeds?

Since all three steps of aerobic cellular respiration depend on the activity of enzymes, proteins whose catalytic activity are highly dependent upon temperature, respiration rates are affected by temperature changes.

Review/discuss dormancy and germination in seeds. Tell the students what you did to the dry seeds to create the germinating seeds. Discuss imbibition (the swelling of the seed) and germination.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare germinating pea seeds: Buy Alaska pea seeds from a biological supply company or lima beans from the grocery store. Three to four days before the lab, soak the pea seeds in a small pan of water overnight. Each group will need at least 75 germinating pea seeds. Twenty four hours later, remove the seeds from the water and place on a moist paper towel. Place the seeds and the towel in a plastic bag (or plastic, lidded storage container) and leave in a warm, dry place for another 24 hours. Do not seal the bag or plastic storage container.
2. On the day of the lab, prepare the boiled pea seeds. Remove 25 germinating pea seeds per group and place them in boiling water for 5 minutes. After removing them from the water, allow them to cool, pat them dry and place them in a beaker labeled "boiled peas".
3. On the day of the lab, prepare the chilled pea seeds. Remove 25 germinating pea seeds per group and place them in a beaker labeled "chilled peas". Place the beaker in an ice water bath. Keep in the refrigerator until ready for use.
4. You can buy glass beads from any biological supply store. The glass beads will take up the same volume in the sampling bottle as the pea seeds, but will not consume carbon dioxide. They make the perfect control group. If you do not have glass beads, you can use any non-living item that is the same size and would take up the same volume as 25 seeds. Marbles and beads make good substitutes.
5. The Metabolism Chamber is designed for use with the CO_2 and O_2 gas sensors. If you do not have both CO_2 and O_2 sensors, use only one sensor and the sampling bottle that comes with the sensor.

Safety

Follow all standard laboratory procedures.

Procedure

Part 1 – Trial using glass beads

Set Up

1. Start a new experiment on the data collection system.
2. Connect a carbon dioxide gas sensor and an oxygen gas sensor to the data collection system. Use the sensor extension cable to connect the carbon dioxide gas sensor to the data collection system.
3. Calibrate the carbon dioxide gas sensor.
4. Calibrate the oxygen gas sensor.
5. Change the unit of measurement for O_2 from % to ppm.
6. Display two graphs simultaneously. On one graph, display CO_2 concentration on the y-axis with Time on the x-axis. On the second graph, display O_2 concentration on the y-axis with Time on the x-axis.

7. Place 25 glass beads into the Metabolism Chamber.

Collect Data

8. Insert the CO_2 sensor into the top of the bottle and the O_2 sensor into the side of the bottle. Firmly press the rubber stoppers of the sensors into the bottle. The bottle must remain vertical; do not lay it on its side.

9. Start data recording (data run #1).

10. Adjust the scale of the graphs to show all data.

11. After 10 minutes, stop data recording.

12. Name this data run, "Glass Beads".

13. Remove the sensors and the glass beads from the bottle.

14. Wave the bottle so that the air in the bottle mixes with the air in the room.

15. What is the purpose of the glass beads?

The glass beads should not produce carbon dioxide gas or consume oxygen gas. This control run using glass beads establishes the baseline against which to measure changes in CO_2 and O_2 gas concentrations.

Part 2 – Trial using dry pea seeds

Set Up

16. Place 25 dry pea seeds into the Metabolism Chamber.

Collect Data

17. Insert the CO_2 sensor into the top of the bottle and the O_2 sensor into the side of the bottle. Firmly press the rubber stoppers of the sensors into the bottle. The bottle must remain vertical, do not lay it on its side.

18. Start data recording (data run #2) on the same graphs.

19. After 10 minutes, stop data recording.

20. Name this data run, "Dry Peas".

21. Remove the sensors and the dry pea seeds from the bottle.

22. Wave the bottle so that the air in the bottle mixes with the air in the room.

23. What is the purpose of testing carbon dioxide production in dry pea seeds?

To determine whether dormant seeds undergo respiration. Because the seeds are dormant, they will undergo respiration at a much lower rate than germinating seeds.

Part 3 – Trial using germinating pea seeds at room temperature

Set Up

24. Place 25 germinating pea seeds at room temperature into the Metabolism Chamber.

Collect Data

25. Insert the CO_2 sensor into the top of the bottle and the O_2 sensor into the side of the bottle. Firmly press the rubber stoppers of the sensors into the bottle. The bottle must remain vertical, do not lay it on its side.

26. Start data recording (data run #3) on the same graphs.

27. After 10 minutes, stop data recording.

28. Name this data run, "Germinating Room Temp".

29. Remove the sensors and the pea seeds from the bottle.

30. Wave the bottle so that the air in the bottle mixes with the air in the room.

Part 4 – Trial using boiled germinating pea seeds

Set Up

31. Place 25 boiled germinating pea seeds into the Metabolism Chamber.

32. Do you think that boiling the germinating pea seeds will have any effect on the respiration rate? Defend your answer.

Students should understand that boiling the pea seeds will destroy the enzymes involved in cell respiration and the respiration rate will be minimal.

Collect Data

33. Insert the CO_2 sensor into the top of the bottle and the O_2 sensor into the side of the bottle. Firmly press the rubber stopper of the sensors into the bottle. The bottle must remain vertical, do not lay it on its side.

34. Start data recording (data run #4) on the same graphs.

35. After 10 minutes, stop data recording.

36. Name this run, "Boiled Germinating".

37. Remove the sensors and the pea seeds from the bottle.

38. Wave the bottle so that the air in the bottle mixes with the air in the room.

Part 5 – Trial using chilled germinating pea seeds

Set Up

39. Place 25 chilled germinating pea seeds into the Metabolism Chamber. Place the chamber into a beaker of ice to ensure that the peas remain cold during data collection.

40. Do you think that the chilled germinating pea seeds will show similar result to the boiled germinating pea seeds? Defend your answer.

Students should understand that chilling the pea seeds will slow down enzymatic activity but not to the same extent as boiling the pea seeds. Boiling the pea seeds destroyed the enzymes involved in cell respiration and caused the respiration rate to be minimal. Chilling the pea seeds should only lower respiration rates slightly.

Collect Data

41. Insert the CO_2 sensor into the top of the bottle and the O_2 sensor into the side of the bottle. Firmly press the rubber stopper of the sensors into the bottle. The bottle must remain vertical, do not lay it on its side.

42. Start data recording (data run #5) on the same graphs.

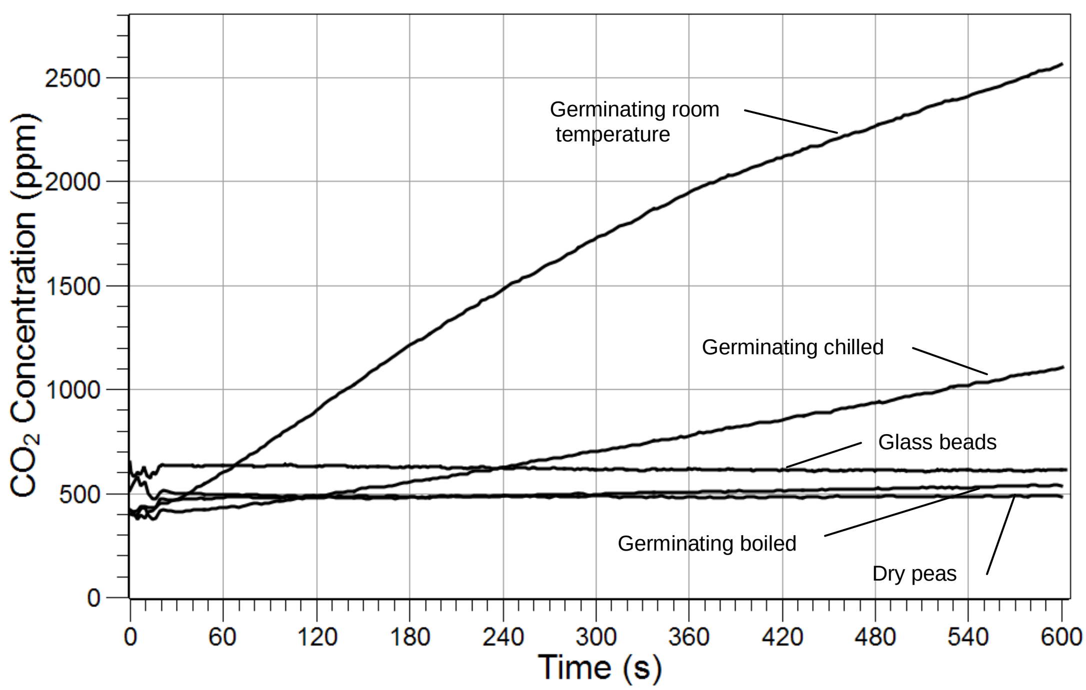
43. After ten minutes, stop data recording.

44. Name these runs, "Chilled Germinating".

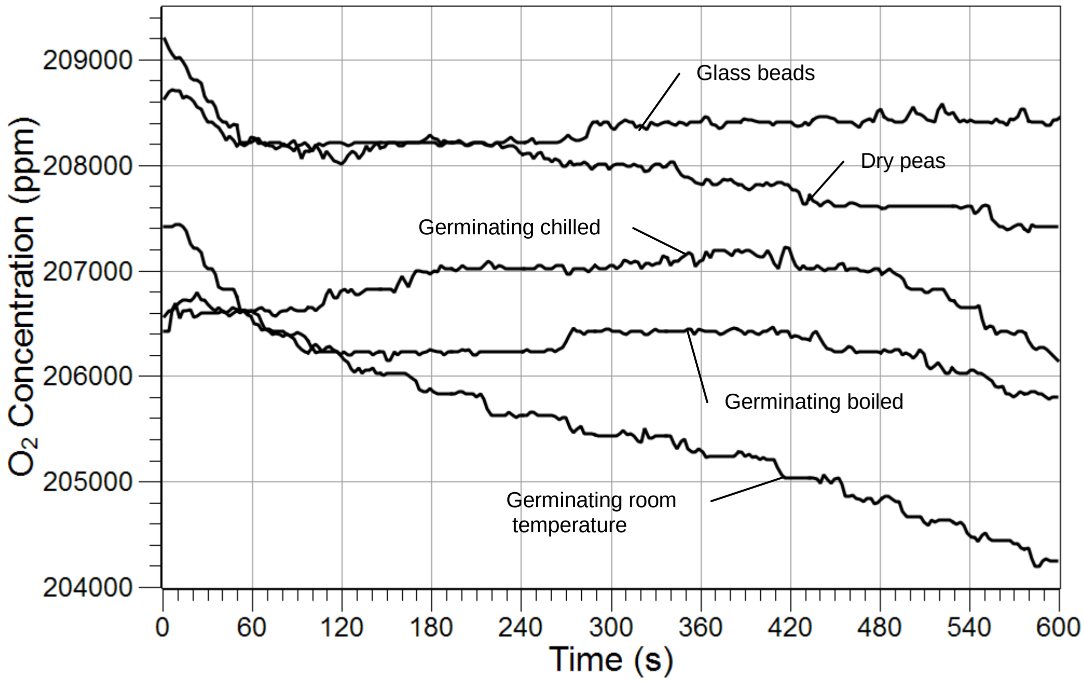
45. Remove the sensors and the pea seeds from the bottle.

46. Save your experiment and clean up according to your instructor's instructions.

Sample Data



Carbon dioxide concentration



Oxygen concentration

Data Analysis

1. Find the minimum and maximum CO_2 concentrations in all five data runs.

2. Record in Table 5.1.

Table 5.1: Carbon dioxide gas production

Data Run #	Set Up	Minimum [CO_2] (ppm)	Maximum [CO_2] (ppm)	CO_2 Gas Production Rate (ppm/s)
1	Glass Beads	570	649	0.13
2	Dry Pea Seeds	471	585	0.19
3	Germinating (room temp)	371	2560	3.65
4	Germinating (boiled)	403	540	0.23
5	Germinating (chilled)	370	1105	1.23

3. Using the following formula, calculate the CO_2 gas production rate in ppm/s.

R = (Maximum [CO_2] – Minimum [CO_2]) ÷ Time (s)

Run #1: 649 – 570 / 300 s = 0.13 ppm/s

Run #2: 585 – 471 / 300 s = 0.19 ppm/s

Run #3: 2,560 – 371 / 300 s = 3.65 ppm/s

Run #4: 540 – 403 / 300 s = 0.23 ppm/s

Run #5: 1105 – 370 / 300 s = 1.23 ppm/s

4. Record your results in Table 5.1.

5. Find the minimum and maximum O_2 concentration in all five data runs.

6. Record in Table 5.2.

Table 5.2: Oxygen gas consumption

Data Run	Set Up	Minimum [O_2] (ppm)	Maximum [O_2] (ppm)	O_2 Gas Consumption Rate (ppm/s)
1	Glass Beads	208,008	209,397	–2.32
2	Dry Pea Seeds	207,017	208,802	–2.98
3	Germinating (room temp)	203,844	207,612	–6.28
4	Germinating (boiled)	205,629	206,819	–1.98
5	Germinating (chilled)	205,827	207,612	–2.98

7. Using the following formula, calculate the rate of oxygen consumption in ppm/s.

R = (Minimum [O_2] – Maximum [O_2]) ÷ Time (s)

Run #1: 208,008 – 209,397 / 300 s = -2.32 ppm/s

Run #2: 207,017 – 208,802 / 300 s = -2.98 ppm/s

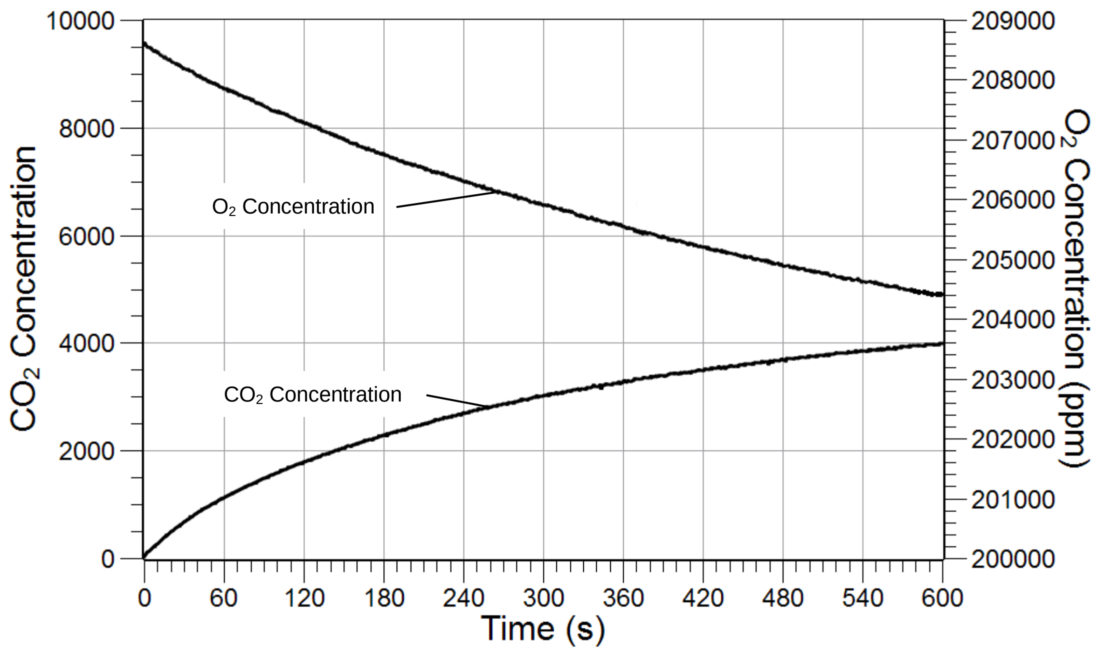
Run #3: 203,844 – 207,612/ 300 s = -6.28 ppm/s

Run #4: 205,629 – 206,819 / 300 s = -1.98 ppm/s

Run #5: 205,827 – 207,612 / 300 s = -2.98 ppm/s

8. Record your results in Table 5.2.

9. Sketch the data for carbon dioxide gas and oxygen gas concentrations on the graph below. Use only the data runs of the germinating peas at room temperature. To create an appropriate scale for your graph, select a range of numbers and fill in the values on the x and y axes.



Analysis Questions

1. Compare the rate of respiration in germinating and non-germinating seeds.

Students should use their data to determine that germinating seeds have higher rates of respiration. Since non-germinating seeds are dormant, their respiration rates are much lower than germinating seeds.

2. Compare the rate of respiration in germinating seeds that have been chilled and boiled.

Students should use their data to determine that lowering the temperature of germinating seeds reduces the rate of respiration. Boiling germinating seeds completely inhibits respiration.

3. Explain the reasons for the difference in the respiration rates between the boiled germinating seeds and chilled germinating seeds.

The rate of respiration in the boiled germinating seeds should have been significantly less than that of the chilled germinating seeds. Since cell respiration is an enzyme-dependent process, the high temperatures associated with boiling denatured the enzymes and rendered them nonfunctional. The lower temperatures experienced by the chilled germinating seeds only decreased the kinetic energy of the molecules within the cells. This caused a reduction in enzymatic activity and, as a result, a decrease in the respiration rate. However, the temperatures were not low enough to denature or damage the enzymes.

4. Explain why carbon dioxide production can be used to determine respiration rate.

Carbon dioxide gas is a byproduct of cellular respiration. Specifically, it is created during the Krebs cycle.

5. Compare the rates of O_2 consumption in germinating seeds versus non-germinating seeds.

Students should use their data to determine that germinating seeds have higher rates of oxygen consumption than non-germinating seeds. Since non-germinating seeds are dormant, their respiration rates are much lower than germinating seeds.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Imagine that we conducted this experiment with lima beans, kidney beans, and pea seeds. Would you anticipate that all seeds would show similar rates of respiration?

No, you would anticipate that larger seeds would have higher rates of respiration than smaller seeds. Students may also comment that different types of seeds (depending on the species) may also have varying respiration rates.

2. Imagine that we measured respiration rates in animals instead of seeds. Would you expect that respiration rates in animals would be affected by temperature in a similar manner?

Because an exothermic animal's body temperature is largely determined by ambient temperature, you would anticipate that respiration rates measured in exothermic organisms would be dramatically affected by temperature. Endothermic organisms on the other hand can regulate their own body temperature to compensate for changes in ambient temperature so they would not show dramatic changes in respiration in relation to temperature changes.

3. After completing this experiment, a student decides to take the germinating seeds and plant them in a pot full of soil. The student nurtures the seed until it grows into a small seedling with tiny green leaves. To satisfy her curiosity, the student places the seedling in a small airtight chamber and measures the O2 consumption and CO2 production. Do you think that she will observe the same data with the seedling that she did with the germinating seeds?

No. The seed has grown into a small plant. The fact that the plant has leaves means that the chloroplasts within the leaf are photosynthesizing. The seed no longer needs to depend on the stored nutrition for energy; it can create its own food through photosynthesis. The student would observe O_2 production and CO_2 consumption. Although the plant is still producing CO_2 through respiration, that CO_2 is now being consumed by the Calvin Cycle of photosynthesis. Aerobic respiration within the plant still requires O_2, but more O_2 is produced through photosynthesis than is used by respiration. Therefore, there is a net consumption of CO_2 and a net production of O_2.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. The function of cellular respiration is to:

- **A.** Reduce CO_2
- **B.** Extract CO_2 from the atmosphere
- **C. Extract usable energy from simple sugars**
- **D.** Synthesize macromolecules from monomers
- **E.** Produce carbohydrates

2. In cell respiration, the largest number of ATP molecules is produced during:

- **A.** Glycolysis
- **B.** The Krebs cycle
- **C. Oxidative phosphorylation**
- **D.** The Calvin cycle
- **E.** The light reactions

3. The function of O_2 in cell respiration is to:

- **A.** Absorb and harness light energy
- **B.** Act as a building block for organic molecules consumed in glycolysis
- **C.** Accept electrons and form CO_2 in the electron transport chain
- **D. Serve as the final electron acceptor during oxidative phosphorylation**
- **E.** Provide electrons to the electron transport chain

4. Most CO_2 from cell respiration is released during:

- **A.** Glycolysis
- **B. The Krebs cycle**
- **C.** Oxidative phosphorylation
- **D.** Lactic acid fermentation
- **E.** The electron transport chain

Extended Inquiry Suggestions

- ♦ This experiment could be conducted long-term by examining respiration rates of germinating seeds over several days.
- ♦ This experiment could also be extended to plants, insects, or small animals such as pillbugs or earthworms.

10. Measuring Aerobic Cellular Respiration in Yeast

Objectives

In this experiment, students investigate how to:

- Calculate the rate of oxygen consumption of yeast during aerobic cellular respiration
- Use rate of oxygen consumption as a measure of the rate of aerobic cellular respiration in yeast at varying temperatures
- Identify a control group, and independent, dependent, and constant variables in an experiment

Procedural Overview

Students will gain experience conducting the following procedures:

- Measuring the dissolved oxygen concentration of a sugar solution before and after adding an activated yeast solution.
- Measuring the dissolved oxygen concentration of diluted sugar solutions at varying temperatures. They will use the temperature probe before and after adding the activated yeast solution.

Time Requirement

Preparation time	20 minutes
Pre-lab discussion and experiment	20 minutes
Lab experiment	45 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Optical dissolved oxygen sensor
- Fast response temperature probe
- Beaker, 250-mL (3)
- Graduated cylinder, 100-mL
- Activated yeast solution, 45 mL [1]
- Sugar, 30 g
- Distilled or deionized water, 1 L
- Ice bath (1-L beaker filled with ice)
- Weighing papers (3)
- Balance
- Stirring rod
- Hot plate
- Labeling marker
- Labeling tape

[1] To formulate activated yeast using dry yeast, sucrose, and water, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Prokaryotic versus eukaryotic cells
- Enzyme structure and function
- Basics of aerobic and anaerobic cellular respiration

Related Labs in This Guide

Labs conceptually related to this one include:

- Exploring the Effects of pH on Amylase Activity
- Fermentation in Yeast
- Cellular Respiration
- Dissolved Oxygen and Primary Productivity

Background

Are yeast aerobic or anaerobic organisms? Yeasts are actually facultative anaerobes, organisms that have the ability to undergo aerobic respiration and anaerobic respiration. With oxygen present, yeast will preferentially undergo aerobic respiration because they can make 36 ATP per glucose molecule through aerobic respiration compared to making 2 ATP through anaerobic respiration.

There are three major parts of aerobic cellular respiration: 1) glycolysis, 2) the Krebs cycle, and 3) the electron transport chain and oxidative phosphorylation. Glycolysis is a series of enzyme-catalyzed reactions that occur in the cytosol. In glycolysis, 1 molecule of glucose oxidizes to 2 molecules of pyruvate. The electrons and protons stripped from glucose are stored in the high energy carrier, NAD^+. Glycolysis produces 2 NADH and 2 net ATP molecules. If oxygen is present, pyruvate is transported across the outer mitochondrial membrane and is converted to acetyl-CoA. Acetyl-CoA then becomes a reactant in the Krebs cycle. Within the mitochondrial matrix, another series of enzymatic reactions oxidizes acetyl-CoA and stores the electrons and protons in NAD^+ and FADH, reducing them to NADH and $FADH_2$. The Krebs cycle produces 2 net ATP molecules. NADH and $FADH_2$ molecules carry those stored electrons and protons to the inner mitochondrial membrane where they donate the electrons to the first protein in the electron transport chain.

If aerobic cellular respiration makes more ATP than anaerobic respiration, then why have only 4 ATP been produced so far? Most of the ATP produced by aerobic cell respiration is produced by the electron transport chain, a series of easily oxidized and reduced proteins in the inner mitochondrial membrane. As electrons pass from protein to protein in the chain, the energy of the electrons pumps protons from the inner membrane space into the mitochondrial matrix. This creates a large proton gradient that drives the production of ATP through the membrane-bound enzyme, ATP synthetase. When the electron reaches the end of the electron transport chain, it reduces the terminal electron acceptor, oxygen, and, with the addition of protons, produces water. This process, known as oxidative phosphorylation, produces 32 ATP. This is the only step of aerobic cellular respiration requiring oxygen.

Pre-lab Discussion and Experiment

Ask students to answer the following questions:

1. What is the basic formula that summarizes the chemical reactions for aerobic cellular respiration? What is the basic formula that summarizes the chemical reactions for anaerobic respiration and ethyl alcohol fermentation)? Does anaerobic or aerobic respiration produce more ATP molecules?

The basic formula that summarizes the chemical reactions for aerobic cellular respiration is
$C_6H_{12}O_6$ (glucose) + $6O_2 \rightarrow 6CO_2 + 6H_2O$ + energy in stored in ATP molecules

The basic formula that summarizes the chemical reactions for anaerobic respiration by yeasts is
$C_6H_{12}O_6$ (glucose) $\rightarrow 6CO_2 + 6H_2O + C_2H_5OH$ (ethanol) + energy in stored in ATP molecules

Aerobic respiration produces many more ATP molecules than anaerobic respiration.

2. Are yeasts aerobic or anaerobic organisms?

Yeasts are facultative anaerobes, organisms that have the ability to undergo aerobic respiration in the presence of oxygen and anaerobic respiration in the absence of oxygen.

3. How can the rate of aerobic cellular respiration be measured in organisms like yeast?

The rate of aerobic cellular respiration can be measured by measuring the amount of carbon dioxide produced as a byproduct of cell respiration, or by measuring the amount of oxygen consumed by the organism during respiration.

4. What part of aerobic cell respiration produces carbon dioxide? What part uses oxygen?

Carbon dioxide is released during the Krebs cycle. Oxygen is used in the electron transport chain.

5. What is glycolysis?

Glycolysis is a series of enzyme-catalyzed reactions that occur in the cytosol. In glycolysis, 1 molecule of glucose oxidizes to 2 molecules of pyruvate. The electrons and protons stripped from glucose are stored in the high energy carrier, NAD+. Glycolysis produces 2 NADH and 2 net ATP molecules.

6. What is the fate of pyruvate?

If oxygen is present, pyruvate moves across the outer mitochondrial membrane and converts to acetyl-CoA. Acetyl-CoA then becomes a reactant in the Krebs cycle.

7. What happens in the Krebs cycle?

Within the mitochondrial matrix, another series of enzymatic reactions oxidizes acetyl-CoA and stores the electrons and protons in NAD^+ and FADH, reducing them to NADH and $FADH_2$. The Krebs cycle produces 2 net ATP molecules. NADH and $FADH_2$ molecules carry those stored electrons and protons to the inner mitochondrial membrane where they donate the electrons to the first protein in the electron transport chain.

8. Where is most of the ATP made?

Most of the ATP produced by aerobic cell respiration is produced by the electron transport chain, a series of easily oxidized and reduced proteins in the inner mitochondrial membrane.

9. Can you measure respiration rates in yeast?

You can measure cell respiration rates in a diluted sugar solution containing yeast by measuring the amount of dissolved oxygen present in the yeast solution.

10. What do you expect to observe when you combine the yeast solution with sugar?

The dissolved oxygen in the yeast solution will decrease as the yeast use the oxygen for aerobic cellular respiration.

11. Does temperature affect respiration rates in yeast?

Because all three steps of aerobic cellular respiration depend on the activity of enzymes, respiration rates would certainly be affected by changes in enzymatic activity.

Review with your students that enzymes are catalytic proteins.

Proteins are organic molecules that have specific three-dimensional configurations. The specificity of an enzyme depends on the shape of its active site, which is the location on the protein where the enzyme will bind to its substrate. Enzymes have an optimal pH and temperature at which they work most efficiently. When pH and temperature deviate too far past optimal, the enzyme will denature and the active site shape will change, no longer specifically fitting the substrate.

Ask your students to make a connection between these concepts and the effects of temperature on respiration activity in yeast.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Start a culture of active yeast approximately 30 minutes before class begins. To do this for a class of eight groups,

   a. Add approximately 450 mL of warm water (30 to 35 °C) to 3 packages of dry yeast. Unexpired baker's yeast from the grocery store works very well.

   b. Add 5 g of sucrose (table sugar), and mix well to thoroughly wet the yeast. You will know that you have living, active yeast if the solution has a foamy head after 10 to 15 minutes.

2. The yeast will become active in 5 to 10 minutes and will begin to metabolize the sugar you have added. You can use the suspension for the remainder of the day. However you should not store it overnight because it might not be active after 24 hours.

3. If you need to make yeast solutions for more than one class, make a larger batch by adjusting the recipe proportionately. To ensure maximum yeast activity for the class, mix the yeast and water at the start of the day, but add only 1 gram of sugar initially. Add 1 more gram of sugar 10 to 15 minutes before each class. This will ensure that each class uses yeast with the same level of activity.

4. After you make the initial yeast solution, ensure that it stays between 20 °C and 45 °C.

5. You cannot use a stainless steel temperature probe at the same time you are using a dissolved oxygen sensor to gather your data. Electrical interference will occur, and your data will not be accurate. Instead use a fast response temperature probe. However, if you are using a dissolved oxygen probe with a Water Quality Sensor, you may use either type of temperature probe.

Safety

Follow all standard laboratory procedures.

Procedure

Set-up

1. Start a new experiment on the data collection system.
2. Connect the optical dissolved oxygen (ODO) and temperature sensors to the device.
3. Calibrate the ODO sensor.
4. Display dissolved oxygen (mg/L) on the y-axis of a graph with Time on the x-axis.
5. Label the 250-mL beakers from "1" to "3" using the labeling tape and marker.
6. Create three 10-gram aliquots of sucrose using the 3 weighing papers.
7. Add 200 mL of room-temperature distilled water and 10 g of sucrose in each of the 3 beakers.
8. Mix using the stirring rod until the sugar dissolves, stirring the water vigorously to saturate it with air.
9. Leave beaker #1 at room temperature.
10. Place beaker #2 in the ice bath to cool until ready for use.
11. Place beaker #3 on the hot plate on a low setting, and allow it to warm to approximately 45 °C.

Collect Data

Part 1 – Room-temperature solution

12. Place the end of the temperature probe into the sugar solution in beaker #1.
13. Measure the temperature and record the temperature in Table 1.
14. Remove and rinse the temperature probe.
15. Make a prediction about how the dissolved oxygen concentration will change after the addition of yeast to each beaker under the three conditions: room-temperature, chilled, and warmed. What background information are you using to support your prediction?

Answers will vary.

Beaker #1: The dissolved oxygen concentration should decrease after the addition of the yeast. The yeast will begin to use the sugar as a reactant in aerobic cellular respiration.

Beaker #2: At decreased temperatures, the rate of dissolved oxygen consumption by the yeast should be slower. At decreased temperatures, molecular motion is slower and enzymatic reactions, like cellular respiration, will occur at slower rates.

Beaker #3: Increased temperatures will also decrease the rate of dissolved oxygen consumption by the yeast. The dissolved oxygen levels will decrease, but they should plateau more quickly than at room temperature. The yeast begin to die when temperatures get above 40 °C.

16. Immerse the end of the DO probe into the sugar solution and gently stir the solution with the probe.

The silver circle on the side of the DO probe should be immersed in water, but the probe should not touch the bottom of the beaker.

17. When the DO stabilizes, begin collecting the DO of the solution for data run #1. Continue to gently stir with the DO probe throughout the data-collection period.

18. After 30 seconds, pour 15 mL of the activated yeast solution into the sugar solution.

19. After 500 seconds, stop collecting data.

20. Rinse the DO probe with the wash bottle or small beaker of clean water.

21. Return the probe to the storage bottle.

22. Why do you have to measure the dissolved oxygen concentration of the sugar solution for 30 seconds before you add the yeast solution?

Measuring the dissolved oxygen before adding yeast allows you to observe conditions in the solution prior to manipulation. It would be difficult to understand the change in dissolved oxygen without knowing the initial concentration.

23. Would it be sufficient to just measure the DO in the sugar solution for 1 minute and use it as your control group for all three parts of the lab? Why or why not?

No. This measurement could be compared with the DO in the yeast solution at room temperature. It could not be compared to the DO in yeast at hot or cold temperatures, because the initial DO will vary with temperature.

Part 2 – Decreased-temperature solution

24. Immerse the end of the temperature probe in the sugar solution in beaker #2. The beaker should remain on ice throughout this part of the lab.

25. Measure the temperature, and record the temperature in Table 1.

26. Remove and rinse the temperature probe.

27. Immerse the end of the DO probe into the cold sugar solution and gently stir the solution with the probe.

The silver ring on the DO probe should be immersed in water, but the probe should not touch the bottom of the beaker.

28. Begin collecting the DO of the solution for data run #2. Continue to gently stir with the DO probe throughout the data-collection period.

29. After 30 seconds, pour 15 mL of the activated yeast solution into the sugar solution.

30. After 500 seconds, stop collecting data run #2.

31. Rinse the DO probe with the wash bottle or small beaker of clean water.

32. Return the probe to the storage bottle.

Part 3 – Increased-temperature solution

33. Place the end of the temperature probe into the sugar solution in beaker #3. The beaker should remain on the hotplate throughout this part of the lab.

34. Measure the temperature and record the temperature in Table 1.

If the temperature rises above 45 °C, remove the dissolved oxygen probe because it can be damaged.

35. Immerse the end of the DO probe into the warm sugar solution and gently stir the solution with the probe.

The silver ring on the DO probe should be immersed in water, but the probe should not touch the bottom of the beaker.

36. Begin collecting the DO of the solution for data run #3.Continue to gently stir with the DO probe throughout the data-collection period.

37. After 30 seconds, pour 15 mL of the activated yeast solution into the sugar solution.

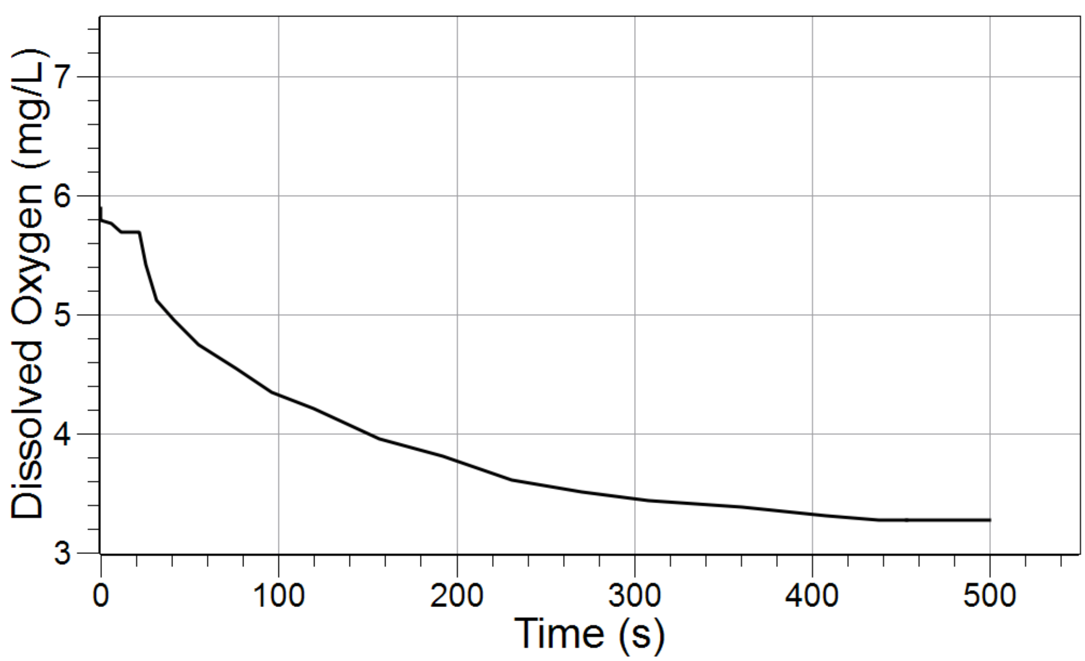
38. After 500 seconds, stop collecting data run #3.

39. Rinse the DO and temperature probes with the wash bottle or small beaker of clean water, and

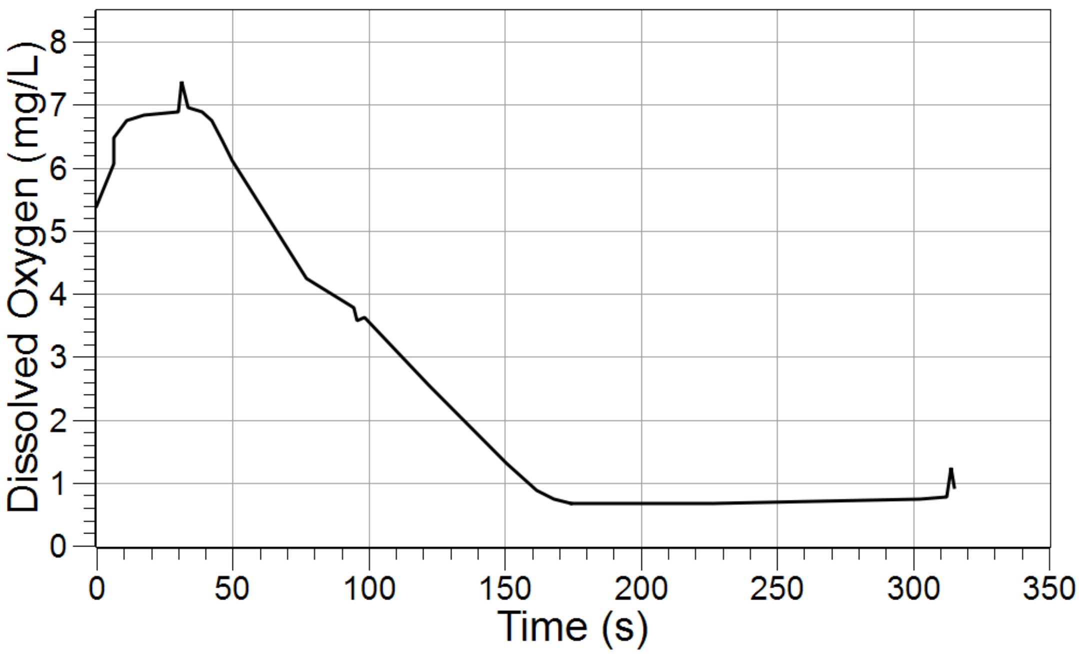
40. Return the DO probe to the probe cover.

41. Save your experiment and clean up according to your instructor's instructions.

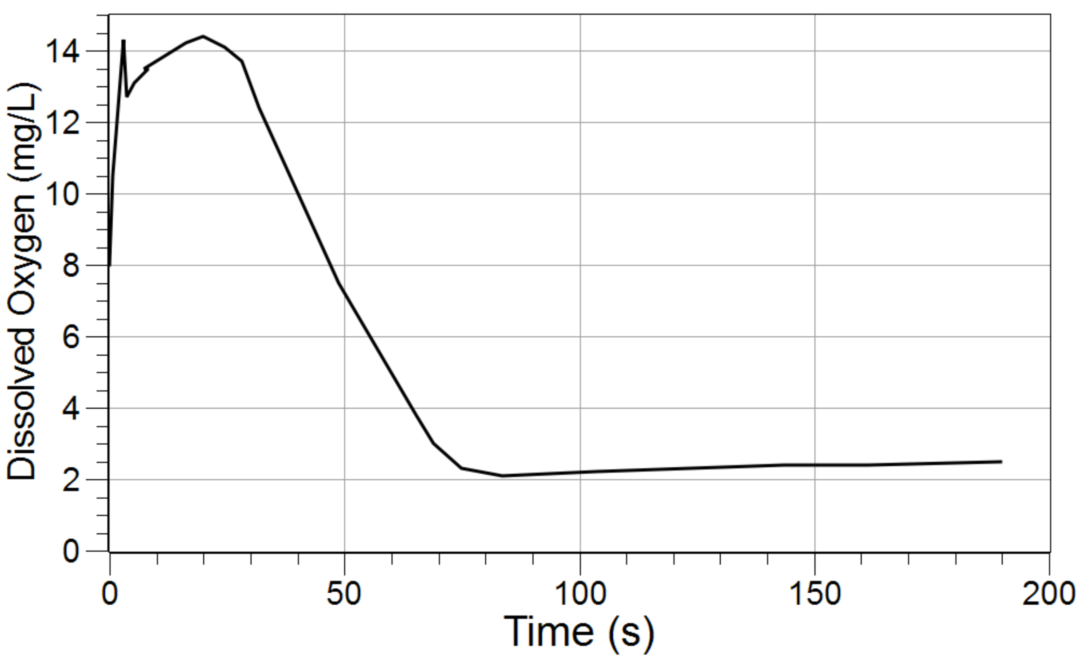
Sample Data



DO concentration (mg/L) versus Time (s)—room temperature



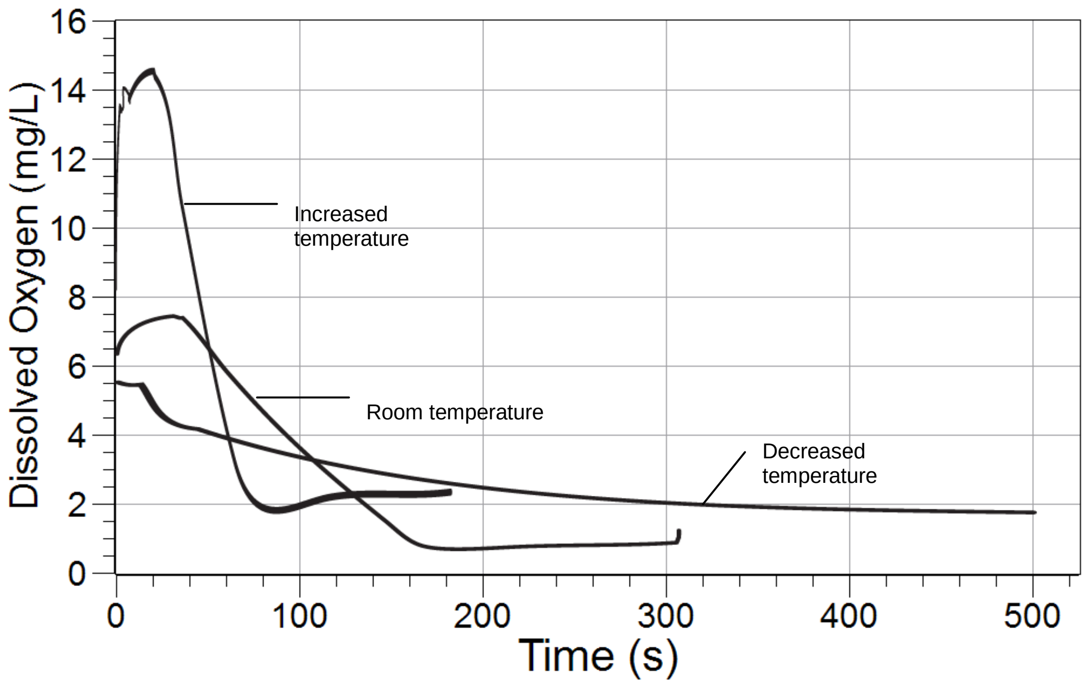
DO concentration (mg/L) versus Time (s)—decreased temperature



DO concentration (mg/L) versus Time (s)—increased temperature

Data Analysis

1. Draw a graph of all three data runs. Label the overall graph, the x-axis, the y-axis, and include units on the axes.


16
14
12
10
8
6
4
2
0
Dissolved Oxygen (mg/L)
0
100
200
300
400
500
Time (s)
Increased temperature
Room temperature
Decreased temperature

2. Find the minimum and maximum DO concentration on all three data runs, and record them in Table 1.

Table 1: Dissolved Oxygen and Temperature

Run	Condition	Temperature (°C)	Maximum DO (mg/L)	Minimum DO (mg/L)	Rate of DO consumption ($mg \cdot L^{-1} \cdot s^{-1}$)
#1	Room Temperature	27.4	7.80	0.70	0.0142
#2	Decreased Temperature	5.90	5.80	3.20	0.0052
#3	Increased Temperature	44.1	14.4	2.50	0.0238

3. Use this information to calculate the rate of oxygen consumption by the yeast during all three data runs.

Rate of DO Consumption = Maximum [DO] - Minimum [DO] ÷ Time (s)

4. List at least three controls in this experiment.

1) The volume of yeast solution tested. 2) The amount of time the dissolved oxygen was measured. 3) The amount of sugar added to the yeast solution.

5. What are the independent and dependent variables for the three data collection parts of the experiment?

In the three data collection parts, the independent variable was temperature (°C), and the dependent variable was the rate of change of dissolved oxygen concentration ($mg\ L^{-1}s^{-1}$).

Analysis Questions

1. Compare your results for each part of the lab with your initial predictions. Were your predictions similar to your results? If not, can you think of a reason why they may not have been similar?

Answers will vary. The decreased-temperature condition of Part 2 should have a negative effect on the rate of respiration in the yeast, thereby decreasing the oxygen consumption rate compared to the room-temperature condition of Part 1. The rate of oxygen consumption would be the greatest for increased-temperature condition of Part 3. The optimal temperature for the environment of yeast is 32 to 38 °C. However, yeasts begin to die when the temperature exceeds approximately 48 to 50 °C.

2. Why is oxygen consumption a good measure of respiration rate in yeast?

Oxygen consumption is used to measure respiration rates in yeast because oxygen is a reactant in the reactions of aerobic cellular respiration. Specifically, oxygen is the final electron acceptor in the electron transport chain. A decrease in dissolved oxygen concentration in the solution is an indirect way to measure cell respiration rates in yeast. If the yeast use the sugar in the solution to make ATP through aerobic cellular respiration, then the dissolved oxygen concentration of the solution will decrease.

3. What would you expect to happen to the results if you forgot to add sugar to the warm solution in beaker #3? What if you forgot to add yeast? Would the results differ from the data you collected in Part 1?

Answers will vary. Students should understand that failure to add the yeast solution would produce very different results than if the student had correctly added the yeast. Without adding the yeast, the dissolved oxygen concentration of the solution would remain relatively constant. Forgetting to add sugar would also affect the dissolved oxygen concentration. Sugar provides a food source for the yeast. To use the sugar during aerobic cellular respiration requires oxygen. Without sugar, cellular respiration would not occur.

However, the results would still differ from those at room temperature, even without the addition of the yeast. The initial temperatures of the two sugar solutions differed. Therefore, the initial dissolved oxygen concentrations would differ. Students should understand that increased temperatures in a solution correlate with a decrease in dissolved gases like oxygen.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Write out the summary equation for aerobic cellular respiration.

Glucose + $O_2 \rightarrow CO_2 + H_2O$ + ATP

2. How much more ATP does aerobic cellular respiration generate than anaerobic respiration? Why?

Aerobic cellular respiration generates 38 ATP per glucose molecule. Fermentation produces 2 ATP per glucose. Most of the ATP produced through aerobic cellular respiration comes from the electron transport chain and oxidative phosphorylation. Without oxygen, pyruvate does not enter the mitochondria to continue to the Krebs cycle, and eventually the electron transport chain. Instead, it is reduced to lactate or ethyl alcohol, processes yielding no additional ATP.

3. What are the three pathways involved in the complete oxidation of glucose? Where in the cell does each pathway occur?

Glycolysis occurs in the cytosol. The Krebs cycle occurs in the mitochondrial matrix. The electron transport chain and oxidative phosphorylation occur in the inner mitochondrial membrane.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Cellular respiration takes place in

- **A.** Plants only.
- **B.** Animals only.
- **C.** Plants and animals only.
- **D. All eukaryotic organisms.**

2. Cellular respiration is the process by which organisms

- **A. Release energy from sugar for metabolic use.**
- **B.** Create complex organic molecules from simple ones.
- **C.** Covert heat to chemical energy for metabolic work.
- **D.** Do more than one of the above.

3. Oxygen consumption can be used as a measure of metabolic rate because oxygen is:

- **A. Necessary for ATP synthesis by oxidative phosphorylation.**

B. Necessary to replenish glycogen levels.

C. Necessary for fermentation to take place.

D. Required for all living things.

4. By the process of aerobic cellular respiration, ________ is/are released into the environment.

A. Oxygen

B. Carbon dioxide

C. Water

D. **Both B and C**

Extended Inquiry Suggestions

Ask students to design an experiment in which they test aerobic cell respiration rates in an organism like a grasshopper.

Ask students to design an experiment in which they test anaerobic respiration rates in bacteria or yeast.

11. Fermentation in Yeast

Objectives

Yeast cells can serve as simple models for studying both aerobic respiration and fermentation. During this investigation, students

- Gather evidence of aerobic respiration and fermentation by yeast
- Determine the rates of aerobic respiration and fermentation in yeast

Procedural Overview

Students gain experience conducting the following procedures:

- Use the oxygen gas and ethanol sensors to gather evidence of aerobic respiration and fermentation by yeast
- Calculate and compare the rates of oxygen consumption and ethanol production by yeast

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	75 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Oxygen gas sensor
- Ethanol sensor
- Beaker, 1000-mL
- Beaker, 500-mL
- Yeast solution, 1 liter[1]
- 0.5 M sucrose solution, 500 mL[2]
- EcoChamber
- Magnetic stirrer and stir bar

[1] To formulate using dry yeast and distilled water, refer to the Lab Preparation section.

[2] To formulate using sucrose and distilled water, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ Prokaryotic versus eukaryotic cells
- ♦ Enzyme structure and function
- ♦ Basics of aerobic and anaerobic cellular respiration

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Exploring the Effects of pH on Amylase Activity
- ♦ Measuring Aerobic Cellular Respiration in Yeast
- ♦ Cellular Respiration
- ♦ Dissolved Oxygen and Primary Productivity

Background

Yeast cells are excellent models to demonstrate cellular respiration by eukaryotic cells. Yeast cells can get the energy they need under both aerobic and anaerobic conditions. If oxygen is present, they use aerobic chemical pathways. In a series of biochemical reactions, the carbon atoms that are bound in the energy-storage molecules combine with oxygen gas to produce carbon dioxide gas, water vapor, and energy. These reactions are summarized as follows:

glucose + oxygen gas +ADP + phosphate→ carbon dioxide gas + water vapor + ATP

If no oxygen is present, yeast cells use anaerobic chemical pathways to obtain energy from food. These reactions are summarized as follows:

glucose + ADP → carbon dioxide gas + ethanol + ATP

During cellular respiration, part of the energy in glucose is captured as chemical energy in a molecule called ATP. The remainder of the energy is released as heat energy into the cell and, ultimately, into the surrounding environment. The ATP molecules provide energy to fuel life processes of growth and reproduction.

Pre-lab Discussion and Experiment

Engage your students in a discussion about the historical role that yeast has played in the progress of science.

You may be able to find a multimedia presentation on the discoveries of Louis Pasteur as related to the French beer and wine industries in the 1860s. If not, you can present the following information:

Only 150 years ago, nobody knew how important the single-celled fungi called yeasts are to humankind. We now know that yeast cells are involved in common medical conditions and are promising genetic research tools. Yeast also turns grape juice into wine; converts sugar, water, and hops into beer; and causes bread dough to rise. However, until 1860, people thought that magic or “the grace of God” was responsible. Beer makers knew that they needed to stir the wort (the sugar, hops, and water) with a special stick that they handed down from generation to generation in order to get the proper fermenting action. Bread makers knew they had to preserve a culture that they carefully kept fresh with regular additions of sugar. Wine makers knew that when they crushed the grapes and let the juice sit in vats with the grape skins for awhile, the juice would ferment and turn into wine.

Louis Pasteur was the scientific genius who discovered that yeast cells caused these processes. When the French government called on him to figure out why the wine and beer industries were literally going sour, he used classic microbiological methods to determine that rogue bacteria had invaded the beer and wine, preventing the yeast cells from growing and doing their job of fermenting sugar into alcohol. He invented the pasteurization process to kill the bacteria, and then he inoculated the sterile solutions with the proper kinds of yeast cells.

Review the chemical reactions involved in aerobic respiration and anaerobic respiration as it occurs in yeast cells. Point out that the process of aerobic respiration in yeast cells is virtually identical to that of human cells.

Despite the similarities, the process of anaerobic respiration in yeast cells is different from that occurring in human muscle cells. In yeast cells, anaerobic respiration produces the waste product ethanol. In human muscle cells, anaerobic respiration produces the waste product lactic acid.

Ask students:

1. Do you think your cells undergo anaerobic respiration?

Students may be surprised to learn that every time they exercise until their muscles get sore, their muscle cells have undergone anaerobic respiration. The lactic acid that is produced as a byproduct of anaerobic respiration makes their muscles feel sore.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare the yeast solution at least 30 minutes before class begins. To make 1 liter of solution, add 20 g of dry baker's yeast to 1 L of warm water (30 to 35 °C). Alter the proportions if you need to make more than 1 L. Baker's yeast from the grocery store works very well, but check the expiration date. Once you have made the initial solution, there is no need to maintain the yeast at 30 to 35 °C. It can be stored at room temperature until ready for use. Just make sure that it stays between 20 and 45 °C.
2. To prepare the 0.5 M sucrose solution:
   a. Measure 171.2 g of sucrose (table sugar).
   b. Place the sugar in a beaker or bottle.
   c. Add distilled water to make 1 liter of solution.

   One liter is enough for 2 groups, so alter the proportions so that each group receives 500 mL of solution. You can store the solution at room temperature.
3. You will need 1% ethanol solution to calibrate the ethanol sensor.
4. You may want to connect the ethanol sensor to a powered-on data collection system before starting the lab so that it will function maximally.
5. If you have access to CO_2 sensors, make them the third sensor in this lab. Students will be able to see CO_2 production in addition to ethanol production. Be sure to calibrate the CO_2 sensors.

Safety

Follow all standard laboratory procedures.

Procedure

Set Up

1. Start a new experiment on the data collection system.

2. Connect the oxygen gas sensor and the ethanol sensor to the data collection system.

3. Change the unit of measure for O_2 from % to ppm.

4. Calibrate the oxygen sensor and ethanol sensor.

Note: The ethanol sensor needs to be connected to a powered-on data collection system for 5 to 10 minutes to function maximally.

5. Obtain one EcoChamber.

6. Place the sensors into the holes in the top of the lid.

7. Push the probe of the ethanol sensor as far down as it will go.

8. Make sure that all other holes in the lid are sealed with a rubber stopper.

9. Place the EcoChamber on top of a magnetic stirrer, and put a magnetic stirrer in the bottom of the EcoChamber.

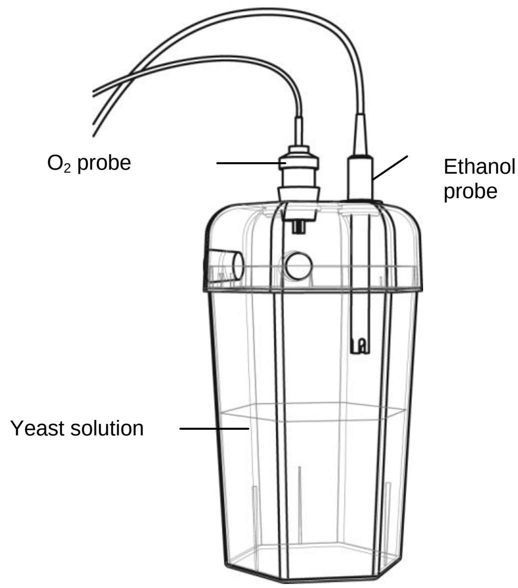
10. Pour 500 mL of 0.5 M sucrose solution into the EcoChamber.

11. Turn the magnetic stirrer on to a medium setting.

Collect Data

12. What do you think will happen to the levels of oxygen and ethanol?

Answers will vary. However, students should mention that they expectethanol levels to rise, and the O_2 level to fall.



13. Start data recording.

14. Pour 1 L of yeast solution into the EcoChamber.

15. Carefully place the lid with the sensors onto the EcoChamber.

16. Press firmly on the lid to ensure an airtight seal.

Note: None of the sensors should be immersed in liquid. They should all be suspended above the solutions. Do not allow the sensors to get wet.

17. Display O_2 concentration on the y-axis of a graph with Time on the x-axis.

18. Display ethanol concentration on the y-axis of another graph with Time on the x-axis.

19. Why is it important to tightly seal the EcoChamber?

You seal the chamber to create a closed system so that all of the gases produced and consumed by the yeast cells will remain in the chamber and be measured.

20. Collect data for 60 minutes, and then stop collecting data.

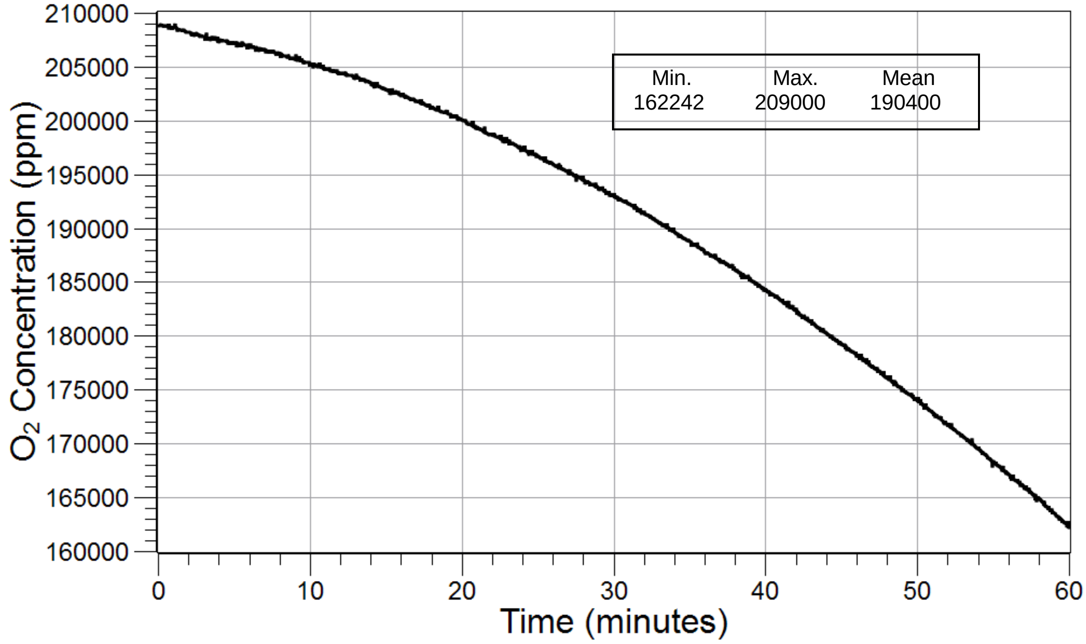
Analyze Data

21. Find the initial and final concentration for oxygen and ethanol of the data run.

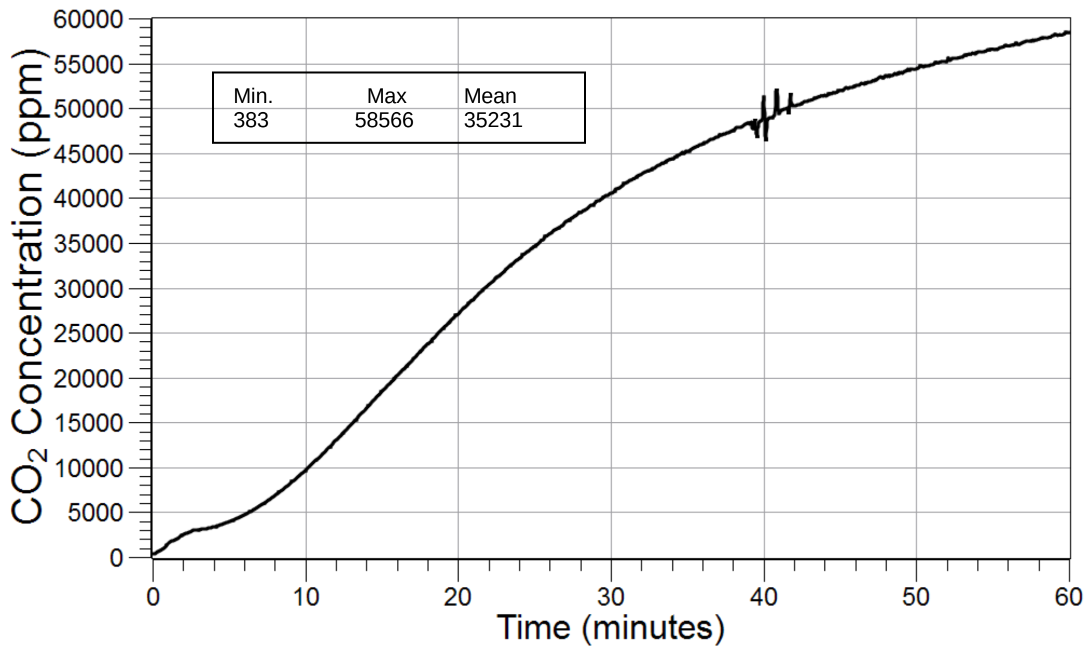
22. Record the results in Table 1.

23. Save your experiment and clean up according to your instructor's instructions.

Sample Data

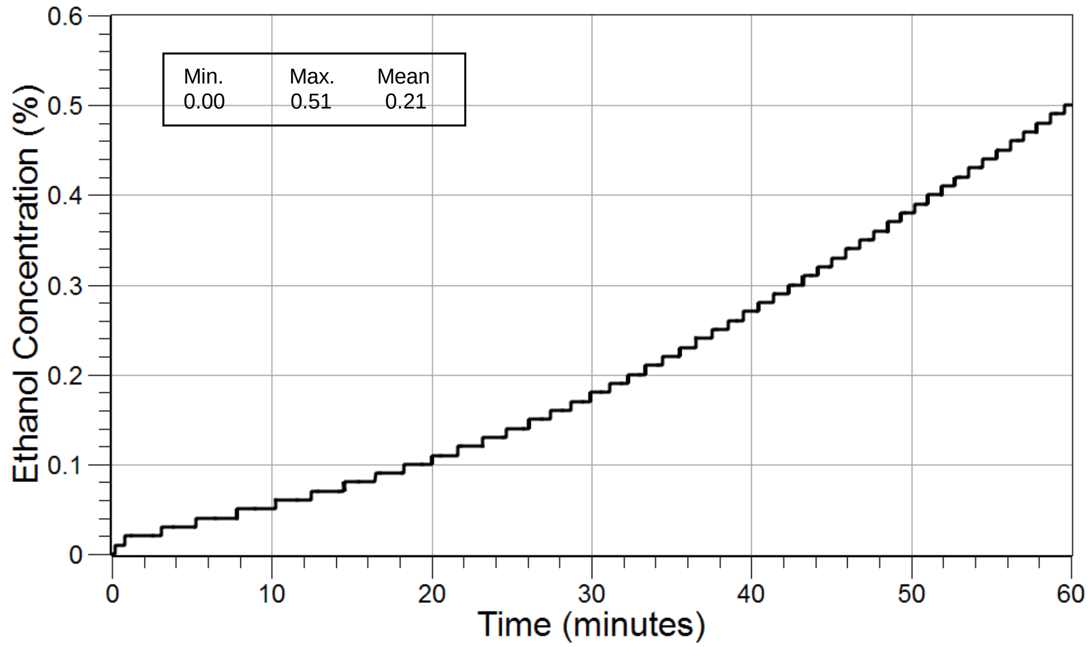


Oxygen consumption



Carbon dioxide production

Note: Data included for your information. If you have a carbon dioxide gas sensor, it is instructive to include it in the experimental setup.



Ethanol production

Data Analysis

1. Calculate the O_2 consumption, and record your answer in Table 1.

Final O_2 concentration – Initial O_2 concentration

2. Calculate the ethanol production, and record your answer in Table 1.

Final ethanol concentration – Initial ethanol concentration

3. Calculate the rate of O_2 consumption, and the rate of ethanol production, and record your answer in Table 1.

Change in concentration ÷ Time (s)

Table 1: Changes in O_2, CO_2, and ethanol concentration

	Initial Concentration	Final Concentration	Change (Final – Initial)	Rate
O_2	209,000 ppm	162,242 ppm	–46,758 ppm	–12.99 ppm/s
CO_2*	383 ppm	58,566 ppm	58,183 ppm	16.16 ppm/s
Ethanol (%)	0.00%	0.51%	0.51%	0.00014 %/s

*If you have a CO_2 sensor, it is instructive to include it in the experiment. The example CO_2 measurements are included for your information to illustrate the utility of this additional evidence of fermentation.

Analysis Questions

1. How do the results compare with your predictions?

Answers will vary. However, students should specifically mention what actually happened to carbon dioxide, oxygen, and ethanol concentrations compared with what they predicted.

2. The following summarizes the chemical reactions during aerobic cellular respiration: O_2 **+ glucose →** CO_2 **+ water + ATP**

What evidence did you gather that shows aerobic cellular respiration took place within the EcoChamber?

Students observed oxygen gas consumption. If you used carbon dioxide gas sensors, then students also observed carbon dioxide gas production.

3. The following summarizes the chemical reactions during anaerobic cellular respiration: **glucose → CO_2 + ethanol + ATP**

What evidence did you gather that shows fermentation took place within the EcoChamber?

Students observed ethanol production. If you used CO_2 sensors, then students also observed carbon dioxide production.

4. If you had access to CO_2 sensors during this experiment, you may have noticed the levels of CO_2 begin to plateau at the end of the experiment, while the yeast continued to consume O_2 and produce ethanol. Give a possible explanation for these trends.

The continued oxygen consumption and ethanol production indicate that both aerobic respiration and fermentation occurred within the EcoChamber. The decrease in CO2 can be attributed to the difference in CO2 produced by aerobic respiration and fermentation. Six CO2 molecules are produced per glucose through aerobic respiration, compared to the two produced by fermentation. The trends in the data indicate that fermentation is occurring in a greater number of yeast than aerobic respiration.

Synthesis Questions

Use available resources to help you answer the following questions.

1. How do you think the data would be affected if you did the exact same experiment, but used a yeast solution that had been stored in the refrigerator all night?

Catalytic proteins called enzymes control the reactions involved in both aerobic and anaerobic respiration. These proteins function best at specific temperatures, and the respiration enzymes in yeast function best at warmer temperatures. Therefore, the rates of O2 consumption, and CO2 and ethanol production would all decrease.

2. List several ways in which fermentation is important to the food and medical industries.

Answers might include one of the following: Fermentation is used to create bread, wine, beer, cheese, tempeh, kimchi, yogurt, sauerkraut, and vinegar. Yeast cells are currently being used in genetic engineering studies, toxicology studies, and molecular biology studies.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Yeasts are

- **A.** Single-celled organisms containing chlorophyll
- **B.** Single-celled organisms that do not have a nucleus
- **C. Single-celled organisms that have a nucleus**
- **D.** Single-celled bacteria
- **E.** Multicellular organisms

2. Yeasts get their energy for life processes from

- **A.** Aerobic cellular respiration
- **B.** Anaerobic cellular respiration
- **C.** Alcoholic fermentation
- **D.** Only A and B are correct
- **E. A, B, and C are all correct**

3. Yeasts release the following as byproducts of cellular respiration:

- **A.** Oxygen gas
- **B.** Carbon dioxide gas
- **C.** Ethanol
- **D.** Water
- **E.** Only B and C are correct
- **F. Only B, C, and D are correct**

Extended Inquiry Suggestions

Have students investigate the role of yeast in the creation of biofuels.

Test the effects of varying sugar concentrations on respiration in yeast. Try solutions that are more and less concentrated.

Biotechnology

12. Bacterial Transformation

Objectives

In this lab, students transform *E. coli* bacteria and screen them for the presence of antibiotic resistance. Students:

- Investigate transformation as a mechanism of genetic exchange
- Create competent *E. coli* cells by chemically and thermally treating them
- Screen the transformed *E. coli* cells to determine which ones have been genetically altered
- Calculate the efficiency of transformation

Procedural Overview

Students will gain experience conducting the following procedures:

- Creating competent *E. coli* cells
- Using a plasmid, pAMP, as the vector to transform competent *E. coli* cells
- Selecting for transformed cells by selecting for ampicillin resistance

Time Requirement

♦ Preparation time	45 minutes
♦ Pre-lab discussion and experiment	20 minutes
♦ Lab experiment	Day one: 55 minutes Day two: 15 minutes

Materials and Equipment

For each student or group:

- Sterile, glass, 15-mL test tubes (2)[1]
- Sterile, graduated, 1-mL transfer pipets (4) or micropipettor with sterile tips
- Sterile, glass spreading rod (1)[1]
- *E. coli* starter plate
- Wire inoculating loop (1)[1]
- Luria Bertani (LB) agar plates[2] (2)
- LB/Amp plates[3] (2)
- pAMP plasmid (10-µL)
- 0.05 M $CaCl_2$ (ice cold) (500 µL)
- Test tube rack
- Ice bath
- Clear tape
- Wax labeling pencil
- Water bath, 42 ºC (1 per class)

[1]Sterile, disposable alternatives are available commercially

[2]To prepare LB agar plates, refer to the Lab Preparation Section.

[3]To prepare LB/Amp agar plates using LB agar and ampicillin, refer to the Lab Preparation Section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Basics of prokaryotic cell structure
- Basic principles of bacterial reproduction
- Basic principles of bacterial transformation
- How a plasmid can be engineered to include a piece of foreign DNA
- How plasmids are used as vectors to transfer genes between cells

Related Labs in this Manual

Labs conceptually related to this one include:

- Mitochondrial Genetics and Biotechnology
- Genetics of Organisms with *Drosophila melanogaster*

Background

The bacterium *Escherichia coli* (*E. coli*) is commonly found in the large intestines of mammals, including humans. Most strains of *E. coli* are harmless, but some cause serious, life-threatening, food-borne illnesses. Over the past few decades, *E. coli* has become one of the most commonly used organisms in biotechnology. This bacterium has become so popular because it is easy to grow and genetically manipulate and its genome has been mapped by geneticists.

E. coli contains a single circular chromosome composed of approximately five million DNA base pairs. In addition to its own genome, most *E. coli* cells contain plasmids, small, circular DNA molecules that carry genetic information. Plasmids often carry genes that would confer some selective advantage to a bacterium that gained the plasmid. For example, some plasmids confer antibiotic resistance or the ability to metabolize certain carbohydrates.

Bacterial genes can be transferred between bacteria in three different ways. In transduction, a virus acts as the vector that transfers the DNA between bacteria. Bacteria can also perform a version of sexual reproduction called conjugation. In conjugation, genetic material transfers from one bacterium of a certain mating type to another of a different mating type. The type of genetic transfer that we explore in this lab is bacterial transformation. Transformation is the direct uptake of foreign DNA, usually a plasmid, leading to its expression within the bacterium. Once these new genes are inside the bacterium, they can be inserted into the bacterial genome, or they can remain autonomous. Scientists use bacterial enzymes called restriction endonucleases to cut DNA at specific sites and insert pieces of foreign DNA into the plasmid. The plasmid can then be used as a vector to insert specific genes into bacterial cells. When cloned, these cells can be used to produce a wide variety of protein products like insulin and growth hormone. Transformation can occur naturally, but very few bacteria are naturally transformed. Most bacteria can be chemically stimulated into taking up foreign DNA. Bacteria that will take up foreign DNA are called competent cells.

Ampicillin is an antibiotic in the penicillin family. Members of this family all contain a beta-lactam ring within their structure. Many bacteria have the gene for an enzyme called beta-lactamase. This enzyme breaks the beta-lactam ring of ampicillin down, and the antibiotic cannot kill the bacteria. This is referred to as antibiotic resistance. The gene for ampicillin resistance can be carried on a plasmid—pAMP. The beta-lactamase gene is called a selectable marker because in the presence of ampicillin, it allows you to select for cells that have been successfully transformed with the pAMP plasmid. Bacteria that have not been transformed and do not contain the plasmid and its gene will not be able to grow in the presence of ampicillin.

Pre-lab Discussion and Experiment

Lead the class through a discussion of the following questions before the lab.

1. Imagine that you are geneticists and are trying to genetically transform an organism. In order to do this, you must insert the new gene into every cell in the organism that they are studying. Which organism is better suited for total genetic transformation: one that is composed of many cells, or one composed of a single cell?

Students should decide that a single-celled organism would be better suited for genetic transformation because it contains only one cell that needs to transform the new gene. Transforming a multicellular organism would require that every single cell in the organism take up the plasmid.

2. As a geneticist, you want to know whether or not the genetically transformed organism can pass its new traits on to its offspring. In order to tell if it successfully passed on the trait, you have to detect the phenotype in some way. Which type of organism is a better candidate for this study: one that develops and reproduces slowly, or one that develops and reproduces more quickly?

Students should determine that a single-celled organism that develops and reproduces quickly is a better candidate for this study. The faster the organism produces offspring, the faster you can assess the phenotype of the offspring.

3. Considering your answers to questions 1 and 2, which organism would be the best choice for a genetic transformation: a bacterium, an earthworm, a fish, or a mouse?

Students should decide that a bacterium is the best candidate for transformation because it is small, is single-celled, and reproduces quickly.

Review the following procedures of aseptic technique:

- ♦ Wipe down lab tables with bleach water before and after the lab.
- ♦ Wash your hands before and after lab.
- ♦ Always keep bacterial plates closed.
- ♦ If you have to open the plate, do not set the lid down on the lab table.
- ♦ Flame inoculating loops before and after touching bacteria or setting the loop down.
- ♦ Do not touch agar or bacteria with a hot loop.
- ♦ Put all material that came into contact with bacteria in the proper disposal bag.
- ♦ Put long hair back in a ponytail.
- ♦ Wear lab goggles and aprons.

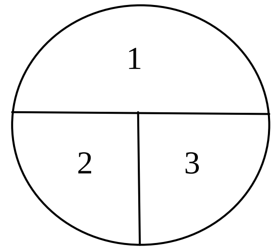
The results of this lab are highly dependent upon proper lab technique. Review the lab procedures with students before they begin the lab.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare *E. coli* starter plates: Buy *E. coli* from a biological supply company. Keep it frozen until you are ready to use it. To create one starter plate per group, you will need one LB agar plate per group (see instructions following). Also, set up a Bunsen burner in an accessible area.

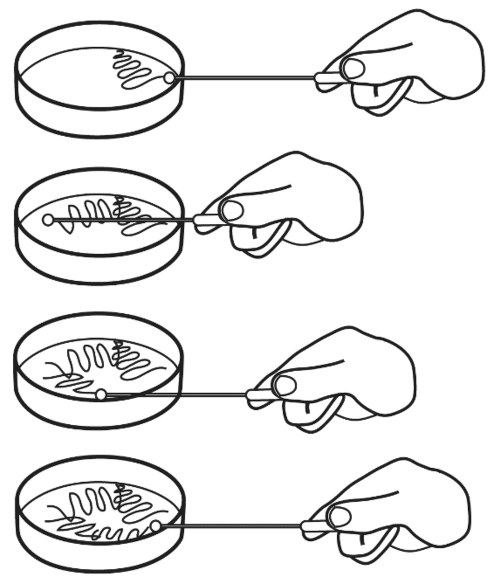
   a. Once the agar has cooled, remove a loopful of bacteria from the *E. coli* culture, using aseptic techniques.



b. Lift the lid of the petri dish out of the way, but keep it angled over the dish.

c. Place the loop gently on the agar surface in the corner of section 1 (see diagram.) Never put enough pressure to dig into the agar surface.

d. Gently sweep the loop back and forth across the agar surface, spreading the sample out in section 1 as shown.



e. Sterilize the loop by placing it in the flame of a Bunsen burner for several seconds. (If you are using sterile, disposable inoculating loops, dispose of this loop and obtain a new one.) Allow the loop to cool before proceeding, but do not set the loop down on the lab bench. If you do set the loop down, flame and cool it again before use.

f. After flaming and cooling the loop, touch it to a sterile part of the agar in section 2. (See diagram above.)

g. Sweep the loop across the agar surface from section 2 into section 1, then back to 2, then back to 1, and so on. Take care not to double back over any streaks in section 2. Stop when you run out of area in section 2, and lift the loop.

h. Sterilize the loop by placing it in the flame of a Bunsen burner for several seconds. (If you are using sterile, disposable inoculating loops, dispose of this loop and obtain a new one.) Allow the loop to cool before moving on, but do not set the loop down on the lab bench. If you do set the loop down, flame and cool again before use.

i. After flaming and cooling the loop, turn the dish so that section 3 (See diagram above.) is accessible.

j. Place the loop in a sterile section of section 3, and then gently stroke the agar surface, returning several times into section 2. Do not double back over any streaks in section 3. Continue in section 3 even though there is not space available for access to section 2. Be careful not to re-enter section 1.

k. When you're finished, re-cover the dish, sterilize or dispose of the loop, invert the plates, and incubate at 37 ºC for 24 to 36 hours (or at room temperature for 48 to 72 hours).

2. Prepare 0.05 M $CaCl_2$: Dissolve 5.55 g of $CaCl_2$ in 75 mL of distilled water.

a. Add distilled water to bring the volume to 100 mL.

b. Cover the top with aluminum foil.

c. Autoclave for 15 minutes at 121 ºC, and then cool to room temperature.

d. Store at room temperature until one day before the lab, and then place the calcium chloride solution in the freezer. If you bought a kit that provided the calcium chloride, place it in the freezer one day before the lab.

3. Prepare Luria Bertani broth.

a. Add 10 g of tryptone, 5 g yeast extract, and 5 g NaCl to a 2-L flask with 1 L of distilled water.

b. Heat gently and swirl for a few minutes to help the ingredients dissolve.

c. Next dispense the solution into five 200-mL bottles.

d. Cap the bottles loosely, and cover them with aluminum foil.

e. Autoclave for 15 minutes at 121 ºC, and then let them cool.

f. Store the bottles at room temperature.

4. Prepare Luria-Bertani agar.

a. Add 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 15 g of agar to a 2-L flask with 1 L of distilled water.

b. Swirl and heat the solution gently for a few minutes to dissolve any lumps.

c. Then cover the flask with aluminum foil, and autoclave for 15 minutes at 121 ºC. Let the flask cool until you can pick up it with your bare hands. If lumps are present, autoclave the solution again.

5. Prepare LB/Amp agar. Add 0.03 g ampicillin to 1 L of autoclaved LB agar. Only add the ampicillin after the agar has cooled enough to hold the flask in your hand. If you bought prepared LB agar in a kit, melt the LB agar in the microwave. To do this,

a. Fill a large beaker (at least 1000-mL) half-way full of water.

b. Remove the cap from the bottle of agar, and place the bottle into the water.

c. Place the beaker and agar into the microwave.

d. Heat the agar in very short increments of time. Every microwave is different, so times will vary. However, you should not exceed 45 seconds of heating at a time. Heat for 30 to 45 seconds.

e. Then, remove the bottle from the water, and swirl the agar.

f. Repeat heating and swirling until the agar is melted. Do not stir or shake the agar excessively because bubbles will form. This process may take 10 to 15 minutes.

g. When the agar is completely melted, remove the bottle from the beaker, and lay the cap on the top of the bottle. Do not seal the bottle.

h. Allow the agar to cool. Do not add ampicillin or pour LB agar plates until the bottle is cool enough to hold with your bare hands.

i. When the agar is cool enough, pour half of the agar into a separate, sterile bottle. Then, add 0.03 g of ampicillin, and swirl to mix the agar.

6. Pour LB agar plates:

a. Place 2 sterile petri dishes per group on a flat surface where the plates can solidify undisturbed.

b. Remove the cover, but do not set it down.

c. Pour the LB agar into the plate, and place the cover over half of the plate. Do not completely cover the plate or else condensation will develop as it cools. However, the cover should cover as much of the dish as possible so that the agar does not become contaminated.

d. Repeat this step for the remaining petri dishes.

e. After the agar has solidified, invert the plates, write "LB" on the bottom of each dish, and store them upside down in the refrigerator.

7. Prepare LB/Amp agar plates.

a. Place 2 sterile petri dishes per group on a flat surface where the plates can solidify undisturbed.

b. Remove the cover, but do not set it down.

c. Pour the LB/Amp agar into the plate, and place the cover over half of the plate. The cover should not completely cover the plate or else condensation will develop as it cools. However, the cover should cover as much of the dish as possible so that the agar does not become contaminated.

d. Repeat this step for the remaining petri dishes.

e. After the agar has solidified, invert the plates, write "LB/Amp" on the bottom of each dish, and store them upside down in the refrigerator.

8. Prepare a 42 °C water bath, and place a test tube rack inside the water bath. Make sure that students will be able to place their 15-mL test tubes in the rack without water entering the test tubes.

9. Prepare an ice bath for each group. This can be a large beaker or cup filled to the top with ice with a little bit of water.

Safety

Add these important safety precautions to your normal laboratory procedures:

- The strain of *E. coli* used in this experiment is a laboratory strain and is relatively harmless under normal conditions. It can, however, cause moderate eye infections, so do not touch your face during the lab.
- When handling any bacterial species, follow aseptic techniques.
- Always flame inoculating loops and spreaders before setting them down on the lab bench.
- Pipetting suspension cultures can create an aerosol. Keep your nose and mouth away from the tip of the pipet to avoid inhaling any aerosol
- Wash your hands before and after entering the lab.
- Any materials that come into contact with the cultures must be disinfected after use. Discard all contaminated materials as directed.
- Absolutely no food or drink is allowed in the lab.

Procedure

Set Up

Make Competent Cells

1. Obtain two sterile 15-mL tubes.

2. Label one tube "+" because it will contain plasmid.

3. Label the other tube "–" because it will not contain plasmid.

4. Use a sterile micropipet to add 250 microliters (µL) of cold (from the freezer) calcium chloride solution to each tube.

5. Place both test tubes into the ice bath.

6. Perform the following steps for each test tube:

a. Use a sterile inoculating loop to transfer a large colony (about the size of a pencil eraser) of *E. coli* from your starter plate to the "+" tube. Make sure not to transfer any agar when transferring *the E. coli*.

b. Spin the inoculating loop vigorously in the solution to dislodge the *E. coli*.

c. Use the micropipet to "re-suspend" the *E. coli* cells into the solution by pulling the solution in and out in the tube.

d. Examine the tube against light to check for any clumps of cells that might remain in the tube or the transfer pipet. The suspension should be milky white.

e. Return the tube to ice bath.

At this point, both tubes should be on ice with *E. coli* suspended in each.

7. Use a clean, sterile micropipet to transfer 10 µL of plasmid DNA directly into the cell suspension in the "+" tube. You can estimate the volume of the plasmid using a sterile inoculating loop. 10 µL of plasmid is approximately the amount that would bubble across the loop opening.

8. Return the "+" tube to the ice and

9. Incubate both tubes on ice for 15 minutes while continuing with the next steps before you "heat shock" the cells. Remember, the "–" tube will not receive plasmid DNA.

Plate Competent Cells

10. Obtain 2 LB agar plates and 2 LB/Amp agar plates from your instructor.

11. Label the bottom of the four plates: label the LB agar plates, one with "+" and the other with "–" and the LB/Amp agar plates, one with "+" and the other "–" along with your initials on the bottom of all four plates.

12. Which of the four plates would you consider the experimental plate? Which one would you consider the negative control?

The experimental plate is LB/Amp + and the negative control is LB/Amp –

13. "Heat shock" the cells after the 15-minute incubation by placing them for 90 seconds in the test tube rack that is in a 42 °C water bath, and gently agitating the tubes.

An abrupt heat shock is critical, so carry your ice bath over to the water bath, and transfer your tubes directly from the ice to the water bath. (You can also hold the tubes in the water for 90 seconds.)

14. Transfer the tubes from the water bath after 90 seconds directly into the ice, and incubate on ice for 2 more minutes.

15. Add 250 μL of room temperature Luria (LB) broth to each tube.

16. Gently tap the tubes with your fingers to mix the suspension.

17. Place the tubes in a test tube rack at room temperature for 10 minutes.

18. What trait are you "selecting for" in this experiment?

You are "selecting for" the ampicillin-resistance trait.

19. Use a sterile pipet to transfer 100 μL of "+" cells to the LB "+" plate, and 100 μL to the LB/Amp "+" plate.

20. Use another sterile pipet to transfer 100 μL of "–" cells to the LB "–" plate, and 100 μL to the LB/Amp "–" plate.

21. Spread the cells over the agar immediately using the sterile spreading rod or glass spreading beads, repeating the procedure for all four plates.

22. Use clear tape to secure the lid to the bottom of the dishes.

23. Stack your plates on top of each other, and turn them upside down.

24. Incubate the plates at 37 °C for 24 to 36 hours (or at room temperature for 48 to 72 hours).

25. Predict the growth of the bacteria on each of the four plates.

Lawns will appear for the LB+ and LB– plates. No colonies will appear on the LB/Amp– plate. Some colonies will appear on the LB/Amp+ plates.

Collect Data

26. Wait until the second day of the lab, and then find your four plates.

27. Hold the plates up to the light, and observe the colonies through the bottom of the plate. Do not open the plates.

28. Count the number of colonies that are present in each plate. If you find it difficult to keep count, use a permanent marker to mark each colony as it is counted. If the cell growth is too dense to count each colony, then you can call the growth a "lawn."

29. Record your data in Table 6A.1.

Data Analysis

Table 6A.1: Number of colonies

Plate	Observed Number of Colonies
LB+	Lawn
LB–	Lawn
LB/Amp+	25
LB/Amp–	0

Transformation efficiency is expressed as the number of antibiotic-resistant colonies per µg of plasmid DNA. Calculate the transformation efficiency of your experiment:

1. Determine the total mass (µg) of plasmid used. To find the mass, multiply the volume of plasmid that you used (10 µL) by the concentration of the plasmid, (0.005 µg/µL).

Total mass = Volume x Concentration

10 µL x 0.005 µg/µL = 0.05 µg

2. Calculate the total volume of cell suspension that you prepared. To do this, add together the volumes of all of the fluids that you added to the plasmid suspension: $CaCl_2$ solution, pAMP solution, and Luria Broth.

250 µL $CaCl_2$ solution + 10 µL pAMP solution + 250 µL LB = 510 µL cell suspension

3. Calculate the fraction of the total cell suspension that you spread on the plate. To do this, divide the volume of suspension that you spread on each plate by the total amount of cell suspension that you prepared (calculated in b.)

Volume of suspension spread ÷ Total volume suspension = Fraction spread

100 µL spread/510 µL total = 0.19

4. Now that you know how much cell suspension was spread on the plate, determine how much that suspension was composed of plasmid. To determine the mass of plasmid in the cell suspension spread, multiply the total mass of the plasmid (calculated in a.) by the fraction of suspension that you spread on the plate (calculated in c.)

Total mass plasmid x Fraction spread = Mass plasmid DNA spread

0.05 µg x 0.19 = 0.0095 µg

5. You can now figure out how many colonies of antibiotic resistant bacteria were found per µg pAMP used. Use scientific notation to express your answer. To do this, divide the number of colonies on the plate by the total mass of plasmid used (calculated in d.).

Colonies observed ÷ Mass plasmid spread = Transformation efficiency

25 colonies ÷ 0.0095 µg pAMP = 2.63 x 10^3 colonies/µg pAMP

Analysis Questions

1. Compare and contrast the number of colonies of the following pairs of plates. What does each pair indicate about the experiment?

Plates	Indications
LB+ and LB–	Because there is no antibiotic in the agar, both appear as lawns.
LB/Amp– and LB–	The LB/Amp– plate does not contain the plasmid, so there is no growth on plate; while in the LB– plate, there isn't any plasmid or ampicillin, so a lawn occurs.
LB/Amp+ and LB/Amp–	Both plates have ampicillin. No colonies appear in the LB/Amp- plate since it is not ampicillin resistant. (No plasmids were added.) The other plate has some colonies because the plasmids that were added make it resistant to ampicillin.
LB/Amp+ and LB+	The first plate produces some colonies because it contains plasmids and is ampicillin resistant. The second plate has a lawn because there is no ampicillin present.

2. What is the purpose of placing the "+" and "–" cells on both the LB and LB/Amp plates?

Comparing the LB+ and the LB– plates allows students to observe bacterial cells growing viably on LB agar after going through the transformation process, regardless of whether they picked up the plasmid. It demonstrates that the transformation process is not harmful to the bacteria.

Comparing the LB/Amp+ and LB/Amp- plates indicates that at least some of the bacteria that are growing on the LB/Amp+ plate were successfully transformed and are now ampicillin-resistant.

3. What evidence do you need to state that transformation successfully occurred?

You would inspect the LB/Amp + plate first because the only colonies produced on this plate are the ones that were transformed with the ampicillin resistant plasmid. If any growth occurred on this plate, then transformation was successful.

Synthesis Questions

Use available resources to help you answer the following questions.

1. What are some other uses of plasmids in genetic engineering?

Plasmids are used as vectors to transfer desirable genes into bacteria that then act as cloning vectors for the gene. If the gene codes for a protein product like a hormone, then the bacterium becomes a factory for that hormone. Plasmids are also used as DNA libraries to store important genes.

2. You have probably heard about the "Nature versus Nurture" debate, which questions whether traits are solely the result of an organism's genetics, solely the result of its environment and upbringing, or a combination of nature and nurture. Based on what you learned in this lab, do you think that the traits of the transformed *E. coli* were the result of nature, nurture, or both? Explain your answer.

Answers will vary. Students should indicate that the phenotypes exhibited by the bacterium are the result of its genotype. However, phenotypes are often affected by chemical factors in the environment. In the case of this transformed *E. coli*, the antibiotic resistance trait was the result of nature, not nurture.

3. In this experiment, you artificially (or chemically) transformed an *E. coli* bacteria and allowed them to live in the presence of a normally lethal antibiotic. Do you think this process is the reason that antibiotic-resistant bacteria like multiple-resistant *Staphylococcus aureus* (MRSA) are becoming such a big public health problem?

It may seem logical that the antibiotic-resistance problem is a result of bacterial transformation, but that is not the case. In this experiment, we artificially transformed the bacteria. The bacteria would not have taken up the plasmid without the chemical and physical treatments that we subjected them to. Many bacterial species are capable of natural transformation, but circular plasmid DNA does not usually participate in this process. The most likely culprit for the antibiotic-resistance problem is over-prescription of antibiotics leading to artificial selection of antibiotic-resistant bacteria. In every bacterial population, a small number of organisms will be naturally antibiotic-resistant because of natural genetic variation. When an antibiotic is taken by a patient, it kills all susceptible bacteria. However, it cannot kill the bacteria that were naturally resistant to the antibiotic. These resistant bacteria continue to live, reproduce and pass on their genes to their offspring. All future generations arising from these resistant cells will carry the antibiotic resistant gene!

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following must be isolated from a eukaryotic cell prior to cloning in order for a bacterium to produce a eukaryotic protein?

- **A.** The protein's primary RNA transcriptase from the nucleus
- **B. The protein's mRNA from the cytoplasm**
- **C.** The protein from the rough endoplasmic reticulum
- **D.** The introns from the segment of DNA that codes for the protein
- **E.** The segments of DNA that control transcription for this protein

2. During an *E. coli* transformation lab, a student forgot to label the culture tube that received the kanamycin-resistant plasmids. The student proceeds with the lab because he thinks that he will be able to determine from his results which culture tube contained cells that may have undergone transformation. Which plate would be most likely to indicate transformed cells?

A. A plate with a lawn cells growing on LB agar with kanamycin

B. A plate with a lawn cells growing on LB agar without kanamycin

C. **A plate with 100 colonies growing on LB agar with kanamycin**

D. A plate with no colonies growing on LB agar without kanamycin

E. A plate with 100 colonies growing on LB agar without kanamycin

Extended Inquiry Suggestions

Students can investigate an application for bacterial transformation in biotechnology including, but not limited to, medical, environment, and forensics.

Lead a class discussion on the definition of bioethics and how it relates to biotechnology. Make sure students address both sides of issues that reflect ethical problems with biotechnology, such as genetic testing, use of embryonic stem cells, and cloning.

13. Mitochondrial Genetics and Biotechnology

Objectives

This mitochondrial genetics lab uses molecular techniques to answer a question about how a mitochondrial disease is inherited and diagnosed. The lab consists of two essential activities: pedigree construction from patient case histories, and molecular analysis of a point mutation in the mitochondrial genome. Students:

- Use restriction endonuclease digests and agarose gel electrophoresis to diagnose an inherited mitochondrial disease
- Construct pedigrees from patient case histories and correlate patterns of inheritance with molecular diagnosis
- Investigate the relationship between mitochondrial DNA and nuclear DNA in the synthesis of the protein complexes of the electron transport chain

Procedural Overview

Students will gain experience conducting the following procedures:

- Analyzing the products of a restriction digest by gel electrophoresis to detect a point mutation in the mitochondrial genome
- Constructing pedigrees from patient family histories to determine the pattern of inheritance of a genetic disease
- Gathering evidence from both the molecular diagnosis and pedigree construction to confirm the diagnosis of a genetic disease

Time Requirement

♦ Preparation time	60 minutes
♦ Pre-lab discussion and experiment	45 minutes
♦ Lab experiment	Three 45-minute periods

Materials and Equipment

For Lab Preparation:

- ♦ Concentrated electrophoresis buffer (50x), 60 mL
- ♦ Distilled or deionized water
- ♦ Electronic balance
- ♦ Erlenmeyer flask, 500-mL
- ♦ Hot gloves
- ♦ Large Beaker, 3 L (to dilute buffer)
- ♦ Marking pen
- ♦ Microwave or hot plate
- ♦ Scissors
- ♦ UltraSpec-Agarose™ powder, 3 g

For each group:

- ♦ Agarose gel, 0.8%
- ♦ Automatic micropipet[1] (5 to 50 µL), with tips
- ♦ Disposable gloves, 1 pair per student
- ♦ Distilled or deionized water
- ♦ DNA visualization system[2]
- ♦ Electrophoresis buffer (1x), 300-400 mL
- ♦ Horizontal gel electrophoresis apparatus
- ♦ D.C. power supply
- ♦ InstaStain® Blue Card
- ♦ Plastic wrap
- ♦ Small plastic tray (for gel staining)
- ♦ QuickStrip™ DNA samples[3]

[1]Edvotek® Variable Micropipet, 5 to 50 µL or Edvotek Fixed Volume MiniPipets™

[2]Edvotek White Light Box (21.5 x 29 cm Viewing Surface) or Edvotek Midrange Transilluminator

[3]No human materials were used in the creation of these materials.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ Structure and function of DNA, RNA, and proteins
- ♦ Structure and function of mitochondria
- ♦ Cellular respiration, electron transport chain, and oxidative phosphorylation

Related Labs in this Manual

Labs conceptually related to this one include:

- ♦ Bacterial Transformation

Background

Restriction enzymes, or restriction endonucleases, are enzymes produced by bacteria that cut DNA at specific nucleotide sequences. Bacteria synthesize these enzymes to defend themselves against bacteriophages, viruses that infect bacteria. Because these enzymes cut DNA at only one specific sequence, they have become powerful tools in molecular biology and biotechnology. Researchers have isolated roughly 700 different restriction enzymes and use them to isolate and "cut" genes of interest from one organism before transforming another organism (usually bacteria) with a gene of interest. For example, DNA polymerase from the extremophile bacteria *Thermophilus aquaticus* is usually isolated from *E. coli* that have been transformed with the recombinant Taq polymerase gene. The enzyme is then used to amplify other genes by polymerase chain reaction (PCR).

Restriction enzymes have several other applications in the laboratory. They can produce restriction fragment length polymorphisms (RFLPs) from two different DNA samples. Given that a restriction enzyme only cuts DNA at a short, specific sequence, two closely related DNA samples will be cut at virtually the same places and produce the same fragment pattern. The genome of all humans is roughly 99.9% identical; however, RFLP analysis can detect small changes in DNA, even if the cut regions of DNA do not code for protein or produce phenotypic changes. Restriction enzymes can also detect point mutations in DNA that cause genetic diseases, such as sickle cell anemia, PKU, or cystic fibrosis. In most cases, a point mutation "erases" a restriction site.

To analyze patterns generated by restriction enzymes, it is necessary to perform gel electrophoresis on the products of a restriction enzyme digest. When you load DNA into an agarose gel bathed in buffer and apply a uniform charge to the gel, fragments of DNA will migrate to the positive end of the gel. This occurs because the phosphate groups of DNA's sugar-phosphate backbone are negatively charged. The rate of DNA migration is inversely proportional to its length. Longer pieces of DNA have a difficult time moving through the pores in an agarose gel, while smaller pieces of DNA move relatively quickly through the pores in a gel. DNA fragments start at the same place, but the small pieces move further in the gel over a set period of time.

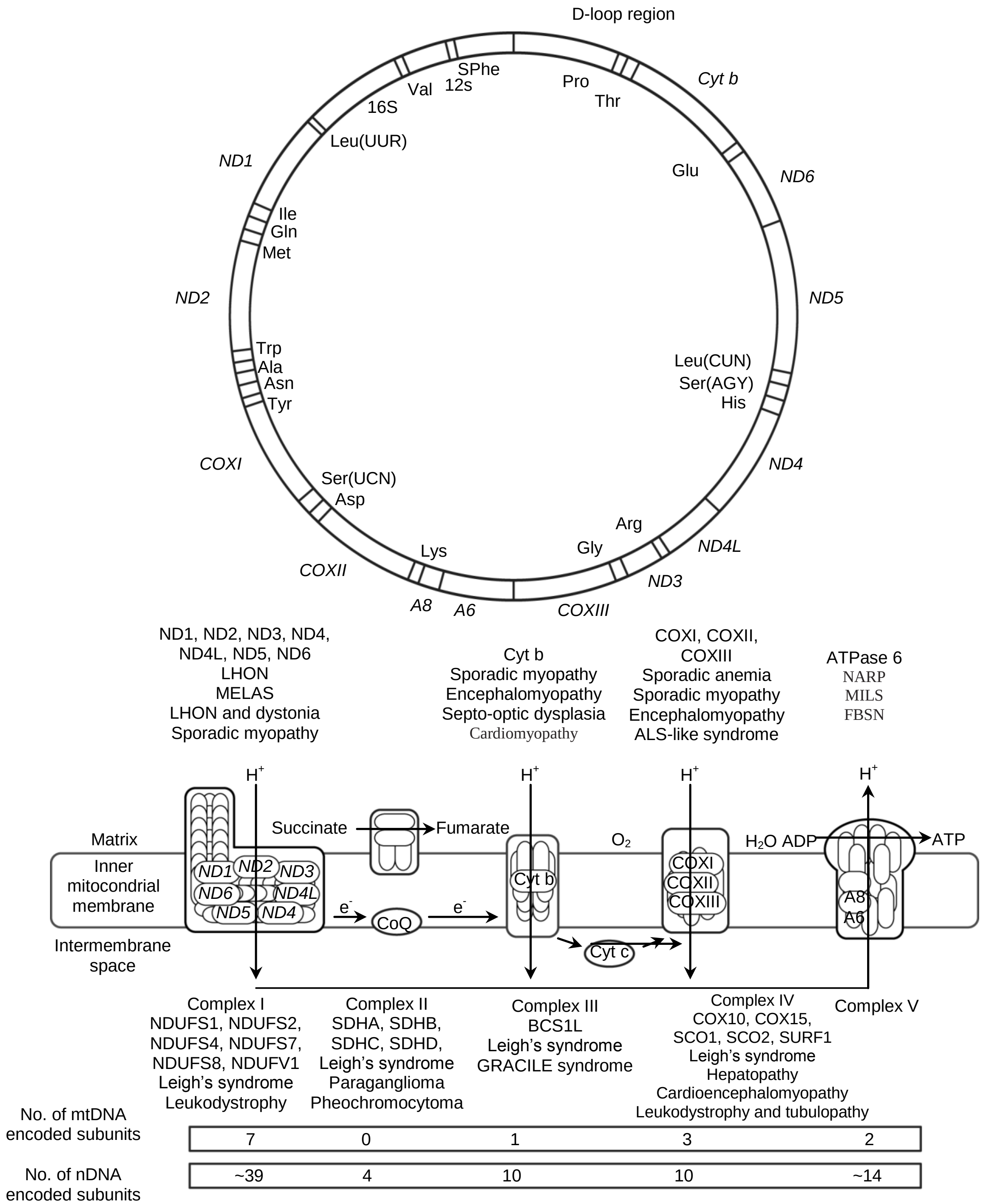
If restriction digests of normal and mutated DNA were run in separate lanes of an agarose gel, the normal DNA would be cut, and the products of the digest would show up as two bands that moved further down the gel. By contrast, the mutated DNA would show up as one band that was closer to the point of origin. This principle is applied to the normal and mutated mitochondrial DNA used in this lab. Normal DNA is "cut." Mutated DNA is not "cut" because a point mutation erases the restriction site.

The mitochondrial genome is often dismissed as a vestigial structure. However, mitochondrial DNA is evidence of the organelle's descent from a free-living, heterotrophic prokaryotic ancestor; it has necessary functions in eukaryotic cells. The mitochondrial genome contains 37 genes, all of which are expressed. Twenty-four of these genes code for RNA molecules used to translate proteins imbedded in the electron transport chain (ETC). Twenty-two of these genes code for transfer-RNAs (tRNAs), exclusive to mitochondria, and two of the genes code for subunits of mitochondrial ribosomes. The remaining genes directly code for 13 proteins of the ETC (see the figure below). The remaining 80 or so proteins of the ETC come from genes contained in nuclear DNA (see the figure below).

Over 100 point mutations have been identified in the mitochondrial genome, and almost all associated with some genetic metabolic disorder. In this lab, students will use molecular techniques and pedigree analysis to diagnose a mitochondrial mutation resulting in an inherited mitochondrial disease called MELAS (mitochondrial myopathy, encephalopathy, lactic-acidosis, and stroke-like episodes). MELAS has several characteristics that are common to many mitochondrial diseases including pattern of inheritance, heteroplasmy, random segregation, and threshold effects. MELAS is inherited maternally because all of the mitochondria in an individual are derived from the mitochondria originally present in the cytoplasm of the egg from which they developed. Interestingly, these mitochondria may not have the same genetic sequence. Some mitochondria may contain normal genomes, and other mitochondria may have mutated genomes. The condition of having more than one mitochondrial genome within cells is known as heteroplasmy. These different populations are randomly distributed throughout an individual's tissues and organs during development and beyond as cells grow and divide. Most cells are packed with hundreds, maybe thousands, of mitochondria. However, because the ETC and phosphorylation of ATP are so important to a cell's survival, cells with a population of diseased mitochondria may die or function improperly. Cells and tissues that have high metabolic demands, and therefore need lots of ATP, include neural tissue, muscle tissue, and renal tissue. These tissue types have a low threshold for mitochondrial dysfunction and often show symptoms of a mitochondrial disease.

In this lab, students will use gel electrophoresis to analyze two different fragments of DNA. These DNA fragments are not actually derived from a mitochondrial genome, but will be used to represent the portion of the mitochondrial genome that contains the gene Leu (UUR). This gene codes for a tRNA molecule used to translate the mRNA that codes for protein complexes of the ETC, and mutations in this gene are associated with MELAS. One fragment is normal, and the other contains a point mutation (a substitution from A to G). The point mutation in Lue (UUR) compromises protein-complex synthesis in the ETC and decreases oxidative phosphorylation of ATP. The normal gene has been cut by the restriction enzyme, and the mutated DNA has not been cut by the restriction enzyme. Because of this substitution, the ApaI restriction site is created, and the fragment can be recognized and digested by this restriction enzyme. The products of the restriction digest will be analyzed by gel electrophoresis.

See http://www.ncbi.nlm.nih.gov/pubmed/2102678 for more information.



Relating the mitochondrial genome with the protein complexes of the electron transport chain (ETC)

Pre-Lab Discussion and Experiment

Engage students by collectively reviewing mitochondrial structure and function. Draw a picture of mitochondria or project an image of mitochondria on a white board and ask students to tell you what they know about mitochondria. Surround the mitochondria drawing with statements from the students, and link them into a concept map.

1. What do we know about the basic structure and function of mitochondria?

Possible responses include: 1) they provide energy for the cell via cellular respiration; 2) they undergo chemiosmosis; 3) they produce ATP by oxidative phosphorylation; 4) they have an electron transport chain (ETC); 5) they have two membranes; 6) they have their own DNA and ribosomes; 7) they divide on their own, 8) they are divided up randomly during cytokinesis.

Teach students more about the mitochondrial genome and how it relates to the protein complexes of the electron transport chain. If you use the concept map approach, include foundational information about DNA, Protein, and the flow of genetic information (the central dogma of molecular biology). See "Relating the mitochondrial genome with the protein complexes of the electron tranport chain (ETC)" image above. Review with your students how to read a pedigree and how to determine a pattern of inheritance from a pedigree.

Divide students into pairs or groups of three. Draw for or present to the class a series of pedigrees. Start with an autosomal recessive trait and an autosomal dominant trait. Move on to a traditional sex-linked (X-linked) trait like color blindness. Each time you present a new pedigree, each student group should write down the pattern of inheritance (PoI) suggested by the pedigree. After all students have written down the PoI, ask one group to tell the PoI to the class. Ask another group that agrees with the first group to provide the evidence for the PoI. If another group disagrees with the first group, ask them to tell the PoI to the class, and explain why they read the pedigree differently. Continue this experiment until everyone is comfortable interpreting and explaining pedigrees.

Introduce the concept of inherited mitochondrial diseases, in particular MELAS. Give the students the patient case histories, and explain how mitochondrial diseases affect different organ systems. Also explain why inherited mitochondrial diseases are inherited maternally.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

The Mitochondrial Genetics Kit contains the following items (for 6 groups):

- Electrophoresis Buffer, 50x (60 mL)
- Graduated Transer Pipet (1)
- Transfer Pipet (10)
- InstaStain Blue Card (6)
- QuickStrip DNA samples (6 strips, 40 uL per well)
- UltraSpec-Agarose powder (3g)

DNA samples are stable at room temperature. However, if the experiment will not be conducted within one month of receipt, it is recommended that the DNA samples be stored in the refrigerator.

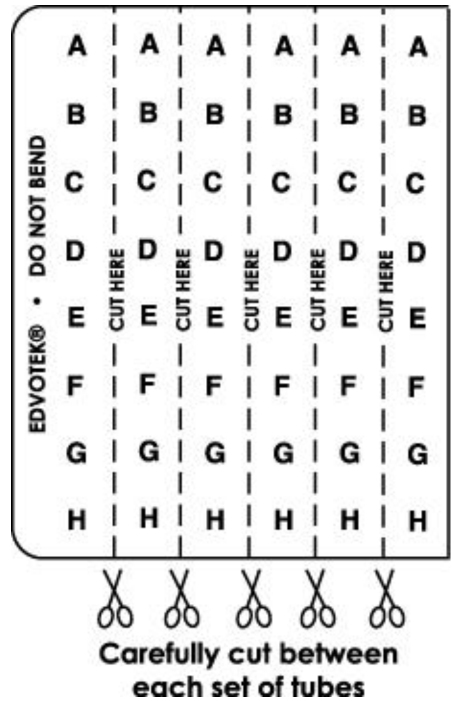
Prepare QuickStrip™ DNA Samples

1. Use sharp scissors to separate the block of QuickStrip samples into individual strips. QuickStrip connected tubes contain pre-aloquited ready-to-load samples. The samples are packaged in a microtiter block of tubes covered with a protective overlay. Cut carefully

between the rows and do not puncture the protective overlay covering the samples. Each gel will require one strip of samples.

Contents of Pre-aliquoted QuickStrip Tubes

Sample	Contents	Size of Fragments (bp)
A	DNA Standard Markers	23,130 9,416 6,557 4,361 3,000 2,322 2,027 725 570
B	+ Control group	3,000 1,300
C	- Control group	4,300
D	Patient #1	3,000 1,300
E	Patient #2	4,300 3,000 1,300
F	Empty	
G	Empty	
H	Empty	



Dilute Electrophoresis Buffer

1. Dilute the concentrated electrophoresis buffer to create diluted electrophoresis buffer.

Concentrated Buffer (50x)	+	Distilled Water	=	Diluted Buffer (1x)
60 mL		2,940		3,000 mL

Prepare 0.8% Agarose Gel

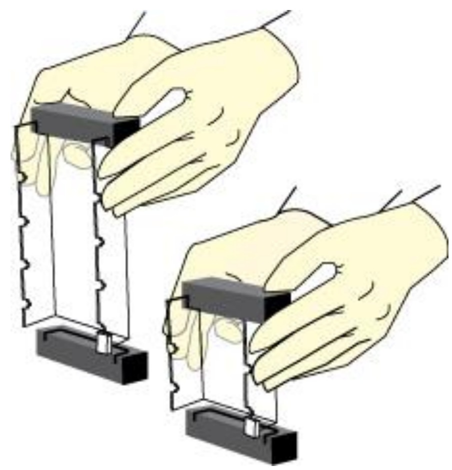
1. Pour 500 mL of diluted buffer into a 500-mL Erlenmeyer flask.
2. Pour 3.0 g of UltraSpec-Agarose™ into the buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the mixture to dissolve the agarose powder.

   a. Microwave Method: Cover the flask with plastic wrap to minimize evaporation. Heat the mixture of high for 1 minute. Swirl the mixture and heat on high in bursts of 25 seconds until all the agarose is completely dissolved. Check the solution carefully. If you see any "crystal" particles, the agarose is not completely dissolved.

   b. Hot Plate Method: Cover the flask with aluminum foil to minimize evaporation. Heat the mixture to boiling. Stir occasionally. Boil until all of the agarose is completely dissolved. Check the solution carefully. If you see any "crystal" particles, the agarose is not completely dissolved.
5. Cool the agarose solution to 60°C. Swirl the flask to promote even dissipation of heat.
6. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask.

Prepare Gel Beds

1. While the agarose is cooling, prepare the gel beds (casting trays).
2. Close off the open ends of a clean, dry gel bed by using rubber dams or tape.
3. Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
4. Place a gel comb in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.

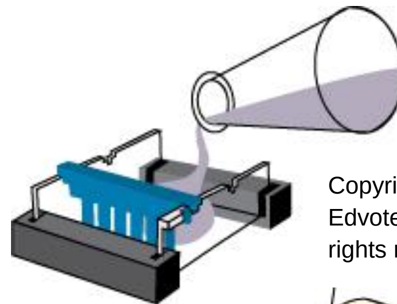


Cast Agarose Gels

1. Determine the volume of your casting tray before pouring the gels. The total volume of agarose solution required per gel can be determined using the volume of the gel bed.

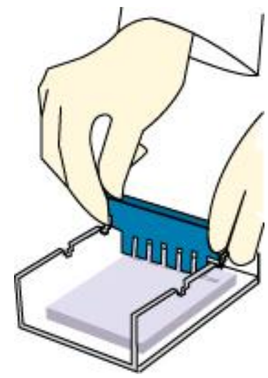
EDVOTEK Model #	Size of Gel (cm)	Total Volume Agarose per Gel (mL)
M36 HexaGel™	7x7	300
M12 Dual	7x7	300
M6Plus	7x10	400
M12	7x14	1,000

2. Place the bed on a level surface and pour the appropriate volume of cooled agarose solution into the bed.
3. Allow the gels to completely solidify. They will become firm and cool to the touch after approximately 20 minutes.



Prepare the Gel for Electrophoresis

1. After the gel is completely solidified, carefully and slowly remove the comb and rubber dams from the gel beds.
2. Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze.



Voltage and Time Recommendations

1. The approximate time for electrophoresis will vary from 15 minutes to 2 hours, depending upon the type of electrophoresis equipment you are using. Generally, the higher the voltage, the faster the fragments should migrate. However, maximum voltage should not exceed the indicated recommendations. Use the table below to determine the time and voltage requirements, and share with your students.

EDVOTEK Electrophoresis Model and Power Supply	75 Volts	150 volts
M6Plus or M12 DuoSource™ Power Supply	40-50 min	
M6Plus or M12 EVT 300 Power Supply	40-50 min	20-30 min

Destaining and Visualization

1. If you don't have a visible light gel visualization system, examine the gel against a white sheet of paper, or on an overhead projector.
2. Use of warmed distilled water at 37°C will accelerate destaining. Exceeding 37°C will soften the gel and cause it to break apart.
3. The volume of distilled water for destaining depends on the size of the tray. Use the smallest tray possible and completely submerge the gel.
4. Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealed plastic bag with water.

Safety

Add these important safety precautions to your normal laboratory procedures:

- Gloves should be worn when working with stain.
- Exercise caution when working with equipment that is used in conjunction with heating and/or melting reagents.
- Exercise caution when using electrical equipment in the lab.
- Always wash hands thoroughly with soap and water after handling reagents in the lab.

Procedure

Part 1 – Pedigree construction

1. Read the following case histories.

a. PATIENT 1: Seven-year old female with a history of normal development until age 2. At that point she developed episodic vomiting, acidosis, epilepsy, general weakness, ataxia (stiff, unsteady gait), and dystonia (movement disorder characterized by involuntary muscle contractions). These symptoms were classified as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome, a classic (but genetically and clinically heterogenous) mitochondrial disorder often associated with 3243A>G. “3243” refers to a particular locus on the mitochondrial genome, the 3243 base pair, and “A>G” represents a point mutation from adenine to guanine. A family history has been taken going back to the patient’s grandparents on her mothers’ side. No similar symptoms have occurred in the patient’s siblings (patient has twin older brothers), patient’s parents, or patient’s mother’s parents. The patient’s grandfather, however, has adult-onset diabetes (diabetes type 2).

b. PATIENT 2: Fifty-year old male with sudden onset headaches and seizures. Patient has a history of diabetes and deafness (which has also been associated with 3243A>G). Magnetic resonance imaging (MRI) detected bitemporal lesions.

(Lesions: dead neurons, and gliosis (dead glial cells), essentially “neural scar tissue”.) Lesions are very common in patients with MELAS that are not due to lack of blood and oxygen (as in a typical stroke) but are due to a breakdown in metabolic function.)

A family history has been taken going back to the patient’s grandparents on his mother’s side. Patient’s brother was found to have asymptomatic mild lactic acidosis. Patient’s mother had diabetes, exercise intolerance, and ptosis (drooping eyelids). Patient’s maternal uncle died of a stroke at age 37, and had multiple health issues (poorly defined). Patient’s maternal grandmother had diabetes and possibly other symptoms. The patient’s father has rheumatoid arthritis, but no history of diabetes or neurological problems.

2. According to the first patient's case history, "MELAS is genetically and clinically heterogeneous." What does that mean?

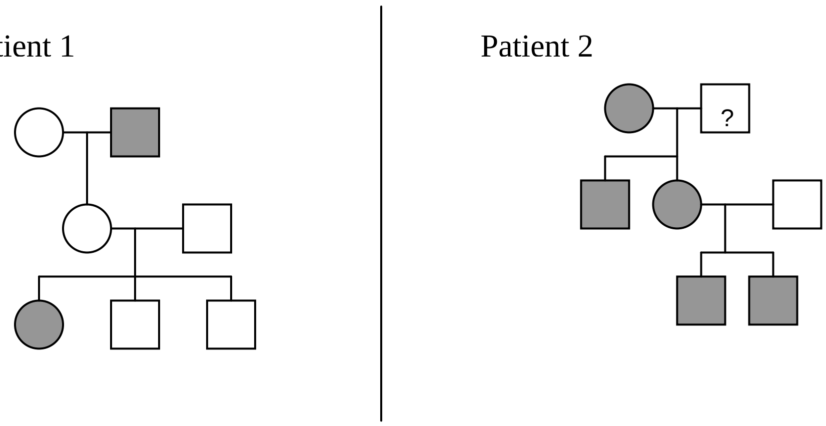
MELAS has been associated with several different mutations on the mitochondrial genome and affects several organ systems. MELAS can present itself clinically in any number of organ systems, or several organ systems at the same time.

3. Why would these organ systems be sensitive to mitochondrial dysfunction?

The cells and tissues of these organ systems have high metabolic demands and therefore require lots of ATP. If the mitochondria within these cells are not working properly, they will not produce enough ATP to drive metabolic processes, and the cells will die. Cell death can lead to tissue breakdown and organ failure.

4. Construct pedigrees for each patient in the space provided below. Shade in any individuals with a metabolic disease or MELAS. Be sure to label the patient in each pedigree.

Patient 1 Patient 2



5. Do the pedigrees suggest a pattern of inheritance for either patient? Do they suggest the same pattern of inheritance?

The pedigree for Patient 1 does not show any strong pattern of inheritance, but the pedigree for Patient 2 shows a maternal pattern of inheritance.

6. Why would a family history of mitochondrial disease show up as a maternal pattern of inheritance?

Mitochondrial diseases usually exhibit maternal patterns of inheritance because children inherit mitochondria from the mother. All the mitochondria in a person are derived from the original population of mitochondria present in the cytoplasm of secondary oocyte (the egg). During fertilization, the sperm only provides genetic material. The cells of the developing embryo contain only mitochondria from the egg.

7. Given the advances in molecular biology and biotechnology, why do geneticists and physicians still use pedigree analysis?

Genetic sequencing and molecular diagnostics are relatively new and are not always practical, especially if a disease does not have a known molecular basis.

8. Read the following excerpt about the restriction digest of patient DNAs

Blood was drawn from both patients in order to isolate and amplify a section of the mitochondrial genome. A 600 base pair (bp) PCR product was obtained from the patients that included the entire tRNA Leu (UUR) gene, (our gene of interest), part of the NO1 gene (which codes for one protein in the ETC complex), and part of the 16s rRNA gene (which codes for the small subunit of the mitochondrial ribosome).

PCR products from both patients were digested with a restriction enzyme for to 2 hours at 37 °C. The restriction enzyme is named "Ddel".

This restriction enzyme allows us to differentiate between normal and mutated tRNA Leu (UUR) genes by uncovering the 3243A>G point mutation associated with MELAS. The normal mitochondrial DNA has an adenine at locus 3243, but the mutated DNA contains a guanine at this locus. Therefore, the restriction site, CTGAG, is the normal genome, which is cut with the restriction enzyme; conversely, the mutated site, CTGGG, is not cut with the restriction enzyme.

In addition to patient DNA samples, control DNA segments that contain either normal or mutated Leu (URR) genes were amplified by PCR. These samples were also digested with the restriction enzyme.

Part 2 –Gel Electrophoresis

9 Table 6B.1 shows the contents of the microcentrifuge tubes your instructor will give you. Predict whether the restriction enzyme will or will not cut the DNA, and predict the number of bands you will see on the electrophoresis gel. Record your predictions in the appropriate cells of Table 6B.1.

Table 6B.1: Contents of restriction enzyme digestion & student predictions regarding the results

Tube	Contents	"Cut" or "Not Cut" by the Restriction Enzyme	Resulting Fragment Pattern in the Agarose Gel
A	DNA Marker	Cut	2 bands
B	Normal DNA	Not cut	1 band
C	Mutated DNA	Cut	2 bands
D	Patient #1	Will cut and not cut: (heteroplasmic)	3 bands
E	Patient #2		

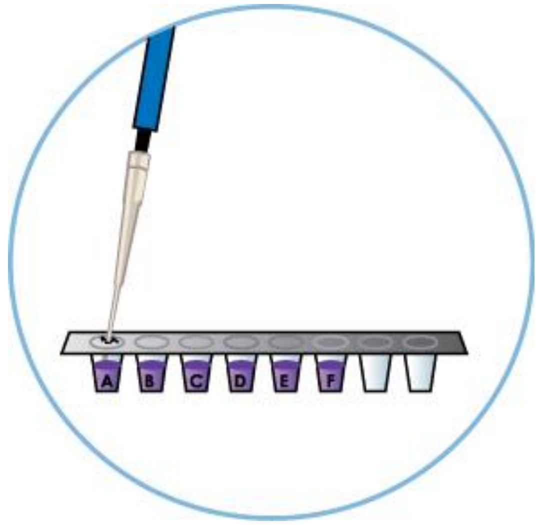
10. Obtain a 0.8% agarose gel and place it (on its bed) into the electrophoresis chamber, properly oriented, centered and leveled on the platform.

Note: During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure that the wells are oriented on the negative side of the chamber.

11. Fill the electrophoresis chamber with 300-400 mL of diluted electrophoresis buffer. Make sure the gel is completely submerged under buffer before proceeding to loading the gels.

Load the Gel

12. Obtain one strip of QuickStrip samples from your instructor. Tap the tubes gently on table, to ensure that the sample is at the bottom of the tubes.



13. Pierce the protective overlay of the QuickStrip with the pipet tip, and draw 35-38 uL of the sample into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.

14. Load the samples in Tubes A-E into the wells in consecutive order. Use a clean pipet tip each time.

Note: Carefully place the tip of the pipet halfway into the well and gently rock the tip around the well to make sure you are "in." Using your thumb, slowly press the button on your micropipet. You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip into a waste receptacle.

Run the Gel

15. Place the top on the electrophoresis chamber after you have loaded all DNA samples and you are sure that the gel is covered in running buffer.

16. Connect the apparatus to the D.C. power supply and set the power source at the required voltage. Ask your instructor what voltage is recommended for your equipment.

17. Turn on the power supply. Check that the current is flowing properly – you should see bubbles forming on the two platinum electrodes.

18. Conduct the electrophoresis for the length of time instructed by your instructor. Turn off the power supply to stop the electrophoresis process.

Stain the Gel

19. Remove the gel from the gel bed and place it on a flat surface covered with plastic wrap.

20. Wearing gloves, place the blue dye side of the InstaStain® card on the gel.

21. Firmly run your fingers several times over the entire surface of the card to establish good contact between the card and the gel.

22. To ensure continuous contact between the gel and the card, place a gel casting tray and weight (such as a small beaker) on top of the card.

23. Remove the card after 5-10 minutes.

24. If the color of the gel appears very light, wet the gel surface with buffer or distilled water and place the InstaStain® card back on the gel for an additional five minutes.

Destain and Visualize the Gel

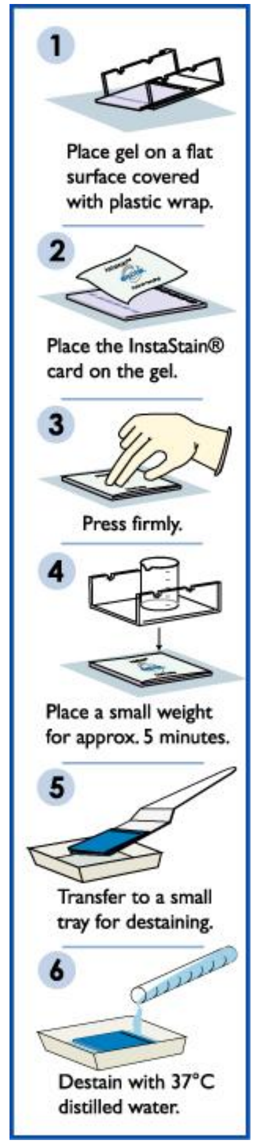
25. Transfer the gel to a large weigh boat or small plastic container for staining.

26. Destain with approximately 100 mL of distilled water (enough to cover the gel).

27. Repeat destaining by changing the water as needed (as it turns blue).

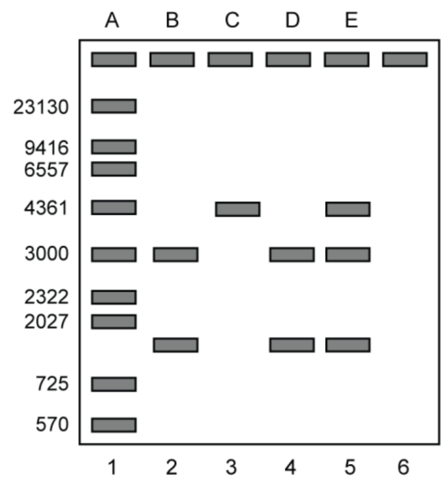
28. Carefully remove the gel from the tray and examine the gel on a Visible Light Gel Visualization System.

Note: If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.



Data Analysis

1. Draw the banding patterns observed in your gel for each lane.



2. Use this "molecular ruler" to determine the sizes of the DNA bands in Lanes 2 through 5. Record that information in Table 6B.2.

Table 6B.2: Agarose gel lane assignments and restriction fragment analysis

Lane Number	Tube	Contents	Predicted Pattern[1]	Actual Pattern	Fragment Size (bp)
1	A	Marker	Answers vary	10 Bands	23,130
					9,416
					6,557
					4,361
					3,000
					2,322
					2,027
					725
					570
2	B	Normal	2 Bands	2 Bands	3,000
					1,300
3	C	Mutated	1 Band	1 Band	4,300
4	D	Patient #1	Answers vary	2 Bands	3,000
					1,300
5	E	Patient #2	Answers vary	3 Bands	4,300
					3,000
					1,300

[1] Transfer your predictions from Table 6B.1.

Analysis Questions

1. Which of the lanes of the gel showed evidence of restriction enzyme cuts?

The following lanes showed evidence of a restriction enzyme cut of the mitochondrial DNA: Lane 1, the positive control, and Lane 5, the treated mitochondrial DNA from Patient 2.

2. Based on the evidence from the gel, does either patient show a point mutation in the Leu (UUR) gene? Cite the specific evidence from the gel.

Yes, Patient 2 has a band in the same place as the control mutated DNA that ran in Lane 3. This suggests that there is a larger, uncut piece of mitochondrial DNA that was the same size as the uncut DNA segment in the negative control (Lane 3).

3. Based on the evidence from the gel, does either patient appear to have a normal (non-mutated) Leu (UUR) gene? Cite the specific evidence from the gel.

Yes, both Patient 1 and Patient 2 appear to have a normal Leu (UUR) gene. Both have DNA fragments at 400 and 200 bp similar to the cut pieces of normal control DNA in Lane 2.

4. Explain why both the normal and mutated DNA patterns that show up in Patient 2?

Patient 2 has a condition called heteroplasmy, meaning there are two populations of mitochondrial DNA: one with a normal Lue (UUR) gene (that is cut by the restriction enzyme) and the other with a mutated Leu (UUR) gene (that is not cut by the restriction enzyme).

5. Could the banding patterns observed in the DNA of Patient 1 and 2 be the result of an error during the restriction digest, sample preparation or electrophoresis? Defend your answer.

Answers may vary. Certainly, errors could occur in the experimental procedure. One possible error might be that the DNA from patient 2 was normal, but the restriction enzyme did not cut all of DNA the molecules. Another possible error would occur if material spilled from Lane 4 to Lane 5, contaminating Lane 5 with cut segments of mitochondrial DNA.

6. MELAS is an acronym for mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. In the table below, define these terms, and identify the organ system(s) affected in each of these conditions. (Hint: think about the function of mitochondria.) Pick out two other symptoms presented by either patient (or the patient's family) and define them also.

Note: Answers provided in this table represent one of many answers students might record.

Condition	Definition	Affected Organ System
Myopathy	Fatigue and atrophy in skeletal muscle	Skeletal muscle
Encephalopathy	A term used to describe the cerebral pathology of neurons and glial cells	Central nervous system
Lactic-acidosis	A surplus of lactic acid building up in cells and tissues caused by a decrease	Widespread throughout organ

	in oxidative phosphorylation	systems
Stroke-like episodes	Memory lapses, temporary blindness, deafness, and paralysis	Central nervous system
Other symptom 1: (ataxia)	A stiff and unsteady gait caused by the uncoordinated contraction of antagonistic skeletal muscles	Skeletal muscles
Other symptom 2: (ptosis)	Dropping eyelids	Skeletal muscles

Synthesis Questions

1. Based on the pedigree analysis and gel electrophoresis, which patient is likely to have MELAS caused by a point mutation in the Leu (UUR) gene? How does the evidence you cite explain a mitochondrial disease?

Patient 2 is likely to have MELAS caused by a point mutation in the Leu (UUR) gene. His pedigree indicates a maternal line of inheritance which is common to mitochondrial diseases. The gel indicates a point mutation in the Leu (UUR) gene because some of her DNA was not cut by the restriction enzyme. Having more than one mitochondrial genome does occur in patients with MELAS.

2. What does the Leu (UUR) gene do?

It is a gene that codes for a transfer RNA (tRNA) that links the amino acid Leucine with the proper codon on the mitochondrial messenger RNA (mRNA).

3. How would a mutation in the Leu (UUR) gene affect the synthesis of protein complexes in the electron transport chain?

A mutated gene may not place the correct amino acid in the correct position in a polypeptide chain, altering the primary secondary, tertiary, and quaternary structure of the proteins. Or the mitochondria with these mutations may synthesize these proteins more slowly than mitochondria with a normal genome.

4. What do you think is more damaging to mitochondrial function: a mutation in a gene that codes for a protein in the electron transport chain (ETC), or a gene that codes for an RNA molecule used to translate proteins of the ETC? Explain your reasoning.

A gene coding for an RNA molecule used in translation would be more damaging because 13 proteins of the ETC might be affected. A gene coding for a specific protein will only affect one protein.

5. Does the pedigree analysis or gel analysis suggest that one of the patients does not have MELAS because of a point mutation in the Leu (UUR) gene?

Yes, Patient 1 is homoplasmic for normal DNA, and her pedigree does not suggest a maternal pattern of inheritance.

6. What is an alternate explanation for this patient's disease?

Patient 1 could have a mutation in another gene in the mitochondrial genome, or there could be a mutation in the nuclear genome that codes for a protein of the ETC. Further, this patient could have inherited normal mitochondrial DNA, and nuclear DNA could have mutated during the patient's development.

7. Explain why cells and tissues that have high metabolic demands would be sensitive to mutations in protein complexes of the ETC.

Cells with high metabolic demands need ATP from oxidative phosphorylation by the ETC and ATP synthase bound to the inner membrane of the mitochondria. If any of the proteins are mutated, ATP synthesis could be decreased, and the cells may not function, grow, or divide as well as normal cells.

8. What is heteroplasmy? Why are some cells more sensitive (have a lower threshold) for heteroplasmy?

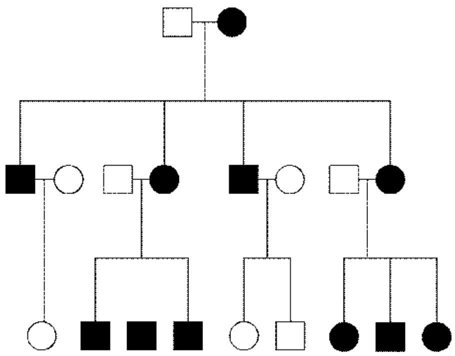
Heteroplasmy is the coexistence of wild-type and mutated mitochondrial DNA in the same cell. Cells with high metabolic demands and that need a lot of ATP from oxidative will be more sensitive to mutated DNA than cells with lower metabolic demands.

Multiple Choice Questions

1. Which of the following statements is false?

- **A.** Because of the role of the mitochondria in producing cellular energy, mitochondrial diseases often affect the muscles and nervous system.
- **B.** Because mitochondria are present in the cytoplasm, mitochondrial diseases are transmitted maternally.
- **C. Like nuclear genes, mitochondrial genes usually follow Mendelian patterns of inheritance.**
- **D.** Mitochondria contain circular DNA molecules that code for proteins and RNAs.
- **E.** Many mitochondrial genes encode proteins that play roles in the electron transport chain and ATP synthesis.

2. The pedigree below shows the transmission of a trait in a family. The trait is most likely:



- **A.** Mitochondrial
- **B.** Autosomal recessive
- **C.** Sex-linked dominant
- **D.** Sex-linked recessive
- **E.** Autosomal dominant
- **F. Cannot be determined with the information given.**

3. What is the function of restriction enzymes?

- **A.** To add new nucleotides to the growing strand of DNA
- **B.** To join nucleotides during replication
- **C.** To join nucleotides during transcription
- **D. To cut nucleic acids at specific sites**
- **E.** To repair breaks in sugar-phosphate backbones

4. Which of the following changes is least likely to alter the rate at which a DNA fragment moves through a gel during electrophoresis?

- **A. Altering the nucleotide sequence of the DNA fragment**
- **B.** Methylating the cytosine bases within the DNA fragment
- **C.** Increasing the length of the DNA fragment
- **D.** Decreasing the length of the DNA fragment
- **E.** Neutralizing the negative charges within the DNA fragment

5. Restriction fragment length polymorphism (RFLP) analysis can be used to distinguish between alleles based on differences in

- **A. Restriction enzyme recognition sites between the alleles**
- **B.** The amount of DNA amplified from the alleles during polymerase chain reaction (PCR)
- **C.** The ability of the alleles to be replicated in bacterial cells
- **D.** The proteins expressed from the alleles
- **E.** The ability of nucleic acid probes to hybridize to the alleles

Extended Inquiry Suggestions

Research other inherited mitochondrial diseases besides MELAS. What are some of the symptoms of these diseases? If possible, find the sequences of genes that are mutated in these diseases, and find a restriction enzyme that could be used to uncover a point mutation in this gene.

Obtain the sequences of the DNA fragments used in this lab; order primers for these fragments; and use polymerase chain reaction to amplify these sequences before setting up and performing restriction digest and electrophoresis on these fragments.

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    - David Marsland, Smithsonian Institution
    - Henry Milne, Smithsonian Institution
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- University of Alabama, School of Medicine
    - J. Daniel Sharer, Ph.D.
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Genetics

14. Mitosis and Meiosis

Objectives

Students investigate the processes of mitosis and meiosis by:

- Stating and recognizing the stages of cell division
- Distinguishing between nuclear division and cytokinesis
- Differentiating between plant and animal cytokinesis
- Comparing and contrasting mitosis and meiosis

Procedural Overview

Students gain experience conducting the following procedures:

- Observing prepared onion root tip and whitefish blastula slides under a microscope
- Calculating the relative amount of time for each stage of mitosis
- Simulating the stages of meiosis
- Estimating the percentage of crossing over that occurs between the centromere and the gene that controls spore color in *Sordaria*
- Observing the arrangement of ascospores in the asci of an organism resulting from a cross between a wild-type and a mutant (for tan spore coat color) *Sordaria*

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	30 minutes
♦ Lab experiment: Part 1	60 minutes
♦ Lab experiment: Part 2	60 minutes

Materials and Equipment

For each group:

- Microscope
- Prepared whitefish blastula slide
- Prepared onion root tip slide
- Prepared *Sordaria* ascospore slides
- Chromosome simulation kit[1]

[1]To create chromosome simulation kits using commonly available items, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Mitosis in plants and animals

- ♦ Gametogenesis in animals
- ♦ Sporogenesis in plants
- ♦ The major differences between mitosis and meiosis

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Genetics of Organisms with *Drosophila melanogaster*

Background

The cell cycle is an ordered set of events that makes up the life of the cell. Most cells finish the cell cycle by dividing into daughter cells. The first stage of the cell cycle, called interphase, consists of the substages Gap 1 (G1), synthesis (S), and Gap 2 (G2). During interphase the cell is grows, organelles are duplicated, proteins are synthesized, and the DNA is copied. A new cell enters interphase after it is created by the division of a pre-existing cell.

When the cell is ready, it enters into the mitotic (M) phase where division of the duplicated chromosomes through nuclear division occurs. Mitosis involves four stages: prophase, metaphase, anaphase, and telophase. The final phase of the cell cycle is called cytokinesis, which occurs during or directly after telophase. Cytokinesis is the division of the cytoplasm and the actual separation of the cell into its daughter cells.

The four stages of mitosis have distinct and recognizable events that help differentiate them from one another. During prophase, changes are occurring in both the nucleus and the cytoplasm. Chromatin fibers in the nucleus begin to condense until they form visible chromosomes. At this stage, each chromosome is composed of two chromatids that are joined together at the centromere. Around the chromosomes, the nuclear envelope disintegrates and the nucleoli disappear. Towards the end of prophase, the spindle apparatus begins to form in the cytoplasm. The spindle apparatus is composed of microtubules that provide the mechanism for which chromatids move towards opposite poles during anaphase.

At the end of prophase, the cell enters metaphase. In metaphase, the chromosomes, which are attached to the spindle apparatus at their centromeres, are pulled to an imaginary line (the metaphase plate) equidistant from the two poles of the cell. The chromosomes begin to move towards opposite poles in anaphase, the third stage of mitosis.

During the final stage of mitosis, telophase, the chromosomes at each pole begin to unravel and the nuclear envelopes begin to reform around the two new daughter nuclei. The nucleoli also reappear. The division of the cytosol, cytokinesis, may also occur.

In animal cells cytokinesis, occurs when creation of the cleavage furrow pinches the parent cell into two identical daughter cells. Cytokinesis of plant cells differs from that of animals cells. In plant cells, a cell plate forms and new cell wall material is laid down between the two new cells, eventually separating the cytoplasm of the two cells.

The formation of gametes (sperm and egg cells), is accomplished using an alternate form of cell division called meiosis. Meiosis involves two successive nuclear divisions to produce four haploid cells. The first division, meiosis I, reduces the chromosome number in half and separates the homologous chromosomes. Meiosis II, the second division, separates the sister chromatids.

Meiosis I is quite different from mitosis. In prophase I, homologous chromosomes pair up in a process called synapsis; this forms a tetrad of four chromatids. They may exchange genetic material in a process called crossing over. Crossing over produces new combinations of genes on chromosomes (which are now recombinant chromosomes.) Thus, genetic variation is increased.

During metaphase I, the tetrads randomly line up on the metaphase plate. During anaphase, the homologous chromosomes are separated. At the end of meiosis I, two haploid cells have formed. DNA replication does NOT occur between meiosis I and meiosis II. The stages of meiosis II are similar to mitosis except that only one homolog from each homologous pair of chromosomes is present in each daughter cell.

Pre-Lab Discussion and Experiment

Part 1 – Mitosis

1. Ask your students to tell you what they were doing at 5:00 p.m., 6:00 p.m., 7:00 p.m., and 8:00 p.m. the night before. What percentage was studying? Working? Participating in sports? Eating? Reading? Watching TV? Playing video games? What do these activities say about you? How would the type and frequency of activities change if it was a different time of year (fall, winter, spring, summer)?

Student answers will vary, indicating that some activities occur frequently while others are not very common. Some activities may only be allowed at certain times of the day/week while some activities may not ever be allowed by parents/guardians.

2. Ask students to find at least one other person whose activities were similar to their own the night before. From the pairs, pick one group and ask them to share how their activities were similar.

Maybe they ran for cross country at 5:00, ate dinner at 6:00, studied at 7:00, and were still studying at 8:00. From looking at these two individuals, it may give an indication of other student's schedules.

3. Ask students to discuss with their partner what types of limitations and assumptions come from this type of inference. Poll the class for their thoughts.

Students should conclude that looking at time in this manner (only snapshots of time) can represent a relative occurrence of activities. You may want to be sure that students understand that these activities represented are limited to those occurring at the sampled times. Thus, some activities may be underrepresented or absent even though they may occur in the evening.

4. Ask students if the same activity is occurring at multiple times, can it be assumed that the same activity is occurring between the times (that is, if you were studying at 7:00 and also at 8:00, can one assume that you were studying at the times between 7 and 8).

Point out that this is an inference and that many scientists make inferences based on the data they collect. It will also be useful to mention that limitations and assumptions are a part of science, since science is a human endeavor.

Review proper use of the microscope.

Parts 2 and 3 – Meiosis

Review with students the differences between mitosis and meiosis

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Each group needs one chromosome kit. You can order these kits from a biological supply company or you can make them. Each kit should contain four strands of beads: two of one color and two of another; for example, red and yellow beads. Pop-beads work well for this experiment because they can be easily removed from the string. To make the kits, buy a roll

of string and two bags of plastic beads (two colors). Have your students help you create the kits. Give each student string, scissors, red beads, and yellow beads. Let the students put the beads on the string and then tie both ends into a knot to keep the beads from falling off the string. Each group will need four strings of beads. The knots should be tied loosely enough that they can be un-tied easily during the lab. Make sure that all strings have equal quantities of beads.

Each chromosome kit will also need two centromeres. There are many common items that can be used as centromeres. Any item that can hold the two strands of beads together can act as the centromere. Some suggestions include: two magnets, a chip clip, paper clips, binder clips, different colored string, clay or silly putty.

2. Order prepared slides from a biological supply company.

3. The mitosis portion of the lab is written for use with a compound light microscope. However, if you have access to digital microscopes and laptop computers, use them to make this lab more interesting. View the prepared slides under the digital microscope and take a picture of three fields of view. Students can then view the pictures on the computer screen and count their cells with ease. If you only have one digital microscope and an interactive whiteboard, plug the microscope into your "instructor" computer and project the image of the slides onto the interactive whiteboard.

Safety

Follow all standard laboratory procedures.

Procedure

Part 1 – Mitosis: observation of stages of the mitotic cell cycle

In part 1, you will be observing plant and animal cells undergoing mitosis using prepared slides of onion root tip and whitefish blastula.

Onion root tip is used in the study of plant mitosis because it is an actively growing tissue that contains a high percentage of cells undergoing mitosis. A whitefish blastula is an embryo in very early stages of development. This ball of undifferentiated cells is actively dividing, making it a great candidate for the study of animal cell mitosis.

Set Up

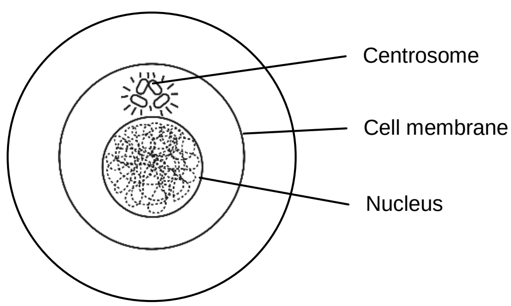
1. Obtain a microscope and prepared slides of an onion root tip and a whitefish blastula.

2. Observe the prepared slide of a whitefish blastula using the microscope. Using the 10X objective, find a field of view with a variety of cells in various stages of mitosis. Switch to the 40X objective to study individual cells.

Collect Data

3. Locate a cell in INTERPHASE.

4. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.

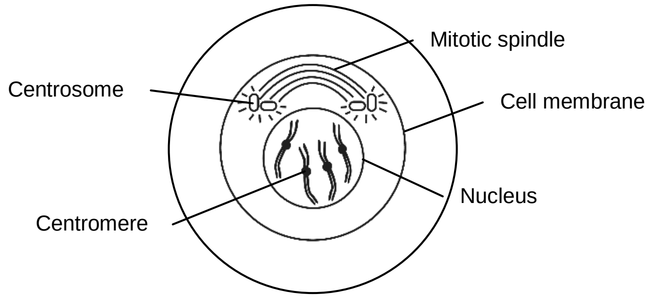


5. Briefly describe the events that occur during interphase.

Interphase consists of Gap 1 (G1), synthesis (S), and Gap 2 (G2). During interphase, the cell is growing, organelles are duplicated, proteins are synthesized, and the DNA is copied. When the cell is ready, it enters into the mitotic (M) phase. The M phase consists of nuclear division (of the chromosomes) and cytoplasmic division.

6. Locate a cell in PROPHASE.

7. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.

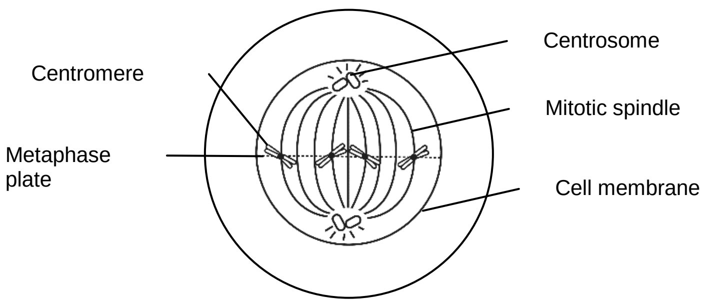


8. Briefly describe the events that occur during prophase.

During prophase changes are occurring in both the nucleus as well as the cytoplasm. First, the chromatin fibers begin to condense until they form visible chromosomes. At this stage, each chromosome is composed of 2 chromatids and is joined together at the centromere. During prophase, the nucleoli disappear and the nuclear envelope disintegrates. Towards the end of prophase, the spindle apparatus begins to form in the cytoplasm.

9. ☐ Locate a cell in METAPHASE.

10. ☐ Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.

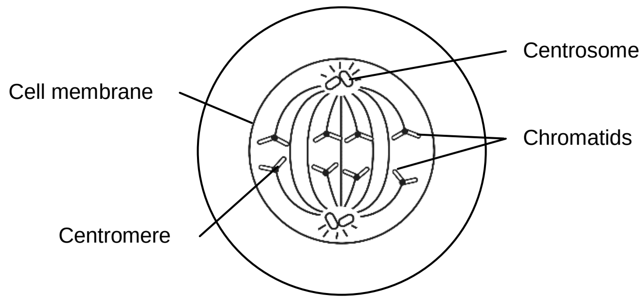


11. Briefly describe the events that occur during metaphase.

The chromosomes line up at an imaginary line (the metaphase plate) equidistant from the two poles of the cell. The chromosomes attach to the spindle apparatus at the centromere.

12. Locate a cell in ANAPHASE.

13. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.

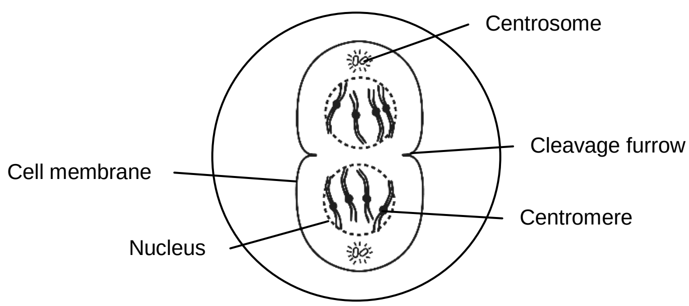


14. Briefly describe the events that occur during anaphase.

The chromosomes begin to move towards opposite poles.

15. Locate a cell in TELOPHASE.

16. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.

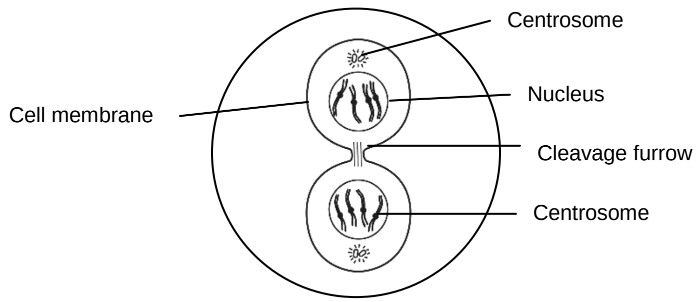


17. Briefly describe the events that occur during telophase.

The chromosomes begin to unravel and the nuclear envelopes begin to reform around the two new daughter nuclei. The nucleoli also reappear.

18. Locate a cell undergoing CYTOKINESIS.

19. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.



20. Briefly describe the events that occur during cytokinesis.

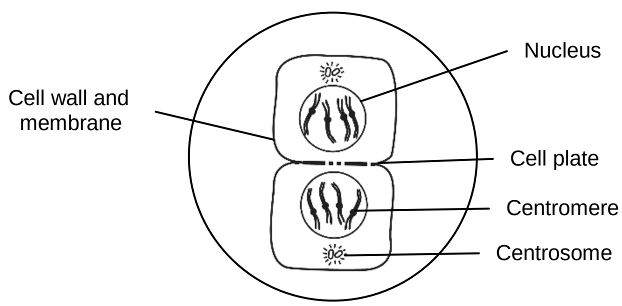
The cytoplasm of the cell is dividing. It looks like the cell is splitting into two new cells.

21. Remove the whitefish blastula slide and insert the onion root tip slide. Find a field of view where you can clearly see a variety of cells in various stages of mitosis.

22. Locate cells undergoing prophase, metaphase, anaphase, and telophase. Are the stages similar or different than what you observed with the onion slide? Why do you think so?

The stages of plant and animal mitosis are the same, but many of the stages may look different.

23. Locate a cell undergoing CYTOKINESIS. Draw what you see in the area below. Label your drawing with any of the following organelles that may be present: nucleus, cell membrane, cell wall, chromatids, metaphase plate, mitotic spindle, centrosome, cleavage furrow or cell plate.



24. Compare and contrast cytokinesis in plant and animal cells. Explain the difference you observed between plant and animal cells.

In animal cells, cytokinesis is accomplished by the formation of a cleavage furrow, and the two new cells will pinch off between their middle. In plant cells, a cell plate forms, and new cell wall material is laid down between the two new cells, eventually completely separating the cells.

25. Remove the whitefish blastula slide from the microscope and replace it with an onion root tip slide, Use the 10X objective to find a field of view with a variety of cells.

26. Using the 40X objective, count the number of cells in each phase of mitosis and interphase. Count all cells in the field of view.

27. Record these counts in Table 3.1.

28. Repeat this step two more times, moving the field of view and counting the total number of cells in each phase for each view.

29. Record these counts in Table 3.1.

30. Turn off the microscope and place the slides in their proper location.

31. Total the number of cells counted in each phase of mitosis and interphase for the three fields of view.

32. Record this figure under "Total" in Table 3.1.

33. Add the total number of cells viewed in each phase and interphase to get the total number of cells counted. Record this in Table 3.1.

Part 2 – Meiosis simulation

34. Obtain a chromosome simulation kit. You will be working with the chromosomes of an imaginary organism with a diploid number of two. The kit should contain four strands of beads, two of one color and two of another; for example, red and yellow beads.

35. Place one strand of red and one strand yellow beads in front of you. This is how the nucleus would look during G1 of interphase. The beads represent one pair of unduplicated homologous chromosomes.

36. Simulate DNA replication during S phase by taking the other red strand and allowing it to stick to its partner at the centromere region.

37. Repeat with the yellow chromosomes.

38. Simulate the movement of chromosomes through the stages of meiosis using these duplicated chromosomes. Remember to go through both meiosis I and meiosis II. While you are in meiosis I, simulate one cross-over event by exchanging pieces of the chromosomes

Note: If you are using pop-beads, you can simply un-pop the beads and pop them back together. If you are using regular beads, untie the knot, remove and swap the beads, and re-tie the knots.

39. When you are confident that you can simulate meiosis and thoroughly explain the events in each stage, call your instructor over and do the simulation for him/her.

Part 3 – Meiosis: observation of stages of meiosis

In the meiosis lab, we will be working with *Sordaria,* a fungus that shows the results of crossing over in meiosis. Like all ascomycotes, *Sordaria* is haploid for the majority of its life cycle. It becomes diploid only when mycelia of two separate strains come into contact and fuse. The fusion process produces a diploid nucleus which must then undergo meiosis to restore the haploid state. Right after meiosis, there is one mitotic division which produces eight ascospores (which are haploid.) The ascospores are contained in the ascus, a sac structure. There are many asci contained within one ascocarp, the fruiting body of the fungus. When the ascospores mature, the asci rupture, releasing the ascospores into the environment. When the ascospores land in a favorable environment, they can develop into a new haploid fungus.

Sordaria hybrids are useful for observing crossing over during meiosis. Wild type *Sordaria* have black ascospores, and mutant *Sordaria* have tan ascospores. When mycelia of the two strains come together and undergo meiosis, the asci that develop will contain four black ascospores and four tan ascospores. The arrangement of these ascospores will tell us whether or not crossing over has occurred. If there are 4 black ascospores in a row and 4 tan ascospores in a row, forming a 4:4 pattern, no crossing over has occurred. However, if there is any other arrangement of the ascospores (i.e. 2 black then 2 tan, then 2 black and 2 tan), then crossing over has occurred.

Set Up

40. Obtain a microscope and a prepared slide *of Sordaria*.

41. Observe the prepared slide of *Sordaria* using the microscope. Using the 10X objective, find a field of view where you can see a group of hybrid asci (they have both tan and black ascospores).

Collect Data

42. Using the 40X objective, count the number of wild-type and mutant asci that **do not** exhibit crossing over.

43. Record your count in Table 3.2.

44. Count the number of hybrid asci in the field of view that **do** exhibit crossing over.

45. Record your count in Table 3.2.

Data Analysis

Part 1 – Estimating the time required for each stage of mitosis

1. Calculate the percentage of cells in each phase and record the percentage in Table 3.1.

Total # of cells in the phase / total # cells counted

Interphase: 1534/1743 = 88%

Prophase: 104/1743 = 6%

Metaphase: 52/1743 = 3%

Anaphase: 35/1743 = 2%

Telophase: 17/1743 = 1%

2. ☐ Calculate the time spent in each stage and record this in Table 3.1. It takes, on average, 24 hours for an onion root tip cell to complete the cell cycle. Using the following equation, you can calculate the amount of time spent in each stage:

Percentage of cells in stage x 1,440 minutes = _________ minutes of cell cycle spent in stage.

Interphase: 0.88 x 1,440 = 21 hours and 7 minutes

Prophase: 0.06 x 1,440 = 1 hour and 26 minutes

Metaphase: 0.03 x 1,440 = 43 minutes

Anaphase: 0.02 x 1,440 = 29 minutes

Telophase: 0.01 x 1,440 = 14 minutes

Table 3.1: The mitotic cell cycle

	Number of Cells Counted				Percentage of Total Cells Counted	Time in Estimated Time For Each Stage
	Onion1	Onion2	Onion3	TOTAL		
Interphase	502	523	509	1534	88	21 hours 7 minutes
Prophase	37	32	35	104	6	1 hour, 26 minutes
Metaphase	17	19	16	52	3	43 minutes
Anaphase	12	11	12	35	2	29 minutes
Telophase	6	5	6	17	1	14 minutes
Total Cells Counted				1743		

Part 3 –% of crossing over during meiosis

3. ☐ Determine the percentage of asci that show evidence of crossing over and record this in Table 3.2.

% asci showing crossover = # hybrid asci counted / total # asci counted

% asci showing crossover = 329 / 640 = 48.6%

Table 3.2: Crossing over in *Sordaria*

# Mutant and Wild Type (No Crossing Over) (4:4 Arrangement of Black to Tan)	# Hybrid Asci (Crossing Over) (2:2:2:2 or 2:4:2)	Total Asci	% Showing Crossing Over
311	329	640	48.6

Analysis Questions

1. What can you infer about the relative length of time an onion root tip cell spends in each stage of mitosis?

The shortest stage is telophase. Metaphase and anaphase are intermediate, with metaphase being slightly longer and prophase the longest stage.

2. Draw and label a pie chart of the onion root tip cell cycle using the data from your experiment. Indicate how much of the pie is dedicated to each phase.

From the sample data, 88% interphase, 6% prophase, 3% metaphase, 2% anaphase, and 1% telophase.

3. What is occurring in each stage of meiosis? Give a brief summary.

In prophase I, homologous chromosomes pair up in a process called synapsis; this forms a tetrad of four chromatids. They may exchange genetic material in a process called crossing over. Crossing over produces new combinations of genes on chromosomes (which are now recombinant chromosomes.) Thus, genetic variation is increased. During metaphase I, the tetrads randomly line up on the metaphase plate. During anaphase, the homologous chromosomes are separated. At the end of meiosis I, two haploid cells have formed. During interphase II, DNA replication does NOT occur. The stages of meiosis II are similar to mitosis except that only one homolog from each homologous pair of chromosomes is present in each daughter cell undergoing meiosis II.

4. List three major differences between the events of meiosis and mitosis.

Mitosis has one nuclear division where meiosis has two.

Meiosis produces four haploid cells where mitosis produces two diploid cells.

Crossing over occurs in meiosis and not mitosis.

Tetrads line up along the metaphase plate in meiosis, where chromatids align on the metaphase plate in mitosis.

Meiosis produces gametes; mitosis is used for regeneration, growth, and development.

5. What are the differences between meiosis I and meiosis II?

In meiosis I chromosome number is cut in half when homologous chromosomes are separated. During meiosis II, sister chromatids of individual chromosomes separate. In meiosis I, synapsis and crossing over occur, resulting in the creation of new combination of alleles on chromosomes. Finally, meiosis I is preceded by the duplication of chromosomes during Interphase, while meiosis II is not.

Synthesis Questions

Use available resources to help you answer the following questions.

1. If you examined a part of the onion plant other than the root tip, how would you expect your results to compare to the root tip?

Other than apical meristems, the cells in the plant are not actively dividing and are in interphase. I would not have seen cells in the various stages of mitosis.

2. Cells of the adult nervous system remain in G0 and never complete the cell cycle. What are the implications of this for someone who has experienced a spinal cord injury?

A cell that remains in G0 will never divide. This explains why people who have spinal cord injuries can never re-grow the damaged tissue.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Why is crossing over important?

- **A.** It creates new combinations of alleles that never previously existed.
- **B.** It allows organisms to increase chances of survival.
- **C.** It facilitates the mixing of maternal and paternal chromosomes.
- **D.** It is one mechanism for increasing genetic variation.
- **E. All of the above are correct.**

2. All of the cells produced during meiosis are different from the parent cell. Which of the following is a difference seen between the pre-meiotic cells and the post-meiotic cells?

- **A.** Post-meiotic cells are larger than pre-meiotic cells.
- **B. Crossing over of the chromosomes has occurred in post-meiotic cells.**
- **C.** Pre-meiotic cells are haploid, post-meiotic cells are diploid.
- **D.** Pre-meiotic cells are flat and post-meiotic cells are round.
- **E.** All of the above are correct.

3. Eukaryotic cell division requires:

- **A.** Only partial passage through the cell cycle
- **B.** No energy, because it is a passive process
- **C.** **Accurate replication and equal division of the genetic information encoded in the cell's DNA**
- **D.** Equal division of the cell's DNA prior to DNA replication
- **E.** Accurate replication of only the DNA that will be expressed in the daughter cells

Extended Inquiry Suggestions

You may choose to grow your own *Sordaria* for the lab. Students could then make their own wet mounts of the ascospores. A *Sordaria* kit can be ordered from biological supply companies.

In animal cells cytokinesis, occurs when creation of the cleavage furrow pinches the parent cell into two identical daughter cells. Cytokinesis of plant cells differs from that of animals cells. In plant cells, a cell plate forms and new cell wall material is laid down between the two new cells, eventually separating the cytoplasm of the two cells.

The formation of gametes (sperm and egg cells), is accomplished using an alternate form of cell division called meiosis. Meiosis involves two successive nuclear divisions to produce four haploid cells. The first division, meiosis I, reduces the chromosome number in half and separates the homologous chromosomes. Meiosis II, the second division, separates the sister chromatids.

Meiosis I is quite different from mitosis. In prophase I, homologous chromosomes pair up in a process called synapsis; this forms a tetrad of four chromatids. They may exchange genetic material in a process called crossing over. Crossing over produces new combinations of genes on chromosomes (which are now recombinant chromosomes.) Thus, genetic variation is increased.

During metaphase I, the tetrads randomly line up on the metaphase plate. During anaphase, the homologous chromosomes are separated. At the end of meiosis I, two haploid cells have formed. DNA replication does NOT occur between meiosis I and meiosis II. The stages of meiosis II are similar to mitosis except that only one homolog from each homologous pair of chromosomes is present in each daughter cell.

15. Genetics of Organisms with *Drosophila melanogaster*

Objectives

This lab allows students to use the common fruit fly, *Drosophila melanogaster*, to perform genetic (monohybrid, dihybrid, and sex-linked) crosses. This lab provides an excellent opportunity to work with and manipulate live organisms in a laboratory setting. During this investigation, students explore:

- The relationship between the genotype and phenotype of wild-type and mutant fruit flies
- The relationship between F_1 and F_2 generations in monohybrid, dihybrid, and sex-linked crosses
- The life-cycle of a metamorphosing insect
- Statistical analysis of genetic crosses

Procedural Overview

Students gain experience conducting the following procedures:

- Culturing and anesthetizing fruit flies
- Determining the sex of fruit flies
- Observing phenotype of wild-type and mutant fruit flies
- Statistically analyzing results using the chi-square test

Time Requirement

♦ Preparation time	30 minutes
♦ Pre-lab discussion and experiment	30 minutes
♦ Lab experiment	30 to 60 minutes several times over a 4-week period

Materials and Equipment

For each group:

- Instant *Drosophila* culturing medium
- Anesthetizing materials[1]
- One vial per class, wild-type flies
- 3 vials per class, mutant flies[2]
- One vial per class, sex-linked F_1 cross[3]
- One vial per class, autosomal monohybrid F_1 cross[4]
- One vial per class, autosomal recessive dihybrid F_1 cross[5]
- Fly morgue[6]
- Petri dish
- Culture vial label or lab tape
- Dissecting microscope (or digital microscope with computer)
- Culture vial with foam plug and screen
- Small, thin camel-haired paintbrush (sorting brush)
- Index cards (2)

[1] For anesthetizing materials, refer to the Lab Preparation section.
[2] For procedures to create vial of mutant flies, refer to the Lab Preparation section.
[3] For procedures to create a sex-linked cross, refer to the Lab Preparation section.
[4] For procedures to create an autosomal monohybrid cross, refer to the Lab Preparation section.
[5] For procedures to create an autosomal recessive dihybrid cross, refer to the Lab Preparation section.
[6] For procedures to create a fly morgue, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Dominant versus recessive traits
- Autosomal versus sex-linked traits
- The relationship between genotype and phenotype
- Monohybrid, dihybrid, and sex-linked crosses
- How to use Punnett squares to predict potential offspring of genetic crosses

Related Labs in This Guide

Labs conceptually related to this one include:

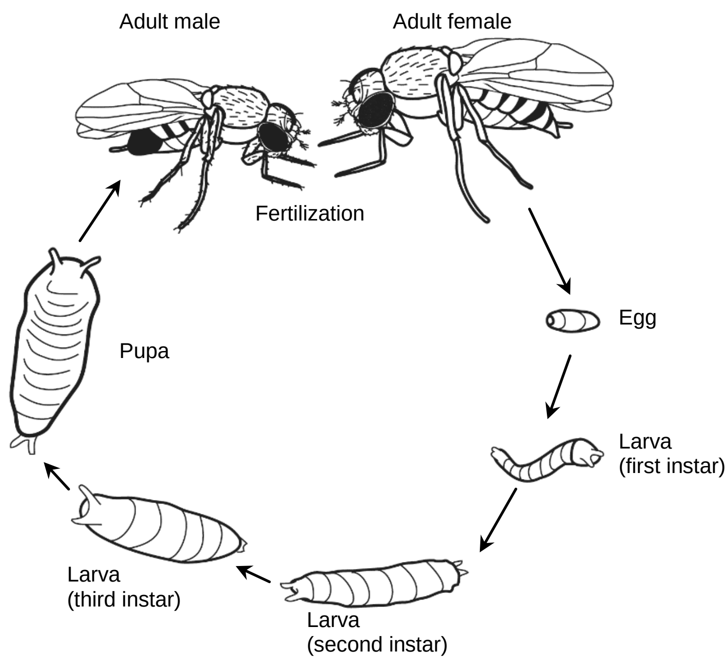
- Mitosis and Meiosis
- Evolution and Population Genetics
- Animal Behavior

Background

Drosophila melanogaster is otherwise known as the common fruit fly. You might have even seen them in your house flying around some unripe or spoiled fruit. *Drosophila* were first used by the geneticist, Thomas Hunt Morgan. He was the first to discover sex linkage and genetic recombination. These organisms have been used for almost 100 years for research purposes, and they remain model organisms used in genetics and developmental research. Fruit flies are commonly used in genetic research because they are small, have a short generation time, are easy to grow and breed in a lab, have minimal nutritional requirements, are easily anesthetized for observation, and have extremely high fecundity (ability to produce many offspring). Additionally, their entire genome has been sequenced, and many genes have been identified.

Fruit flies have four pairs of chromosomes, three pairs of autosomes, and one pair of sex chromosomes. Like humans, a fruit fly with two X chromosomes is a female, and ones with an X and a Y chromosome is male. Traits that are on the X chromosome are said to be sex-linked, or more specifically, X-linked. Male and female flies are relatively easy to distinguish from one another because females are larger than males and have a striped abdomen. Males have a darkened abdomen tip and sex combs (little black bristles) on their front forelegs. Normal fruit flies are said to have the wild-type phenotype. There have been many observed phenotypic deviations from the wild-type, and these flies are called mutants.

Drosophila undergo complete metamorphosis during their short two-week life cycle. At room temperature (25 °C) a female lays up to 100 eggs 24 to 48 hours after fertilization. The worm-like larvae hatch from the eggs approximately 24 hours later and can be easily observed in the culture vial. The larvae eat and grow continuously, molting a total of three times after hatching (first, second, and third instars). During the third and final instar stage (2 days long), the larvae molt to form an immobile pupa. Over the next four days, the larvae tissue degenerates and reorganizes to give rise to the adult winged form, which then hatches from the pupal case. Within 12 hours, the flies become fully mature and fertile.



Pre-Lab Discussion and Experiment

Introduce students to working with *Drosophila* in a lab setting.

Show students images of male and female *Drosophila* and point out the anatomical differences. Point out that females are generally larger than the males, and the females have a striped abdomen and a pointed "bottom". Males have a more rounded, black tip to their abdomen. Males also have "sex combs" on the uppermost joint of their forelimbs. These sex combs look like bristles or hairs.

Discuss the basics of *Drosophila* genetics with your students. The normal fly is considered to be wild-type. Any fly that exhibits a phenotype other than the wild-type is called mutant. Students will need to be familiar with these phenotypes when observing the flies. Some common wild-type traits are normal wing condition, black and tan striped bodies, and red eyes. Some common mutant traits are short wings, curled wings, ebony bodies, and white eyes.

Show your students how to correctly denote fruit fly mutations. Mutant flies are named and denoted by the type of mutation they exhibits. For example, the mutant, ebony, has a darker body than wild-type flies. Mutations are also given letter codes, for example, ebony flies are denoted with an "e". Wild-type flies are denoted by a + next to the mutant letter code. So, e^+ denotes a wild-type fly for the ebony body trait, meaning it has normal body color (not ebony).

The naming technique used above only applies to traits associated with genes found on autosomes. If the gene for the trait is located on a sex chromosome, it is called a sex-linked trait, and a different notation is used. The lower case letter becomes associated with the sex chromosome it is found on, usually the X. For example, the notation for a white-eyed female is X^wX^w, while a white-eyed male is X^wY.

Now that your students know how to denote a mutation and how to denote a sex-linked trait, ask them what the genotypic notation of a red-eyed ($X^{w+}X^w$ or $X^{w+}X^{w+}$) female and red-eyed ($X^{w+}Y$) male.

Demonstrate the procedure you choose for anesthetizing the flies, and then show students how to work with immobilized flies without harming them.

Review monohybrid, dihybrid, and sex-linked crosses and the predicted F_1 and F_2 phenotypic ratios.

Each group needs a microscope to observe and sex the flies. You can use a dissecting microscope or a digital microscope to observe the flies. Be sure that your students know how to properly use the equipment prior to the lab.

Lab Preparation

The following table lists some fruit fly mutants that are commonly available from biological supply stores:

Examples of commonly available *Drosophila* mutants

Mutation type	Example mutants (genetic symbol) and description of mutation
Sex-linked	white (w) – eyes are white yellow (y) – yellow body, wing hairs and veins scalloped – wing margins scalloped and veins thickened
Autosomal dominant	Antennapedia (Antp) – antennae are replaced by fully developed legs Curly wing (Cy) Wrinkled – wrinkled wings
Autosomal recessive	vestigial (vg) – small/reduced wing apterous (ap) – no wings dumpy (dp) – wings truncated curved (c) – wings divergent, thin, uplifted and curving down ebony (e) – body gradually turns black in adults sepia (s) – eyes change from brownish red to black with age

1. Anesthetize the flies: Prior to Part 1, anesthetize the wild-type and mutant flies.

   There are many commercial anesthetizers available for use with *Drosophila*. If you use a product like "Fly-nap," be sure to follow instructions carefully.

   Another option is the "Cooling method", which requires ice and petri dishes. This method will do no harm to the flies, and they will recover quickly once allowed to warm up. Use this method with caution. Flies kept in the freezer for too long may not "wake up." To anesthetize the flies, place the culture vial in the freezer until the flies are immobile, generally 8 to 10 minutes. Next, place the flies onto a chilled surface. Construct this by putting crushed ice into the top of a petri dish, and then place the bottom of the petri dish on top. This will keep the flies immobile long enough to do the experiment. Place the flies back into the culture vial when finished.

2. Create petri dishes for Part 1: Choose one wild-type fly, one sex-linked mutant, and two autosomal mutants. There are many mutant varieties to chose from, including vestigial wings, apterous wings, sepia eyes, eyeless, and ebony body color. Sex-linked traits include white eyes, or yellow body color. Place these anesthetized flies in a petri dish. Each group needs one dish. Be sure the place the lid on the petri dish in case the flies wake up.

3. Create a fly morgue: Make sure each group has a fly morgue where they can deposit flies they wish to dispose of. A fly morgue is a small jar with a lid that is filled with alcohol.

4. If you are not purchasing your F_1 flies, start your mating crosses 7 to 10 days before you plan to do Part 2. The offspring of these crosses will be used by the students to create the generation in Part 2. You will be creating three crosses and assigning one of the crosses to each group. You will need to tell each group the phenotypes of the parental generation.

5. Create the sex-linked cross that produces the F_1 offspring needed for Part 2. This cross can be purchased from a biological supply company or can be created by mating a parental generation consisting of white-eyed females (X^wX^w) with wild-type males ($X^{w+}Y$). The resulting F_1 generation will be white-eyed males (X^wY) red-eyed, heterologous females ($X^{w+}X^w$). Students will use these F_1 flies to perform the F_2 cross during Part 2. Create the mating cross 7 to 10 days before you plan to do Part 2. When the F_1 flies emerge, anesthetize them, and place them in petri dishes for the student groups who have been assigned this cross.

6. Create petri dishes of the autosomal monohybrid cross for Part 2. This cross can be purchased from a biological supply company or created by mating parental flies. The parents of this monohybrid autosomal cross should consist of one homozygous recessive and the other homozygous dominant for the particular trait you choose to observe. (Some examples of monohybrid autosomal F_1 crosses that are available from biological supply houses include F_1 apterous x wild (autosomal dominant) and F_1 sepia x wild (autosomal recessive).) Thus, the F_1 flies that the students will cross will all be heterozygous. Create the mating cross 7 to 10 days before you plan to do Part 2. When the F_1 flies emerge, anesthetize them, and place them in petri dishes for the student groups who have been assigned this cross.

7. Create petri dishes of the autosomal recessive dihybrid cross for Part 2: This cross can be purchased from a biological supply company (examples: F_1 vestigial x sepia or F_1 vestigial x ebony) or created by mating parental flies. The first parent should be homozygous recessive for trait #1 and homozygous dominant for trait #2. The second parent should be homozygous dominant for trait #1 and homozygous recessive for trait #2. Thus, all the F_1 flies will be heterozygous. Create the mating cross 10 to 12 days before you plan to do Part 2. When the F_1 flies emerge, anesthetize them, and place them in petri dishes for the student groups who have been assigned this cross.

8. Begin to look for the emergence of the F_2 flies two or three days after the students removed the F_1 flies in Part 3. When the F_2 flies emerge, create the petri dishes for your students. Anesthetize the flies in each vial and place them in petri dishes. Be sure to label the petri dishes so that you give each group their F_2 flies.

Safety

Add these important safety precautions to your normal laboratory procedures:

- Do not eat, drink, or inhale the *Drosophila* medium or any anesthetizing material you may use.

Procedure

Part 1 – Sexing *Drosophila* and observing the parental generation

Set Up

1. Obtain a sorting brush, two index cards, a fly morgue, a microscope, and a petri dish containing a wild-type fly and three different mutants. The sample of flies should consist of both males and females.

Note: Your instructor will have already anesthetized the flies and made petri dishes containing some of each type of fly to be observed. Place the index cards under the petri dish for better contrast to make seeing the flies easier.

2. Name one way to distinguish between male and female fruit flies.

Males are smaller than females. Males have a prominently black abdomen tip while females have a striped abdomen. Males have sex combs on their forelimbs, while females do not.

Collect Data

3. Observe the wild type and mutant flies under the microscope. Compare the mutants and the wild-type flies. Notice their similarities and differences.

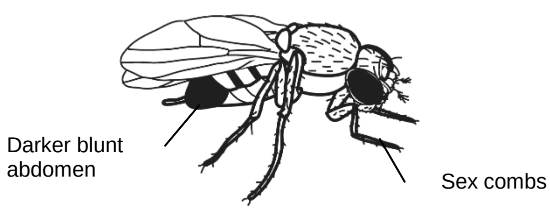
4. Record your observations of the phenotype of each of the four flies in Table 7.1 in the Data Analysis section. Be as descriptive as possible because you will use this data when observing the results of your crosses.

5. Record the suspected genotype of each of the four flies in Table 7.1.

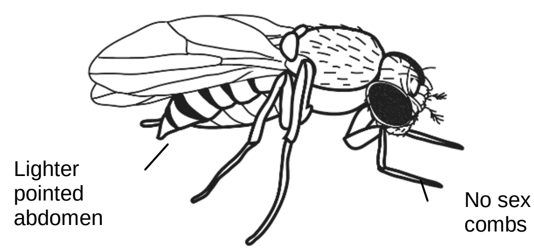
6. Practice sexing the *Drosophila*, and try to distinguish between males and females.

7. Make a drawing of a wild-type male and wild-type female fruit fly in the space below. Be sure to include color where applicable, and be as detailed as possible.

Wild-type Male



Wild-type Female



8. Dispose of all of the flies in the fly morgue.

Part 2 – Observing and scoring the F_1 phenotypes, and setting up the F_1 crosses

Set Up

9. Obtain a sorting brush, two index cards, a fly morgue, a microscope, a petri dish of the F_1 flies, and a culture vial with screen and plug.

Note: You will be assigned to observe the offspring (F_1 generation) of a cross between two of the four *Drosophila* strains that you observed in Part A. Because one of the traits is X-linked, you will be given the phenotypes of the parental generation. Your instructor will have already anesthetized the flies and made petri dishes containing some of F_1 flies. Place the index cards under the petri dish for better contrast to make seeing the flies easier.

10. Complete the following statement: This lab investigates the cross of:
___s+s+___ (Male Parent) & ___ss___ (Female Parent)

Collect Data

11. Use the microscope to observe the F_1 flies in the petri dish.

12. Record the sex and phenotype of each fly (this is called the "scoring" of the flies) in Table 7.2.

13. Set up a culture vial for your F_1 cross.

a. Create culture medium according to your instructor's instructions.

b. Place one screen in the jar on top of the medium after the medium has set.

14. Count out 6 male and 6 female F_1 flies, and place them in your culture vial.

Note: To prevent the flies from sticking in the medium, you may want to place the culture vial on its side, and put the flies in the vial while it is on its side. (The flies will be lying on the glass, not on top of the medium.) When the flies have "woken up," you can turn the vial right-side up.

15. Label your culture vial with your group names, the date, the number of male and female flies and the phenotype of the male and female flies, and "F_1 cross."

16. Put the remaining flies in the fly morgue when you finish scoring and sorting the flies in the vials.

17. Use Table 7.2, your results from Part 1, and the information you know about the Parental generation of your F_1 flies to hypothesize how the particular phenotypes you are studying are inherited. In other words, do you think the trait is dominant or recessive, and autosomal or sex-linked?

Answers will vary. For a simple monohybrid cross between a sepia parent and a wild-type parent, a hypothesis could be, "It is hypothesized that the trait for sepia eye color is autosomal and recessive."

18. Construct two Punnett squares to show the results of the parental and F_1 crosses.

Include the symbols and a key for each allele involved in your cross.

Answers will vary depending on the type of cross given. For example, for a simple monohybrid cross between a homozygous sepia female (ss) and a wild-type male (s^+s^+), the following Punnett squares will be constructed:

For this example:

Parental Cross:

Female gametes

Male gametes	S	S
S+	S+S	S+S
S+	S+S	S+S

Note: All the F_1 flies will be heterozygous

F_1 Cross:

Female gametes

Male gametes	S+	S
S+	S+S+	S+S
S	S+S	SS

19. Indicate the predicted genotypic and phenotypic ratios for the F_2 generation you will obtain from your F_1 cross.

Using the example above, the genotypic ratio would be 1:2:1, $s^+s^+:s^+s:ss$. The phenotypic ratio would be 3:1; wild eyes: sepia eyes.

Part 3 – Removing the F_1 generation

Approximately 7 days after completing Part 2, perform the following procedures.

20. Obtain a sorting brush, two index cards, a fly morgue, a petri dish of the F_1 flies, and a culture vial with screen and plug.

Note: Place the index cards under the petri dish for better contrast to make seeing the flies easier.

21. Check the vial for the presence of larvae in the medium and on the side of the vial. These larvae are the F_2 generation, the offspring of the F_1 flies.

22. Anesthetize the F_1 flies from your "F_1 cross" culture vial. Once the flies have been immobilized, remove them from the culture vial and place them into the morgue.

23. Why do you think it is necessary to remove the F_1 flies from your culture vial?

It is necessary to remove the F_1 flies from the culture vial because they can alter the F_2 data. If the F_1 flies are allowed to remain living, they will remain in the vial as the F_2 flies begin to emerge. When it is time to collect data and score the F_2 flies, the F_1 flies would also be counted. This may alter the data and provide for erroneous results.

Part 4 – Observing and scoring the F_2 generation

Observe the flies every two days until they completely emerge from the pupae.

Set Up

24. Obtain a sorting brush, two index cards, a fly morgue, a microscope, a petri dish of the F_2 flies, and a culture vial with screen and plug.

Note: Your instructor will have already anesthetized the flies and made petri dishes containing your F2 flies. Place the index cards under the petri dish for better contrast to make seeing the flies easier.

Collect Data

25. Observe the F_2 flies under the microscope. The more F_2 flies collected, the more valid the data will be.

26. Note and record in Table 7.3 the sex and presence or absence of the phenotypes observed in the Parental generation.

27. After you count and observe a fly, put it in the fly morgue. Do not return them to the petri dish or culture vial.

28. Obtain class totals for the F_2 flies and their phenotypes, and record the data in Table 7.4.

Data Analysis

Table 7.1: Phenotypes of parental strains of *Drosophila*

Phenotype	Suspected Genotype
Wild-type: Red eyes, normal wings	s^+s^+ (red-eyed female) a^+a^+ (normal wings)
Mutant 1: White eyes, normal wings	X^wX^w (white-eyed female) a^+a^+ (normal wings)
Mutant 2: No wings, red eyes	aa (apterous wings) s^+s^+ (red eyed male)
Mutant 3: Sepia eyes, normal wings	ss (sepia eyed female) a^+a^+ (normal wings)

Table 7.2: F_1 *Drosophila* data, for the cross of ____ss a+a+______ & _____s+s+ a+a+_____

Phenotype	# Males	# Females	TOTAL (females + males)
Red eyes, normal wings	16	14	30

Table 7.3: Phenotypes of F_2 flies, for the cross of ______s+s a+a+_____ & ______s+s a+a+________

Phenotype	Total
Red eyes, normal Wings	77
Sepia eyes, normal Wings	23
TOTAL scored: ______100___	

Table 7.4: Class data for the three F_2 crosses

CROSS 1:

Phenotypes of F_2 flies, for the cross of s+s a+a+ & s+s+a+a+

Phenotype	Total
Red eyes, normal wings	450
Sepia eyes, normal wings	150

TOTAL scored: 600

CROSS 2:

Phenotypes of F_2 flies, for the cross of s+s a+a & s+s a+a .

Phenotype	Total
Red eyes, normal wings	340
Red eyes, no (apterous) wings	100
Sepia eyes, normal wings	115
Sepia eyes, no (apterous) wings	45

TOTAL scored: 600

CROSS 3:

Phenotypes of F_2 flies, for the cross of Xw+Xw & XwY

Phenotype	Total
Red-eyed female	155
Red-eyed male	140
White-eyed female	152
White-eyed male	153

TOTAL scored: 600

Using the chi-square goodness-of-fit test to analyze F_2 data

How does your actual data compare with the predicted data? Are the ratios close to each other or not? How can you determine if your data is statistically similar to the predicted data? Genetics is much like the roll of a die, they both deal with the likelihood, or probability, of one outcome occurring over another. When you roll a die, there is a 1/6 probability that you will roll any particular number, one through six. If you roll a die 600 times, you would expect that you will get approximately 100 rolls of each number. But in real life what if your data look like this: 98 rolls of side 1, 102 rolls of side 2, 101 rolls of side 3, 97 rolls of side 4, 99 rolls of side 5 and 103 rolls of side 6. This seems close enough to that 1:1:1:1:1:1 ratio that you expected, right? But what if you got 300 rolls of side 4 and 200 rolls of side 2? That would raise a red flag and you would most likely expect that something besides chance is affecting the outcome of the roll of the die. If you are a gambler and want to know if a die is fair or biased in some way, how would you be able to test this hypothesis? If you are a geneticist and want to know if the results from the F_2 generation of a dihybrid cross are consistent with Mendel's laws, how would you test this?

In this lab, you hypothesized that your observed data would be close to the expected phenotypic ratios for each type of cross. This is known as the null hypothesis, a hypothesis of no difference between actual results and expected results. In order for you to make this assumption, you suppose that only chance during independent assortment and recombination of the alleles will affect the outcome of your crosses. Hence, any variation of the actual data as compared to the expected data will minimal and occur only due to chance.

To determine if observed data support the null hypothesis, you will use an inferential statistical test known as the chi-square goodness-of-fit test. Many geneticists use this test to compare actual results with predicted Mendelian, and non-Mendelian, phenotypic ratios. The chi-square statistic (X^2) formula is below:

$$X^2 = \sum [(\text{observed} - \text{expected})^2] \div \text{expected}$$

The difference between the number observed and the number expected is squared and the divided by the number expected. This is repeated for each observed phenotype and then all the numbers are added together to determine the calculated chi-square (X^2) value.

Next, the degrees of freedom must be determined. The degrees of freedom (df) are equal to the number of different phenotype classes (n) minus 1. For example, if you are studying a monohybrid cross, you would expect to see two different phenotypes in the F_2 generation. Thus, your n would equal 2 and the degrees of freedom would be $n - 1 = 2 - 1 = 1$. By statistical convention, you should use the 0.05 probability level as your critical value. If the calculated chi-square value is less than the 0.05 table value, you accept the null hypothesis. If the value is greater than the value, you reject the null hypothesis.

If the monohybrid cross you are studying deals with sepia and red eyes and you obtain 66 red eyed flies and 34 sepia eyed flies in an F_2 population of 100 flies, then:

$$X^2 = \sum [(\text{observed} - \text{expected})^2] \div \text{expected}$$

$$= [(66 - 75)^2/75] + [(34 - 25)^2/25] = 4.32$$

Hence, since the calculated chi-square value of 4.32 is greater than the table value of 3.84 (using a df = 1), the observed data are statistically different than the expected phenotypic ratio and the differences are due to something other than random chance.

Table 7.5: Chi-square table

	Probability				
Degrees of Freedom	**0.9**	**0.5**	**0.1**	**0.05**	**0.01**
1	0.02	0.46	2.71	3.84	6.64
2	0.21	1.39	4.61	5.99	9.21
3	0.58	2.37	6.25	7.82	11.35
4	1.06	3.36	7.78	9.49	13.28
5	1.61	4.35	9.24	11.07	15.09

1. ☐ Using the class data, calculate the chi-square value for each cross. Show your work. For each of the crosses, determine if the observed data is statistically similar to or different from the expected ratios.

Calculations for Cross 1

Cross 1: s+s a+a+ x s+ a+a+

Expected F_2 phenotypes: red and sepia

Expected F_2 phenotypic ratio: 3:1

Degrees of freedom (n – 1) = 1

Use the table value of: 3.84

The calculated X^2 = 0.213

$X^2 = \sum [(\text{observed} - \text{expected})^2] \div \text{expected}$

2. ☐ Do you Accept or Reject the null hypothesis? (Circle Accept or Reject)

3. ☐ If you reject the null hypothesis, list the possible sources of error that could have affected the outcome and altered your data.

Fail to Reject the null hypothesis since the calculated chi-square value of 0.213 is less than the table value of 3.84. This means that there is no statistical difference between the observed number of flies for each phenotype when compared to the expected number of flies for each phenotype.

Calculations for Cross 2

Cross 2: s+s a+a x s a+a

Expected F_2 phenotypes: red eyes and normal wings; red eyes and apterous wings; sepia eyes and normal wings; sepia eyes and apterous wings

Expected F_2 phenotypic ratio: 9:3:3:1

Degrees of freedom (n – 1) = 3

Use the table value of: 7.82

The calculated X^2 = 3.32

$X^2 = \sum [(\text{observed} - \text{expected})^2] \div \text{expected}$

4. ☐ Do you Accept or Reject the null hypothesis? (Circle Accept or Reject)

5. ☐ If you reject the null hypothesis list, the possible sources of error that could have affected the outcome and altered your data.

Fail to Reject the null hypothesis since the calculated chi-square value of 3.32 is less than the table value of 7.82. This means that there is no statistical difference between the observed number of flies for each phenotype when compared to the expected number of flies for each phenotype.

Calculations for Cross 3

Cross 3: XwXw x Xw+Y

Expected F_2 phenotypes: female red eyes, female white eyes, male red eyes, male white eyes

Expected F_2 phenotypic ratio: 1:1:1:1

Degrees of freedom (n – 1) = 3

Use the table value of: 7.82

The calculated X^2 = 0.9237

$X^2 = \sum [(\text{observed} - \text{expected})^2] \div \text{expected}$

6. ☐ Do you Accept or Reject the null hypothesis? (Circle Accept or Reject)

7. ☐ If you reject the null hypothesis, list the possible sources of error that could have affected the outcome and altered your data.

Fail to Reject the null hypothesis since the calculated chi-square value of 0.9237 is less than the table value of 7.82. This means that there is no statistical difference between the observed number of flies for each phenotype when compared to the expected number of flies for each phenotype.

Some possible sources of error could be: (1) sample size (the number of flies counted - the smaller the sample size, the greater likelihood that random chance could alter the outcome;) (2) counting error; (3) did not take all the F_1 flies out of the jar, etc.

Analysis Questions

1. Use the results in Table 7.4 to determine the phenotypic ratios for the F_2 flies of each of the three types of crosses. Record your results in the appropriate place below.

Phenotype Ratio for Cross 1: 3:1

Phenotype Ratio for Cross 2: 9:3:3:1

Phenotype Ratio for Cross 3: 1:1:1:1

Answers will vary depending on the actual numbers the student groups counted.

2. How do the results from your assigned cross compare with your initial predicted phenotypic ratios for the F_2 generation? (Refer to your initial prediction in Part 2.)

Answers will vary. The ratios should be close to the expected ratios of 3:1 for the monohybrid cross; 9:3:3:1 for the dihybrid cross; and 1:1:1:1 for the sex-linked cross.

3. Refer to your predicted genotypic ratios from Part 2. Copy your initial hypothesis in the space below.

Answers will vary. The ratios should be close to the expected ratios of 3:1 for the monohybrid cross; 9:3:3:1 for the dihybrid cross; and 1:1:1:1 for the sex-linked cross.

4. Does your data support your initial hypothesis? If not, modify your hypothesis to fit what you know about the inheritance patterns of the trait you are studying.

Answers will vary. An example: Our data does support our hypothesis because we saw close to a 3:1 ratio in the F_2 generation and did not see the trait show differences depending on the sex of the fly.

5. Review your data for the sex-linked trait. Do you think this trait is X-linked or Y-linked? Explain your answer using the actual data you collected with expected data if it was X-linked or Y-linked.

If using the white-eye trait, the trait is X-linked. The results of the F_2 cross are approximately 1:1:1:1 with equal numbers of males and females with red and white eyes. If the trait was Y-linked, then only males will show the recessive trait because females do not have Y chromosomes.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Purple corn kernel color is dominant and yellow corn kernel color is recessive. Consider a cross between two purple heterozygous corn plants. If you count 1000 offspring and 747 have purple kernels and 253 have yellow kernels, is your data statistically similar to the expected results? Show your work.

$X^2 = \sum (\text{observed} - \text{expected})^2 \div \text{expected}$

$= [(747 - 750)^2/750] + [(253 - 250)^2/250] = 0.012 + 0.036 = 0.048$

Because $0.048 < 3.84$, you can accept the null hypothesis that there is no statistically significant difference between the observed results and the expected results.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. What would be the phenotypic ratios of a cross between a heterozygous red eyed female (she carries a white allele) and a white-eyed male?

- **A.** 1; All offspring will be wild-type (red-eyed)
- **B.** 1:1; All females will be white-eyed and all males will be red-eyed
- **C. 1:1:1:1; Red female: White female: Red male: White male**
- **D.** 1:1:1; Red female: White female: White male

2. What would the F_1 generation look like between the parental cross of a sepia-eyed male (normal wings) and an apterous female (red-eyes). Both are autosomal traits

- **A.** All red eyes and apterous wings
- **B.** All sepia eyes and apterous wings
- **C.** All sepia eyes and normal wings
- **D. All red eyes and normal wings**

Extended Inquiry Suggestions

The salivary glands of the larvae contain easily visible chromosomes. Allow students to extract and observe these chromosomes.

Additionally, *Drosophila* can be used to create chromosome linkage maps. By choosing traits that are on the same chromosome and performing simple crosses, interesting results can be obtained. It can be of note to students that female chromosomes do cross over, but male *Drosophila* chromosomes do not cross over.

16. Evolution and Population Genetics

Objectives

Students investigate Hardy-Weinberg equilibrium and deviations from Hardy-Weinberg equilibrium. Students:

- ♦ Calculate the frequencies of alleles and genotypes in a gene pool of a population using the Hardy-Weinberg equations
- ♦ Using the class as a sample population, study the relationship between microevolution and changes in allele frequency

Procedural Overview

Students will gain experience conducting the following procedure:

- ♦ Using the Hardy-Weinberg equations to answer genetics problems

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	20 minutes
♦ Lab experiment	60 minutes

Materials and Equipment

For each student:

- ♦ Calculator with square root function
- ♦ 3" x 5" index card labeled "A" (2)
- ♦ 3" x 5" index card labeled "a" (2)
- ♦ Coin

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ The causes of microevolution including: natural selection, genetic drift, non-random mating, mutation, and small population and that these all can alter allelic frequencies in a population
- ♦ The Hardy-Weinberg formula and its use in determining the frequency of alleles and genotypes in a population
- ♦ The effects of allelic frequencies on selection against the homozygous recessive or other genotypes

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Population Ecology

- ♦ Mitosis and Meiosis
- ♦ Genetics of Organisms with *Drosophila Melanogaster*

Background

In the early 1900s, G. Hardy and W. Weinberg, both mathematicians, used their knowledge of binomial expansion and applied it to population genetics (microevolution). Microevolution involves the change in allele frequency over time. A population that is in Hardy-Weinberg equilibrium shows no change in allele frequency over time; it is a non-evolving population. In order for a population to comply with Hardy-Weinberg equilibrium, the following five conditions must be met:

1. The population is infinitely large. This reduces the probability that genetic drift (random change in allele frequency) will occur.

2. Mating is random. No sexual selection.

3. No natural selection. Thus, all genotypes will have the same chance of survival.

4. No mutation of alleles. There are no new alleles entering the population.

5. No gene flow. **No emigration or immigration.**

A Hardy-Weinberg population cannot exist in the real world because it is impossible to meet all five of the conditions simultaneously. The Hardy-Weinberg principle simply provides a baseline to determine whether or not allele frequencies have changed in a population and, thus, whether evolution has occurred.

There are two equations to use when working with the Hardy-Weinberg theorem:

1. $p + q = 1$, where

p = the frequency of the dominant allele in the population;

q = the frequency of the recessive allele in the population.

2. $p^2 + 2pq + q^2 = 1$, where

p^2 = the frequency of the homozygous dominant genotype in a population;

$2pq$ = the frequency of the heterozygous genotype in a population;

q^2 = the frequency of the homozygous recessive genotype in a population.

Pre-lab Discussion and Experiment

Have the class review the Hardy-Weinberg principle and the conditions that must be met for populations to be in Hardy-Weinberg equilibrium. Then, go over the practice problems below.

1. Given that the genetic frequencies of a population are 0.49 AA and 0.09 aa, what are the gene frequencies of A and a?

Use the equation $p^2 + 2pq + q^2 = 1$ to solve this problem. Take the square root of 0.49 to get the gene frequency of 0.7 for A. Take the square root of .0.9 to get the gene frequency of 0.3 for a.

2. Tay-Sachs disease, which is inherited as a Mendelian recessive gene, occurs in Ashkenazic Jews in 1 of every 3600 births. Estimate the percentage of this population that carries the allele without manifesting the disease (heterozygous).

1/3600 individuals is homozygous recessive (aa). So, if you take the square root of 1/3600, you get 0.0167, which is the frequency of the recessive allele, a. Using the equation $p + q = 1$, you can calculate that the frequency of the dominant allele, A, is 0.9833. Because we need to calculate the frequency of heterozygous carriers (Aa), we need to calculate $2pq = 2(0.0167)(0.9833) = 0.033$. To calculate the percentage, multiply 0.033 by 100 = 3.3%.

3. A population of individuals living in Brazil was surveyed for blood type with the following results: 10 individuals were found with type M blood (genotype L^ML^M), 180 had type MN blood (genotype L^ML^N), and 810 had type N blood (genotype L^NL^N). What is the frequency of the L^M allele in this population?

p = frequency of the L^N allele; q = frequency of the L^M allele. Calculate the number of individuals in the population (1000.) Then calculate the frequency of the L^ML^M individuals = 10/1000 = 0.01. Then, take the square root of this number, 0.1, which is the frequency of the L^M allele in this population.

Instructor Tip: Work the first problem in front of the class to review how to work through the problems. Then allow students to work either alone or in small groups to answer the remaining problems. You can then go over the answers with the class to ensure confidence in their ability to work with these types of problems.

Procedure

Part 1 – Estimating allele frequencies of a specific trait within a sample population

Instructor Tip: You may also choose any other easily observable trait that is controlled by a single gene, such as a widow's peak or hand clasping.

1. Look at the earlobes of the person next to you to see if they have attached or free earlobes? Make sure you can tell the difference.

The class will become a sample population to estimate the frequency of the gene that controls whether or not a person has an attached earlobe. The dominant trait is for the earlobe to hang free (for there to be some earlobe hanging past the point of attachment to the head). If you have the dominant phenotype, a free earlobe, you are either homozygous dominant (EE) or heterozygous (Ee). The recessive phenotype (ee) will have a completely attached earlobe.

2. Count how many individuals in the class have attached earlobes (ee) and the number of individuals that have unattached earlobes (Ee or EE). Record your results in Table 8.1.

3. In the space below, calculate the frequency of individuals with attached earlobes (ee) by dividing the number of attached earlobes by the total number of people in the class. This gives you q^2.

4. In the space below, calculate the frequency of individuals with free earlobes (EE and Ee) by dividing the number of free earlobes by the total number of people in the class. This gives
you $p^2 + 2pq$. Record your results in Table 8.1.

5. To estimate the frequency of the dominant allele in the population (E), you must find p. To find p, q (the frequency of the recessive allele) must be determined first, because only the genotype of the homozygous recessive individuals is known with certainty. (Those individuals who show the dominant phenotype could be EE or Ee.) Use the Hardy-Weinberg equation to determine the frequencies of the dominant and recessive alleles (p & q) in the class population. To do this,

a. ☐ Find q by taking the square root of q^2.

b. ☐ Determine p using the equation $p + q = 1$ and rearranging it to solve for p.

c. ☐ Record these values in Table 8.1.

6. In the space below, calculate and record the values of p and q for the expected values of an ideal population in Table 8.1.

Part 2 – Case studies

Case 1 – Testing of an ideal Hardy-Weinberg population

7. Choose another student at random as your partner to simulate random mating.

The entire class will be used as a breeding population, and you will need sufficient room to move around. In this simulation, gender and genotype are irrelevant to mate selection. The class will simulate a population of randomly mating heterozygous individuals with an initial frequency of 0.5 for the dominant allele A and 0.5 for the recessive allele a. The initial genotype frequencies will be 0.25 AA, 0.5 Aa, and 0.25 aa. So, your initial genotype is Aa.

8. Obtain four cards from your instructor. Two will have "A" printed on them, and two will have "a" on them. These four cards represent the products of meiosis. Each "parent" contributes only a haploid set of chromosomes to their offspring.

9. Decide between you and your partner which one of you will be "Number 1" and "Number 2."

10. Turn your four cards face down so that the letters do not show, and then shuffle the cards.

11 Pick up the card on the top and look at the letter. This is the allele that you contribute to the next generation.

12. Have your partner now go through the same process.

13. Put the two cards together, and you have the two alleles of the first "offspring" of the students.

The student who is assigned to "Number 1" should record this as their Initial Genotype in the Case I portion of the Data Analysis section.

14. The student who is "Number 2" needs to produce an offspring. (You and your partner need to produce a total of two offspring.) So, all four cards must be reshuffled and the process repeated to produce a second offspring.

15. Student "Number 2" should now record this as their Initial Genotype in the Case I portion of the Data Analysis section.

This marks the end of the short reproductive career of the first generation. You and your partner now should assume the genotype of each of your offspring, respectively. That is, Number 1 assumes the genotype of the first offspring and Number 2 assumes the genotype of the second offspring.

16. *Randomly* find another partner to "mate" with to produce offspring for the next generation.

Remember, the sex and genotype of your mate is irrelevant. Follow the same mating procedure as you did for the first generation to produce a total of five generations, being sure to record your new genotype after each mating event.

17. Collect class data after each generation for five generations and record in Table 8.2 in the Data Analysis section.

Case II – Selection

In this case, you will modify the simulation followed in Case I to make it more realistic. In the natural environment, not all genotypes have the same rate of survival. In nature, some genotypes confer an advantage over others, which are selected against. Infantile Tay Sach's disease is an example of a human disorder that is selected against in nature. This condition is caused by a mutation on the allele that codes for a lysososmal enzyme, which breaks down fatty acid derivatives. When this enzyme is nonfunctional, lipids aggregate in the brain of affected individuals, eventually causing death. Individuals who are homozygous recessive do not survive past the age of 5, and hence, do not reach reproductive maturity.

For this simulation, you will assume that the homozygous recessive individuals are selected against and do not survive. It will also be assumed that the heterozygous and homozygous dominant individuals survive 100 percent of the time.

18. Follow the procedure for Case I to produce offspring through five generations with your initial genotype as *Aa*. But, this time there is one important difference. Whenever your offspring receives two recessive alleles, *aa*, it does not survive (and does not reproduce). If you produce an offspring that is *aa*, then you and your partner must keep trying to reproduce until you produce a viable offspring (to maintain the same population size). Remember that the homozygous recessive genotype is selected against all (100%) of the time.

19. Record your viable offspring in Table 8.3 in the Data Analysis section.

Case III – Heterozygote advantage

In this simulation, you will investigate the effect that the heterozygote advantage will have on allele frequencies. In some populations, individuals who are heterozygous have a selective advantage over the individuals who have other genotypes. This is exactly the case with the sickle-cell anemia allele in certain populations. Sickle-cell anemia is a caused by a mutation on a single allele. Homozygous recessive individuals do not produce normal hemoglobin proteins. These proteins can crystallize in the red blood cells, causing them to form a sickle shape. Individuals with this disorder are chronically anemic and experience periodic episodes of pain. Many of these people do not survive to reach reproductive maturity. One would expect that the recessive allele would have a relatively low frequency in all populations. However, certain populations show an unexpectedly high frequency of the recessive allele due to the fact that heterozygous individuals are slightly more resistant to malaria when compared to homozygous individuals.

20. Follow the procedure for Case II with your initial genotype as Aa. Record your initial genotype in the Case III section of the Data Analysis section below. Except this time, when you produce an offspring with the AA genotype, you will flip a coin. If the coin lands on heads, the AA offspring survives, but if it lands on tails, the offspring dies before reproductive maturity. Simulate 5 generations. Remember that whenever your offspring receives two recessive alleles, aa, it does not survive, and that the homozygous dominant offspring will only survive if, when you flip a coin, you land on tails. Again, if you produce an offspring that is aa, or if your AA offspring dies, then you and your partner must keep trying to reproduce until you produce a viable offspring (to maintain the same population size.) Be sure to record your viable offspring in Table 8.4 in the Data Analysis section.

Case IV – Genetic drift

21. We will investigate the effect of small population size on allele frequencies. To do this, the class should be divided into several small "populations." For example, if your class has 30 people, it can be divided into three populations of ten each. These new populations do not interact and, thus, will not interbreed.

22. Follow the procedure for Case I to produce offspring through 5 generations with your initial genotype as Aa.

a. Record Aa your initial genotype in the Case IV section of the Data Analysis section below.

b. Record the genotype of your offspring for five generations in Table 8.5.

Data Analysis

Part 1

Table 8.1: Phenotypic proportions of attached and free earlobes and frequencies of the dominant (E) and recessive (e) alleles

	Phenotypes				Allele frequency based on H-W equation	
	Free Earlobes (p^2+ 2pq)		Attached Earlobes (q^2)		p	q
Class population	#	Frequency	#	Frequency	0.55	.045
	24	0.8	6	0.2		
Expected frequency from an ideal population	0.75		0.25		0.5	0.5

Part II

Case I: Testing of an ideal Hardy-Weinberg population

My initial genotype: Aa

Generation 1 genotype: aa

Generation 2 genotype: Aa

Generation 3 genotype: AA

Generation 4 genotype: Aa

Generation 5 genotype: Aa

1. Gather class data. Count the number of offspring in each generation with the following genotypes: AA, Aa, and aa. Record in Table 8.2.

Table 8.2: Case I – Testing of an ideal Hardy-Weinberg population

Generation	Class totals for each genotype		
	AA	Aa	aa
1	6	16	8
2	6	17	7
3	7	18	5
4	6	18	6
5	7	16	7

2. Using the genotypes recorded for the first generation in Table 8.2, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 6/(6 + 16 + 8) = 0.20

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 16/(6 + 16 + 8) = 0.53

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 8/(6 + 16 + 8) = 0.27

3. Using the genotypes recorded for the fifth generation in Table 8.2, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 7/(7 + 16 + 7) = 0.23

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 16/(7 + 16 + 7) = 0.53

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 7/(7 + 16 + 7) = 0.23

4. Calculate the total number of alleles in the class data of the fifth generation.

Total number of alleles in population = Total population 30 x 2 = 60

5. Calculate the total number of dominant alleles (A) occurring in the fifth generation using the equations below.

Number of offspring with genotype AA 7 x 2 = 14 A alleles

Number of offspring with genotype Aa 16 x 1 = 16 A alleles

TOTAL = 30 Total A alleles

6. Calculate p, the frequency of the dominant allele.

p = Total number of A alleles ÷ Total number of alleles in the population* = 30/60 = 0.50

7. Calculate the total number of recessive alleles (a) occurring in the fifth generation using the equations below.

Number of offspring with genotype aa 7 x 2 = 14 a alleles

Number of offspring with genotype Aa 16 x 1 = 16 a alleles

TOTAL = 30 a alleles

8. Calculate q, the frequency of the recessive allele.

q = Total number of a alleles ÷ Total number of alleles in the population = 30/60 = 0.50

Case II – Selection

My Initial Genotype: Aa

Generation 1 genotype: aa

Generation 2 genotype: Aa

Generation 3 genotype: Aa

Generation 4 genotype: Aa

Generation 5 genotype: AA

9. Gather class data. Count the number of offspring in each generation with the following genotypes: AA, Aa, and aa. Record in Table 8.3.

Table 8.3: Case II – Selection

Generation	Class totals for each genotype		
	AA	**Aa**	**aa**
F_1	13	17	0
F_2	15	15	0
F_3	17	13	0
F_4	16	14	0
F_5	19	11	0

10. Using the genotypes recorded for the first generation in Table 8.3, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 13/(13 + 17 + 0) = 0.43

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 17/(13 + 17 + 0) = 0.57

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0/(13 + 17 + 0) = 0.00

11. Using the genotypes recorded for the fifth generation in Table 8.3, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 19/(19 + 11 + 0) = 0.63

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 11/(19 + 11 + 0) = 0.37

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0/(19 + 11 + 0) = 0.00

12. Calculate the total number of alleles in the class data of the fifth generation.

Total number of alleles in population = Total population 30 x 2 = 60

13. Calculate the total number of dominant alleles (A) occurring in the fifth generation using the equations below.

Number of offspring with genotype AA 19 x 2 = 38 A alleles

Number of offspring with genotype Aa 11 x 1 = 11 A alleles

TOTAL = 49 A alleles

14. Calculate p, the frequency of the dominant allele.

p = TOTAL number of A alleles ÷ TOTAL number of alleles in the population* = 49/60 = 0.82

15. Calculate the total number of recessive alleles (a) occurring in the fifth generation using the equations below

Number of offspring with genotype aa 0 x 2 = 0 a alleles

Number of offspring with genotype Aa 11 x 1 = 11 a alleles

TOTAL = 11 a alleles

16. Calculate q, the frequency of the recessive allele.

q = TOTAL number of a alleles ÷ TOTAL number of alleles in the population* = 11/60 = 0.18

Case III – Heterozygous advantage

My initial genotype: Aa

Generation 1 genotype: AA

Generation 2 genotype: Aa

Generation 3 genotype: Aa

Generation 4 genotype: Aa

Generation 5 genotype: AA

17. Gather class data. Count the number of offspring in each generation with the following genotypes: AA, Aa, and aa. Record in Table 8.4.

Table 8.4: Case III – Heterozygous advantage

Generation	Class totals for each genotype		
	AA	Aa	aa
1	7	23	0
2	10	20	0
3	11	19	0
4	10	20	0
5	12	18	0

18. Using the genotypes recorded for the first generation in Table 8.4, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 0.23

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 0.77

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0.0

19. Using the genotypes recorded for the fifth generation in Table 8.4, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 0.4

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 0.6

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0.0

20. Calculate the total number of alleles in the class data of the fifth generation.

Total number of alleles in population = Total population 30 x 2 = 60

21. Calculate the total number of dominant alleles (A) occurring in the fifth generation using the equations below.

Number of offspring with genotype AA ____12____ x 2 = ____24____ A alleles

Number of offspring with genotype Aa ____18____ x 1 = ____18____ A alleles

TOTAL = ____42____ Total A alleles

22. Calculate p, the frequency of the dominant allele.

p = Total number of A alleles ÷ Total number of alleles in the population* = ____0.7____

23. Calculate the total number of recessive alleles (a) occurring in the fifth generation using the equations below

Number of offspring with genotype aa ____0____ x 2 = ____0____ a alleles

Number of offspring with genotype Aa ____18____ x 1 = ____18____ a alleles

TOTAL = ____18____ a alleles

24. Calculate q, the frequency of the recessive allele.

q = Total number of a alleles ÷ Total number of alleles in the population* = ____0.3____

Case IV – Genetic drift

My initial genotype: ____Aa____

Generation 1 genotype: ____Aa____

Generation 2 genotype: ____aa____

Generation 3 genotype: ____Aa____

Generation 4 genotype: ____AA____

Generation 5 genotype: ____Aa____

25. Gather class data. Count the number of offspring in each generation with the following genotypes: AA, Aa, and aa. Record in Table 8.5.

Table 8.5: Case IV – Genetic drift

Generation	Class totals for each genotype		
	AA	**Aa**	**aa**
1	5	4	1
2	3	5	2
3	6	4	0
4	7	3	0
5	9	1	0

26. Using the genotypes recorded for the first generation in Table 8.5, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 0.5

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 0.4

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0.1

27. Using the genotypes recorded for the fifth generation in Table 8.5, calculate the genotype frequencies for the class.

Frequency of AA = Total AA ÷ (Total AA + Total Aa + Total aa) = 0.9

Frequency of Aa = Total Aa ÷ (Total AA + Total Aa + Total aa) = 0.1

Frequency of aa = Total aa ÷ (Total AA + Total Aa + Total aa) = 0.0

28. Calculate the total number of alleles in the class data of the fifth generation.

Total number of alleles in population = Total population 10 x 2 = 20

29. Calculate the total number of dominant alleles (A) occurring in the fifth generation using the equations below.

Number of offspring with genotype AA 9 x 2 = 18 A alleles

Number of offspring with genotype Aa 1 x 1 = 1 A alleles

TOTAL = 19 Total A alleles

30. Calculate *p*, the frequency of the dominant allele.

p = Total number of A alleles ÷ Total number of alleles in the population* = 0.95

31. Calculate the total number of recessive alleles (a) occurring in the fifth generation using the equations below

Number of offspring with genotype aa ___0___ x 2 = ___0___ a alleles

Number of offspring with genotype Aa ___1___ x 1 = ___1___ a alleles

TOTAL = ___1___ a alleles

32. Calculate q, the frequency of the recessive allele.

q = Total number of a alleles ÷ Total number of alleles in the population* = ___0.05___

Analysis Questions

1. What percentage of individuals in your class is heterozygous for free earlobes? Hint: You need to find 2*pq*.

In a class of 18 students where 12 students have free earlobes and 6 have attached earlobes:

Frequency of attached earlobes (q^2) = 6/18 = 0.33

q = √0.33 = 0.58

p = 1 – q = 1 – 0.58 = 0.42

Frequency of heterozygous earlobes = 2pq = 2 x 0.58 x 0.42 = 0.49

Percentage of heterozygous earlobes = 49%

2. What percentage of individuals in your class are homozygous dominant for free earlobes?

In a class of 18 students where 12 students have free earlobes and 6 have attached earlobes:

p = 0.42

p^2 = 0.18

18% of the students in this class are homozygous dominant for free earlobes.

3. What percentage of individuals in an ideal population is heterozygous for free earlobes?

q^2 = 0.25

q = 0.5

p = .1 – q = 0.5

2pq = 2 x 0.5 x 0.5 = 0.5

% heterozygous for free earlobes = 0.5 x 100 = 50%

4. What does the Hardy-Weinberg equation predict for the values of *p* and *q* in the ideal population?

They should be the same, p = 0.50 and q = 0.50.

5. Do the results of the class simulation agree with the predicted values? Explain your answer.

Yes, they should agree. If they do not agree, one of the rules of the Hardy-Weinberg equilibrium was violated (possibly due to the fact that the breeding population was not sufficiently large).

6. What assumption(s) of Hardy-Weinberg equilibrium were not followed in this simulation?

The breeding population was not large.

7. Compare the new values of *p* and *q* to the initial frequencies in Case I. Explain any differences seen.

The p value is much higher and the q value is much lower. This is due to the fact that the homozygous recessive genotype is selected against and will not survive to reproduce.

8. What assumption(s) of Hardy-Weinberg equilibrium were not followed in the simulation in Case I?

The breeding population was not large and there was selection.

9. Compare the new values of *p* and *q* to the frequencies in Case II. Explain any differences seen.

The *p* value is lower and the *q* value is higher. This is due to the fact that the heterozygotes have a selective advantage over the homozygous dominant individuals, which raises the frequency of the a allele in the population.

10. Do you think that the recessive allele would be completely removed from the populations of Case II or Case III? Why?

It would not be removed in Case II because it is hidden in the heterozygotes. In Case III, the heterozygotes are favored and the recessive allele would be selected for.

11. How do the initial allelic frequencies in Case I and Case IV compare?

They are the same.

12. Compare the new values of p and q in Case IV to the frequencies at the end of Case I. Explain any differences seen.

The *p* value is much higher in Case IV and the *q* value is much lower in Case IV. The recessive allele is disappearing.

Synthesis Questions

1. Explain the importance of a large population size in maintaining genetic diversity.

Small populations are more at risk for random chance to cause an allele to disappear completely from the genetic pool. Large populations have more individuals to help reduce the effect of chance.

2. Is there a difference between your class frequencies of the E and e alleles when compared to the frequencies from the ideal population? If so, why do you think this happened?

If there is a difference, it may be due to the fact that the class sample size is very small which increases the chance that it may not be representative of the whole population.

3. Is it possible to eradicate a lethal recessive allele in a very large population? Why or why not?

No, there would be no way to completely rid a population of a lethal recessive allele. It would always be present in a population in the heterozygous individuals (who would survive since they have a normal dominant allele.)

4. Why is the heterozygous condition important in populations for maintaining genetic variation?

Since both alleles are present in heterozygote individuals, they provide a population with more variation and hence more possible genotypes (thus phenotypes) with which to tackle variation in the environment.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. In a certain population, the recessive phenotype of a certain trait occurs 16% of the time. What is the frequency of the dominant allele?

- **A.** 0.30
- **B.** 0.40
- **C.** 0.50
- **D.** **0.60**
- **E.** 0.70

Instructor Tip: You arrive at answer D by: $\sqrt{(0.16)} = 0.40 = q$ $\quad p = 1 - .4 = 0.60$

2. In *Drosophila melanogaster*, the allele for normal wings is dominant over the allele for apterous wings (when a fly has the apterous genotype, it has no wings.) In a population of 2,000 flies, 1300 show the dominant phenotype. How many individuals would you expect to be homozygous dominant, homozygous recessive, and heterozygous for this trait?

A. homozygous dominant: 340; homozygous recessive: 700; heterozygous: 960

B. homozygous dominant: 960; homozygous recessive: 700; heterozygous: 340

C. homozygous dominant: 1000; homozygous recessive: 500; heterozygous: 500

D. homozygous dominant: 535; homozygous recessive: 500; heterozygous: 965

E. homozygous dominant: 300; homozygous recessive: 700; heterozygous: 1000

Instructor Tip: You arrive at answer A by:

2000 -–1300 = # homozygous recessive individuals = 700

700/2000 = q^2 = .35 $q = \sqrt{(.35)} = 0.59$ $p = 1 – 0.59 = 0.41$ $p^2 = 0.17$ $2pq = .48$

homozygous dominant = 0.17 * 2000 = 340

homozygous recessive = 0.35 * 2000 = 700

heterozygous = 0.48 * 2000 = 960

3. Phenylketonuria is a severe form of mental retardation due to a rare autosomal recessive. About 1 in 10,000 newborn Caucasians are affected with the disease. About what percent of individuals are carriers?

A. 0.05%

B. 2.0 %

C. 10%

D. 20%

E. 45%

Instructor Tip: You arrive at answer B by: 1/10000 = q^2 $q = \sqrt{(0.0001)} = 0.01$ $p = 1 – 0.01 = 0.99$
$2pq = 0.0198$ or about 2%

Extended Inquiry Suggestions

You could have the class research history in human disorders to uncover which ones have conferred a possible heterozygote advantage to certain populations. You could also have the class calculate the allelic frequencies of other human traits that are determined by one gene.

Animal and Plant Physiology

17. Transpiration

Objectives

Students observe the rate of transpiration in a plant and then test the effects of various environmental factors on that rate. Students also observe the differences between monocot and dicot stems and relate their structure to their functions.

♦ Students measure the effects of wind, light, and humidity on transpiration rate.

♦ Students relate the properties of water to water transport in plants.

♦ Students study the organization of the xylem in a plant stem as it relates to its function.

Procedural Overview

Students gain experience conducting the following procedures:

♦ Setting up a potometer and a barometer sensor to measure the rate of transpiration in a plant

♦ Measuring the rate of transpiration under different environmental factors (wind, light, and humidity)

♦ Calculating the surface area of the leaves used in the transpiration experiment

♦ Observing prepared slides of monocot and dicot stems

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment: Part 1	60 minutes
♦ Lab experiment: Part 2	20 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Barometer/low pressure sensor
- Sensor extension cable
- Compound light microscope
- Electronic balance (1 per class)
- Disposable pipet
- Wide, shallow bowl or tub filled with water
- Heat sink - large beaker or aquarium filled with water
- Plant seedling, 12 to 15 cm tall
- Prepared slides: monocot and dicot stem
- Large base and support rod
- Utility clamp
- Three-finger clamp
- Glycerin, few drops
- Spray bottle filled with water
- 100-watt light source
- Knife (or single-edge razor blade)
- Petroleum jelly, 2 to 3 g
- Transparent plastic bag
- Scissors
- Fan

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- The role of transpiration, guttation, and the properties of water in the transport of water within a plant.
- How the properties of water result in the movement of water from the soil into the plant root and then up the stem, et cetera
- Basic differences between xylem and phloem
- Basic differences between monocots and dicots

Related Labs in this Guide

Labs conceptually related to this one include:

- Diffusion and Osmosis
- Plant Pigments and Photosynthesis
- Dissolved Oxygen and Primary Productivity

Background

Plants lose an astonishing amount of water by transpiration, the loss of water vapor from leaves. The average maple tree loses more than 200 liters of water per hour during the summer. If plants lose so much water, then how do they survive?

The water that is lost to the atmosphere is replaced by the flow of water transported up the xylem. At night, when transpiration is very low, the root cells are still expending energy to pump minerals into the xylem. The accumulation of minerals in the stele lowers water potential there, generating a positive pressure called root pressure. This pressure forces fluid up the xylem and results in guttation, the exudation of water droplets that can be seen in the morning on the tips of leaves.

In most plants, root pressure is not the major driving force of water in the xylem. At most, root pressure can force water upward only a few meters. For the most part, fluid in the xylem is not pushed from below by root pressure but is pulled upward. Transpiration provides this pull, and the cohesion of water due to hydrogen bonding transmits the upward pull along the entire length of the xylem to the roots.

Transpiration depends on the generation of negative pressure (tension) in the leaf. As water transpires from the leaf, water from the leaf xylem is drawn into that space. The tension generated by adhesion and surface tension lowers the water potential, drawing water from where its potential is higher to where it is lower. The transpirational pull on the fluid in the xylem is transmitted all the way from the leaves to the root tips and even into the soil solution.

The amount of water and gases that are allowed to enter and exit the leaf are controlled by the stomata (also called stomates), small openings in the leaf surface which open into air spaces that surround the mesophyll cells of the leaf. When the stomata are open, carbon dioxide and oxygen gas can be exchanged between the inner leaf space and the atmosphere, a process that is beneficial to photosynthesis and carbohydrate synthesis.

However, this poses a problem. If the stomata are open on a hot, sunny day, a tremendous amount of water can evaporate through transpiration. Because of this, plants must maintain a compromise to deal with open stomata that promote water loss and closed stomata that prevent gas exchange. The opening and closing of the stomata is influenced by temperature, light intensity, air currents, and humidity.

In the second part of this lab, students study the differences between the xylem in the stems of two different types of plants, monocots and dicots. The stems of both monocots and dicots contain three parts: the epidermis, the cortex, and the stele. The epidermis of the stem contains epidermal cells that are covered with a waxy substance called cutin. Cutin forms the protective layer known as the cuticle. The cortex consists of various ground tissue types that lie between the epidermis and the stele. Many of these cells contain chloroplasts. The stele consists of xylem, phloem, and pith. Unlike their central position in a root, the vascular tissue runs the length of a stem in strands called vascular bundles. The stem's vascular bundles meet the root's vascular cylinder and this is the place where water from the root enters the stem.

Each vascular bundle of the stem is surrounded by ground tissue. The arrangement varies among species, but monocots and dicots exhibit distinct differences. The vascular bundles of most dicots are arranged in a ring with pith on the inside and cortex on the outside of the ring. The vascular bundles have their xylem facing the pith and their phloem facing the cortex. Thin rays of ground tissue between the vascular bundles connect the two parts of the ground tissue system, the pith and cortex. Monocot stems have vascular bundles scattered throughout the ground tissue.

Pre-Lab Discussion and Experiment

Setting up the potometer can be difficult. It may be helpful to have one potometer set up so that students can see it.

1. Ask the students how much they think the pressure inside of the tube will change during a 10-minute period.

Students should predict change in terms of hPa s well as the direction of the change (positive or negative). Depending upon the type of plant and the environmental conditions, the pressure could drop 10 or more hPa.

2. Ask students to predict how other factors such as air movement, light intensity, and humidity might change the rate of transpiration?

Students may predict that: 1), the rate of transpiration will be greater when the air blows across the leaves than when the leaves are in still air, 2) high light intensity would increase the rate of transpiration; and 3) high humidity would reduce the rate of transpiration.

3. Tell students that some scientists think that people with allergies and other respiratory ailments benefit from keeping plants with high transpiration rates in their homes. Ask students to brainstorm some ideas on why this might be true.

Plants with high transpiration rates exchange larger amounts of gases with the environment than those with lower rates. Scientists think that plants like pothos, a vine plant, clean the air of impurities and release larger amounts of oxygen than other plants.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Each student will need a small plant or seedling for this lab. You can buy plants or grow them yourself. If you plan it right, you can grow the plants yourself and use the growing seedlings as examples during your plant anatomy unit. To grow bean seedlings, fill small pots with soil and place a bean seed on top of the soil. Cover the seed with soil and immediately water. Place the pot in a well-lit area for two weeks. Water when the soil feels dry.

   If you would prefer to buy the plant, here are some suggestions for plants that work well with this lab: tomato plants, honeysuckle, vinca, chrysanthemums, coleus, bush bean seedlings, and geraniums.

   Above all, you are looking for a plant whose stem fits TIGHTLY into the tubing.

2. To save time, have each group complete only one set up. For example, have one group do the ambient temperature setup, another group the light, et cetera. Then have the students share their data.

Safety

Add this important safety precaution to your normal laboratory procedures:

♦ Be careful when using a knife or razor blade to trim the plant stem.

Procedure

Part 1 – Measuring transpiration rates under different conditions

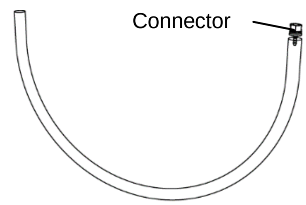
Set Up – Room Conditions

1. Start a new experiment on the data collection system. Connect a barometer sensor to your data collection system using the sensor extension cable.

2. Display Barometric Pressure (hPa) on the y-axis of a graph with Time on the x-axis.

3. If it has not been done already, cut the plastic tubing to a length of approximately 20 inches.

4. Put a drop of glycerin on the barb end of the quick-release connector and insert the barb into one end of the plastic tubing. The barb will not insert all the way into the tubing; however, be sure that it is in far enough to not fall out easily.



5. Use a knife or single edge razor blade to cut the stem 2 to 3 cm above the soil. Immediately immerse the cut end of the seedling in the bowl of water.

6. While keeping the cut end of the stem submerged in the water, shave the stem to a 45-degree angle. Continue holding the stem under water.

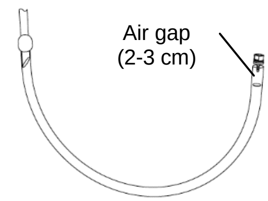
7. Place the tubing into the bowl of water.

8. Place the pipet into the open end of the tubing and pull water into the tubing using the pipet. If there are any bubbles in the tubing, use the pipet to remove them.

9. Once the tube is completely filled with water and has no air bubbles, insert the plant stem (still under water), cut-end first, into the tubing. **AVOID CREATING ANY AIR BUBBLES IN THE TUBING.**

10. Raise the tubing out of the water in a U-shape.

11. Adjust the level of the tubing so that there is a 2- or 3-cm air gap between the barb end and the water. Water will spill out of the tubing to achieve this level. Be sure that water in the tubing always stays in contact with the plant.



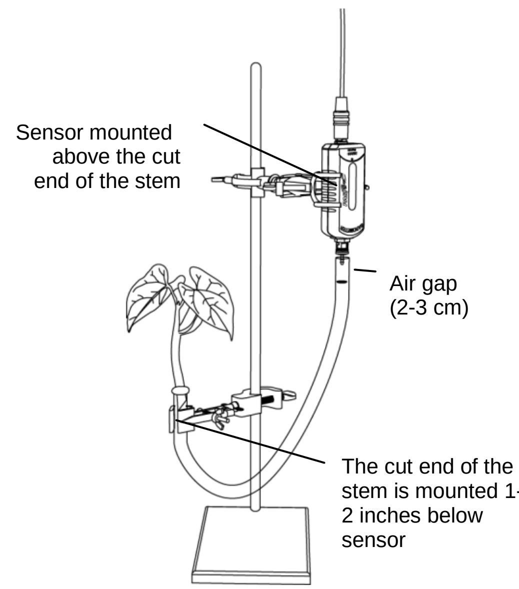
12. Spread petroleum jelly around the end of the tube to create an airtight seal between the top edge of the plastic tubing and the plant stem. If you see any water leaking out of the end of the tubing near the plant, add more petroleum jelly. Be sure that the petroleum jelly does not come in contact with the cut end of the plant stem.

Note: If air bubbles do form around the cut end of the stem, pull the tubing away from the stem. Use the pipet to refill the open end of the tubing with water. Put the stem back into the water in the tubing.

13. Secure the plant seedling in an upright position with the utility clamp, base, and support rod.

14. Mount the barometer/low pressure sensor to the support rod with the three-finger clamp. The pressure port should be above the cut end of the stem. This will prevent water from entering the pressure sensor.

15. Align the quick-release connector on the tubing with the connector on the pressure port of the sensor. Push the connector onto the port and then turn the connector clockwise until it clicks (about one-eighth turn). Make sure that no water enters the sensor. There should be a 2- to 3-cm air pocket between the water level and the pressure port.



Note: Do not move the barometer sensor up or down on the support rod while recording data.

16. Why is the change in barometric pressure being measured in the experiment?

Change in barometric pressure is being measured to show water loss by the plant.

Collect Data – Room Conditions

17. Start recording data.

18. Adjust the scale of the graph to show all data.

19. Record data for 600 seconds (10 minutes). After 10 minutes, stop recording data.

20. Name this data run "Room Conditions".

21. Explain the importance of collecting data at room condition?

Room condition is the experiment control group that shows the plant in normal condition.

Set Up – Increased air movement

22. Restore the pressure in the tubing to the initial amount by carefully disconnecting and then reconnecting the tubing to the sensor.

23. Place the fan about 1 meter from the plant seedling. Put the fan on a low setting so it blows a light breeze over the seedling.

Collect Data – Increased air movement

24. Start recording data. Adjust the scale of the graph to show all data.

25. Record for 600 seconds (10 minutes). After 10 minutes, stop recording data.

26. Name this data run "Wind".

27. What are the independent and dependent variables in this experiment?

The independent variable is air movement and the dependent variable is the change in barometric pressure.

Set Up – Increased light intensity

28. Remove the fan.

29. Restore the pressure in the tubing to the initial amount by carefully disconnecting and then reconnecting the tubing to the sensor.

30. Place the light (100-watt) about 1 meter from the plant seedling, and place the heat sink between the light and the plant.

31. What is the purpose of the heat sink?

The heat sink absorbs any heat associated with the light. Since we are testing only the effects of light in this part of the experiment, not light and heat, we must have a way to shine the light but remove the heat. The water in the heat sink does that for us.

Collect Data – Increased light intensity

32. Start recording data. Adjust the scale of the graph to show all data.

33. Record for 600 seconds (10 minutes). After 10 minutes, stop recording data.

34. Name this data run "Light Intensity".

Set Up – Increased humidity

35. Remove the floodlight.

36. Restore the pressure in the tubing to the initial amount by carefully disconnecting and then reconnecting the tubing to the sensor.

37. Mist the plant with the spray bottle and then cover the plant with a transparent bag. Make sure to leave the bottom of the bag open.

Collect Data – Increased humidity

38. Start recording data. Adjust the scale of the graph to show all data.

39. Record for 600 seconds (10 minutes). After 10 minutes, stop recording data.

40. Name this data run "Humidity".

41. Save your experiment.

42. What other environmental conditions can be tested to see their effect on transpiration?

Other environmental conditions include air temperature and soil conditions.

Set Up – Determining Leaf Surface

43. Remove your plant from the tubing.

44. Cut or pick off all of the leaves.

Note: You must use the plant that was used in the potometer.

Collect Data – Determining Leaf Surface

45. Use a balance to measure the mass all of the leaves. Use only the leaves, not the stems.

46. Record the mass in Table 9.1.

47. Cut out a section from one leaf that is 5 cm x 5 cm.

Note: If none of the leaves are big enough, use sections from multiple leaves to equal a 5 cm x 5 cm leaf area.

48. Measure the mass of this leaf section. Record the mass in Table 9.1.

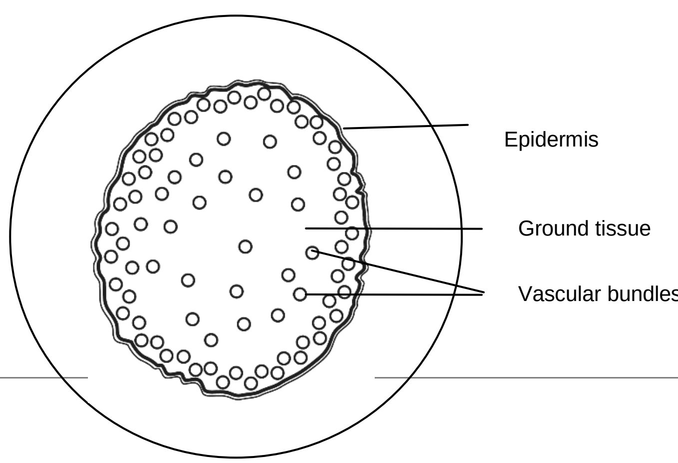
Part 2 – Stem Structure

Set Up

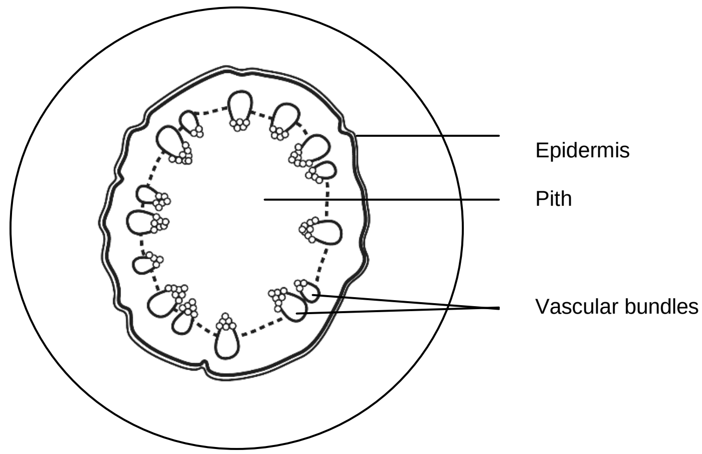
49. Obtain a microscope, a monocot stem prepared slide, and a dicot stem prepared slide.

Collect Data

50. Observe the monocot stem slide and make a drawing of your observations in the space below. Identify and label the vascular bundles, epidermis and ground tissue.



51. Observe the dicot stem slide and make a drawing of your observations in the space below. Identify and label the vascular bundles, epidermis, and pith.



Data Analysis

Table 9.1: Leaf surface area

Total mass of leaves (g)	1.60
Mass of 5 x 5 cm section (g)	1.50
Mass per cm^2 of leaf (g/ cm^2)	0.06
Leaf surface area (cm^2)	26.67

1. Use the space below to calculate the mass of a 1 cm^2 piece of the leaf

Mass per cm^2 of leaf = Mass of 5 x 5 cm section / 25 cm^2 1.5 g / 25 cm2 = 0.06 g/cm^2

2. Record the mass in Table 9.1.

3. Use the space below to calculate the total leaf surface area. Record in Tables 9.1 and 9.2.

Total leaf surface area = Total mass of the leaves (g) / Mass per cm^2 of leaf (g/cm^2

1.60 g / 0.06 g/cm^2 = 26.67 cm^2

Table 9.2: Individual transpiration rate

Run	Initial Pressure (hPa)	Final Pressure (hPa)	Change in Pressure (hPa)	Rate of Transpiration (hPa/s)	Surface Area of Leaves (cm^2)	Rate of Transpiration per Area [(hPa/s)/cm^2]
#1 Room	1018.4	1011.6	–6.8	–0.011	26.67	-0.0004
#2 Wind	1018.8	1010.8.	–8.0	–0.013	26.67	-0.0005
#3 Light	1018.3	1008.1	–10.2	–0.017	26.67	-0.0006
#4 Humidity	1018.7	1016.5	–2.2	–0.004	26.67	-0.0002

4. Find the initial and final pressure values (hPa) for all four runs. Record in Table 9.2.

5. Calculate the change in pressure and the rate of transpiration for all four runs.

Change in pressure = Initial pressure (hPa) – Final pressure (hPa)

Rate of Transpiration = [Initial pressure (hPa) – Final pressure (hPa)] ÷ time (s)

Change in pressure Run #1: 1018.4 – 1011.6 = -6.8 hPa
Change in pressure Run #2: 1018.8 – 1010.8 = -8.0 hPa
Change in pressure Run #3: 1018.3 – 1008.1 = -10.2 hPa
Change in pressure Run #4: 1018.7 – 1016.5 = -2.2 hPa

Rate of Transpiration Run #1: -6.8 / 600 s = -0.011 hPa/s
Rate of Transpiration Run #2: -8.0 / 600 s = -0.013 hPa/s
Rate of Transpiration Run #3: -10.2 / 600 s = -0.017 hPa/s
Rate of Transpiration Run #4: -2.2 / 600 s = -0.004 hPa/s

6. Record in Table 9.2.

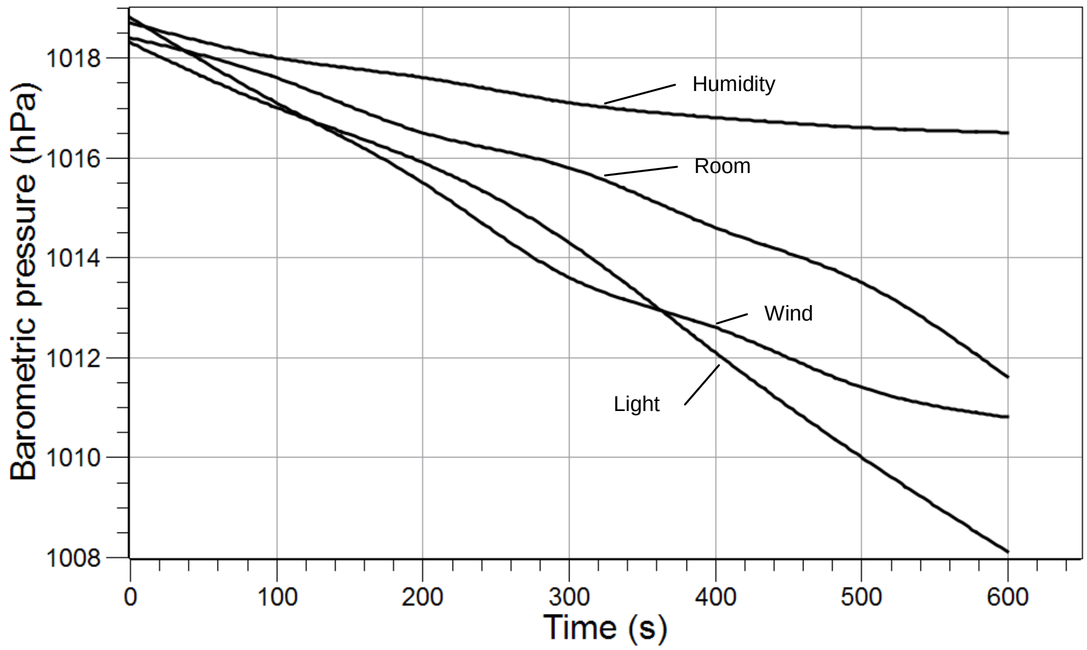
7. Calculate the rate of transpiration per area for all four runs.

Rate of transpiration per area = Rate of transpiration ÷ Surface areas of leaves

Rate of Transpiration per area Run #1: -0.011 / 26.67 =-0.0004 [(hPa/s)/cm^2]
Rate of Transpiration per area Run #2: -0.013 / 26.67 = -0.0005 [(hPa/s)/cm^2]
Rate of Transpiration per area Run #3: -0.017 / 26.67 = -0.0006 [(hPa/s)/cm^2]
Rate of Transpiration per area Run #4: -0.004 / 26.67 = -0.0002 [(hPa/s)/cm^2]

8. Record in Table 9.2.

9. Draw a graph of all four data runs showing the change in Barometric pressure versus Time. Use a key to differentiate your four runs. Label the overall graph, the x-axis, the y-axis, and include units on your axes.



Analysis Questions

1. How did the pressure change in the plastic tubing? Does a decrease in pressure in the tubing correspond to an increase or a decrease in water loss through the seedling's stomata? Explain.

The pressure inside of the tubing decreased in all setups. A decrease in pressure inside the tubing corresponds to an increase in water loss through the stomata. As water is lost through the stomata, more water is drawn out of the tubing and into the plant's xylem by transpiration, causing the volume of water in the tubing to decrease. The trapped air pocket will expand to fill the space vacated by the water, so the pressure of the air will decrease because volume is inversely proportional to pressure.

2. State whether wind, light, and humidity increase or decrease transpiration when compared to the control. Explain each of the conditions and their role in transpiration.

Wind – An increase in wind speed causes an increase in the rate of leaf water loss because it decreases the boundary layer of still air at the leaf surface. This boundary layer acts to slow leaf water loss. Increased wind also causes the rapid removal of evaporating water molecules form the leaf surface. This results in a low water potential in the air immediately surrounding the leaf, which leads to increased rates of water loss from the leaf.

Light – Absorption of light resulted in an increase in leaf temperature. Since the rate of water evaporation increases as temperature increases, the increase in leaf temperature results in an increased rate of leaf water loss.

Humidity – An increase in humidity results in a decrease in the rate of water loss from the leaf. When the water content of the air surrounding the leaf is increased, the concentration gradient between the inner leaf space and the environment is decreased. A decreased concentration gradient means that less water will diffuse from the inside of the leaf into the environment.

3. Why is it important to calculate the leaf surface area?

Because leaves are all different in size; reporting the water loss without considering a unit area would provide non-comparable data.

4. How does gas exchange affect transpiration?

The supply of CO_2 for photosynthesis drives the delicate balance between the opening and closing of stomata. If the stomata remain open too long, the abundant supply of CO_2 can be traded for a significant evaporative loss of water through transpiration. However, if the stomata remain closed to minimize transpiration, CO_2 supply can be greatly diminished by the Calvin cycle without being replaced.

The O_2 levels can also affect the rate of transpiration. The enzyme rubisco fixes CO_2 and brings it into the Calvin cycle. Since the shape of the O_2 molecule is similar to that of the CO_2 molecule, rubisco cannot differentiate between them and will fix O_2 as often as it fixes CO_2. When rubisco fixes O_2 instead of CO_2, the carbohydrate output of photosynthesis is cut in half, a process referred to as photorespiration. The plant balance closing stomata to reduce transpiration and opening the stomata to reduce photorespiration.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Water potential plays an important role in the movement of water from soil through the plant and into the air. Explain the role of water potential in this process. In your discussion include how the anatomy of vascular plants and the properties of water contribute.

At each step of transpiration, water moves from an area of high water potential to an area of lower water potential. Students may discuss various plant structures including stomata/guard cells, spongy mesophyll, xylem/tubes/tracheids/vessel element, and specific root structures (root hairs and casparian strips). They might mention some water properties including polarity/hydrogen bonding, cohesion, adhesion/capillarity, and high heat of vaporization (water vapor exiting leaf).

2. When water is in short supply the plant closes its stomata. What are the advantages and disadvantages for the plant?

Disadvantage— Closed stomata prevent carbon dioxide from entering, and oxygen limits the light-independent reactions of photosynthesis. A decrease in the carbon dioxide with the increase in oxygen in the air spaces of the leaf can result in the cells converting to photorespiration.

Advantage— Closed stomata prevents excessive water loss. This prevents the plant from wilting and/or dying. Lack of water in the air space of the leaf is a limiting factor in the light-dependent reactions of photosynthesis.

3. Name some of the structural and physiological adaptations that plants possess to reduce water loss from their leaves. Explain how they are beneficial to the plant.

There are a number of adaptations that reduce water loss. These include reduced number of stomata, an increase in the thickness of the leaf cuticle, a decrease in leaf surface area, and adaptations that decrease air movements around stomata such as dense hair and sunken stomata. Students may also mention the adaptations in plants that use C_4 photosynthesis and/or CAM (crassulacean acid metabolism).

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following adaptations do not prevent dehydration in land plants?

- **A.** Many guard cells in the flaccid condition
- **B. Many stomata on the top leaf surface**
- **C.** Water-resistant cuticle
- **D.** The presence of many epidermal hairs
- **E.** Recessed stomata

2. The movement of materials in the phloem of plants is driven by which of the following forces?

- **A.** Gravity
- **B. A difference in osmotic potential between the source and the sink**
- **C.** Root pressure
- **D.** Transpiration of water through the stomata
- **E.** Adhesion of water to vessel elements

3. Which of the following regulates the rate of flow of water through the xylem?

- **A.** Passive transport by the pith
- **B. The force of transpirational pull**
- **C.** The number of companion cells in the phloem
- **D.** Active transport by the sieve-tube members
- **E.** Active transport by tracheid and vessel cells

4. The closing of stomata in plant leaves during a sunny day results in which of the following situations?

- **A. A decrease in CO_2 intake**
- **B.** A shift from C_3 photosynthesis to C_4 photosynthesis
- **C.** An increase in transpiration
- **D.** An increase in the concentration of CO_2 in mesophyll cells
- **E.** An increase in the rate of production of starch

Extended Inquiry Suggestions

To investigate the stomata structure further, paint the lower surface of the leaf with clear fingernail polish, peel, and then mount the dried polish on a slide to view stomatal structure and distribution.

Investigate the transpiration rate of various plant species in your community. Have students bring in various plant species to determine transpiration rates under the various environmental conditions on. Then compare the data of the various plant species.

18. Reflex versus Reaction

Objectives

Students investigate somatic and autonomic reflexes in their own bodies and learn to distinguish reflexes from reactions. Students

- Distinguish between a reflex and a reaction
- Describe the differences between a stretch reflex and the pupillary reflex

Procedural Overview

Students gain experience conducting the following procedures:

- Measuring reaction rate using a meter ruler and stop watch
- Testing the patellar reflex using a reflex hammer
- Testing the pupillary reflex

Time Requirement

♦ Preparation time	5 minutes
♦ Pre-lab discussion and experiment	15 to 20 minutes
♦ Lab experiment	30 minutes

Materials and Equipment

For each student or group:

- Stop watch
- Meter ruler
- Reflex hammer

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Basic organization of the mammalian nervous system
- Basic structure and function of the mammalian neuron
- Differences between sensory and motor neurons
- Difference between voluntary and involuntary movements

Related Labs in This Guide

Labs conceptually related to this one include:

- Physiology of the Circulatory System
- Animal Behavior

Background

A reflex is a rapid, predictable, involuntary, self-protective movement that does not involve the brain. A reflex arc consists of all of the neurons involved in transmitting the impulses of the reflex. A typical reflex arc consists of a sensory receptor, a sensory neuron, an interneuron, a motor neuron, and an effector cell.

The sensory receptor, usually a dendrite housed within a sensory organ, reacts to the stimulus. The sensory neuron carries the message from the sensory receptor towards the spinal cord. The interneuron, located within the spinal cord, connects the sensory neurons to the motor neurons. The interneuron allows the impulse to synapse with a motor neuron without sending the message to the brain. The motor neuron carries the message from the spinal cord to the effector cell. Finally, the effector cell is a muscle, organ, or gland that will produce the effect of the reflex.

A reaction is a voluntary response to the reception of a stimulus. Unlike a reflex, a reaction requires integration of the sensory information by the brain. The stimulus gathered by the sensory receptor must travel up the sensory neuron to the brain. The message then travels down a motor neuron to its final destination, the effector cell. Because of this extra travel, a reaction requires more time than a reflex.

The simplest type of reflex is the stretch reflex. In the reflex arc of a stretch reflex, the sensory and motor neurons directly synapse with each other; interneurons are not involved. Stretch reflexes are important in controlling balance and complex movements like walking. One of the stretch reflexes commonly tested by physicians is the patellar reflex. In the patellar reflex, the sensory receptors are mostly in the muscle spindle in the quadriceps muscle in the thigh. When the physician taps the patellar ligament just below the knee, the patellar ligament is stretched, stretching the patellar tendon, which then stretches the quadriceps muscle. The stretch receptors in the muscle fibers of the quadriceps muscle trigger an impulse in the afferent nerve leading to the spinal cord. The nerve signal then passes directly to the motor nerve fiber that serves the quadriceps muscle, stimulating contraction of the muscle fibers there. The reflex results in a leg kick, which essentially is contraction of the quadriceps muscle.

The pupillary reflex controls the amount of light entering the eye. The pupillary reflex is an autonomic reflex. Its effector cells are smooth muscle, controlled involuntarily by the autonomic nervous system. The iris is a diaphragm that gives your eye its color and controls the amount of light entering the eye. In the middle of the iris is a small opening called the pupil, through which light enters the eye. Tiny muscles in the iris regulate the size of the pupil. In dim light, the iris' muscles relax, increasing the size of the pupil and the amount of light entering the eye. Conversely, in bright light, the muscles contract which decreases pupil size and the amount of light entering the eye. The pupillary reflex protects the eye from intense light.

Pre-Lab Discussion and Experiment

Engage your students in a discussion about reactions and reflexes. Ask the following series of questions.

1. What are some examples of reflexes? List them on the board for all students to see.

Some answers will include knee jerk reflex, blink reflex, gag reflex. Students may list actions that are not reflexes, like blocking your face from a blow. List these even though they are not truly reflexes. Suggest other reflexes like the Achilles, salivary, triceps, and biceps reflex.

2. What are the characteristics of a reflex?

Prompt students to define a reflex as a rapid, predictable, self-protective, involuntary response of the nervous system.

3. What is the function or purpose of a reflex?

Prompt students to determine that all reflexes provide a self-protective function for the organism. For example, the blink reflex protects the eye from particulate matter.

4. Do we have control over our reflexes?

Help students understand that there are two types of reflexes, somatic and autonomic. All reflexes are involuntary, but somatic reflexes occur on muscles that are normally controlled voluntarily. For example, the knee jerk reflex is involuntary, but kicking your leg can be a voluntary movement.

5. What is the difference between a reaction and a reflex? Go through the list that you created and determine which actions are reflexes and those that are reactions.

Students should understand that a reflex is an involuntary response to a stimulus and a reaction is a voluntary response to a stimulus.

Safety

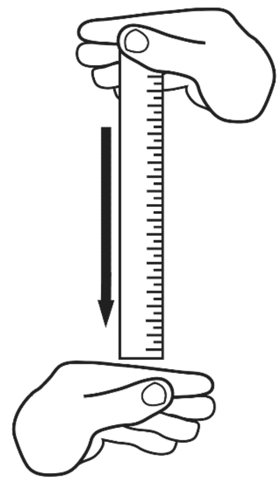
Add this important safety precaution to your normal laboratory procedures:

- ♦ Carefully use the reflex hammer while conducting the stretch reflex.

Procedure

Part 1 – Determining reaction time

Set Up



1. Designate one lab partner as #1 and the other as #2.

2. Partner #1 – Sit in a chair and place the forearm of your dominant hand flat on the surface of a desk or table in front of you. Your entire hand should extend over the edge of the desk. Spread the thumb and index finger of your hand 6 cm apart.

3. Partner #2 – Hold a meter ruler above your partner's hand so that when dropped, it will fall directly between their index finger and thumb. Hold it with the 0 cm end level with the top of his or her fingers.

Collect Data

4. Partner #2 – Drop the meter ruler between your partner's thumb and finger.

5. Partner #1 – Catch the meter ruler between your thumb and finger.

6. Observe the distance the meter ruler dropped and record in Table 1.

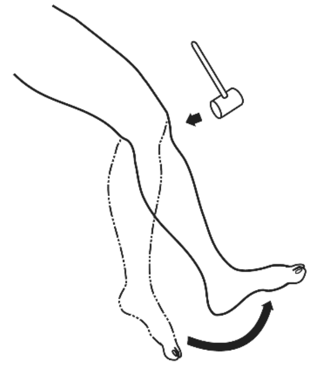
7. Repeat the procedure 9 more times and record in Table 1.

8. Why is it necessary to repeat the experiment 10 times?

Repeating the experiment will prove the reliability of the experiment and its results.

Part 2 – Observing the stretch reflex

Set Up



9. Partner #1 – Sit on the edge of your desk. You must be high enough so your feet are not touching the floor. Relax your leg.

10. Partner #2 - Locate your partner's kneecap and feel the large tendon below the midline of the kneecap. This is the patellar ligament.

Collect Data

11. Partner #2 – Using the flat end of the reflex hammer, gently tap the patella ligament. If you are having trouble getting a response from your lab partner, distract your partner by having them countdown from 10. While they are counting, tap the patella ligament.

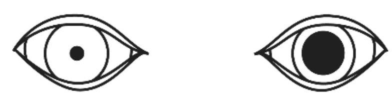
12. What is the response?

Most students will say that their lab partner's leg kicked when they tapped the patellar tendon with the reflex hammer. Some students will not have a patellar reflex.

Part 3 – Observing the pupillary reflex

Set Up

13. Partner #1 – Close your eyes and cover one eye with your hand. Keep both eyes closed for 1 minute.



Collect Data

14. Partner #2 - After 1 minute, tell your partner to open his or her eyes and remove their hand.

15. Describe the changes you observed in your partner's pupils.

Most people's pupils will dramatically shrink in size.

Data Analysis

Part 1 – Determining reaction time

Table 1: Reaction lengths and reaction time

Trial	Reaction Length (cm)
1	5
2	4
3	4
4	6
5	7
6	4
7	4
8	5
9	5
10	5
Average reaction length (cm)	4.9
Reaction time	0.1 s

16. Use the equation below to determine partner #1's reaction time (t). Show your work in the space below.

$$t = \sqrt{\frac{2d}{g}}$$ where:

g = acceleration due to gravity, 9.8 m/s^2

d = reaction length (m)

t = reaction rate (s)

$$t = \sqrt{\frac{2d}{g}}$$

$$t = \sqrt{\frac{2 \times 0.049\ \text{m}}{9.8\ \text{m} / \text{s}^2}}$$

$$t = \sqrt{0.01\ \text{s}^2}$$

$$t = 0.1\ \text{s}$$

Analysis Questions

1. Create a diagram of the four parts of a stretch reflex.

Sensory receptor → Sensor neurons detect and send signal→ Motor neurons send signal for response → Effector cell.

2. Swallowing several times in quick succession is not easy. Why do you think this is? Remember to think about stimulus.

Swallowing is a complex reflex of many reflex arcs and interneurons. This reflex is triggered by saliva, food or drink moving into the posterior oral cavity. Thus, trying to swallow without these stimuli is difficult.

3. Why was it necessary for partner #1 to always be the "patient" and partner #2 to always take the measurements?

The patient was a constant in this experiment. If both partners served as patient, then we would not be able to compare the reflexes in one section to the reactions in another section.

Synthesis Questions

Use available resources to help you answer the following questions.

1. As you learned in this lab, organisms are not always consciously aware of activities occurring in their nervous system. Why might this be advantageous?

Consciously controlled activities involve the brain and therefore take longer to occur. Reflexes occur so quickly because they do not involve the brain; they are simply self-protective movements.

2. How would your reaction time change in the experiment if it was a true reflex? Explain your reasons.

The reaction time would decrease if it was a true reflex since it would be an involuntary movement and not have to be controlled by the individual.

3. What would happen to a reflex if the motor neuron in its reflex arc was severed?

The sensory receptor would detect the stimulus, and the sensory neuron would send the message towards the spinal cord, but the effector cells would not be stimulated. Therefore, the action or movement normally associated with the reflex would not occur.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following statements about the reflex arc is true?

- **A.** It does not involve the brain and spinal cord
- **B.** It always consists of only a sensory and motor neuron.
- **C. It always consists of a sensory neuron, motor neuron, and interneuron.**
- **D.** It always consists of a sensory neuron, motor neuron, the brain, and the spinal cord.

2. Which type of neuron is not directly involved in the stretch reflex?

- **A.** Sensory neuron
- **B.** Motor neuron
- **C.** Multipolar neuron
- **D. Interneuron**

3. A voluntary response to a stimulus is a(n)

- **A.** Reflex
- **B. Reaction**
- **C.** Effector
- **D.** Receptor

Extended Inquiry Suggestions

For the average reaction time, calculate the average time for males and females in the class. Then repeat the meter ruler experiment but have the students use their non-dominant hand. After these experiments, determine if there is a difference in the results. Discuss whether there is a plausible, logical reason for any differences.

Have students design an experiment to test for reaction time of an individual using a motion detector.

Have students dip their hands into ice water and then run the experiment to test the effect of temperature on reflex and reaction.

19. Endotherms and Ectotherms: Temperature Regulation in Animals

Objectives

Students explore the different ways that endotherms and ectotherms respond to temperature change by:

- Examining the relationship between the external temperature and an organism's respiration rate
- Observing an organism's behavior as it responds to a temperature change

Procedural Overview

Students gain experience conducting the following procedures:

- Measuring the respiration rate of two different organisms
- Correlating their observations of an organism's behavior to the organism's respiration rate

Time Requirement

♦ Preparation time	5 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment	60 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Carbon dioxide gas sensor
- Stainless steel temperature probe or fast-response temperature probe
- Quad temperature sensor or temperature sensor[1]
- Electronic balance (1 per class)
- Sensor extension cable
- Beaker, 2-L or similarly sized container
- Sampling bottle (included with the carbon dioxide gas sensor)
- Ring stand
- Ring clamp
- Mouse, 5 to 10-g
- Crickets, approximately 10,
- Ice

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Basic concepts of aerobic cellular respiration
- The difference between endothermic and ectothermic animals

Related Labs in This Guide

Labs conceptually related to this one include:

- Measuring Aerobic Cellular Respiration in Yeast
- Fermentation in Yeast
- Exploring the Effects of pH on Amylase Activity
- Cell Respiration

Background

Animals can be classified into two general types of temperature regulators: ectotherms and endotherms. Ectothermic animals, such as reptiles, amphibians, insects, and fish, do not spend a significant amount of their energy budget maintaining a constant internal body temperature. Endothermic animals, such as birds and mammals, maintain a fairly constant internal body temperature regardless of the external temperature.

The internal body temperature of ectotherms is largely determined by their surroundings. They rely on external sources of heat for warmth so their metabolic rate is controlled by the external temperature. Conversely, an endotherm can maintain its internal body temperature in a narrow range by burning more or less fuel (food calories) depending on the external temperature.

Internally, as an organism consumes food, it produces heat energy and carbon dioxide as by-products. The rate at which this occurs is known as the metabolic rate. The metabolic rate can be measured by the amount of oxygen consumed or the amount of carbon dioxide produced during respiration in a given time period.

Many factors affect metabolic rate including external temperature, body size, activity level, age, diet, and time of day. In the winter, humans living in the northern latitudes tend to eat more calories than they do in the summer so they can stay warm at the lower temperatures. On summer days when it is hot, people tend to be less active. Likewise, a marathon runner or tri-athlete must consume large amounts of food calories to sustain their bodies during these long races.

Pre-Lab Discussion and Experiment

Engage your students in a discussion about how animals respond to external temperature changes. Ask the following series of questions.

1. How do you react and what changes do you make when the temperature becomes colder than normal?

Some responses might include: shivering, getting goose bumps, increasing physical activity to warm up, huddling with other people, putting on more clothing, or covering up with a blanket.

2. How do you react and what changes do you make when the temperature becomes warmer than normal?

Some responses might include: sweating, reducing physical activity, retreating to a shady area, taking a cold shower or a swim, or reducing clothing or covering.

3. Ask students if they think other animals respond the way humans do to temperature changes? What kind of changes do other endothermic animals make in response to temperature changes?

Students should understand that most endothermic animals will react in a similar way as humans. They will change their physical activity, retreat to shade, and may even sweat. However, some animals have physiological reactions or behaviors that humans do not. For instance, a dog will pant to cool down since it is covered in hair. Sweating through the skin is not an efficient way to lose heat if you are covered in hair. Animals like bears have a response to cooler temperatures that is much more drastic than putting on a sweater. These animals actually hibernate to make it through the time of reduced food supply.

4. Ask students whether the reactions that they listed in questions 1 and 2 are physiological responses or behaviors.

Examples of physiological responses: sweating, panting, shivering, goose bumps.

Examples of behaviors: moving into shade, putting on a sweater, taking a swim, huddling with other organisms.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Purchase the crickets and mice at a local pet store, or order them from a biological supply company. Note the size of the mouth of the sampling bottle before you buy the mice. The mice must be small enough to fit into the bottle. You need very small mice.
2. What should you do with the crickets before and after the lab? Keep them in a small bucket or classroom aquarium with a small amount of mulch or other plant material in the bottom. The crickets can be kept alive for quite some time with dry pet food and an occasional spritz of water. Search the internet for labs that use crickets.
3. What should you do with the mice before and after the lab? Mice make great pets, especially for younger children. Keep the mice in a small aquarium or cage with a secure mesh lid. Use paper shreds for the bedding, and change them often, as they can get very smelly. You can buy rodent food at a pet store and supplement with carrots or apples. Most mice will not drink from a water bottle, so you must give them moist food. Search the internet for experiments that include mice.
4. If you do not want to keep animals in the classroom, donate them to a classroom that will take care of them. Most pet stores will also gladly take the animals back.
5. Obtain ice for the cold water bath and place it in an accessible location.

Instructor Tip: If you do not teach on a block schedule, you may not be able to finish the entire lab in one class period. If this is the case, complete the cricket activity during one period and the mouse activity during the next period.

Safety

Add these important safety precautions to your normal laboratory procedures:

- Use caution and compassion when handling the animals.
- Follow the time limits carefully when the animals are in the sealed containers. If the animals appear to be suffering remove them quickly from the containers.

Procedure

Set Up

1. Prepare a cold water bath in the 2-L beaker by filling it ¾ full with water that is 8 to 12 °C. Test the water bath by placing the beaker on the platform of the ring stand and the closed sample bottle into the water. The neck of the sample bottle should be submerged without the water overflowing.

2. Start a new experiment on the data collection system.

3. Connect a carbon dioxide gas sensor and a temperature sensor to the data collection system. Use a sensor extension cable to connect the CO_2 sensor.

4. Calibrate the carbon dioxide gas sensor.

5. Display two graphs simultaneously. On one graph, display CO_2 concentration on the y-axis with Time on the x-axis. On the second graph, display Temperature on the y-axis with Time on the x-axis.

Part 1 – Crickets

Collect Data

6. Measure the mass of the sampling bottle.

7. Record the mass in Table 1.

8. Place the carbon dioxide sensor and the fast response temperature sensor into the empty sampling bottle.

9. Start data recording (run #1) on the empty bottle.

10. Adjust the scale of the graphs to show all data.

11. Record the CO_2 concentration and temperature in the empty bottle for 5 minutes (300 seconds).

12. What is the purpose of measuring the CO_2 and temperature in the empty bottle?

The empty bottle at room temperature serves as our control group. It allows us to view and record the environment without manipulating the independent variable, temperature.

13. While you are waiting, obtain approximately ten crickets and observe the cricket's activity for several minutes.

14. Record your observations of the crickets' activity level in Table 2.

15. After 5 minutes of data collection, remove the CO_2 and temperature sensors from the empty bottle and add approximately 10 crickets to the bottle. **Do not stop recording data**.

16. Quickly measure the mass of the bottle and the crickets.

17. Record the mass in Table 1.

18. Securely place the carbon dioxide and temperature sensors into the bottle with the crickets. Continue recording data while you move the sensors.

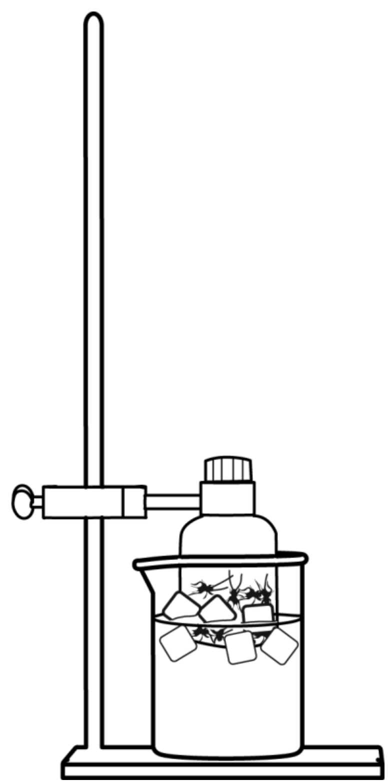
19. Use the clamp to securely attach the bottle with the crickets to the ring stand.

20. Lower the bottle into the water bath so the water surrounds the bottle up to the neck.

21. Leave this bottle in the cold water bath to allow the temperature inside the bottle to decrease. This will take about 10 minutes.

22. Do you predict that the CO_2 levels in the bottle will increase or decrease when the temperature of the bottle decreases?

Students should predict that CO_2 levels will be higher than in the empty bottle because the crickets are respiring and creating CO_2. However, CO_2 levels may stop rising or the rate of increase may slow as temperature decreases, and the rate of increase will be greater as temperature increases.



23. Record temperature and CO_2 data for another 5 minutes after the point that temperature stabilizes.

24. While waiting, observe the crickets' activity.

25. Record your observations of the crickets' activity level in Table 2.

26. After recording data in the cold bottle for 5 minutes, carefully lift the bottle completely out of the cold water. Keep the sensors in the bottle and continue to record data. Make sure the bottle is still securely fastened to the ring stand so it does not fall over.

27. Continue to record data in the bottle at room temperature for another 5 minutes.

28. While waiting, observe the cricket's activity.

29. Record your observations of the crickets' activity level in Table 2.

30. After recording data for 5 minutes, stop data recording. Name the data run "cricket".

31. Remove the sensors from the bottle.

32. Observe the activity level of the crickets for the last time.

33. Record their activity level in Table 2.

34. Return the crickets to their container and make sure that the bottle is clean.

35. Shake the bottle to remove any excess carbon dioxide.

Part 2 – Mouse

Collect Data

36. Measure the mass of the sampling bottle again.

37. Record the mass in Table 1.

38. Place the carbon dioxide gas sensor and the fast response temperature sensor into the empty sampling bottle.

39. Start data recording (run #2) on the empty bottle.

40. Adjust the scale of the graphs to show all data.

41. Record the CO_2 concentration and temperature inside the empty bottle for 5 minutes (300 seconds).

42. After 5 minutes of data recording, remove the carbon dioxide and temperature sensors from the bottle. **Do not stop recording data**.

43. Add one small mouse to the bottle.

44. Measure the mass of the bottle and mouse.

45. Record the mass in Table 1.

46. Observe the mouse's activity for several minutes.

47. Record your observations of the mouse's activity level in Table 2.

48. What do you think is the purpose of measuring the mass of the organism before and after the experiment? What do you think an increase or decrease in mass will tell you about the way the organism responds to temperature changes?

By measuring the mass of the crickets and calculating an average mass, we can determine the average rate of CO_2 production per gram. If we do the same with the mouse, we can compare the rate of respiration per gram of body mass in a cricket versus a mouse.

49. Do you think that the mouse will respond to decreased temperature in the same manner as the crickets? Why or why not?

Students should predict that the mouse will expend more energy maintaining its body temperature since it is an endotherm. The mouse will use more energy, respire more, and produce more CO_2.

50. Securely place the carbon dioxide and temperature sensors into the bottle with the mouse.

51. Use the clamp to securely attach the bottle with the mouse to the ring stand.

52. Lower the bottle into the water bath so the water surrounds the bottle up to the neck.

Note: Add more ice to the water bath if necessary.

53. Leave this bottle in the cold water bath to allow the temperature inside the bottle to decrease. This will take about 10 minutes.

54. Record temperature and CO_2 data in the bottle with the mouse in cold water for another five minutes.

55. While waiting, observe the mouse's activity.

56. Record your observations of the mouse's activity level in Table 2.

57. List the independent and dependent variable in this part of the experiment.

Independent: temperature

Dependent: CO_2 level

58. After recording data in the cold water for 5 minutes, carefully lift the bottle completely out of the cold water. Keep the sensors in the bottle and continue to record data. Make sure the bottle is still securely fastened to the ring stand so it does not fall over.

59. Continue to record data at room temperature for another 5 minutes.

60. While waiting, observe the mouse's activity.

61. Record your observations of the mouse's activity level in Table 2.

62. After 5 minutes, stop data recording. Name the data run "mouse."

63. Remove the sensors from the bottle.

64. Observe the activity level of the mouse for the last time.

65. Record the activity level in Table 2.

66. Return the mouse to its container.

Analyze Data

67. Apply a linear fit to determine the slope of the CO_2 runs from 1 to 5 minutes. Disregard the area of large fluctuations when the sensors were placed into the bottle.

68. Record the slope as the rate in Table 3.

69. Apply a linear fit to determine the slope of the CO_2 runs from 5 to 10 minutes. \ Disregard the areas of large fluctuations when the sensors were placed into the bottle and when the bottle was removed from the cold water bath.

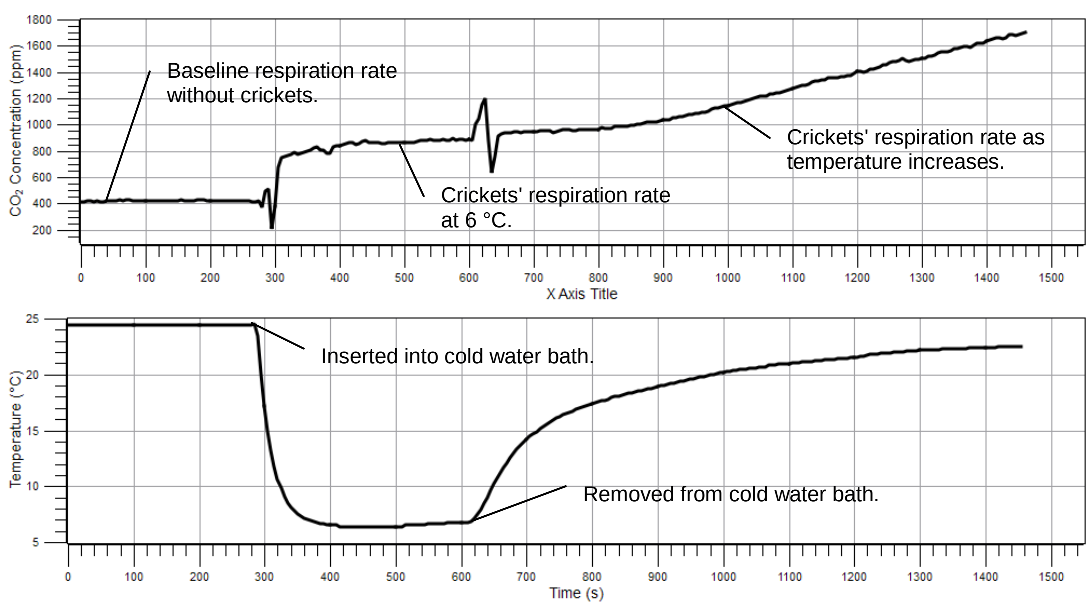
70. Record the slope as the rate in Table 3.

71. Apply a linear fit to determine the slope of the CO_2 runs from 10 to 20 minutes. Disregard the area of large fluctuations when the bottle was removed from the cold water bath.

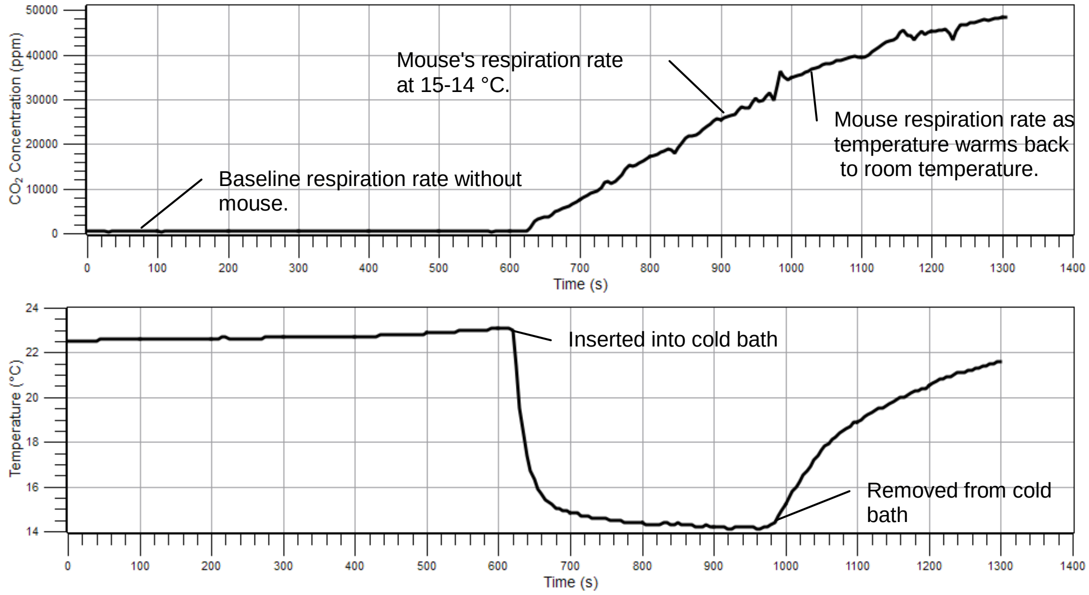
72. Record the slope as the rate in Table 3.

73. Save your experiment and clean up according to your instructor's instruction.

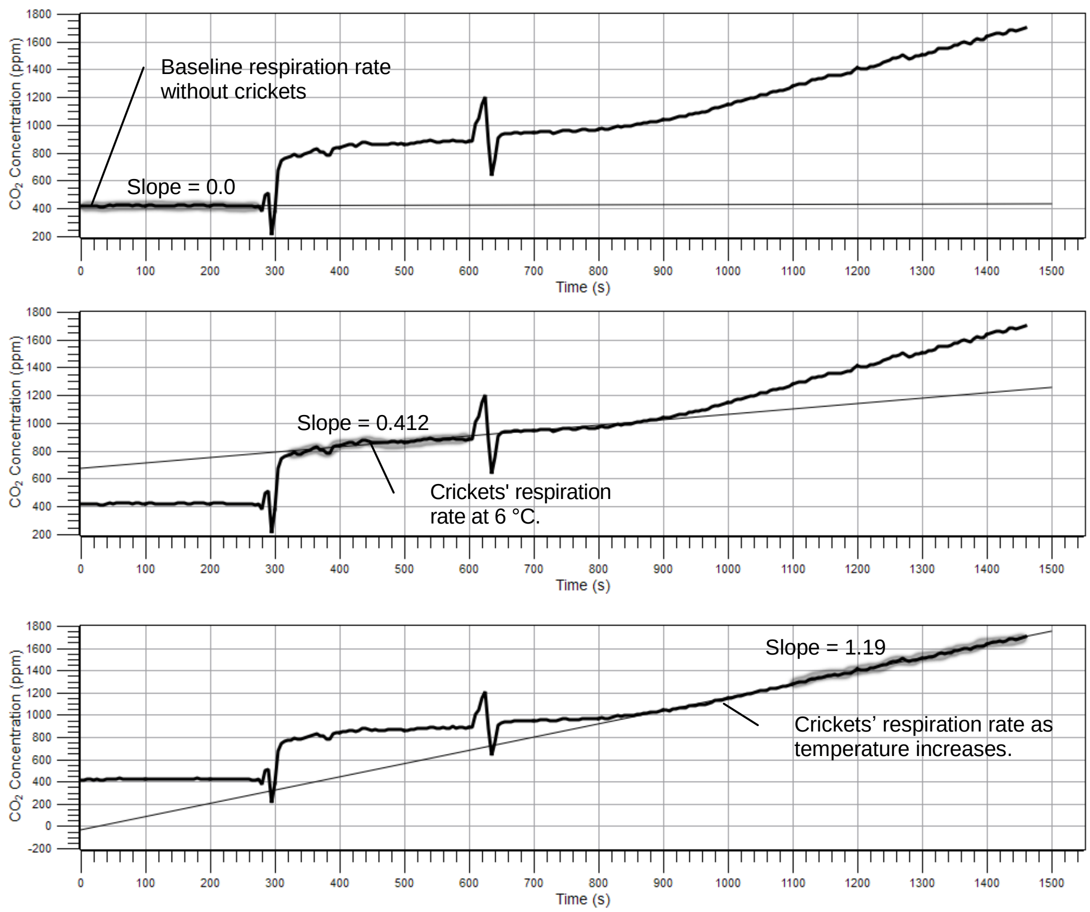
Sample Data



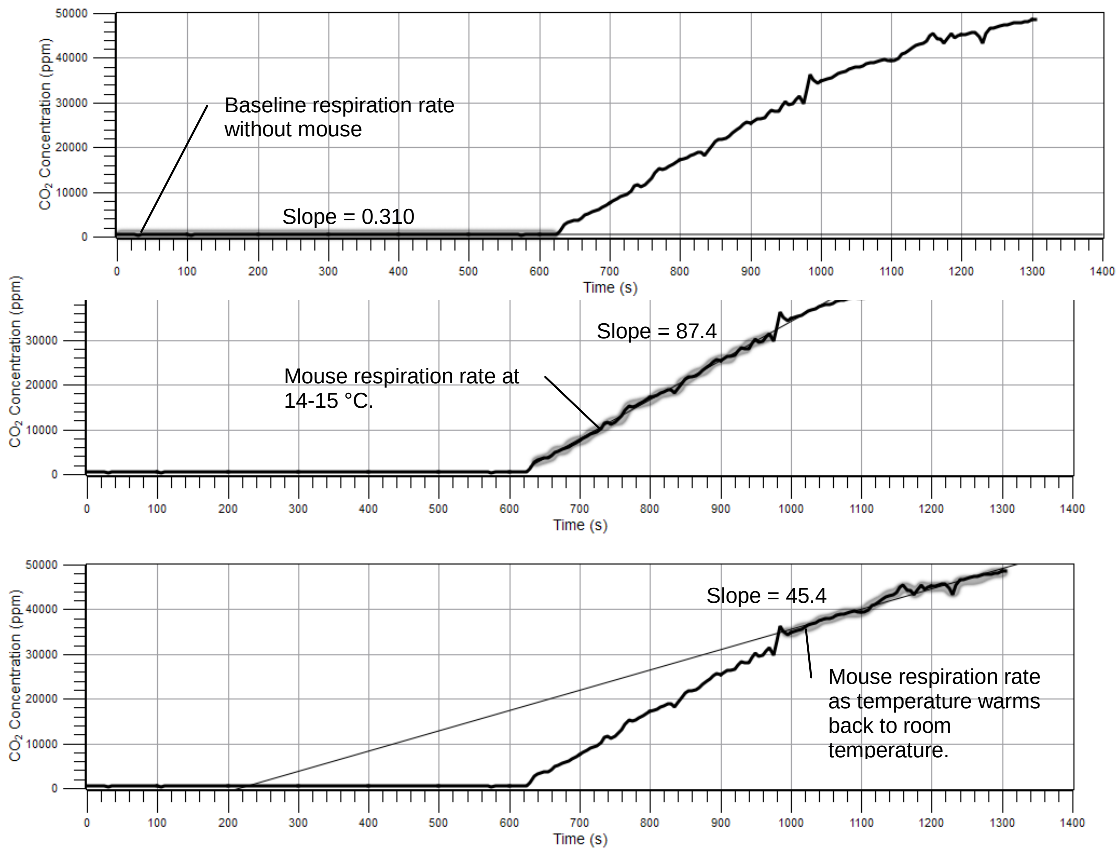
Crickets: Temperature and CO_2 levels as container is moved from room temperature into ice water, and then back to room temperature.



Mouse: Temperature and CO_2 levels as container is moved from room temperature into ice water, and then into a warm water bath.



Crickets: Determination of respiration rates at different temperatures from the slope of the best-fit line



Mouse: Determination of respiration rates at different temperatures from the slope of the best-fit line

Data Analysis

Table 1: Mass

Animal	Number of Animals	Mass of bottle	Mass of bottle and animals	Mass of animals	Average mass of animals
Cricket	9	55.6 g	58.9 g	3.3 g	0.37 g
Mouse	1	56.1 g	63.6 g	7.5 g	7.5 g

Table 2: Activity level

Animal	Activity at start	Activity after 10 min. in cold water bath	Activity after 15 min. in cold water bath	Activity at the end of data collecting
Cricket	Moving around	Slight movement	No movement	Starting to move again
Mouse	Moving around	Moving around, washing face	Seems to be shaking	Moving around

Table 3: Respiration rate

Animal	Rate at 1 to 5 minutes	Rate at 5 to 10 minutes	Rate at 10 to 20 minutes
Cricket	0	0.41 ppm/s	1.2 ppm/s
Mouse	0	87 ppm/s	45 ppm/s

1. Use the average body mass of the crickets and mouse from Table 1 to determine the production of CO_2 in $\text{ppm}\cdot\text{s}^{-1}\text{g}^{-1}$ of body mass.

Example: If the rate (slope of the graph of CO_2) is 80 ppm/s and the average body mass is 7.5 grams, then the rate/gram of body mass would be:

$80/7.5 = 10.7\ \text{ppm}\cdot\text{s}^{-1}\ \text{g}^{-1}$

2. Record your results in Table 4.

Table 4: Rate of respiration per mass

Animal	Rate at 1 to 5 minutes per gram	Rate at 5 to 10 minutes per gram	Rate at 10 to 20 minutes per gram
Cricket	0	$0.12\ \text{ppm}\cdot\text{s}^{-1}\text{g}^{-1}$	$0.36\ \text{ppm}\cdot\text{s}^{-1}\text{g}^{-1}$
Mouse	0	$12\ \text{ppm}\cdot\text{s}^{-1}\text{g}^{-1}$	$6.0\ \text{ppm}\cdot\text{s}^{-1}\text{g}^{-1}$

Analysis Questions

1. What differences did you observe between the activities of the crickets and the activity of the mouse during each time interval in Table 2?

The crickets' activity slowed down as the temperature dropped but the mouse's activity level was fairly constant, and it seemed to be shivering in the cold environment.

2. How did your observations of activity compare to the rates of respiration you observed in Table 3?

As the temperature dropped for the cricket, its level of activity and respiration rate both dropped. However, the mouse showed an increased respiration and increased activity level, including shivering, as temperature decreased.

3. As the temperature in the sampling bottle increased during the last 5 minutes, how did the respiration rate of the cricket change? How did the respiration rate of the mouse change?

As temperature increased, the respiration rate of the cricket increased and the respiration rate of the mouse decreased.

4. Why is the respiration rate per gram higher for the mouse than for the crickets?

Mice are endothermic animals. When temperature decreases, they spend a significant amount of energy trying to maintain their internal body temperature.

5. Based on your results which animal do you think is the ectotherm and which is the endotherm? Provide evidence from your experiment.

Crickets are ectotherms and the mouse is an endotherm. Students should cite the respiration rate per gram as the most decisive evidence. The mouse has a higher respiration rate per gram, which means it expends more energy per gram than the cricket to maintain its body temperature.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Ectothermic animals cannot maintain a stable internal body temperature. What behavioral adaptations do these animals have that help them maintain their body temperature when external temperatures are extremely high or low?

They can move to the shade or sun, burrow into the ground, or cover themselves with mud. They may also have colorations that help them to stay warm or cool.

2. Endothermic animals can maintain a fairly stable internal body temperature, but pay a high energy price to do so. What adaptations do mammals have that help them conserve fuel in low temperatures?

Mammals have fur or hair to help insulate them from the cold. Also, in the winter they will retain more fat to provide insulation and food. They perspire or pant, which is a cooling mechanism.

3. Speculate which type of organism, ectotherm or endotherm, would be better suited to a change in climate.

Based on an article in the June 6th 2008 issue of Science (Vol. 320, page 1296), ectotherms have a narrow thermal tolerance and therefore would be more vulnerable to climate changes.

4. Many physiological processes are controlled by enzymes whose activities are temperature sensitive. How would the enzymes in endotherms and ectotherms differ?

Since endotherms can maintain an internal body temperature with in a narrow range their enzymes function within that range. Conversely, for ectotherms to survive, some of their enzymes must be able to function in a wider range of temperatures. They do slow down their metabolic rate in colder temperatures but certain enzymes must still be functioning to keep the organism alive.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. For an ectotherm, when the external or surrounding temperature drops, what happens to their level of activity and their internal temperature?

- **A.** Activity increases and internal temperature increases
- **B. Activity decreases and internal temperature decreases**
- **C.** Activity increases and internal temperature remains constant
- **D.** Activity stays constant and internal temperature remains constant
- **E.** Activity stays constant and internal temperature increases

2. Which of the following behavioral adaptations would help an ectotherm survive a low temperature?

- **A.** Eat more food
- **B.** Increase its level of activity
- **C. Move from the shade to the sun**
- **D.** Go for a swim
- **E.** Increase the number of offspring/litter

3. Which of the following behavioral adaptations would help an endotherm survive a high temperature?

- **A.** Eat more food
- **B.** Increase its level of activity
- **C.** Move from the shade to the sun
- **D. Go for a swim**
- **E.** Increase the number of offspring/litter

Extended Inquiry Suggestions

Repeat the experiment with other animals, such as lizards or salamanders. Compare the respiration rates per gram of body mass.

Ask the students to design and carry out an experiment to measure the effect of higher temperatures (30 to 35 °C) on the ectotherms and endotherms.

20. Physiology of the Circulatory System

Objectives

Students will explore the various cardiovascular responses of endothermic and ectothermic animals to changes in environmental conditions. Students:

- Explore the effects of body position and exercise on pulse rate and blood pressure
- Determine a human's relative cardiac fitness
- Observe the effects of temperature on the pulse rate of an ectothermic organism

Procedural Overview

Students gain experience conducting the following procedures:

- Using a blood pressure sensor to measure changes in a subject's blood pressure and pulse rate in response to various body positions.
- Measuring and analyzing blood pressure and pulse rate an index of relative cardiac fitness.
- Measuring the changes in pulse rate of a *Daphnia magna* (an ectothermic animal) under different temperature conditions.

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment: Part 1	15 minutes
♦ Lab experiment: Part 2	45 minutes
♦ Lab experiment: Part 3	30 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Blood pressure sensor
- Kena™ (USB or dissecting microscope)
- Temperature sensor
- Fast response temperature probe
- Disposable pipet
- *Daphnia magna*, living, large-size (1 to 2)
- Depression slide (2)
- Petri dish
- Small rubber band (2)
- Small container of crushed ice or an ice pack
- Container of warm tap water
- Container of room-temperature tap water

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- The circulatory system and its general functions
- The structure of the mammalian heart and the pathway of blood flow through the heart
- The differences between ectothermic and endothermic animals

Related Labs in this Guide

- Endotherms and Ectotherms: Temperature Regulation in Animals
- Cell Respiration

Background

The circulatory system circulates an aqueous fluid around the human body. This fluid contains vital substances including oxygen, sugar, fats, proteins, vitamins, minerals, and heat.

The circulatory system consists of blood, blood vessels, and the heart. The regular contractions of the heart push blood through the blood vessels delivering nutrients to the entire body. The rhythmic contractions of the heart are controlled by the action of the pacemaker within the heart as well as the autonomic nervous system. As blood passes through the vessels, it exerts pressure, causing their walls to expand slightly. As blood moves away from the heart through the arteries to smaller blood vessels, the pressure decreases.

Blood pressure is usually measured in the brachial artery in the upper arm. Blood pressure has two main components: systolic and diastolic pressure. Systolic pressure is the pressure in the brachial artery when the ventricles (the two lower chambers) of the heart contract. Diastolic pressure is the pressure in the artery when the ventricles are relaxed. Blood pressure is reported using these two measurements (expressed as a fraction of systolic to diastolic pressure in mm Hg).

For every contraction of the heart, the arteries expand and relax. This can be observed where they pass near the surface of the skin. This is the pulse. Measuring a person's pulse allows you to measure their pulse rate – the number of times the heart contracts per minute.

The circulatory systems of humans and other endotherms are relatively unaffected by changes in environmental temperatures. The pulse rate of ectotherms, however, is significantly affected by external temperature. In this lab, you will be exploring the effects of temperature on the pulse rate of the water flea, *Daphnia magna*. Most biological reactions occur at faster rates as temperature increases. This effect is greatest in ectotherms between 5 °C and 35 °C. The increased metabolic rate observed in ectotherms within this temperature range is expressed as a value called "Q_{10}." Q_{10} is the ratio of the metabolic rate of an organism at one temperature compared to the rate at a temperature 10 °C lower. A Q_{10} of 3 indicates that the metabolic rate of the organisms triples with a 10 °C increase in temperature.

Pre-Lab Discussion and Experiment

Engage your students in a discussion about the human circulatory system, blood pressure, pulse rate, and pulse. Ask the following series of questions.

1. What is the function of the circulatory system? How does it accomplish this?

The circulatory system circulates an aqueous fluid around the human body. This fluid contains vital substances such as oxygen, sugar, fats, proteins, vitamins, minerals, and heat. The heart is the muscular pump that pushes the blood through the blood vessels.

2. What are the major components of the circulatory system?

The heart, blood, and blood vessels

3. What is blood pressure?

Blood pressure is the force that blood exerts on the walls of blood vessels. This pressure is caused by the contraction of the heart and by muscles that surround the blood vessels.

Blood pressure is expressed as systolic pressure over diastolic pressure. Systolic pressure is the pressure of the blood when it leaves the ventricles at peak ventricular contraction. It is normally 120mm Hg for males and 110 mm Hg for females. Diastolic pressure is the pressure of the blood on the artery walls when the ventricles relax. Normal diastolic pressure is 80 mm Hg for males and 70 mm Hg for females.

4. What is pulse rate? How is it measured?

Pulse rate is a measurement of the number of times the heart beats per minute. Pulse can be measured manually at the wrist, neck, temple, groin, behind the knees, or on top of the foot. In these areas, the arteries pass close to the skin.

5. How are blood pressure and pulse rate monitored and controlled in the human body?

Blood pressure and pulse rate are maintained two ways: (1) the nervous system, which can speed up or slow down the pulse rate; (2) the kidneys, which regulate blood pressure by the amount of fluid in our blood.

Blood pressure depends on peripheral resistance – the amount of friction encountered by blood as it flows through the vessels. Peripheral resistance is influenced by narrowing of the blood vessels and changes in blood volume. Blood vessels can be narrowed by sympathetic stimulation.

6. What other factors could affect the blood pressure and pulse rate of an individual?

Other factors include stress, sickness, and chemical substances like caffeine, nicotine, et cetera. Many other factors can affect blood pressure, including the volume of water in the body; salt content of the body; condition of the kidneys, nervous system, or blood vessels; and levels of various hormones in the body.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. If an aerobics step is not available for the cardiac fitness test, use a short stool, bleachers or the bottom steps of the building stairs.
2. To save time, have only one partner complete the fitness tests.
3. *Daphnia* cultures can be purchased from a biological supply company.
4. Create three water baths before the beginning of the lab. The first bath should include cold water and ice water (0 to 5 °C), the second should be room temperature (15 to 20 °C) and the third should be warm (but not hot) water (25 to 30 °C). Make the water baths accessible to students.

Safety

Add this important safety precaution to your normal laboratory procedures:

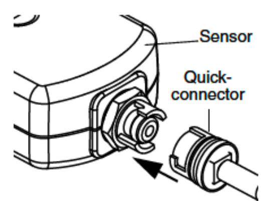
- Students with health concerns affected by strenuous activity should not do the fitness tests.
- Students should not pump the pressure in the blood pressure cuff above 200 mmHg. If there is serious discomfort, deflate the cuff and remove it.
- One student may act a “spotter” during the step portion of the fitness test, in case the step tester takes a stumble.

Procedure

Part 1 – Measuring blood pressure

Set Up

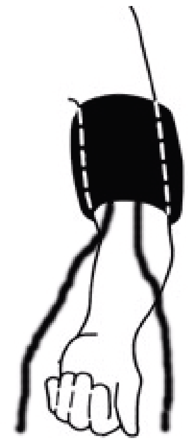
1. Designate one lab partner as #1 and the other as #2.
2. Partner #1 should sit in a comfortable chair with feet flat on floor, legs not crossed. Remove any constrictive clothing (roll up sleeves) or jewelry that may interfere with the cuff placement.
3. Partner #2 should connect the blood pressure sensor to the blood pressure cuff by aligning the quick connector at the end of the tube from the cuff with the quick connector port on the sensor. Push the quick connector onto the port and turn the connector clockwise until the connector clicks into place on the port.



4. Start a new experiment on the data collection system.

5. Connect the blood pressure sensor to the data collection system.

6. Partner #2 should help partner #1 wrap the blood pressure cuff around partner #1's upper left arm.



7. The two tubes should hang down (one on each side of the arm) and you should be able to read the word "ARTERY" right above the elbow pit.

8. Partner #1 should rest his or her left elbow and forearm on a solid surface with palm facing upward. The cuff should be slightly lower than the heart.

9. Partner # 2 should press and hold the push-button release valve to make sure that all of the air in the cuff has been released.

Collect Data

10. Display pulse rate, pressure, diastolic pressure and systolic pressure in digits displays.

11. Partner #2 should start data recordingand begin squeezing the bulb to pump air into the cuff. Partner #1 should stay as still as possible during the blood pressure measurement.

12. Partner #2 should monitor the pressure in the digits display. When the pressure reaches approximately 150 mmHg, stop pumping and let go of the bulb. The pressure in the cuff will decrease automatically and the cuff will slowly deflate by itself. You may hear a slight "hissing" noise as air slowly leaks from the cuff. This may take about one minute.

CAUTION: Do not pump above 200 mmHg. If there is serious discomfort, deflate the cuff and remove it.

13. After about one minute, the systolic pressure, diastolic pressure, and pulse rate of partner #1 will appear in the digits display.

14. When the measurements appear, stop data recording and release the remaining air in the cuff by pressing the push-button release valve on the bulb and holding it for several seconds.

15. Record the systolic and diastolic pressure in Table 10.1.

16. Repeat the measurement two more times, and record in Table 10.1. Wait a few minutes in between each measurement.

Part 2 – Cardiac fitness test

Determine the relative cardiac fitness level for partner #1 by conducting the following fitness tests. If time permits, repeat for partner #2.

Reclining blood pressure and pulse rate

17. Partner #1 should recline (lay down) for 5 minutes, with the blood pressure cuff still in place around the upper left arm.

18. After 5 minutes, partner #2 should use the blood pressure sensor to determine partner #1's reclining systolic pressure and pulse rate.

19. When the measurements appear, stop data recording and release the remaining air in the cuff by pressing the push-button release valve on the bulb and holding it for several seconds.

20. Record the reclining systolic pressure and reclining pulse rate in the Table 10.2.

Standing blood pressure and pulse rate

21. Partner #1 should stand up and partner #2 should immediately start data recording to measure partner #1's standing systolic pressure and pulse rate.

22. When the measurements appear, stop data recording and release the remaining air in the cuff by pressing the push-button release valve on the bulb and holding it for several seconds.

23. Record the standing systolic pressure and pulse rate in Table 10.2.

Blood pressure and pulse rate after exercise

24. With the blood pressure cuff still on the arm, partner #1 should climb up and down a step (with an approximate height of 18 inches) 5 times, allowing three seconds for each step up/down.

25. Immediately after the completion of the 5th step, partner #2 should start data recording \ to measure partner #1's pulse rate after exercise.

26. When the measurements appear, stop data recording and release the remaining air in the cuff by pressing the push-button release valve on the bulb and holding it for several seconds.

27. Record the pulse rate after exercise in Table 10.2.

Part 3 – *Daphnia* pulse rate and temperature

Set Up

28. Remove the blood pressure sensor and connect the temperature probe to the data collection system.

29. Display Temperature in a digits display.

30. Using a pipet with a large opening, pick up a *Daphnia* from the culture fluid and place it in on a depression slide.

31. Place the second depression slide over the first.

32. Hold the slides together using a rubber band on either side of the slide. Be sure to wrap the rubber bands with one strand between the slides to allow for air flow.

33. Place the slide in a Petri dish with crushed ice.

34. Place the temperature probe into the ice.

Collect Data

35. Start data recording.

36. Record the temperature in Table 10.3.

37. Remove the temperature probe and stop data recording.

38. Observe the *Daphnia* under the microscope.

39. Locate the heart, and count the number of times the heart beats for 10 seconds.

40. Multiply this number by 6 to determine the number of beats per minute and record the pulse rate in Table 10.3.

Part 4 – *Daphnia* pulse rate at increased temperature

Set Up

41. Place the slide in the next warmest temperature bath provided by your instructor.

42. Place the temperature probe into the water. Let the system equilibrate for several minutes.

Collect Data

43. Start data recording.

44. Record the temperature in Table 10.3.

45. Remove the temperature probe and stop data recording.

46. Observe the *Daphnia* under the microscope.

47. Locate its heart. Count the number of times the heart beats for 10 seconds.

48. Multiply this number by 6 to determine the number of beats per minute and record the pulse rate in the Table 10.3.

49. Move the *Daphnia* slide to one more of the warm water baths provided by your instructor, determine the temperature of the water bath, and record in Table 10.9.

50. Observe the *Daphnia* under the microscope in the warm water bath, calculate its pulse rate, and record in Table 10.3.

51. Save your experiment and clean up according to your instructor's instructions.

Data Analysis

Table 10.1: Blood pressure data

Measurement	1	2	3	Average
Systolic Pressure (mm HG)	122	120	118	120
Diastolic Pressure (mm HG)	80	80	79	79.7

Table 10.2: Cardiac fitness data

Fitness Test	Systolic Pressure (mm Hg)	Pulse Rate (beats/min)	Cardiac Fitness Points
Reclining pulse rate	-	65	3
Standing pulse rate	-	72	3
Pulse rate change upon standing	-	-7	3
Reclining systolic pressure	118	-	-
Standing systolic pressure	114	-	-
Pressure change upon standing	-4	-	0
Pulse rate after exercise	-	112	-
Pulse rate change after exercise	-	40	1
		TOTAL	

Table 10.3: Temperature and Daphnia pulse rate data

Reading	Temperature (°C)	Pulse rate (beats/min)
1	5	103
2	15	214
3	25	391

1. Calculate the average systolic and diastolic pressure and record in Table 10.1.

2. Assign cardiac fitness points for the reclining and standing pulse rate measurements and record in Table 10.2.

Reclining Pulse Rate (beats/min)	Cardiac Fitness Points
50 to 60	3
61 to 70	3
71 to 80	2
81 to 90	1
91 to 100	0
101 to 110	–1

Standing Pulse Rate (beats/min)	Cardiac Fitness Points
60 to 70	3
71 to 80	3
81 to 90	2
91 to 100	1
101 to 110	1
111 to 120	0
121 to 130	0
131 to 140	–1

3. The baroreceptor reflex is the increase in pulse rate resulting from a drop in blood pressure in the carotid artery and/or the aortic arch. For example, when the body moves from a reclining to a standing position, gravity causes blood in the arteries to fall downward, pooling in lower parts of the body, resulting in a drop in pressure in the arteries in the chest.

Calculate the change in pulse rate upon standing subtract the reclining pulse rate from the standing pulse rate and record in Table 10.2.

65 beats/min (reclining pulse rate) – 72 beats/min (standing pulse rate) = -7 beats/min

4. Assign cardiac fitness points for the change in pulse rate upon standing and record in Table 10.2.

Reclining Pulse Rate (beats/min)	**Cardiac Fitness Points for Change in Pulse rate upon Standing (# beats)**				
	0 to 10	**11 to 18**	**19 to 26**	**27 to 34**	**35 to 43**
50 to 60	3	3	2	1	0
61 to 70	3	2	1	0	–1
71 to 80	3	2	0	–1	–2
81 to 90	2	1	–1	–2	–3
91 to 100	1	0	–2	–3	–3
101 to 110	0	–1	–3	–3	–3

5. Calculate the change in systolic pressure from reclining to standing and record in Table 10.2.

114 mm Hg (reclining systolic pressure) – 118 mm Hg (standing systolic pressure) = -4 mm Hg

6. Assign cardiac fitness points for the change in systolic pressure upon standing and record in Table 10.2.

Change in Systolic Pressure upon Standing (mm Hg)	**Cardiac Fitness Points**
Rise of 8 or more	3
Rise of 2 to 7	2
No rise	1
Fall of 2 to 5	0
Fall of 6 or more	–1

7. Calculate the change in pulse rate after exercise and record in Table 10.2.

112 beats/min (after exercise pulse rate) – 72 beats/min (standing pulse rate) = 40 beats/min

8. Assign cardiac fitness points for the change in pulse rate after exercise and record in Table 10.2.

Standing Pulse Rate (beats/min)	**Cardiac Fitness Points for Change in Pulse Rate After Exercise (# beats)**				
	0 to 10	**11 to 20**	**21 to 30**	**31 to 40**	**41+**
60 to 70	3	3	2	1	0
71 to 80	3	2	1	0	–1
81 to 90	3	2	1	–1	–2
91 to 100	2	1	0	–2	–3
101 to 110	1	0	–1	–3	–3
111 to 120	1	–1	–2	–3	–3
121 to 130	0	–2	–3	–3	–3
131 to 140	0	–3	–3	–3	–3

9. Sum the cardiac fitness points in Table 10.2 and use the table below to see how fit your cardiovascular system is compared to the average person.

Total Score	**Relative Cardiac Fitness**
14 to 13	Excellent
12 to 10	Good
9 to 4	Fair
3 or less	Poor

10. Calculate the Q_{10} of the *Daphnia magna* that you observed, using the pulse rates gathered at two temperature measurements using the following formula.

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{10/(t_2 - t_1)}$$

where:

t_1 = lower temperature

t_2 = higher temperature

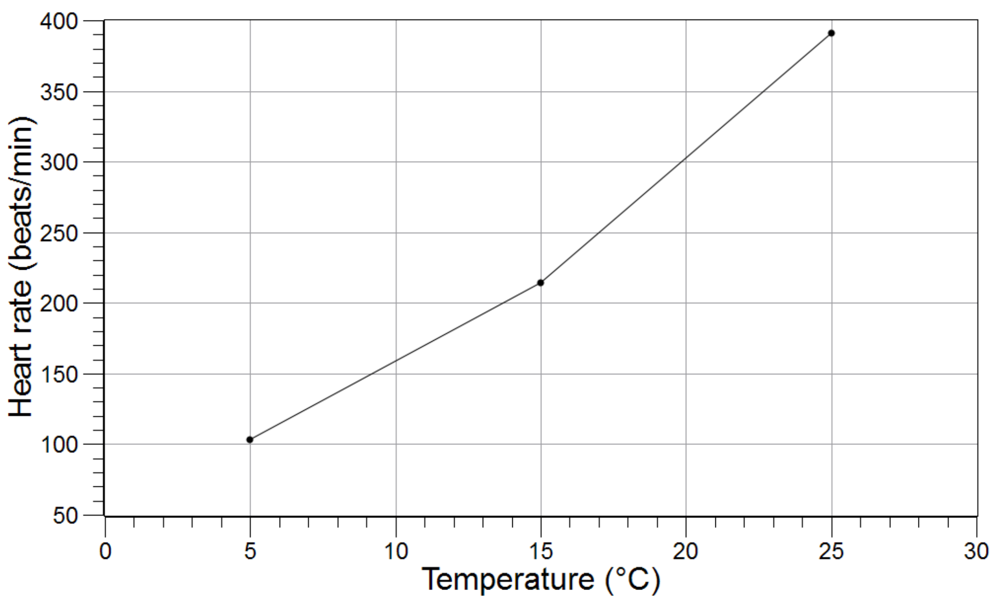
k_1 = pulse rate at lower temperature

k_2 = pulse rate at higher temperature

$$Q_{10} = \left(\frac{391\text{ bpm}}{241\text{ bpm}}\right)^{10/(25-15)}$$

$$Q_{10} = 1.83$$

11. Using the data in Table 10.3, plot a graph of Temperature versus *Daphnia* pulse rate. Label the overall graph, the x-axis, the y-axis and include units on the axes.



Analysis Questions

1. How did changing from a standing to a reclining position affect pulse rate? Explain the results you observed.

The subject's pulse rate should be lower in a reclining position because the heart is not working against the effect of gravity on the flow of blood. In a reclining position, the head is at the same position as the rest of the body.

2. How did the pulse rate change after the baroreceptor reflex? What might explain this change in pulse rate?

As a person moves from a lying to a standing position, the pulse rate increases. When a person is standing the heart has to pump harder to counter the affect of gravity on blood flowing toward the upper portion of the body.

3. If the participant had exercised for double the amount of time, how might his or her pulse rate have changed?

Depending on the physical fitness of the individual, the pulse rate would continue to increase but eventually would level off with continued exercise.

4. From the data you collected, how would you define an individual that is fit?

A fit individual would have a low resting blood pressure and pulse rate. A fit individual would also have a small baroreceptor reflex as well as a rapid recovery rate after exercise.

5. In Parts 3 and 4, you determined the affect of temperature on an ectothermic organism. How would the results of the experiment change if the participant was an endothermic organism?

Endothermic organisms are able to regulate their body temperatures in response to changes in ambient temperature by using a number of strategies including increasing pulse rate and muscle contraction which increases blood flow.

6. How does temperature affect the pulse rate of an ectothermic organism such as *Daphnia magna*? Why does temperature affect ectotherms in this way?

As the temperature increases, the pulse rate also increases. The body temperature of ectothermic organisms is determined by ambient temperature. As environmental temperatures increase their body temperature also increases causing an increase in metabolic activity. Conversely, as the ambient temperature decreases, the pulse rate decreases and causing decreased metabolic activity.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Why would a person with a high fitness rating need to exercise for longer periods of time in order to achieve a target pulse rate than a person with a lower fitness rating?

The heart of a person with a high fitness rating is able to pump more blood for every beat of the heart than a person with a lower fitness rating. The large blood volume of the fit individual allows the tissues of the body to receive more oxygen than for a less fit individual. A fit person will have to exercise longer and harder to increase oxygen demand in the tissues.

2. Why do some people feel faint when quickly standing after reclining?

A person may feel faint when moving to a standing position as the blood flows away from the upper portion of the body due to gravity.

3. An ectothermic organism's body temperature is largely determined by ambient temperature. How might the behavior of an ectothermic organism change its body temperature?

An ectothermic organism could change its body temperature by moving to an area that is warmer or cooler or by changing its body position to increase or decrease its surface area.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following would have the least effect on the blood pressure of an endothermic subject?

- **A. Ambient temperature**
- **B.** Body position
- **C.** Physical fitness
- **D.** Constant stress
- **E.** Caffeine intake

2. If an individual's blood pressure was determined to be 120/70, which of the following is true?

- **A.** The pressure during the contraction phase of the heart is 70, and the pressure during the relaxation phase is 120.
- **B. The systolic pressure is 120 and diastolic pressure is 70.**
- **C.** The pulse is 120 during exercise and 70 when at rest.
- **D.** The individual shows possible borderline high blood pressure.
- **E.** The diastolic pressure is 120 and systolic pressure is 70.

3. Which of the following organisms would show the most drastic change in internal temperature throughout a normal day in its natural habitat?

- **A.** A whale living in the ocean.
- **B.** A rat living in an urban area.
- **C.** A tropical marine fish.
- **D. A lizard living in the desert.**
- **E.** A human living in an urban area.

Extended Inquiry Suggestions

Have students use a rate sensor to measure changes in pulse rate during different physical activities.

Compare the data between different genders and age groups and discuss reasons for any differences.

Take an anonymous survey of the health and physical activity levels of the class and compare fitness levels.

21. Animal Behavior

Objectives

- ♦ Students design and carry out a controlled experiment to investigate an organism's response to environmental variables.
- ♦ Students explore different types of animal behavior such as orientation behavior, agonistic behavior, or dominance display.

Procedural Overview

Students gain experience conducting the following procedures:

- ♦ Designing a controlled experiment.
- ♦ Measuring an organism's preference for environmental factors.

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	10 minutes
♦ Lab experiment: - Part 1	20 minutes
♦ Lab experiment :- Part 2	55 minutes

Materials and Equipment

For each student or group:

- ♦ Plastic Petri dishes with lids (2)
- ♦ Pillbugs (10 to 15)
- ♦ Several sheets of filter paper
- ♦ Adhesive tape
- ♦ Scissors
- ♦ Additional stimulus agents[1]

[1]Assortment of physical or chemical agents, such as sugar, salt, vinegar, household ammonia, lamps, noise makers, or heaters, to serve as independent variables in student-designed experiments.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- ♦ The requirements for conducting a controlled experiment
- ♦ An organism's population density varies based on resource availability
- ♦ The difference between taxis and kinesis
- ♦ Basic knowledge of pillbug anatomy

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Endotherms and Ectotherms: Temperature Regulation in Animals
- ♦ Physiology of the Circulatory System

Background

Animal behavior relates to what an animal does and why it does it. These behaviors are triggered by either internal or external stimuli. The responses to these stimuli are either an instinct (based on the organism's genes) or a learned behavior acquired by the interaction of the organism with its parents or surrounding.

There are a variety of examples of animal behaviors including, long distance migration of birds and fish, mating rituals like the Blue-footed Booby's dance, and agonistic behavior like the aggressive response that Siamese fighting fish display when placed closed to each other. These responses can protect the organism from predators, attract mates, or secure food resources.

Some of the simplest behaviors are those related to an organism's reaction to environmental factors such as light, sound, or moisture. If an organism changes its behavior in response to a stimulus but is not directed by the stimulus, it is called kinesis. However if the organism responds positively (towards) or negatively (away), the movement is called taxis.

Chemicals can also act as stimuli and can be used to influence an organism's behavior. Ants are attracted to sugar, and crab spiders to a flower fragrance. Chemicals released by a species that trigger a behavioral response in other members of that species are called pheromones. A female of a species could release a chemical to attract a male when she is ovulating.

This experiment will use pillbugs, which are isopods, an order of the subphylum Crustacea. They can be found in both terrestrial and aquatic environments, yet all members of the order have similar characteristics. For example, they all have seven pairs of legs and they all possess gills.

Pre-Lab Discussion and Experiment

In order to prepare the students for the lab experiment, have them observe pillbugs for 5 to 10 minutes before starting the experiment. For each lab group, place 10 pillbugs in a Petri dish with some of the material from the pillbug container. Students should make notes on how the organism moves, whether they prefer a particular part of the dish, and whether or not the organisms clump together.

It would be helpful not to introduce external stimuli such as bright lights or loud sounds into this experiment.

1. After the observation time, ask the students to share their observations with their classmates.

2. Ask students to give their ideas about why the pillbugs behaved the way that they did. Talk about some of the behaviors that humans have and why we engage in these behaviors.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Construct the choice chambers: Cut a small (1 to 2 cm) opening on the side wall of each dish. Place the two dishes side by side with the two opening adjacent to one another. Tape the two dishes together.
2. Make sure the filter paper fits inside the Petri dish.
3. Buy pillbugs from a biological supply company or take your students outside and have them dig them up. Pillbugs are also known as roly-polies, sow bugs, or wood lice.

Safety

Add these important safety precautions to your normal laboratory procedures:

- In the student-designed part of the experiment, limit the use of dangerous or harmful chemicals.
- Always treat the animals with respect and handle them carefully.

Procedure

Part 1 – Pillbug behavior

Set Up

1. Obtain a choice chamber. Place a damp piece of filter paper in one chamber and a dry one on the other side.
2. Carefully transfer 10 pillbugs to the choice chamber, placing 5 on each side.
3. Cover the chambers with the Petri dish lids.
4. Predict what you think will happen to the distribution of the pillbugs.

Students should back up their prediction with reasons supporting it.

Collect Data

5. Count how many pillbugs are in each chamber every 30 seconds for the next 10 minutes. Record your results in Table 11.1

6. Return the pillbugs to their original container.

Part 2 – Student-designed experiment

Set Up:

7. Design an experiment to investigate the pillbugs' response to temperature, pH, background color, light, or some other variable. Complete the steps below to help you organize your investigation.

8. State the question that you wish to explore in this experiment.

An example: How does light affect pillbug behavior?

9. State your hypothesis. It should indicate to the reader your prediction on how the independent variable will affect the dependent variable.

An example: If pillbugs prefer dark environments, then there will be more pillbugs in the dark choice chamber than the light choice chamber.

10. Define the variables that you will be testing. Indicate the independent and dependent variables.

An example: The independent variable is the amount of light and the dependent variable is the number of pillbugs in each chamber.

11. List the material you will need to carry out the experiment.

An example: 15 pillbugs, choice chamber, black construction paper

12. Decide what data you will collect and how you will collect the data.

An example: The data collected will be the number of pillbugs; it will be collected visually and recorded manually.

13. Decide how you will organize that data. For example: in a table or by graph.

An example: The number of pillbugs will be recorded in a table

14. Provide a detailed outline of your procedure. Your instructor must approve your procedure before you begin your experiment.

An example: Place 15 pillbugs in one side of the chamber. Cover the other side of the chamber with a piece of black construction paper. Count how many pillbugs are on the light side of the chamber every 30 seconds. Record in the table. Compare these data to the data collected in part one.

Collect Data

15. Conduct the experiment.

16. When you have collected, recorded, and organized all of your data, present it in a form that will help others to understand it.

17. Write a conclusion and be sure to note if your experiment verified your hypothesis.

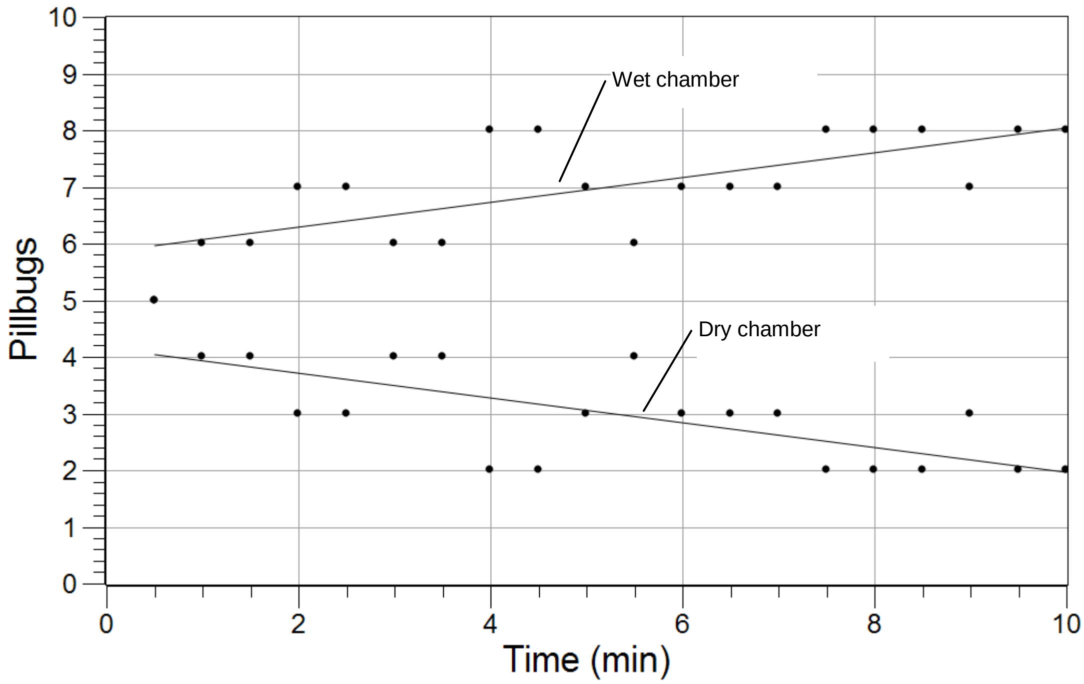
An example: From this experiment, it can be concluded that pillbugs prefer dark environments to light environments.

Data Analysis

Table 11.1: Pillbug behavior

Time (min)	Number in Wet Chamber	Number in Dry Chamber	Observations/Notes
0	5	5	Will vary
0.5	5	5	
1.0	6	4	
1.5	6	4	
2.0	7	3	
2.5	7	3	
3.0	6	4	
3.5	6	4	
4.0	8	2	
4.5	8	2	
5.0	7	3	
5.5	6	4	
6.0	7	3	
6.5	7	3	
7.0	7	3	
7.5	8	2	
8.0	8	2	
8.5	8	2	
9.0	7	3	
9.5	8	2	
10.0	8	2	

Plot a graph of the data in Table 11.1 showing the number of pillbugs in the dry chamber and the number in the wet chamber versus time. Draw a line of best fit line for each trial.



Analysis Questions

1. What is the independent variable in part one of the experiment?

The independent variable is the presence or absence of water on the filter paper of one side of the choice chamber.

2. On which axis of the graph did you put the dependent variable?

The dependent variable is shown on the y-axis.

3. In both parts of the lab, which variable is the dependent variable?

The dependent variable is the number of pillbugs in the chamber.

4. Form a conclusion about pillbug environmental preferences based on your data from both experiments. Can you think of any physiological or behavioral reasons that might explain the pillbugs' movements?

Usually the pillbugs have a preference for the moist filter paper, but sometimes you get a random distribution. The pillbugs are attracted to the moisture because they have gills which need to stay moist.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Pillbugs are usually found under rocks. Give several reasons for this type of behavior.

They need the moisture for their gills and they would avoid the light because the sun would dry their gills out.

2. Pillbugs are also called roly-polies because when they are touched, they roll into a ball. Explain the purpose of this behavior.

This would be a defense mechanism. The underside of the pillbug is soft and the top surface is hard, so by rolling itself up into a ball it protects the soft underside. This could also help it survive dry conditions by reducing the amount of moisture lost from the underside.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Which of the following would be an example of kinesis?

- **A.** A moth attracted to a light
- **B.** A salmon swimming upstream to spawn
- **C.** A mosquito biting you
- **D. Random movement of plankton in the water at night**
- **E.** A dead fish on a beach

2. From your work in this lab, which variable should go on the y-axis of a graph?

- **A.** Light intensity
- **B.** pH
- **C.** Time
- **D. Number of pillbugs**
- **E.** Moisture content of filter paper

3. Which of the following would be an example of learned behavior?

- **A. Running from a bear**
- **B.** A tadpole turning into a frog
- **C.** A newborn taking its first breath
- **D.** Becoming jet lagged from long flights
- **E.** A tree having green leaves

Extended Inquiry Suggestions

Students could examine fruit fly mating behavior. Studies have shown that there are as many as 14 different types of behaviors that occur during the mating process. Students should propose a hypothesis involving one of the behaviors and design an experiment to prove their hypothesis. An example would be males will only demonstrate courtship behaviors in the presence of females. Have the students follow the outlined procedure from part 2 of the Procedures to help them organize their work.

Ecology

22. Air Pollution and Acid Rain

Objectives

In this experiment, students investigate chemical reactions that are important in the formation of acid rain to better understand the relationship between certain types of anthropogenic (man-made) emissions and problems arising from acid rain. During this investigation, students:

- Determine the effect of different anthropogenic gases on the pH of water
- Explore the effects of changes in the pH of water on the environment
- Describe the effect of changes in the pH of water on living things

Procedural Overview

Students will gain experience conducting the following procedures:

- Learning the chemical reactions involved in generating three types of gases, carbon dioxide (CO_2), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2), that are common anthropogenic emissions
- Measuring the effect of CO_2, SO_2, and NO_2 on the pH of water using a pH sensor
- Using radish plants exposed to acidic water as a model of the effects of acid rain on living things

Time Requirement

♦ Preparation time	15 minutes
♦ Pre-lab discussion and experiment	30 minutes
♦ Lab experiment: Part 1	50 minutes
♦ Lab experiment: Part 2	15-minute setup, 5 to 10 minutes every other day for 3 weeks

Materials and Equipment

For each student or group:

- Data collection system
- pH sensor
- Erlenmeyer flask, 50-mL
- Beaker, 50-mL
- Graduated disposable pipet
- Graduated cylinder, 25-mL
- Barbed connector
- 1-hole rubber stopper for flask (size 6)
- Flexible tubing, 20 cm
- Sodium bisulfite ($NaHSO_3$), 5 g
- Sodium nitrite ($NaNO_2$), 5 g
- Glycerin, a few drops
- 1 M Hydrochloric acid (HCl), 15 mL[1]
- Sodium bicarbonate ($NaHCO_3$), 5 g
- Vinegar (400 mL)
- Radish seeds (15)
- Planting pots (3), 2" diameter
- Potting soil
- Electronic balance (1 per class)
- Wash bottle containing distilled or deionized water
- Ring stand
- Three-finger clamp
- Safety gloves (1 pair per student)
- Labeling marker and tape

[1] To formulate 1 M HCl using concentrated HCl, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Most cells function best within a narrow range of acidity.
- pH is a logarithmic measurement of the concentration of hydrogen ions in water. ($pH = -\log_{10}[H^+]$)
- pH measurements can range from 0 through 14. The lower the value, the higher the concentration of hydrogen ions.
- pH can be a measure of acidity, with values below 7 becoming increasingly acidic as the value approaches 0. Therefore, the lower the pH, the higher the concentration of hydrogen ions and the higher the acidity.
- A pH of 7 is neutral—neither acidic nor basic.
- pH values greater than 7 are considered basic. Practically speaking, a pH of 6 to 8 is considered to be in the neutral zone.

Related Labs in This Guide

Labs conceptually related to this one include:

- pH and Buffers
- Interrelationships of Plants and Animals
- Exploring the Effects of pH on Amylase Activity

Background

Acid rain is a type of wet deposition. As this acidic water flows over and through the ground, it affects a variety of plants and animals. Acid rain can also accelerate the dissolving of metals and cations in soils and rocks that are essential for plant growth (such as Mg^{2+} and K^+). The strength of these effects depends on many factors, including:

- The acidity of the water;
- the chemistry and buffering capacity of the soils involved; and
- the types of fish, trees, and other living things that rely on the water.

Scientists have discovered that sulfur dioxide (SO_2) and nitrogen oxides (including nitric oxide (NO), nitrogen dioxide (NO_2), and nitrous oxide (N_2O), collectively known as NO_x) are the primary causes of acid rain. Acid rain occurs when these gases react in the atmosphere with water, oxygen, and other airborne chemicals to form various acidic compounds.

Sulfur dioxide and nitrogen oxides go through several complex pathways of chemical reactions in the atmosphere before they become the acids found in acid rain. One of the most important pathways involves the oxidation of sulfur dioxide (SO_2) to sulfur trioxide (SO_3) by ozone (O_3). Sunlight ultraviolet energy increases the rate of most of these reactions by degrading ozone to oxygen gas (O_2) and oxygen radical (O^-), which is a highly reactive oxidizer. The sulfur trioxide then reacts with water vapor to form sulfuric acid. These reactions are shown as follows:

$$2SO_2 + 2O^- \rightarrow 2SO_3$$

$$SO_3 + H_2O \rightarrow H_2SO_4$$

Dust or ice particles can transport this sulfuric acid through the atmosphere before settling on the ground as dry acid deposition. The sulfuric acid can also dissolve in rain or fog and settle on the ground as wet acid deposition. Scientists believe that sulfuric acid is primarily responsible for the formation of acid rain.

Sulfur dioxide also readily dissolves in water, producing bisulfite and hydrogen ions. Students will explore this reaction of sulfur dioxide in this lab.

In the United States, about two-thirds of all SO_2 and one-quarter of all NO_x comes from electric power generation that relies on burning fossil fuels such as coal. Other sources include automobile exhaust, furnaces, paper pulp production, and metal smelters.

The effects of acid rain are widespread. Acid rain causes acidification of lakes and streams. Acid rain also damages trees at high elevations, such as red spruce trees above 600 meters, and damages sensitive forest soils. In addition, acid rain accelerates the decay of building materials (such as limestone and marble), metals (such as bronze), and automotive paint and other coatings. Acid rain results in stressful and sometimes deadly fluctuations in water systems that cause aquatic life to experience chemical "shock" effects. For example, as the pH drops to 5.5, plankton, certain insects, and crustaceans begin to die, and trout eggs do not hatch well.

Acid rain reduces crop productivity and forest growth rates while accelerating the rate at which heavy metals and nutrient cations leach from soils, rocks, and waterway sediments. Scientists believe that acid rain causes increased concentrations of methylmercury in natural waterways. Methylmercury is a neurotoxic molecule that accumulates in fish tissues and can cause birth defects in populations that ingest high concentrations of affected fish.

Pre-Lab Discussion and Experiment

Engage your students by having them research adverse effects of acid rain worldwide. Brainstorm a variety of examples, and record them for class viewing. Local examples focus and stimulate interest best.

Point out that anthropogenic emissions are responsible for some of the acidity in the atmosphere that results in acid rain. Tell students that they will generate some of these gases and test their effect on the pH of water.

Review the chemical reactions that produce the gases they will be studying:

sodium bicarbonate + HCl + water → sodium ion + chloride ion + water + carbon dioxide gas

$$NaHCO_3 + HCl(aq) \rightarrow Na^+ + Cl^- + H_2O + CO_2(g)$$

sodium bisulfite + HCl + water → sodium ion + chloride ion + water + sulfur dioxide gas

$$NaHSO_3 + HCl(aq) \rightarrow Na^+ + Cl^- + H_2O + SO_2(g)$$

sodium nitrite + HCl + water → sodium ion + chloride ion + water + sulfur dioxide gas

$$NaNO_2 + HCl(aq) \rightarrow Na^+ + Cl^- + H_2O + NO_2(g)$$

If necessary, review the pH scale and how it is determined.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare the 1.0 M hydrochloric acid solution by adding 16.6 mL of concentrated HCl per 100.0 mL of solution. Pour the concentrated HCl into about 80 mL of water, bring the solution up to 100 mL with water, and then pour 15 mL into a beaker for each group.

Note: Do not pour water into the concentrated acid.

2. Use tap water unless the water in your area has a high level of dissolved solids, which can produce a significant buffering action. This is the case for some well water, for example. The pH of distilled and deionized water is highly susceptible to large changes in pH as a result of the minute amount of dissolved chemicals in these types of water.
3. Because the pH measurements in this experiment are relative to measurements within this lab, it is not necessary to calibrate the pH sensor.
4. If your classroom does not have a vented hood, student groups or the instructor can generate the SO_2 and NO_2 under the vented hood in the chemistry lab. It is safe for students to generate the CO_2 at their workbenches in the classroom. Each group can then present its results to the class for analysis.
5. For students generating CO_2 at their workbench, you can substitute household white vinegar if you do not have 1 M HCl.
6. If you do not have windows or natural light for Part 2, ensure that students have access to a greenhouse or a constant light source, such as a grow light.
7. Vinegar has an approximate pH of 2.8. This is very close to the pH of the sulfur-dioxide-infused water from Part 1, which is why vinegar is a suitable substitute for sulfur dioxide infused acid rain.

Safety

Add these important safety precautions to your normal laboratory procedures:

- Consult the manufacturer's MSDS for instructions on handling, storage, and disposing of hydrochloric acid, sodium bisulfite, and sodium nitrite. (You can find these on the Internet.) Keep these instructions available in case of accidents.
- Students creating sulfur dioxide and nitrogen dioxide should work under a vented hood.
- Do not touch the hydrochloric acid (HCl). Handle the pipet with HCl with extreme care.
- Do not remove the rubber stopper from the Erlenmeyer flask once the reaction has started.
- After completing the lab, wash your hands.
- Wear safety glasses and lab coats or aprons.

Procedure

Part 1 – Create carbon dioxide (CO_2) gas and measure its effect on the pH of water

Set Up

1. Start a new experiment on the data collection system.

2. Connect the pH sensor to the data collection system.

3. Display pH on the y-axis of a graph with Time in the x-axis.

4. Remove the pH probe from the bottle and thoroughly rinse the probe with distilled water.

5. Use the three-finger clamp to secure the pH probe to the ring stand.

6. With a graduated cylinder, measure 20 mL of water and pour it into the 50-mL beaker.

7. Place the beaker of water under the pH probe.

8. What do you think will happen to the pH of the water when you dissolve carbon, nitrogen and sulfur dioxide gases in it? Which gas will produce the largest change in pH?

Answers will vary according to student predictions.

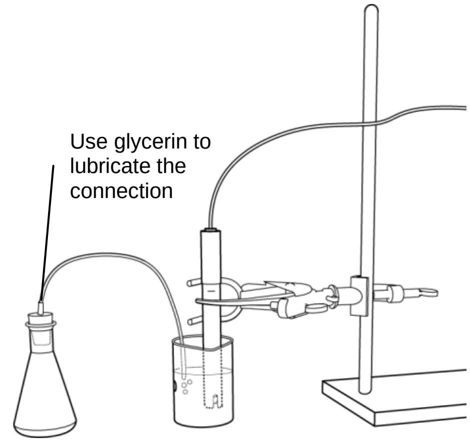
9. Obtain a sample of powdered sodium bicarbonate ($NaHCO_3$) and a sample of hydrochloric acid (HCl) from the instructor.

You will create CO_2 gas by mixing $NaHCO_3$ with HCl.

10. Measure 5 g of $NaHCO_3$.

11. Place the measured $NaHCO_3$ in the Erlenmeyer flask.

12. Press the barbed connector into the hole in the stopper. Connect the tubing to the barbed stopper.



Note: If necessary, use glycerin to lubricate the connection so that the connector or glass tubing is well seated in the rubber stopper

Collect Data

13. Place the free end of the plastic tubing beneath the surface of water in the beaker.

14. Lower the pH probe into the beaker of water.

15. Begin collecting data. \ One group member should make sure that the tubing remains under water, while another quickly pipets 4 mL of 1.0 M hydrochloric acid (HCl) into the Erlenmeyer flask, and immediately stopper the flask.

Caution! Hydrochloric acid is a strong acid. Handle with care. Flush any spillage with a lot of water.

Note: If water starts going backwards into the tubing, or if the bubbling stops, firmly grasp the Erlenmeyer flask in your hand, so that the heat from your hand heats the gas in the flask, creating a higher pressure area in the flask and thus expelling the generated gas into the water.

16. Record data for about 200 seconds (or until the change in pH stops or stabilizes), and then stop recording.

17. Name your run to reflect the sample type, and save your experiment.

18. Dispose of the contents of the flask and beaker as directed by your instructor.

19. Rinse the beaker, flask, probe and tubing with water.

Part 2 – Create sulfur dioxide (SO_2) gas and measure its effect on the pH of water

20. Repeat the steps in Part 1 using 5 g sodium bisulfite ($NaHSO_3$) instead of $NaHCO_3$.

You will create SO_2 gas by mixing $NaHSO_3$ with HCl.

Part 3 – Create nitrogen dioxide (NO_2) gas and measure its effect on the pH of water

21. Repeat the steps in Part 1 using 5 g sodium nitrite ($NaNO_2$) instead of $NaHCO_3$.

You will create NO_2 gas by mixing $NaNO_2$ with HCl.

22. Complete Table 1 in the Data Analysis Section.

Part 4 – Effects of acid rain on living things

Now that you have seen how greenhouse gases affect water pH, you will see how that acidic fluid will affect plant growth. You will be watering radish plants for 3 weeks with liquids of varying pH. One plant will receive pure water with a pH of 7. Another plant will receive water for a week and vinegar for two weeks. The third plant will receive only vinegar.

Set Up

23. What effects, if any, will there be on the radish plants because of the different pH of the liquids used to water them?

Answers will vary. The plants receiving liquids with lower pH will have their growth adversely affected.

24. Label 3 pots "A," "B," and "C" with masking tape and a marker.

25. Fill each pot with potting soil.

26. Make a 4-cm deep hole in the soil with your finger.

27. Place 5 seeds in each hole.

28. Cover the seeds carefully with soil.

29. Water the seeds in pots A and B with 50 mL of water.

30. Water the seeds in pot C with 50 mL of vinegar.

31. Place your pots in the indoor growing area designated by your instructor.

Collect Data

32. Check your plants each day.

33. Measure their growth (in centimeters) every two days, and write this information on your chart.

34. For one week add 20 mL of water every other day to pots A and B, and 20 mL of vinegar to pot C.

35. After one week:

a. ☐ Water only pot A with 20 mL of water every other day.

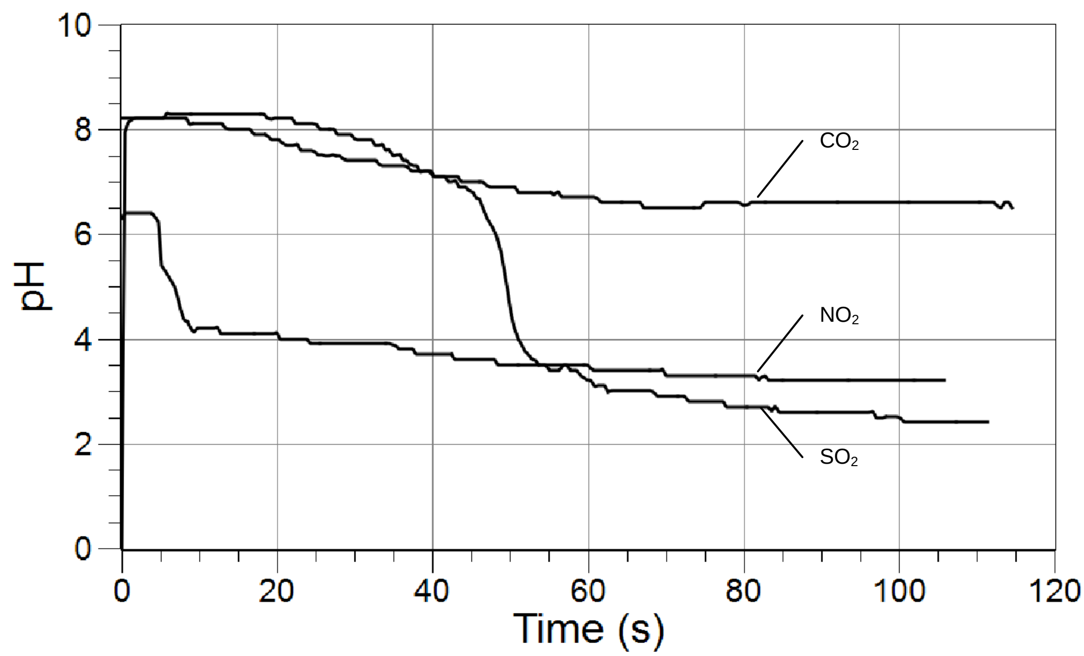
b. ☐ Water pots B and C with 20 mL of vinegar every other day.

36. Continue measuring and recording plant growth every other day.

37. Take the last measurements on the 21st day, and measure the pH of the soil in each of the pots.

Sample Data

The following graph shows pH versus Time following bubbling of CO_2, SO_2, and NO_2 into distilled water.



Data Analysis

1. Find the maximum and minimum pH values, and record them in Table 1.

2. Calculate the change in pH for all three gases, and record each in Table 1.
Change in pH = final pH – initial pH.

Table 1: pH change due to gases dissolved in water

Gas	Maximum pH	Minimum pH	Change in pH
Carbon dioxide	8.2	6.5	1.7
Sulfur dioxide	8.3	2.9	5.4
Nitrogen dioxide	6.4	3.2	3.2

3. Fill in Table 2 with the plant heights and appearance. Include the color of the leaves and stem, the quantity of leaves, and the overall health of the plant. You will collect data for 3 weeks. Follow the watering procedures carefully.

Table 2: Comparison of plants watered with liquids of varying pH

Day	Fluid Added (mL)	Height (cm)	Appearance (color, health, leaf quantity)
1	A : 50 mL water	0	No data yet
	B : 50 mL water	0	No data yet
	C : 50 mL vinegar	0	No data yet
3	A : 20 mL water	0	No data yet
	B : 20 mL water	0	No data yet
	C : 20 mL vinegar	0	No data yet
5	A : 20 mL water	1.5	Healthy and green
	B : 20 mL water	1.5	Healthy and green
	C : 20 mL vinegar	0	No growth
7	A : 20 mL water	2.0	Healthy and green
	B : 20 mL water	2.0	Healthy and green
	C : 20 mL vinegar	0	No growth
9	A : 20 mL water	5	Healthy and green
	B : 20 mL vinegar	5	Healthy and green
	C : 20 mL vinegar	0	No growth
11	A : 20 mL water	6	Healthy and green
	B : 20mL vinegar	5	Limp and turning yellow
	C : 20 mL vinegar	0	No growth
13	A : 20 mL water	5.5	Healthy and green
	B : 20 mL vinegar	5	Limp and turning yellow
	C : 20 mL vinegar	0	No growth
15	A : 20 mL water	6	Healthy and green

9	A : 20 mL water	5	Healthy and green
	B : 20 mL vinegar	4	Limp and turning yellow
	C : 20 mL vinegar	0	No growth
17	A : 20 mL water	6	Healthy and green
	B : 20 mL vinegar	4	Some leaves falling off
	C : 20 mL vinegar	0	No growth
19	A : 20 mL water	6	Healthy and green
	B : 20 mL vinegar	4	No leaves
	C : 20 mL vinegar	0	No growth
21	A : 20 mL water	6	Healthy and green
	B : 20 mL vinegar	3.5	No leaves
	C : 20 mL vinegar	0	No growth

Analysis Questions

1. Was your prediction in Part 1 correct regarding what would happen to the pH when you dissolved the gases in it? Why or why not?

Answers will depend on what students predicted. They should briefly discuss why they were correct or not correct.

2. The following chemical reactions are involved in this lab. Write each formula using chemical notation.

a) One molecule of carbon dioxide gas dissolves in water to form one bicarbonate ion and one hydrogen ion.

$CO_2 + H_2O \rightarrow HCO_3^- + H^+$

b) Two nitrogen dioxide gas molecules dissolve in water to form one nitrate ion, one nitrite ion, and two hydrogen ions.

$2NO_2 + H_2O^- \rightarrow 2H^+ + NO_3^- + NO_2^-$

c) One sulfur dioxide gas molecule dissolves in water to form one bisulfite ion and one hydrogen ion.

$SO_2 + H_2O \rightarrow HSO_3^- + H^+$

3. Which gas created the smallest change in pH of the water?

The carbon dioxide gas produced the smallest decrease in pH.

4. Compare your results with those from other groups. What factors might have caused some of the variability in the change of the observed pH?

Some factors that might contribute to experimental variability include: 1) variations in the mass of reactants used; 2) variations in the amount of water used; 3) variations in the efficiency of collecting the gases; 4) variations in pH sensors, which may not have been calibrated for this experiment.

5. For the three reactions of gas dissolving in water, what caused the reduction of the pH of the water in which these gases are dissolved?

The pH is lowered because hydrogen ions are formed. When the concentration of hydrogen ions increases, the pH decreases.

6. Was your prediction in Part 2 correct regarding the effects on the radish plants because of the different pH of the liquids used to water them? Explain why or why not.

Answers will vary.

Synthesis Questions

Use available resources to help you answer the following questions.

1. What industrial or other anthropogenic gases emitted into the atmosphere are considered the primary gases that cause acid rain? What are some sources of these gases?

The primary gases involved in producing acid rain are sulfur dioxide and the nitrogen oxides. The primary sources of these gases are the burning of fossil fuels, smelting of metals, and paper pulp production.

2. Scientists have found that sulfuric acid is the primary acid that causes acid rain. What are some of the chemical reactions that produce sulfuric acid in the atmosphere? Why does radiation from the sun speed up this reaction?

$2SO_2 + O_2(g) \rightarrow 2SO_3(g)$ (this reaction requires an oxidizer, such as ozone)

$SO_3(g) + H_2O(l) \rightarrow H^+(l) + HSO_4^-(l)$

The sun's radiant energy, particularly ultraviolet energy, produces oxidizing molecules from ozone, water molecules, and oxygen gas. These oxidizing molecules speed the reaction.

3. Coal from states in the western United States, such as Montana and Wyoming, has a lower percentage of sulfur impurities (lower sulfur content) than coal found in the eastern United States. How would burning low-sulfur coal change acid rain?

Burning low-sulfur coal would decrease the amount of acid rain by reducing the amounts of sulfur oxides (the reactants required to produce sulfuric acid) emitted into the atmosphere.

4. What are some ways to treat the effects of acid rain?

Some ways to treat the effects of acid rain include the following: 1) Buffer lakes by adding lime. 2) Add vegetation to the watershed so that water does not run off as rapidly into the body of water. That way, the water seeps into the soil, dissolving buffers contained in the soil. 3) Add large amounts of water that have neutral or basic pH.

5. What are some ways to prevent the formation of acid rain?

Answers will vary. Ways to prevent the formation of acid rain include the following: 1) Burning a lower sulfur-containing fuel will result in less SO_x emissions; 2) Install scrubbers (or other technology) on smoke stacks to reduce SO_x emissions; 3) Install catalytic converters in cars to convert nitric oxide gases to nitrogen gas; 4) Conserve energy, since using less energy will require burning less fossil fuels, which contain sulfur and nitrogen; 5) Develop more fuel-efficient cars, thus reducing NO_x emissions.

Multiple Choice Questions

1. Which of the following is true about acid rain?

- **A.** Acid rain is linked to NO_x and SO_x molecules in the atmosphere.
- **B.** Acid rain can result in the death of many species of water-dwelling organisms when it causes the pH of lakes to decrease to a level outside their tolerance.
- **C.** Acid rain affects soil chemistry and the ability of plant roots to take in nutrients.
- **D.** Acid rain increases the mobility of toxic metals in ecosystems.
- **E. All of the above are true.**
- **F.** Only A, B, and C are true.

2. Which of the following play important roles in the formation of acid rain?

- **A.** Solar radiation
- **B.** Buffers in soils and water
- **C.** Water in the atmosphere
- **D.** Nitrogen gas (N_2) in the atmosphere
- **E.** All of the above
- **F. Only A and C**

3. In general, rain exerts harmful effects on ecosystems when it falls below a pH of

- **A.** 3.6
- **B.** 4.6
- **C. 5.6**
- **D.** 6.6
- **E.** 7.6

4. Acid rain has been linked to

- **A.** Contamination of fish with highly toxic methylmercury
- **B.** Damage to fish through reactions that create high aluminum concentrations in the water
- **C.** Reduced nutrient uptake by tree roots
- **D.** Weakening trees, so they become more susceptible to other types of damage
- **E. All of the above**

Extended Inquiry Suggestions

What is the pH of your local rainwater? Have students design a way to collect rainwater and then measure the pH of the sample using a pH sensor.

What is the pH of a local water system? Have students collect samples from a local pond, stream, lake, or river. Then, determine the pH of the samples using a pH sensor.

Visit a local cemetery and observe the wearing away of the headstones or other grave markers over time. Military cemeteries use limestone markers that are more easily affected by acid rain than the granite markers in some private cemeteries. Use the dates on the marker stones and the condition of the stones to determine which ones acid rain may have damaged. Remember that these materials would naturally deteriorate when exposed to the weather and rain (even unpolluted rain). Acid rain would accelerate this damage.

Have students write, produce, and direct a “weather special” segment for TV on how weather patterns affect the travel of acid rain over large distances. Contact the weather bureau or a local television station's weather department to ask about the wind patterns in your area. This information and data for your area may also be available on the Internet.

Have students contact a local natural resource specialist at your local zoo or park. Ask that person to tell you about the impact, if any, of both acid rain and dry acid deposition in the lakes.

23. Population Ecology

Objectives

Students use the bacteria *E. coli* as a model for studying population growth, growth curves, and factors affecting growth.

Procedural Overview

Students gain experience conducting the following procedures:

- Comparing the growth rate (*r*) of *E. coli* populations in different sized chambers
- Comparing *r* in *E. coli* populations with different nutrient availability
- Comparing *r* in *E. coli* populations with different initial population densities
- Comparing *r* in *E. coli* populations incubated at different temperatures

Time Requirement

♦ Preparation time	120 minutes
♦ Pre-lab discussion and experiment	55 minutes
♦ Lab experiment	90 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Colorimeter
- Access to shaking incubator (optional)
- Cuvettes (4)
- Culture vessels (3), 50-mL
- Culture vessels, 15-mL, 50-mL, 250-mL
- Sterile transfer or Pasteur pipets, toothpicks, or inoculation loops
- 4 mL overnight (O/N) culture of *E. coli*[1] in a culture tube, 15-mL
- 40 mL of Luria-Bertani (LB) broth[2]
- Sterile water
- Bleach, 100%
- Squirt bottle with 10% bleach
- Disposable gloves
- Labeling marker
- Tape
- Lint-free tissue

[1] To prepare overnight (O/N) cultures of *E. coli*, refer to the Lab Preparation section

[2] To prepare Luria-Bertani (LB) broth, refer to the Lab Preparation section

Related Labs in This Guide

Labs conceptually related to this one include:

- ♦ Diffusion
- ♦ Elodea and the Snail
- ♦ Measuring Aerobic Cellular Respiration in Yeast
- ♦ Diffusion and Osmosis
- ♦ Cell Respiration
- ♦ Dissolved Oxygen and Primary Productivity

Background

Typically, a population of organisms will exhibit a logistic or S-shaped growth. When a population is introduced to a new or unoccupied habitat with unlimited nutrients, is unconstrained by competition with other species, and free from predation pressure, the population tends to grow rapidly. In other words, the number of births (B) is greater than the number of deaths (D) and the intrinsic rate of growth (r) is greater than 1.

$$r = B - D$$

However, unlimited population increase does not occur for any species, either in the laboratory or in nature. A small population in a favorable environment may increase rapidly for a while, but eventually, as a result of limited resources and other factors, its rate of increase must slow and eventually stops. At this point B is roughly equal to D, r approaches zero, and the growth curve flattens out.

What is carrying capacity? Ecologists define carrying capacity (K) as the maximum population that a habitat can support. Carrying capacity is not fixed, but varies over space and time with the abundance of limiting factors. As the number of individuals in a population (N) approaches K, the rate of increase slows. In fact r may drop to zero or even decline. Carrying capacity (K) is the reason for logistic growth. Logistic growth incorporates K, and is represented by the formula below:

$$dN/dT = r_{max}N(K\text{–}N)/K$$

This formula represents the following scenario: at any given population density, at any given time (dN/dT), the growth rate of the population is dependent on the maximum rate of growth times the population density (r_{max} x N). However, population growth will slow as population density (N) reaches carrying capacity K. As N approaches K the value of (K–N) gets closer to 0, driving the value of dN/dT to 0.

What are some limiting factors and how do they affect the growth rate and carrying capacity of populations? Limiting factors are anything that slows population growth. Limiting factors can include water availability, mineral nutrients like nitrogen and phosphorus, food availability, soil structure, soil moisture, oxygen, sunlight, shelter, and mating territory. Other limiting factors are known as density-dependent factors such as predation, build up of toxins, spread of disease, and competition for resources. These factors are said to be density-dependent because they affect the population as it gets closer to K.

As populations approach K, there is greater competition for available resources, increased likelihood of predation, increased build up of toxins and metabolic byproducts, and increased risk of disease. All of these increase the number of deaths (D) and limit the number of births (B). Recall that population growth $r = B - D$, and if D is greater than B, then r will be negative and the population will decrease.

Pre-Lab Discussion and Experiment

Engage your students the day before they begin their experiments by showing them how to set up an overnight (O/N) culture of *E. coli*. This will give you an opportunity to talk about what *E. coli* is, where it is normally found, and what its ideal conditions are for growth. You can also allay some fears about working with *E. coli* by discussing aseptic technique. Go over the following procedures of aseptic technique:

- ♦ Wash hands before and after lab.
- ♦ Wipe down lab tables with bleach water before and after lab.
- ♦ Always keep bacterial plates and culture tubes closed.
- ♦ If you have to open a plate or tube, do not set the lid down on the lab table.
- ♦ Put all material that came into contact with bacteria in the proper disposal bag.
- ♦ Put hair back in a ponytail.
- ♦ Wear lab goggles and aprons.

Ask the following series of questions:

1. What environment does the *E. coli* need for optimal growth?

E. coli grows best in the presence of oxygen and at 37 °C.

2. What are some factors that limit the growth of these bacteria?

Oxygen availability, increase in metabolic wastes, nutrient limitation, extremely high or low temperature, competition for nutrients by another organism.

3. What is Luria-Bertani (LB) broth?

Luria-Bertani (LB) broth is an “optimal” growth medium for *E. coli*. It contains protein, carbohydrate, salt, and water.

4. What will the LB broth with the bacterial inoculation look like tomorrow?

It should look “cloudy”.

Demonstrate exponential growth with the following experiment:

Use rice to model *E. coli* and a meter stick to model time (each decimeter representing a generation). Place one grain of rice at the 0-cm mark to represent an initial cell in a population. With each successive decimeter, double the number of rice grains. Continue through the 80-cm mark. Students should work together to count and place the rice grains on each decimeter. Finish the experiment by asking students to graph the results on the board. The x-axis should be time (in generation) and the y-axis should be N (population size) in number of cells.

On the first lab day, show your students a culture tube of sterile LB, and the O/N cultures you prepared. Before students begin their experiments, show them how to use aseptic techniques to transfer 5 mL of LB and O/N culture to separate cuvettes and how to use the colorimeter to measure the absorbance of orange light (610 nm). Students should be able to see and measure what zero bacterial growth looks like (0 cells/mL), and what a saturated liquid culture looks like (2×10^9 cells/mL).

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Scheduling the lab: You need four consecutive lab periods for your students to get the most out of this experience. Use the following table to help plan your schedule.

Day	Instructor Task(s)	Student Task(s)	Time Required
Day Prior to Lab	• Start O/N Cultures • Prepare LB broth • Sterilize culture chambers		2 to 3 hours
Day 1	• Demonstrate starting O/N cultures and aliquot LB broth for students • Review aseptic technique procedures	Perform exponential growth activity	One laboratory period
Day 2	Teach students how to use the colorimeter and data collection system	Perform population growth experiments	One laboratory period, and continuing throughout the day
Day 3		Clean up laboratory, begin data analysis	One laboratory period
Day 4	Lead data analysis	Finish data analysis	One laboratory period

2. Preparing Luria-Bertani (LB) broth. Purchase LB powder from a scientific supply company (Flinn, Bio-Rad, Fisher, Wards, Carolina, et cetera), and mix 12.5 g of LB powder into 500 mL of distilled water. Ideally, you will autoclave this solution for at least 35 minutes at 15 to 17 psi. Alternatively, the LB powder can be dissolved into the water using a microwave oven. The LB broth should be tightly covered to prevent contamination. If you make your LB broth a few days before the lab begins, store it in your refrigerator (20 °C) until you are ready to use it. If you store your LB broth at 20 °C, make sure it is at room temperature before students begin their experiments.

Note: Scientific suppliers also sell sterile LB broth, but it is more expensive.

3. Preparing overnight (O/N) cultures of *E. coli*. *E. coli* can be purchased from any biological supply company. Transfer 3 to 5 mL of sterile LB broth to a clean 15-mL culture tube. Using a sterile toothpick (toothpicks sterilized in an autoclave for 25 minutes at 15 to 17 psi), inoculation loop, or micropipet tip, scrape part of a well defined colony off your *E. coli* starter plate. Drop the scraping device into the culture tube. If using a toothpick or a micropipet tip, leave the scraping device in the tube and cover. If using an inoculation loop, shake the loop vigorously to dislodge the colony of bacteria. You will not “hurt” the cells. Cap the tubes and incubate at 37 °C with aeration overnight.

For best results you need a shaking incubator, or a rotating tray or vortexer in a 37 °C incubator. If you do not have equipment to shake the tubes continuously overnight, vigorously shake the tubes for 10 to 20 seconds before starting the incubation. It is unlikely you will get a saturated population (2×10^9 cells/mL) with this method, but it should yield a dense enough population to perform the experiments.

4. Obtaining culture flasks. Laboratory cultures of *E. coli* are traditionally grown in Erlenmeyer flasks. These are available from your scientific supplier. You can also use plastic disposable centrifuge tubes, 15-mL and 50-mL. They are less expensive than glass, but they should be disposed of at the end of the experiment. (If necessary, they can be cleaned and used the following year). The 15-mL tubes usually come in packs of 50 and the 50-mL tubes in packs of 25. They are available from Fisher Scientific or your preferred scientific supplier.

Note: You may be able to borrow all the equipment. Bacteria, LB broth, and disposables may be available from a local university or college. Contact local universities and colleges and inquire about their science outreach programs. If formal science outreach programs do not exist, possibly you could partner with a biology department or individual faculty member to obtain the equipment, bacteria, and reagents needed to complete this lab. Quite often, they can even autoclave all the equipment when you bring it back.

5. Using an autoclave, or sterilizing equipment: If you have access to an autoclave, use it according to its instructions. In general liquids should be autoclaved for at least 35 minutes at 15 to 17 psi, and solid material should be autoclaved for at least 20 minutes at 15 to 17 psi. If you do not have access to an autoclave, you can use sterilized, disposable chambers and pre-made solutions. Because this lab only runs for 24 hours, you can use non-autoclaved culture chambers and non-sterilized plastic transfer pipets. Clean culture chambers as you normally would, then microwave them for 5 to 10 minutes. This should get equipment clean enough to use in this lab.

Note: Ideally, everything should to be sterile for this lab, but it is OK if you if you get some fungal contamination or other bacterial contamination in your chambers over the course of the experiment. Students will still see an increase in absorbance of orange light. Your students will not know whether it is *E. coli* population growth or "community" growth.

6. It is unlikely all students will perform all procedures. Each student or student group should perform one procedure and present results to the entire class.

7. Cleaning up: If an autoclave is available, place all contaminated disposable equipment (chambers, pipets, glove, et cetera) into a biohazard bag and autoclave. If an autoclave is not available, soak all disposable equipment (excluding gloves) in a 10% bleach solution overnight, then place in a plastic bag. Place the plastic bag in your regular garbage.

Pour 100% bleach into glassware containing *E. coli* cultures, then pour bleached cultures down a sink drain. Allow water to run in the drain for 1 to 2 minutes. Clean glassware as you normally would. All lab benches and other work surfaces should be sprayed down with a 10% bleach solution and cleaned.

Note: If time permits, autoclave all culture chambers, cover them with aluminum foil, and store until needed again.

Safety

Add these important safety precautions to your normal laboratory procedures:

- ♦ Wear safety glasses, gloves, and lab coats or aprons.
- ♦ Absolutely no food or drinks should be consumed during this laboratory.
- ♦ Tape down or secure any electric cables.
- ♦ Keep a biohazard bag or beaker near each student workstation.
- ♦ Place all contaminated disposable material in a biohazard bag.
- ♦ Label all tubes, cuvettes, pipets, and wrappers.
- ♦ Reuse disposable materials to minimize the amount of biohazard waste.
- ♦ Wipe down your workspace with 10% bleach solution after each data collection point to minimize build up of bacteria on lab surfaces.
- ♦ Do not touch your face with gloved hands. If you need to sneeze or scratch, take off your gloves, wash your hands, and then take care of the situation.
- ♦ Wash your hands before and after a laboratory session.
- ♦ Rinse all contaminated glassware with 10 mL of 100% bleach; clean according to your instructor's instructions.

Procedure

Note to students: You will only be completing the setup and data collection sections that are assigned to you by your instructor. Complete the setup and then quickly proceed to your assigned data collection section.

Set Up: Different Container Sizes

1. Obtain 3 sterile, but different-sized culture chambers. One should be 50-mL, one should be larger than 50-mL and the other smaller.

Note: Have multiple different sized chambers available for the students, and let them decide what chambers to use. This lab was piloted using a 15-mL culture tube, a 50-mL culture tube, and a 250-mL Nalgene bottle.

2. Label the 50-mL chamber "A" and label the other two "B" and "C." Record the volumes in the space provided and in the first row of Table 1.

Chamber A: ____50__ (mL)

Chamber B: ____250___ (mL)

Chamber C: ____15___ (mL)

3. Transfer 10 mL of Luria-Bertani (LB) broth to each chamber using a sterile transfer pipet.

4. Using another sterile transfer pipet, transfer 1 mL of overnight (O/N) *E. coli* culture to each chamber. Immediately place the cap on the chamber and dispose of the pipet in the biohazard area designated by your instructor.

5. Gently mix the LB and the *E. coli* culture.

6. Proceed immediately to the Collect Data – All Experiments section.

Set Up: Nutrient Limitation

7. Obtain 3 sterile 50-mL culture chambers; label them "A", "B", and "C" using labeling tape and a marker.

8. Transfer 7 mL of Luria-Bertani (LB) broth to chamber A using a sterile transfer pipet.

9. Now add 3 mL of sterile distilled water (SDH_2O) to make a total of 10 mL of liquids in Chamber A. Write down 70% in the space provided below.

10. Now, work with your lab partners to determine how much you will limit the amount of LB available to the *E. coli*. The amount should be greater than 70% for one chamber and less than 70% for the other, but the total volume of liquid media in each chamber will be 10 mL. Make up the difference in each chamber with SDH_2O.

11. When all 50 mL chambers are set up with 10 mL of liquid culture, label the chambers with their percentages appropriately, and record in the spaces below and in row 1 of Table 2.

Chamber A: ______70_____ %

Chamber B: ______100____ %

Chamber C: ______50_____ %

Note: Let students determine the level of nutrient limitation. Greater nutrient limitation slows growth rate (*r*) and limits carrying capacity (*K*) in each chamber. This lab was piloted with 10 mL LB (100%) in chamber B and 5 mL LB: 5 mL SDH_2O (50%) in Chamber C.

12. Using another sterile transfer pipet, transfer 1 mL of overnight (O/N) *E. coli* culture to each chamber. Immediately place the cap on the chamber and dispose of the pipet in the biohazard area designated by your instructor.

13. Gently mix the LB and the *E. coli* culture.

14. Proceed immediately to the Collect Data – All Experiments section.

Set Up: Initial Population Density

15. Obtain 3 sterile 50 mL culture chambers; label them "A", "B", and "C" using labeling tape and a marker.

16. Transfer 10 mL of Luria-Bertani (LB) broth to each chamber using a sterile transfer pipet.

17. Using another sterile transfer pipet, transfer 1 mL of overnight (O/N) *E. coli* culture to chamber A. Dispose of the pipet in the biohazard area designated by your instructor.

18. Now, work with your lab partners to determine how much *E. coli* you will inoculate chambers B and C with. It is up to your group, but make sure that one chamber has a less dense initial population than A and the other has a more dense population. Record the values in the space provided and in the first row of Table 3.

Chamber A: ___1.0__ mL Initial culture (~ 2 x 10^9/11-mL Total Volume)

Chamber B: ___2.0__ mL Initial culture (___~4x10^9___/11-mL Total Volume)

Chamber C: ___0.5__ mL Initial culture (__~1x10^9_____/11-mL Total Volume)

Note: Let students determine the density of the initial population. Because these cells divide asexually, the growth rates in each chamber should be the same, but the total number of bacteria will be different. This is a great opportunity to talk about population size and exponential growth. Even though *r* is the same, the larger populations will increase in size faster because there are more cells. The larger initial population will reach *K* faster, but all these populations should have roughly the same carrying capacity. This lab was piloted with 0.5 mL O/N culture in Chamber B, and 2.0 mL O/N culture in chamber C.

19. Using a new sterile pipet, transfer the appropriate amounts of *E. coli* to chambers B and C. Be sure to document the chamber codes and initial population volumes in Table 3. Immediately place the cap on the chamber and dispose of the pipet in the biohazard area designated by your instructor.

20. Gently mix the LB and the *E. coli* culture.

21. Proceed immediately to the Collect Data – All Experiments section.

Set Up: Temperature

22. Obtain 3 sterile 50-mL culture chambers; label them “A”, “B”, and "C" using labeling tape and a marker.

23. Transfer 10 mL of Luria-Bertani (LB) broth to each chamber using a sterile transfer pipet.

24. Using another sterile transfer pipet, transfer 1 mL of overnight (O/N) *E. coli* culture to chambers A, B, and C. Immediately place the cap on the chamber and dispose of the pipet in the biohazard area designated by your instructor.

25. Now, work with your lab partners to determine what temperature the *E. coli* populations will grow in. Chamber A will be placed in a 37 °C incubator. The other two are up to your group. Be sure one is colder and one is hotter. Record the values in the spaces provided and in the first row of Table 4.

Chamber A: ___37___°C

Chamber B: __42__ °C

Chamber C: __22__ °C

Note: Let students determine the incubation temperatures. They can pick a tight range (for example, 34 °C, 37 °C, 34 °C, 45 °C) or a broad range (for example 4 °C, 37°C, 45 °C,). This lab was piloted using room temperature (22 °C), optimal temperature (37 °C), and a simulated fever (42 °C).

26. If time permits, place a temperature probe in the location where your *E. coli* will grow so that you can determine whether the temperature gradients were maintained throughout the course of the experiment. (Do not insert a temperature probe into the culture chamber.)

27. Because *E. coli* needs oxygen to grow optimally, the cultures need to shake while they are incubating. If you have access to three shaking incubators all set to different temperatures, use them. Alternatively, you can vigorously shake each culture chamber for 5 seconds before placing the chamber in its experimental temperature. If you have to shake the chambers, shake them after each data point is collected and before the next 30 minute incubation.

28. Why do you think *E. coli* grows so well at 37 °C?

E. coli are adapted to live in the human colon, where the temperature is roughly 37 °C.

29. If time permits, take your temperature with a temperature probe by holding it in your hand. What is your temperature right now? Why is it different from the optimal temperature for *E. coli*?

Possible answers are 33 °C to 35 °C. Human core temperature is 37 °C. Our extremities are cooler because at room temperature we are constantly losing heat to our environment

30. Proceed immediately to the Collect Data – All Experiments section.

Collect Data – All Experiments

31. IMPORTANT: Make sure all samples have been inoculated with an overnight (O/N) culture of *E. coli*. Most samples will have the same total volume consisting of 10 mL of LB and 1 mL of *E. coli* culture.

32. Start a new experiment on the data collection system.

33. Connect a colorimeter to the data collection system.

34. Calibrate the colorimeter using a cuvette of distilled water.

35. Obtain 3 cuvettes. Label the tops “A”, "B", and “C”.

36. Using a sterile transfer pipet, transfer 5 mL of bacterial culture from your experiment’s chamber A to cuvette A.

37. Close the top of the cuvette, and invert slowly 1 time.

38. Wipe the cuvette down with a lint-free tissue to remove liquid, dust and fingerprints.

39. Hold the cuvette by the top and place into the colorimeter. Carefully, but firmly close the lid of the colorimeter. You should hear an audible “click.”

40. On the data collection system, note the value for absorbance of orange light (610 nm).\\ This is the wavelength traditionally used to measure *E. coli* growth. Leave the colorimeter on this setting.

41. Record the value from the colorimeter in the appropriate table (depending upon which part of the experiment you were assigned). If your instructor has an alternate method of collecting data, follow his or her instructions.

42. Transfer the liquid culture back into the correct culture chamber and begin the aerated incubation. If you do not have access to a shaking incubator, your instructor will direct you in how to incubate your samples. Make sure your incubation technique is consistent for all culture chambers.

43. Following the same procedure as with Chamber A, determine the absorbance for your experiment's chambers B and C using cuvettes B and C.

44. Record the colorimeter values in the appropriate table.

45. Transfer the B and C liquid samples back to their respective chambers and incubate as you did with Chamber A.

46. Allow culture chambers to incubate for 30 minutes, and then determine the absorbance for all chambers again.

47. Record the colorimeter values in the appropriate table.

48. Allow the chambers to incubate an additional 30 minutes and determine the absorbance for all chambers again. Repeat this step for the remainder of the laboratory period.

49. Record the colorimeter values in the appropriate table.

Note to students: You will not be able to record all the data necessary to complete the experiment. Other students or your instructor will finish the experiment for you. If possible, check in periodically to see how the bacterial populations are growing.

Note to instructors: Make sure students know these experiments extend past a traditional class or laboratory period. They have performed the most important parts of the experiment, now they are relying on others to continue to collect accurate data.

Before you finish for the day, make some predictions about what will happen in the culture chambers, and think about your group's experimental design.

50. Which population (A, B, or C) do you think will grow the fastest? Why? Be concise, but explain the reasons for your prediction.

Students completing the chamber size experiment may predict that the chamber with the largest volume will experience the fastest growth, because their habitats are less crowded and they complete with fewer organisms for the same nutrients.

Students completing the nutrient limitation experiment may predict that the chamber with the greatest nutrient concentration will experience the fastest growth, because this concentration of nutrients provided a constant supply of energy to a rapidly reproducing population.

In most cases, students completing the initial population density experiment may predict that the chamber with the highest initial population density will experience the most growth. This is because the more bacteria that are present in the population, the more organisms are available to reproduce and create more members of the population. This is true to a limit. If the initial population is so large that there are not enough nutrients to sustain the population, then this chamber will not show the fastest growth.

E. coli can grow in a range of temperatures form 7 °C to 50 °C, but optimal growth occurs at 37 °C. Students completing the temperature experiment should predict that the chamber kept at 37 °C exhibits the fastest growth.

51. Does your group's experiment have a "control"? What is it?

The experiment does not have a control, but it does have optimal conditions, and they are as follows:

Size: 50-mL chamber

Nutrient limitation: 10 mL LB

Initial population density: 1 mL overnight (O/N) culture

Temperature: 37 °C

A true control would be a 10 mL volume of LB measured for absorbance (610 nm) at 0 minutes and after 24 hours. It should be the same.

52. What is the independent variable for your group's experiment?

The independent variable was (depending on the group):

Size: size of the culture chamber

Nutrient limitation: amount of LB for the *E. coli*

Initial population density: the amount of overnight (O/N) culture inoculum

Temperature: temperature the culture chamber was placed in

53. What is the dependent variable for your group's experiment?

For all experiments, the dependent variable was the number of bacteria in culture over time, as indicated by the absorbance of orange light.

54. What are some of the constants for your group's experiment? Name at least three.

The constants in the experiments can include the following:

Size: temperature, volume of inoculums, 10 mL of LB, oxygenation,

Nutrient limitation: size of culture chamber, temperature volume of inoculums, oxygenation

Initial population density: size of culture chamber, 10 mL of LB temperature, oxygenation

Temperature: size of culture chamber, 10 mL of LB, volume of inoculums, oxygenation

55. Save your experiment and clean up according to your instructor's instructions.

Data Analysis

1. This is a "raw data" table. Complete the table by identifying your group's independent variable, filling in how the independent variable was changed for each chamber, the real time for each data collection point, the minutes from time 0, and the Absorbance of orange light (610 nm).

Table 1a: Raw data for the independent variable, container size

Title:____________________			**Chamber A:** __50__ **mL**	**Chamber B:** _250_ **mL**	**Chamber C:** _15__**mL**
Time Point	**Real Time**	**Minutes from Time 0**	**Absorbance of Orange Light (610 nm)**		
0	8:00 AM	0	0.25	0.25	0.25
1	8:30 AM	30	0.30	0.30	0.3
2	9:00 AM	60	0.40	0.40	0.35
3	9:30 AM	90	0.50	0.50	0.4
4	10:00 AM	120	0.60	0.60	0.5
5	10:30 AM	150	0.65	0.70	0.55
6	11:00 AM	180	0.75	0.80	0.6
7	11:30 AM	210	0.80	0.90	0.62
8	12:00 PM	240	0.90	1.00	0.65
9	12:30 PM	270	1.00	1.10	0.67
10	1:00 PM	300	1.10	1.15	0.7
11	1:30 PM	330	1.15	1.20	0.67

Table 1b: Raw data for the independent variable, nutrient limitation

Title:____________________			Chamber A: __70__% LB	Chamber B: _100_% LB	Chamber C: _50__% LB
Time Point	Real Time	Minutes from Time 0	Absorbance of Orange Light (610 nm)		
0	8:00 AM	0	0.15	0.15	0.15
1	8:30 AM	30	0.25	0.25	0.25
2	9:00 AM	60	0.35	0.35	0.35
3	9:30 AM	90	0.45	0.45	0.40
4	10:00 AM	120	0.50	0.50	0.45
5	10:30 AM	150	0.55	0.60	0.50
6	11:00 AM	180	0.65	0.70	0.60
7	11:30 AM	210	0.75	0.80	0.65
8	12:00 PM	240	0.85	0.90	0.75
9	12:30 PM	270	0.90	0.95	0.85
10	1:00 PM	300	1.00	1.10	0.90
11	1:30 PM	330	1.05	1.15	0.90

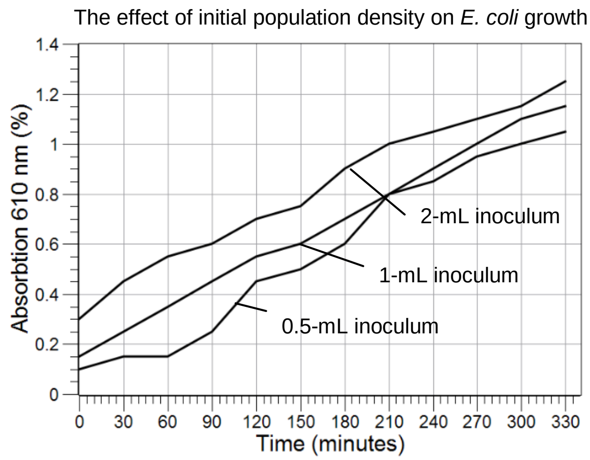
Table 1c: Raw data for the independent variable, initial population density

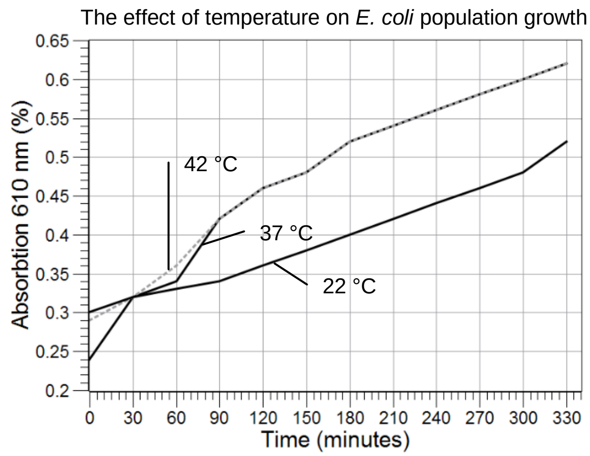
Title:____________________			Chamber A: _1.0__mL	Chamber B: _2.0_ mL	Chamber C: _0.5__mL
Time Point	Real Time	Minutes from Time 0	Absorbance of Orange Light (610 nm)		
0	8:00 AM	0	0.15	0.30	0.10
1	8:30 AM	30	0.25	0.45	0.15
2	9:00 AM	60	0.35	0.55	0.15
3	9:30 AM	90	0.45	0.60	0.25
4	10:00 AM	120	0.55	0.70	0.45
5	10:30 AM	150	0.60	0.75	0.50
6	11:00 AM	180	0.70	0.90	0.60
7	11:30 AM	210	0.80	1.00	0.80
8	12:00 PM	240	0.90	1.05	0.85
9	12:30 PM	270	1.00	1.10	0.95
10	1:00 PM	300	1.10	1.15	1.00
11	1:30 PM	330	1.15	1.25	1.05

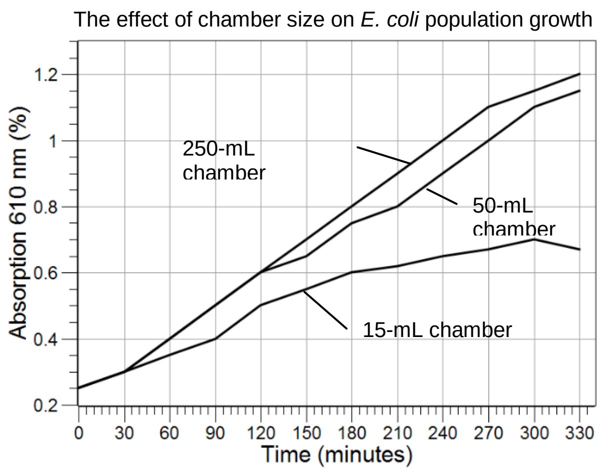
Table 1d: Raw data for the independent variable, temperature

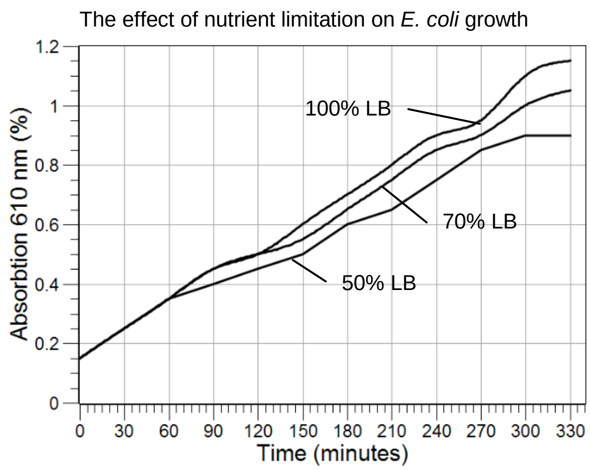
Title:____________________			Chamber A: __37__ °C	Chamber B: __42__ °C	Chamber C: __22__ °C
Time Point	**Real Time**	**Minutes from Time 0**	**Absorbance of Orange Light (610 nm)**		
0	8:00 AM	0	0.24	0.29	0.30
1	8:30 AM	30	0.32	0.32	0.32
2	9:00 AM	60	0.34	0.36	0.33
3	9:30 AM	90	0.42	0.42	0.34
4	10:00 AM	120	0.46	0.46	0.36
5	10:30 AM	150	0.48	0.48	0.38
6	11:00 AM	180	0.52	0.52	0.40
7	11:30 AM	210	0.54	0.54	0.42
8	12:00 PM	240	0.56	0.56	0.44
9	12:30 PM	270	0.58	0.58	0.46
10	1:00 PM	300	0.60	0.60	0.48
11	1:30 PM	330	0.62	0.62	0.52

2. Graph your group's data for Absorbance versus Time. Use a key to differentiate the three chambers. Label the overall graph, the x-axis, the y-axis, and include units on the axes.









3. Calculate the final population size (N_{final}) for your three chambers. Record these in Table 2, and show your work in the space below.

All chambers received 1 mL of the overnight (O/N) culture (except those in the population density experiment). One mL of O/N culture contains approximately 2 x 10^9 organisms, N_{initial}. Using N_{initial} and the initial and final absorbances, find the final population size.

Example: If 1 mL of culture contains approximately 2 x 10^9 organisms and has an initial absorbance of 0.25, what is the size of the population if the final absorbance is 1.20?

$N_{\text{final}} = (N_{\text{initial}} \times \text{Final Absorbance}) \div \text{Initial Absorbance}$

N_{final} = (2 x 10^9 organisms x 1.20)/0.25

N_{final} = 9.6 x 10^9 organisms

4. Calculate the change in the size of the population size (ΔN) for your three chambers. Record these in Table 5, and show your work in the space below.

$\Delta N = N_{\text{final}} - N_{\text{initial}}$

Example: ΔN = 9.6 x 10^9 organisms – 2 x 10^9 organisms

ΔN = 7.6 x 10^9 organisms

5. Calculate the rate of change in the population size (growth rate) for your three chambers. Record these in Table 5, and show your work in the space below.

Growth Rate (R) = $\Delta N/\Delta t$ (time)

Example: Growth Rate = 7.6×10^9 organisms ÷ 1.98×10^4 s

Growth Rate = 3.84×10^5 organisms/s

Table 2a: Absorbance (Abs.), population size, and growth rate for independent variable, container size

Cham-ber	Condition	Initial Abs.	Final Abs.	$N_{initial}$	N_{final}	ΔN	Growth Rate (R)
A	50 mL	0.25	1.15	2×10^9	9.2×10^9	7.2×10^9	3.64×10^5
B	250 mL	0.25	1.20	2×10^9	9.6×10^9	7.6×10^9	3.84×10^5
C	15 mL	0.25	0.67	2×10^9	5.36×10^9	3.36×10^9	1.70×10^5

Table 2b: Absorbance, population size, and growth rate for independent variable, nutrient limitation

Cham-ber	Condition	Initial Abs.	Final Abs.	$N_{initial}$	N_{final}	ΔN	Growth Rate (R)
A	100%	0.15	1.15	2×10^9	1.53×10^{10}	1.33×10^{10}	6.73×10^5
B	70%	0.15	1.05	2×10^9	1.40×10^{10}	1.2×10^{10}	6.06×10^5
C	50%	0.15	0.90	2×10^9	1.20×10^{10}	1×10^{10}	5.05×10^5

Table 2c: Absorbance, population size, and growth rate for independent variable, initial population density

Cham-ber	Condition	Initial Abs.	Final Abs.	$N_{initial}$	N_{final}	ΔN	Growth Rate (R)
A	1.0 mL	0.15	1.15	2×10^9	1.53×10^{10}	1.33×10^{10}	6.73×10^5
B	0.5 mL	0.10	1.05	1×10^9	1.05×10^{10}	0.95×10^{10}	4.80×10^5
C	2.0 mL	0.30	1.25	4×10^9	1.67×10^{10}	1.27×10^{10}	6.40×10^5

Table 2d: Absorbance, population size, and growth rate for independent variable, temperature

Cham-ber	Condition	Initial Abs.	Final Abs.	$N_{initial}$	N_{final}	ΔN	Growth Rate (R)
A	37 °C	0.24	0.62	2×10^9	5.17×10^9	3.17×10^9	1.60×10^5
B	22 °C	0.30	0.44	2×10^9	2.93×10^9	$.93 \times 10^9$	4.71×10^4
C	42 °C	0.29	0.62	2×10^9	4.28×10^9	2.28×10^9	1.15×10^5

Analysis Questions

1. For the experiment that you ran, compare the rate of change of population size (growth rate) for each chamber. Be sure to show the value of the independent variable for your experiment.

Examples:

Growth rates for independent variable, size of chamber:

Chamber A (50 mL): 3.64×10^5 organisms/s

Chamber B (250 mL): 3.84×10^5 organisms/s

Chamber C (15 mL): 1.70×10^5 organisms/s

Growth rates for independent variable, nutrient limitation:

Chamber A (100%): 6.73×10^5 organisms/s

Chamber B (70%): 6.06×10^5 organisms/s

Chamber C (50%): 5.05×10^5 organisms/s

Growth rates for independent variable, initial population density:

Chamber A (1.0 mL of the O/N culture): 6.73×10^5 organisms/s

Chamber B (0.5 mL of the O/N culture): 4.80×10^5 organisms/s

Chamber C (2.0 mL of the O/N culture): 6.40×10^5 organisms/s

Growth rates for independent variable, temperature:

Chamber A (37 °C): 1.60×10^5 organisms/s

Chamber B (22 °C): 4.71×10^4 organisms/s

Chamber C (42 °C): 1.15×10^5 organisms/s

2. Which of the three chambers in your experiment experienced the fastest rate of growth? The slowest rate of growth? Why do you think that your variable affected growth in this way?

In the Size of Chamber Experiment, Chamber B (250 mL) had the fastest rate of growth: 3.84×10^5 organisms/s. Students should indicate that this chamber size provided the bacteria with ample room to live and reproduce. Smaller chambers limited the space available for reproduction.

In the Nutrient Limitation Experiment, Chamber A (100%) had the fastest rate of 6.73×10^5 organisms/s. Students should indicate that this concentration provided optimal nutrient levels for the bacteria to grow and reproduce. Decreasing the nutrient availability caused organisms to compete for food; some organisms were able to obtain nutrients, survive, and reproduce, while others were not.

In the Initial Population Density Experiment, Chamber A (1.0 mL) had the fastest rate of 6.73×10^5 organisms/s. Students should indicate that this initial population was the optimal size for population increase. A smaller population size takes longer show an initial increase in size, and a larger population can become overcrowded and cause competition among the organisms.

In the Temperature Experiment, Chamber A (37°C) had the fastest rate of growth: 1.60×10^5 organisms/s. Students should indicate that this was the optimal temperature for population growth. Increased and decreased temperatures inhibit enzyme activity and limit population growth.

3. Ask other groups to share their data with you and compare their growth rates to the rates that you calculated for your experiment. Which variable positively affected the growth of *E. coli* the most? Why do you think this factor had such a significant effect?

The chambers with 100% nutrient availability and an initial population density of 1.0 mL showed the fastest rates of growth: 6.73×10^5 organisms/s. These bacterial populations grew quickly because the initial low population density decreased competition for nutrients and oxygen. 100% nutrient availability was optimal for population growth.

4. Ask other groups to share their data with you and compare their growth rates to the rates that you calculated for your experiment. Which variable limited the growth of *E. coli* the most? Why do you think this factor had such a significant effect?

The bacteria incubated at room temperature (22 °C) had the slowest rate of growth, 4.71×10^4 organisms/s. Population growth was limited in this chamber because the enzymes necessary for bacterial respiration were operating at suboptimal temperatures.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Why did you measure the absorbance of orange light in the colorimeter?

LB broth transmits 100% of orange light or absorbs 0%; therefore, any increase in absorption is caused by increased bacterial growth.

2. Describe three specific limits on population growth for a real population in its natural habitat.

Some examples:

1) A population of lodgepole pines (*Pinus contorta*) in a mountain ecosystem in Colorado can be limited by herbivory, pine beetles, lack of fire to stimulate germination of pine seeds, and thin soils that are low in nutrients.

2) Moose populations on Isle Royale, Michigan, can be limited by predation from wolves, lack of available food because of harsh winters and a short growing season, and low reproductive rates caused by increased gestation period and increased amount of parental care.

3) Phytoplankton populations in an oligotrophic lake can be limited by available nitrogen and phosphorus in the photic zone, low water temperature during late spring and late fall, and predation by zooplankton.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. A small population of white-footed mice has the same intrinsic rate of increase (*r*) as a large population. If everything else is equal,

- **A. The large population will add more individuals per unit time.**
- **B.** The small population will add more individuals per unit time.
- **C.** The two populations will add equal numbers of individuals per unit time.
- **D.** The J-shaped growth curves will look identical.
- **E.** The growth trajectories of the two populations will proceed in opposite directions.

2. Carrying capacity (*K*)

- **A.** Is calculated as the product of annual per capita birth rate (r)
- **B.** Remains constant in the presence of density-dependent population regulation
- **C.** Differs among species, but does not vary within a given species
- **D. Is often determined by energy limitation**
- **E.** Is always eventually reached in any population

3. As *N* approaches *K* for a certain population, which of the following is predicted by the logistic equation?

- **A.** The growth rate will not change.
- **B. The growth rate will approach zero.**
- **C.** The population will show an allele effect.
- **D.** The population will increase exponentially.
- **E.** The carrying capacity of the environment will increase.

Extended Inquiry Suggestions

Students can design their own experiments to measure growth rate and carrying capacity of *E. coli*. Suggestions include trying a different growth media, adding an antibiotic, adding an antiseptic, or adding a predator, such as a protist.

If you have transformed bacteria from another lab (for example, Lab 6A), you can grow overnight (O/N) cultures of transformed cells and non-transformed cells, then compare growth rates of both populations under standard conditions (37 °C with aeration). Explore whether or not harboring a plasmid slows the growth of the transformed cells. If you have ampicillin or another antibiotic, add it to the culture during the rapid–growth rate phase of the growth curve. Students can compare the effects of the antibiotic on transformed versus non-transformed cells.

24. *Elodea* and the Snail

Objectives

- In this experiment, students investigate the interrelationships that exist between photosynthetic and aerobic organisms. Students explore:
- The processes of cellular respiration and photosynthesis
- The interdependence of respiration and photosynthesis
- How oxygen molecules cycle through respiration and photosynthesis

Procedural Overview

Students gain experience conducting the following procedures:

- Modeling environmental changes using an aquatic snail and an aquatic plant
- Using the pH probe to measure pH changes in a series of simulated closed systems containing various organisms

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	15 to 20 minutes
♦ Lab experiment	Day one: 45 minutes Day two: 15 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Advanced water quality sensor with pH probe
- Large test tubes (4)
- Test tube stoppers (4)
- *Elodea* sprigs (2 to 4)
- Aquatic snails (2)
- Standard buffers pH 4 and pH 10
- Bromothymol blue solution in dropper bottles[1]
- Test tube rack
- De-chlorinated water, 500 mL[2]
- Drinking straw
- Marker
- Labeling tape

[1] To prepare bromothymol blue, see Lab Preparation Section.

[2] To prepare de-chlorinated water, see Lab Preparation Section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- Photosynthesis
- Cellular respiration

- Basic principles of photosynthesis in plants
- Basic principles of respiration in plants and animals
- The relationship between carbon dioxide, pH, and buffers

Related Labs in This Guide

Labs conceptually related to this one include:

- pH and Buffers
- Interrelationship of Plants and Animals

Background

Oxygen gas comprises approximately 21 percent of the earth's atmosphere. Aerobic organisms use oxygen from their environment for aerobic respiration, which releases energy from food. Most aerobic organisms depend on the action of photosynthetic organisms for oxygen. Photosynthesis converts carbon dioxide gas into sugars and splits water, releasing oxygen into the environment. Aerobic organisms use the oxygen for cellular respiration. This respiration produces carbon dioxide gas as a waste product. These reactions are shown as follows:

Photosynthesis: $6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2$

Respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$

Students will create a series of closed systems to observe the interrelationships that exist between photosynthetic and aerobic organisms. In the process, they will discover how each organism affects the level of CO_2 in a test tube of water. They will use bromothymol blue, a pH indicator. Under acidic conditions, this indicator turns yellow. In the presence of a neutral substance, bromothymol blue turns green. In the presence of an alkaline substance, it turns blue. CO_2 is soluble in water and will spontaneously form carbonic acid, H_2CO_3. The acid in turn dissociates, releasing H^+ into the water, causing a decrease in pH as follows:

$$CO_2(g) + H_2O(l) \rightleftharpoons H_2CO_3(aq) \rightleftharpoons H^+(aq) + HCO^{3-}(aq).$$

Pre-Lab Discussion and Experiment

Start the lesson by showing students a picture of a biosphere. Discuss the factors they need to consider for this system to be self-sustained.

Provide a brief overview of the experiment: This lab allows students to discover how plants and animals affect the level of CO_2 of the water, which affects the pH of the water.

Demonstrate to students how bromothymol blue indicates pH: Fill two 50-mL beakers about 1/3 full with water. Measure the pH of the water using the pH sensor. Add about 10 to15 drops of bromothymol blue to each beaker. The water should turn blue. Gently blow through a straw into the solution of one beaker. It should turn yellow. Continue to blow until there is no more color change. Measure the pH of the water again. Ask students to propose their own explanations of what occurred in this demonstration.

Explain that bromothymol blue is an indicator, a substance that allows us to visualize chemical changes by changing color.

Discuss the changes in pH that occurred after blowing into the water. Talk to students about the gases that are found in your exhaled air and how they affect the pH of the water.

Explain to the students that they will be answering the question: "What is the biological relationship between an aquatic snail and an aquatic plant?" They will discover how each organism affects the level of CO_2 in a tube of water.

Discuss the appropriate handling and respect of living organisms.

Ask students to graph their prediction of pH changes over time before beginning the experiment.

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Prepare a 0.1% weight per volume solution of bromothymol blue by adding 0.1 g bromothymol blue per 100 mL of distilled water.
2. Prepare de-chlorinated water by filling a large vessel with tap water and letting it stand, uncovered, on the counter for several days. You can also purchase spring water.
3. Purchase *Elodea* and freshwater aquatic snails from a biological supply company or local aquarium store.
4. For short-term care of the aquatic snails, store them in dechlorinated water with a few sprigs of *Elodea* or an algal culture. For long-term care, transfer the snails to an established aquarium. Their diet can consist of either algae and freshwater plants or lettuce. You can also provide a block of plaster of paris (check that the plaster doesn't contain other chemicals) to enhance shell growth.
5. You can use large test tubes with stoppers, large culture tubes, mason jars, or small plastic bottles with screw tops for this experiment.

Note: The tubes should be left for at least 24 hours, but may be left for up to 3 days for more dramatic changes in pH.

Safety

Add these important safety precautions to your normal laboratory procedures:

♦ Keep water away from electrical outlets.

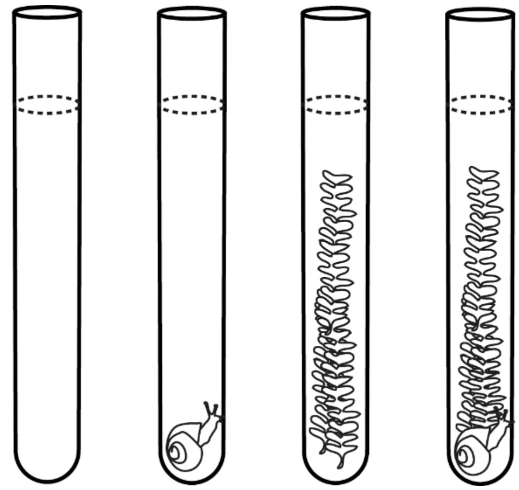
Procedure

Day One

Set Up

1. Start a new experiment on the data collection system.
2. Connect the pH sensor to the data collection system.
3. Use the pH 4 and pH 10 buffer solutions to calibrate the pH sensor.
4. Display pH concentration in a digits display.
5. Label four test tubes "1," "2," "3," and "4."
6. Fill each test tube about three-quarters full with water.
7. Add 10 drops of the bromothymol blue solution to each of the four test tubes.

8. Have one person use the straw to carefully blow into each vial until the color stops changing.



9. Record the color of each in Table 1.

10. Add one snail to tube #2.

11. Add two sprigs of *Elodea* to tube #3.

12. Add two sprigs of *Elodea* and one snail to tube #4.

Note: You will add nothing to test tube #1.

Collect Data

13. Measure the pH of each test tube and record the initial pH measurements in Table 1.

14. Seal all of the tubes so no air can enter or escape.

15. What do the color change and the decrease in pH tell you about the level of CO_2?

The decrease in pH and the color change result from increased CO_2 levels, which in turn increase the amount of carbonic acid in the water. CO_2 is soluble in water and will spontaneously form carbonic acid (H_2CO_3). The carbonic acid, in turn, dissociates, releasing H^+ into the water, causing the decrease in pH.

$$CO_2(g) + H_2O(l) \rightleftharpoons H_2CO_3\ (aq) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

16. Place the test tubes near a light source for at least 24 hours. Make sure that the tubes will not get too hot during the incubation.

Note: If you place your hand in front of the test tube and can feel the warmth of the light, then the light is too close.

17. Predict how the pH may change in each of the tubes over the next 24 hours. Record your predictions in the "pH Prediction." column of Table 1.

Student answers will vary. The test tube containing the snail will show a decrease in pH because of the accumulation of carbon dioxide. The tube with the snail and the *Elodea* should not decrease in pH because the *Elodea* will use the carbon dioxide in photosynthesis.

Day Two

Collect Data

18. Observe the color of the liquid in the test tubes after 24 hours, and record the color in Table 1.

19. Unseal the tubes, measure the pH of each, and record these final pH values in Table 1.

Data Analysis

1. Record the final color and final pH values in Table 1.

2. Calculate the change in pH. Change in pH = final pH – initial pH.

Table 1: Color and pH

Test Tube	Description	Initial Color	Final Color	pH Prediction	Initial pH	Final pH	Δ pH
1	Water only	Yellow	Yellow	Will vary	6.4	6.6	+0.2
2	*Elodea* only	Yellow	Blue/green	Will vary	6.4	7.3	+0.9
3	Snail only	Yellow	Yellow	Will vary	6.4	5.9	–0.5
4	*Elodea* and snail	Yellow	Blue/green	Will vary	6.4	7.6	+1.2

Analysis Questions

1. Use your knowledge of the behavior of CO_2 in water to explain what happened to the pH in each tube.

Tube #1: This tube shows only minor changes in pH because there are no organisms present that produce or consume CO_2. The slight change in pH is a result of the reversal of the reaction that released H^+ ions from carbonic acid.

Tube #2: Through the process of photosynthesis, the *Elodea* absorbs the CO_2 added to the tube, causing a rise in pH.

Note: Students typically neglect to mention that the *Elodea* undergoes cellular respiration and produces CO_2 as well.

Tube #3: The snail produces CO_2 through cellular respiration. The additional CO_2 from the snail causes the pH to decrease.

Tube #4: The snail produces CO_2. The *Elodea* absorbs the CO_2. This causes a fluctuation in the pH, depending on the ratio of photosynthesis rates to respiration rates. Students may miss the fact that the *Elodea* is also producing CO_2.

2. How do snails affect the level of carbon dioxide in the water?

The snail increases the level of CO_2 by producing carbon dioxide through cellular respiration.

3. How does *Elodea* affect the level of carbon dioxide in the water?

The *Elodea* can cause a decrease in CO_2 levels by using the CO_2 for photosynthesis. It is important to note that the *Elodea* also undergoes respiration, and therefore also causes CO_2 levels to rise.

4. What is the purpose of tube #1?

Tube #1 is a control. This tube will show little or no changes in pH because no organism consumes or produces CO_2.

5. If the pH has changed in tube #1, how do you explain it?

The slight change in pH results from the reversal of the reaction that released H^+ ions from carbonic acid:

$CO_2(g) + H_2O(l) \rightleftharpoons H_2CO_3(aq) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$.

6. Which tube best illustrates a balanced system? Why?

Tube #4 is a balanced system when the amount of CO_2 consumed in photosynthesis equals the amount of carbon dioxide produced during respiration.

Synthesis Questions

1. How do plants and animals depend on each other?

Animals depend on plants for oxygen (and as a food source). Students will most likely answer that plants depend on animals for carbon dioxide. It is important to convey that plants can produce sufficient amounts of carbon dioxide through respiration without the assistance of animals.

2. What would happen if the experiment were repeated in the dark?

In the dark, the *Elodea* would only undergo respiration. The light reactions of photosynthesis require sunlight. Without the absorption of CO_2 for photosynthesis, the tubes containing *Elodea* would show decreased pH.

3. What would you expect to happen to the pH of test tube #3 if the snail died shortly after being placed in the tube? Explain your answer.

As soon as the snail dies, decomposition begins to occur. Aquatic microbes will begin to break down the snail's tissue and release CO_2 in the process. As CO_2 levels increase, the pH will decrease.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Carbon dioxide is produced during

- **A.** Glycolysis.
- **B.** The light reactions of photosynthesis.
- **C.** The Calvin cycle.
- **D. The Krebs cycle.**
- **E.** The electron transport chain.

2. What is molecular oxygen (O_2) used for in aerobic respiration?

- **A.** At the end of glycolysis in order to oxidize pyruvate
- **B.** At the end of the citric acid cycle in order to regenerate citric acid
- **C.** Between glycolysis and the citric acid cycle, in order to split a carbon from pyruvate and produce a CO_2 molecule
- **D.** As a source of O_2 in every reaction that produces CO_2
- **E. At the end of the electron transport chain in order to accept electrons and form H_2O**

3. Which of the following statements best describes the relationship between photosynthesis and respiration?

- **A.** Respiration is the exact reversal of the biochemical pathways of photosynthesis.
- **B. Photosynthesis stores energy in complex organic molecules, while respiration releases it.**
- **C.** Photosynthesis occurs only in plants and respiration occurs only in animals.
- **D.** ATP molecules are produced only in photosynthesis and used only in respiration.
- **E.** Respiration is an anabolic process and photosynthesis is a catabolic process.

Extended Inquiry Suggestions

Repeat the experiment with varying amounts of light (or completely in the dark).

Students can also conduct this experiment using the dissolved oxygen (DO) sensor.

Students could alter their experiments to see if the rates of photosynthesis and respiration change with different amounts of *Elodea* and numbers of snails.

25. Interrelationship of Plants and Animals

Objectives

Students explore the relationship between plants and animals in an ecosystem.

- Students explore the roles of producers, consumers, and decomposers and their effects on the cycling of gases in a closed system, the EcoChamber.
- Students monitor the concentrations of oxygen (O_2) and carbon dioxide (CO_2) gas.

Procedural Overview

Students gain experience conducting the following procedures:

- Creating a terrestrial ecosystem in an EcoChamber.
- Measuring changes in O_2 and CO_2 gas levels in the EcoChamber.
- Designing and conducting a controlled experiment.

Time Requirement

♦ Preparation time	20 minutes
♦ Pre-lab discussion and experiment	20 minutes
♦ Lab experiment – Part 1	75 minutes
♦ Lab experiment – Part 2	75 minutes

Materials and Equipment

For each student or group:

- Data collection system
- Oxygen gas sensor
- Carbon dioxide gas sensor
- Sensor extension cable
- EcoChamber
- Small plant, variety [1]
- Small animal, variety[2]
- Soil, potting or other (2 to 3 cups)

[1]See the Lab Preparation section for small plant suggestions.

[2]See the Lab Preparation section for small animal suggestions.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- The basic principles of photosynthesis and cellular respiration
- The basic principles of the carbon and oxygen cycles
- The role of producers, consumers, and decomposers in ecosystems

Related Labs in This Guide

Labs conceptually related to this one include:

- Elodea and the Snail
- Measuring Aerobic Cellular Respiration in Yeast
- Fermentation in Yeast
- Factors that Limit Photosynthetic Activity
- Plant Pigments and Photosynthesis
- Cellular Respiration

Background

Ecosystems involve complex interactions between the nonliving (abiotic) and living (biotic) parts of the environment. The flow of energy and cycling of matter in an ecosystem directly impacts the success of the organisms in the ecosystem. Living things not only respond to their environment but also cause change in the abiotic and biotic factors in their environment.

An EcoChamber is a simplified ecosystem model in which some of the complex interactions that occur within an ecosystem can be simulated. A terrestrial ecosystem, such as the one constructed for this experiment, consists of an energy source, soil, water, a plant, and an animal.

Plants are producers or photosynthetic organisms. Like algae, plants have the ability to harness light energy from the sun. They use this energy, together with carbon dioxide gas from the atmosphere to drive the synthesis of carbohydrates summarized as follows:

$$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2.$$

Cellular respiration breaks down these sugars to generate energy in the form of ATP. During cellular respiration, the energy stored in glucose is released and harnessed to phosphorylate ADP, producing ATP. In the presence of oxygen (a "waste" product in photosynthesis), glucose can be fully oxidized, releasing large amounts of energy, summarized as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}.$$

The process of cellular respiration uses oxygen and produces water and carbon dioxide gas as waste products. Organisms that use oxygen for the breakdown of glucose are called aerobic organisms. Plants and animals are both examples of aerobic organisms.

Pre-Lab Discussion and Experiment

Engage students in discussion about the following topics.

Gauge your students understanding of the cycling of gases in the atmosphere and the roles animals and plants play in the carbon dioxide/oxygen cycle. They should have a good understanding of the carbon and water cycles.

Getting students to predict and explain the changes that will occur in the EcoChamber is essential to the success of the experiment. Students should also know the composition of the atmosphere; particularly, oxygen (21%) and carbon dioxide (0.04%).

To help your students start thinking about what will happen in the EcoChamber, discuss the following questions with them. (There are no right or wrong answers.)

1. What volume of air would be necessary to sustain a plant and some small animals?

2. How would the size, type, or color of the plant influence the concentrations of gases detected in the EcoChamber over time?

3. How many organisms (for example, crickets) does it take to consume all the oxygen produced by the plant in the EcoChamber?

Lab Preparation

These are the materials and equipment to set up prior to the lab.

1. Suggested small plants: pothos, coleus, tomato, basil, or other herbs in small pots. Any plant in a pot small enough to fit into the EcoChamber will suffice. You can buy these plants at a nursery, supermarket, a home improvement store, or even your local grocery store.
2. Suggested animals: crickets, pillbugs, earthworms, land snails, or hissing cockroaches. The animal must be small enough to fit several in the EcoChamber. You can buy crickets, earthworms, and snails at a pet store. You may have to order pillbugs and hissing cockroaches from a biological supply company. Or you can go outside and have your students dig for and collect pillbugs.
3. To make this lab shorter, you could do part one as a demo and then have the students only do part two. If you would like students to do both parts but your class is a 45 or 55 minute block, allow them to set up the experiment, but let the data collection continue even after class has ended. After one hour, you can stop data collection and save their data.

Instructor Tip: Do not release plants and animals into the environment unless you know that they are species native to your area.

Safety

Add this important safety precaution to your normal laboratory procedures:

- Treat plants and animals with respect. When finished with the lab, place them in the area that your instructor designates.

Procedure

Part 1 – Getting to know the EcoChamber

Set Up

1. Start a new experiment on the data collection system.

2. Connect an oxygen gas sensor and a carbon dioxide gas sensor to the data collection system. Use a sensor extension cable to connect the carbon dioxide sensor.

3. Calibrate the oxygen gas sensor.

4. Calibrate the carbon dioxide gas sensor.

5. Display two graphs simultaneously. On one graph, display CO_2 on the y-axis with Time on the x-axis. On the second graph, display O_2 on the y-axis with Time on the x-axis.

6. Place the EcoChamber in an area with natural lighting.

7. Fill the bottom of the EcoChamber with two to three cups of soil. Slightly dampen the soil with water.

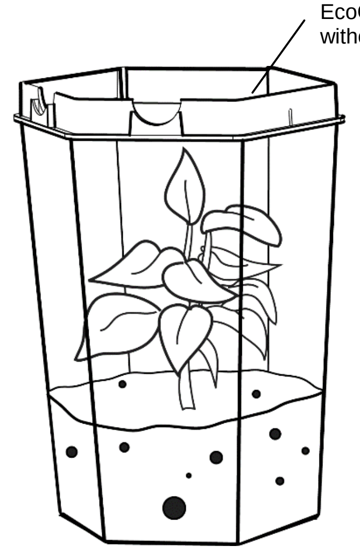
8. Create a depression in the soil large enough to accommodate the plant.

9. Place the plant in the soil.

10. Seal the EcoChamber by placing the top securely on the chamber.

11. Why is it important to seal the EcoChamber?

In order to observe the interactions of the organisms and cycling of matter in the EcoChamber, it is essential that it be closed off from the surrounding environment.



12. Insert the oxygen and carbon dioxide sensors into two of the holes on the top of the EcoChamber. All other holes should be sealed with a rubber stopper.

Note: It is essential that the sensor's rubber stopper fit snuggly into the hole so that no gases escape.

Collect Data

13. Start data recording.

14. Adjust the scale of the graphs to show all data.

15. Record both O_2 and CO_2 gas readings continuously for 1 hour.

16. Predict how the O_2 and CO_2 levels may change over this hour period.

Students should indicate that O_2 will be produced by the plant through photosynthesis and consumed by the animal and the plant during respiration. They should also predict that CO_2 will be produced by the animal and plant during respiration and consumed by the plant during photosynthesis. Since the rate of CO_2 consumption in photosynthesis is greater than the rate of production in respiration, there is a net consumption of CO_2.and students should predict a decrease in CO_2 and an increase in O_2.

17. After 1 hour, stop data recording.

18. Did the CO_2 levels fluctuate? Describe what you observed. Can you explain these changes?

Students might observe a decrease in CO_2 levels as the plant consumes CO_2 during photosynthesis. Plants also produce CO_2 through cell respiration, but the rate of consumption in photosynthesis may be greater than the rate of production in respiration. Therefore, there may be a net consumption of CO_2. Alternatively, CO_2 levels may increase if the rate of cellular respiration by the plant and the soil organisms is greater than the rate of photosynthesis by the plant, as was the case in the sample provided.

19. Did the oxygen levels fluctuate? Describe what you observed. Can you explain these changes?

Students might observe O_2 levels increasing as the plant produces O_2 during photosynthesis. Plants are also consuming O_2 through cell respiration, but the rate of production in photosynthesis may be greater than the rate of consumption in respiration. Therefore, there may be a net consumption of O_2. Alternatively, O_2 levels may decrease if the rate of cellular respiration by the plant and the soil organisms is greater than the rate of photosynthesis by the plant, as was the case in the sample provided.

20. Name the data run, "Plant only".

21. Save your experiment, and clean up according to your instructor's instructions.

Note: If you will complete Part 2 during another class period, label the plant you used with your name or initials. You will need to use the same plant during the next part of the lab.

22. Complete the Data Analysis section for Part 1.

Part 2 – Student-designed experiment

Set Up

In this part of the experiment, you will design a controlled experiment to test your hypothesis for the following question: How does an animal affect the environment of a plant? Complete the steps below to help you organize your investigation.

23. State your hypothesis. It should indicate to the reader that you believe in one particular answer to the question stated above.

Example: If the animal undergoes cell respiration at a rate higher than the photosynthetic rate of the plant, then CO_2 gas levels in the EcoChamber will rise and O_2 gas levels will not.

24. Define the variables that you will be testing. Indicate the independent and dependent variables.

Example: The independent variable is the presence or absence of an animal. The dependent variables are the levels of CO_2 and O_2 gas in the EcoChamber.

25. List the materials you will need to carry out the experiment. You may use any materials in the lab, but you must use all of the following materials: EcoChamber, O_2 and CO_2 gas sensors, your plant from part one, and an animal. Your instructor will provide you with a variety of animal choices.

Example: EcoChamber, CO_2 gas sensor, O_2 gas sensor, sensor extension cable, small plant from Part One, 10 crickets

26. Decide what data you will collect and how you will organize that data.

Example: We will measure O_2 gas and CO_2 gas. We will organize the data in two graphs, CO_2 versus time and O_2 versus time.

27. Provide a detailed outline of your procedure. Your instructor must approve your procedure before you begin your experiment.

Example: Connect the CO_2 sensor and the O_2 sensor to the data collection system. Insert them into the top of the EcoChamber. Place the plant and 5 crickets in the EcoChamber. Tip the EcoChamber slightly so as not to dump the animal(s) straight to the bottom. Put the top on the EcoChamber; ensure the chamber is tightly sealed. Begin collecting data. Collect O_2 and CO_2 gas readings for 1 hour. After 1 hour, stop collecting data.

Collect Data

28. Conduct the experiment.

29. Name this data run, "Plant and animal"

30. Save your experiment, and clean up according to your instructor's instructions.

31. Complete the Data Analysis section for Part 2.

Sample Data

Sample Data Table 1: Rate of change (plant only)

	Rate (1 hour)	**Rate (24 hours)**
O_2 gas (%/s)	–0.04	–0.05
CO_2 gas (ppm/s)	0.24	0.04

Sample Data Table 2: Rate of change (plant and animal)

	Rate (1 hour)	**Rate (24 hours)**

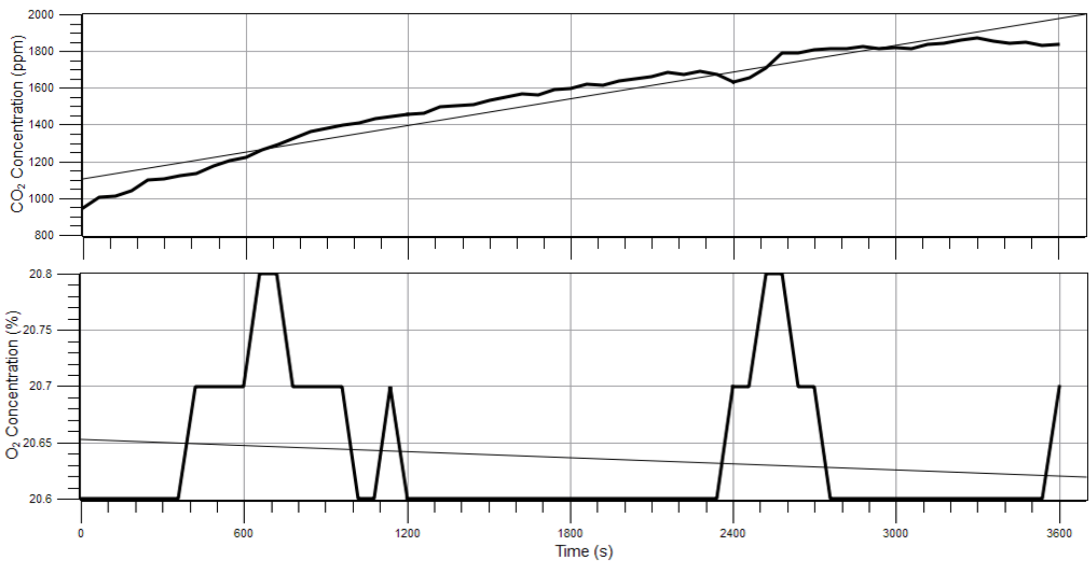
O_2 gas (%/s)	–0.42	–0.13
CO_2 gas (ppm/s)	0.19	0.14

Instructor Note: These tables include for your information, sample data for 1 hour of data collection as well as for 24 hours of data. Gas levels change significantly over a 24-hour period, so it is valuable to see how the data differs.

Data Analysis

Part 1 – CO_2 concentration (ppm) and O_2 concentration (%) for the plant-only setup

1. Sketch graphs of CO_2 gas and O_2 gas versus Time for Part One. To create an appropriate scale for your graph, select a range of numbers and fill in the values on the x and y axes.



CO_2 concentration (ppm) and O_2 concentration (%) for the plant-only setup

2. Apply a linear fit to the O_2 graph of the "Plant only" run, and sketch this line on the graph above.

3. Record the slope as the "rate" in Table 1.

4. Apply a linear fit to the CO_2 graph of the "Plant only" run, and sketch this line on the graph above.

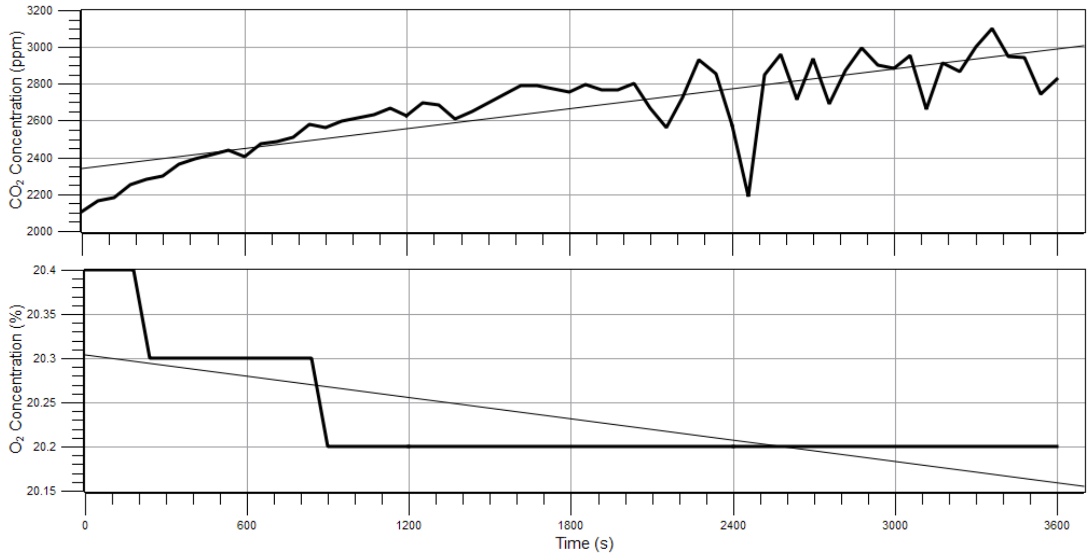
5. Record the slope as the "rate" in Table 1.

Table 1: Rate of change in O_2 and CO_2 gas concentrations during 1 hour of data collection

	Rate of Change	
	Plant only (Part 1)	**Plant and animal (Part 2)**
O_2 gas (%/s)	–0.04	–0.42
CO_2 gas (ppm/s)	0.24	0.19

Part 2 – Student-designed experiment

6. Sketch graphs of CO_2 gas and O_2 gas versus Time for Part Two. To create an appropriate scale for your graph, select a range of numbers and fill in the values on the x and y axes.



CO_2 concentration (ppm) and O_2 concentration (%) plant and 5 crickets (1 hour)

7. Apply a linear fit to the O_2 graph of the "Plant and animal" run, and sketch this line on the graph above.

8. Record the slope as the "rate" in Table 1.

9. When you have collected, recorded, and organized all of your data, present it in a form that will help others to understand it. Your data analysis MUST include an analysis of the O_2 and CO_2 levels in the EcoChamber.

10. Write a conclusion. Be sure you include whether your experiment verified your hypothesis.

Example: The data show that the rate of consumption of O_2 was much faster when crickets were added to the setup than without the crickets. This finding supports our hypothesis that the additional cellular respiration by the added insects would result in a faster rate of oxygen gas consumption. However, the rate of increase in CO_2 gas was slower after the insects were added, which does not support our hypothesis that the rate of CO_2 gas production would increase after the addition of the insects due to increased cellular respiration in the system. Some possible explanations for this include inadequate control of variables, such as temperature and light level, which could affect the CO_2 production rates of all the organisms in the EcoChamber. Another variable that was not controlled was the initial CO_2 gas level (about 950 versus 2100 ppm) in the two trials. Overall, we conclude that adding the insects to the EcoChamber caused oxygen gas levels to reduce faster because of increased amounts of cellular respiration in the system. I also conclude that perhaps some of the variables in the experiment need to be better controlled to understand what the effect on CO_2 gas levels of adding animals to the system is.

Analysis Questions

1. Compare the rates of CO_2 and O_2 levels in the experiment you designed with the rates that you calculated in part one.

Answers will vary based on the experiment that the student designs. In the sample data, the rate of carbon dioxide production was greater in the plant only EcoChamber than in the presence of an animal. In the sample data, the rate of oxygen consumption was greater in the presence of the animal.

2. What can you conclude based on the differences between the rates of CO_2 and O_2 production in Parts 1 and 2?

Students should use their data to make a statement about the rate of change of the two gases. In the sample data, the EcoChamber with the animal showed a slight decrease in the rate of carbon dioxide production over one hour. However, if you observe the data over 24 hours, you should observe an increased rate of CO_2 production in the presence of an animal. The rate of oxygen consumption should increase in the presence of an animal, as they consume greater amounts of oxygen during respiration.

3. Compare your data with other members of the class. How does your data compare with others? Were some EcoChambers more stable than others? Can you explain any differences that you see?

Differences in the stability of the chambers will depend on the number and type of plants and animals used.

4. Compare your model ecosystem to a real ecosystem. List a few similarities and a few differences between the two types of ecosystems.

EcoChambers are obviously smaller and have a much lower species diversity and abundance than a real ecosystem. EcoChambers are also more susceptible to small changes in the microenvironment. The EcoChamber is similar to a real ecosystem in that they both contain biotic and abiotic factors interacting with and depending upon each other.

Synthesis Questions

Use available resources to help you answer the following questions.

1. How large would an EcoChamber need to be to effectively sustain a small mammal like a mouse? How would energy flow and cycling of nutrients be implemented in the design?

Students should describe a model that would allow enough food, water, and shelter for survival of the organism.

2. Do you think the animal was the only source of CO_2 in this system? Could CO_2 have been produced by other organisms in the system?

Although the animal was the largest contributor of CO_2 in the system, it was not the only contributor. Plants photosynthesize, but they also respire, so a small amount of the CO_2 measured may have been produced by the plant. Also, there are a tremendous number of bacteria and fungi living in the soil. They are essential to the survival of the plant, and they play an essential part in the nitrogen cycle. The bacteria in the soil were decomposing small bits of dead organic material and produced CO_2 during this process.

3. Atoms like carbon and oxygen cycle between the living and nonliving components of the environment. Does energy cycle in this same way? Explain your answer.

No. The second law of thermodynamics states that energy is neither created nor destroyed, it is only transferred. The third law of thermodynamics states that all systems move towards disorder. In ecological terms, that means that light energy from the sun is captured by plants in an ordered state and every transfer after this point changes the energy into a less ordered form. For example, at each step of a food chain, energy is lost to the environment as heat, used by the organism in its daily activities, or lost during chemical conversions.

Multiple Choice Questions

Select the best answer or completion to each of the questions or incomplete statements below.

1. Plants and other producers are always at the bottom of ecological pyramids. Why?

- **A.** Plants are producers and get their energy from the top levels.
- **B.** Plants are consumers and get their energy from the top levels.
- **C. Plants are producers and therefore supply energy to the levels above.**
- **D.** Plants are decomposers and get energy from all levels of the pyramid.
- **E.** Plants are consumers and therefore supply energy to the levels above.

2. The maintenance of a self-sustaining ecosystem requires:

- **A.** Constant temperature
- **B.** A greater number of herbivores than producers
- **C. Cycling of materials between organisms and their environment**
- **D.** Soil with an acidic pH
- **E.** Soil with a neutral pH

3. What is the best reason why mostly consumers and very few producers are found at great ocean depths?

- **A.** In deep water, consumers ingest any producer at a rate that prevents reproduction of the producers.
- **B. Photosynthesis requires light.**
- **C.** Increased pressure favors the survival of heterotrophs over autotrophs.
- **D.** Autotrophs are independent of heterotrophs.
- **E.** The temperature at the bottom of the ocean is too cold for plants to survive.

Extended Inquiry Suggestions

Use the weather sensor to measure changes in temperature and humidity in the EcoChamber.

Add a light source and measure the changes in CO_2 and O_2 in the EcoChamber.

Instead of using normal house plants, use crassulacean acid metabolism (CAM) or C_4 carbon-fixing plants like succulents.

Use more than one EcoChamber to compare aquatic and terrestrial ecosystems.

26. Dissolved Oxygen and Primary Productivity

Objectives

In this lab, students will determine the primary production of an aquatic cell culture system. Students will:

- Explore the effect of temperature on the concentration of dissolved oxygen
- Explore the primary productivity of *Chlorella* algae cultures as revealed from changing dissolved oxygen concentrations
- Investigate the effects of changing light intensity on primary productivity of *Chlorella* in a controlled experiment

Procedural Overview

Students gain experience conducting the following procedures:

- Measuring and analyzing the dissolved oxygen concentration in water samples of varying temperatures
- Measuring the concentration of dissolved oxygen of algae cultures incubated in the aquatic productivity bottles, which are designed to simulate the decrease of light with increasing depth
- Using the concentration of dissolved oxygen to calculate net productivity, gross productivity, and respiration rate

Time Requirement

♦ Preparation time	10 minutes
♦ Pre-lab discussion and experiment	15 minutes
♦ Lab experiment: Part 1	20 minutes
♦ Lab experiment: Part 2 (day one)	10 minutes
♦ Lab experiment: Part 2 (day two)	30 minutes

Materials and Equipment

For each group:

- Data collection system
- Optical dissolved oxygen sensor
- Beakers (3), 250-mL
- Large vessel, to hold 2 L of algae
- Aquatic Productivity Bottles (1 set)
- Wash bottle
- *Chlorella*[1] (or other green algae) culture, 2 L
- Ice water, 200 mL
- Warm water, 200 mL
- Room temperature water, 200 mL
- Fluorescent light source
- Wax pencil, or stickers and marker

[1]To prepare algae culture using Alga-Gro®, refer to the Lab Preparation section.

Concepts Students Should Already Know

Students should be familiar with the following concepts:

- The importance of molecules such as carbon and oxygen cycling through ecosystems
- The relationship between primary productivity and the metabolism of autotrophs and heterotrophs in the ecosystem
- The factors that affect gas solubility in aquatic environments
- The relationship between dissolved oxygen and metabolic processes like photosynthesis and respiration
- The relationship between metabolic processes like photosynthesis and respiration and how these processes affect primary productivity

Related Labs in This Guide

Labs conceptually related to this one include:

- *Elodea* and the Snail
- Plant Pigments and Photosynthesis
- Cellular Respiration

Background

Oxygen is necessary for the life processes of most organisms, including aquatic organisms. The abundance of available oxygen varies greatly between terrestrial and aquatic habitats. However, in both terrestrial and aquatic ecosystems, autotrophs like plants and green algae release oxygen gas as a byproduct of photosynthesis. The oxygen gas is then used by many organisms to create ATP through aerobic cellular respiration.

Approximately 21% of the atmosphere is composed of oxygen, whereas the dissolved oxygen (DO) concentration in water is only a fraction of 1%. The concentration of DO in a body of water is often used as a benchmark indicator of water quality because it suggests the number of producers in the ecosystem and the activity levels of the producers and consumers.

An ecosystem's primary production is the amount of light energy converted to chemical energy by autotrophs during a given time period. To measure the primary productivity of an aquatic ecosystem, one could attempt to measure the concentration of organic compounds produced by the autotrophs in a given time period. Since oxygen is a byproduct of photosynthesis, it is easier to use the concentration of DO as a measure of an aquatic ecosystem's primary productivity.

Gross primary production (GPP) is the total primary production by the autotrophs; it is the amount of light energy that autotrophs convert into chemical energy. Net primary production (NPP) is the amount of light energy that autotrophs convert to chemical energy minus the energy used by the autotrophs for cellular respiration (R). Net primary production is a very important measurement because it represents the amount of surplus organic material that will be available to consumers as food.

Respiratory rate is the rate at which energy is consumed through aerobic cellular respiration. Respiratory rate can be found by determining the amount of DO consumed by the autotrophs per unit area per given time. The relationship between GPP, NPP, and R is illustrated by the following equation:

$$GPP = NPP + R.$$

Pre-Lab Discussion and Experiment

Make sure that the students are familiar with the following terms: autotroph, producer, heterotroph, consumer, and decomposer.

Autotrophs produce their organic molecules from CO_2 and other inorganic raw materials obtained from the environment. Autotrophs are the ultimate source of organic compounds for all heterotrophic organisms. Photoautotrophs use light as the energy source. Autotrophs are also called producers.

Heterotrophs live on organic compounds produced by other organisms. These organisms are the consumers of the biosphere. Some heterotrophs feed on plants and other animals while others, referred to as decomposers, decompose and feed on dead organisms and on organic litter, like feces and fallen leaves.

Review photosynthesis and aerobic cellular respiration. Make sure students remember the basic formulas, products, and reactants and the types of organisms that use each process.

Photoautotrophs use a process called photosynthesis to capture light energy from the sun and convert it to chemical energy stored in sugars and other organic molecules.

The equation describing the net process of photosynthesis is:

$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2$

Cellular respiration is the process by which organisms complete the breakdown of a variety of organic molecules in order to produce ATP and other organic molecules. Most of the processes in cellular respiration occur in mitochondria. Both plants and animals undergo cellular respiration. In fact, all organisms from the smallest bacteria to the largest animal undergo some form of cellular respiration.

The overall process of respiration is:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy (ATP + heat)}$

Carbohydrates, fats, and proteins can all be used as the fuel, but it is traditional to start learning with glucose.

Discuss the concept of dissolved oxygen. Talk about how dissolved oxygen gets into and out of the water in an aquatic ecosystem.

Dissolved oxygen (DO) refers to the freely available oxygen content in a solution. In aquatic ecosystems, autotrophs like algae release oxygen gas as a byproduct of photosynthesis. This oxygen is used by other organisms to create ATP through aerobic cellular respiration. The concentration of DO in a body of water is often used as a benchmark indicator of water quality because it suggests the number of producers in the ecosystem and the activity level of the producers and consumers.

Although a large percentage of the DO in an aquatic ecosystem is the product of photosynthesis, some oxygen can enter from the air. The rate at which oxygen enters or leaves the water depends upon several chemical and physical factors. The solubility of oxygen, or its ability to dissolve in water, decreases as temperature increases. Water currents, winds, and tides can all increase the absorption of atmospheric oxygen into the water and affect the spatial distribution of DO throughout a body of water. The partial pressure of the atmospheric oxygen can also affect the concentration of DO of an aquatic ecosystem. In areas of higher elevation where the partial pressure of oxygen in the air is lower, the water will also have a lower concentration of DO.

Oxygen is removed from an aquatic ecosystem through aerobic cellular respiration by producers and decomposition of organic material by consumers, such as bacteria and fungi.

Ask students to predict how they think the concentration of DO will be affected by temperature changes. Have them write these predictions down for later reference.

Students should understand that the solubility of any gas, including oxygen, is inversely related to the temperature of the liquid. Therefore, as temperature increases, the concentration of dissolved oxygen that can be held in the water decreases.

Talk about the effects of pressure on the concentration of DO. A 2-L bottle of soda is a great analogy to use. Ask students why soda goes flat if you leave it in a warm place with the cap off.

Students should understand that the solubility of any gas, including oxygen, is directly related to the pressure on the solution. Therefore, as gas pressure increases, the dissolved oxygen of the solution also increases.

Explain to the students that we tend to keep soda bottles tightly sealed in the refrigerator because of the effects of pressure and temperature on gas solubility. In order to keep the “fizz” in the soda, temperature must be low and pressure must be high. The temperature is maintained by the refrigerator and the pressure is maintained by tightly screwing the cap on. If you left an open bottle of soda on the counter at room temperature for several hours, the solubility of the gases would decrease so much that much of the gas would diffuse out of the solution; in other words, the soda would go flat.

Discuss gross and net primary production.

The amount of light energy converted to chemical energy by an ecosystem’s autotrophs in a given time period is called primary production.

Total primary production is known as gross primary production (GPP). This is the amount of light energy that is converted into chemical energy.

The net primary production (NPP) is equal to gross primary production minus the energy used by the primary producers for respiration (R):

Ask students to think of any other factors that might limit primary production in an aquatic ecosystem. Discuss limiting nutrients.

Students should understand that light, temperature, and limiting nutrients all affect primary production in aquatic ecosystems. A limiting nutrient is a substance that is required for production to increase. Examples include nitrogen, phosphorus, iron, and ammonia.

Ask students if they think all ecosystems have equal primary productivity. Do they think aquatic and terrestrial ecosystems have the same productivity? Require students to defend their answers.

Students should understand that ecosystems vary considerably in their primary productivity. Tropical rain forests, estuaries, and coral reefs have the highest net primary productivities. However, since these ecosystems make up a very small portion of the total area of the biosphere, their contribution to total global productivity is relatively small. The least productive ecosystem is the open ocean. Because the ocean covers the largest surface area of any ecosystem, it contributes as much to total global productivity as the tropical rain forests.

Lab Preparation

Note: there is a video available at www.pasco.com/resources/videos/index.cfm demonstrating this lab that you might find helpful to view before implementing it with students.

These are the materials and equipment to set up prior to the lab.

1. Prepare algae culture (for 8 groups): Each group will need about 2 L of dilute algae culture. If you buy your algae from a biological supply company, immediately remove it from the box when it arrives. Remove the test tube from the box, place it in a test tube rack, and remove the cap. Use a disposable pipet to squirt air bubbles into the test tube and then place the algae in a cool place with indirect light until ready to culture.

   When you are ready to make the culture, obtain a very large, sterile container. Mix all of the algae with 8 L of spring water (or dechlorinated tap water) and 160 mL of Alga-Gro® concentrate. Stir to mix. Place the algae in a cool place in direct light until ready to use. If you are making culture for a different number of groups, use 20 mL of Alga-Gro per liter of water and test tube of algae.

   If you do not have access to an algae culture, you can use local pond water. In this case, no culturing is necessary. Dissolved oxygen data will vary greatly depending upon the algae source. Pond water may have a higher level of heterotrophic and decomposing organisms than pure algae culture. These organisms consume oxygen through respiration and can affect your results.

2. Prepare 2 L of room-temperature water (approximately 20 °C), 2 L of warm water (approximately 30 °C), and 2 L of ice water (approximately 0 °C). To protect the DO sensor, the hot water should not exceed 45 °C. Feel free to use tap water instead of distilled or deionized water. Be sure to get all three water samples from the same source. If your tap water is highly oxygenated, allow all three samples to sit out on the counter for a few hours before heating or cooling them. This will allow any excess dissolved oxygen to dissipate.

3. To prevent students from mixing up their bottles of algae after incubation, they should label the bottom of the bottles. To save time, you can label the bottles prior to the lab. With a wax pencil, sticker or marker, write the percentages of light on the bottom of each bottle.

4. When measuring the DO at differing temperatures, the size of the beaker is important. The water level must be high enough so that the silver ring on the DO sensor is immersed.

5. Set up a light source for incubation of the Aquatic Productivity Bottles: Place the bottles far enough from the light source that they will not be heated. To ensure this, place your hand where you want the bottles to sit. You should not feel any warmth from the light.

   Another option is to create a heat sink by placing a large, clear vessel of water between the light source and the bottles.

6. To shorten the lab, have each group only test the concentration of DO of one temperature of water in Part 1, and share their data with the class. Also, have each group set up only one of the algae bottles and share their data with the class.

Safety

Follow all standard laboratory procedures.

Procedure

Part 1 – Measuring dissolved oxygen

Set Up: Room-temperature water

1. Start a new experiment on the data collection system.

2. Connect the optical dissolved oxygen (DO) sensor to the data collection system.

3. Display Dissolved Oxygen (mg/L) and Temperature (°C) in digits displays.

4. Do you think that the concentration of DO will be affected by temperature changes? If yes, do you think that it will rise at increasing or at decreasing temperatures?

Student should indicate that the concentration of DO will be affected by changes in temperature. Increased temperatures make gases less soluble in water, so it should decrease as temperature increases.

5. Obtain 200 mL of room temperature water (approximately 20°C) in a 250-mL beaker.

6. Calibrate the DO sensor.

7. Place the probe into the water. The silver circle on the side of the DO probe should be immersed in water, but the probe should not be touching the bottom of the beaker.

Collect Data: Room-temperature water

8. Begin data recording.

9. Record the temperature of the room temperature water in Table 12.1.

10. Observe the [DO] of the room temperature water while continuously stirring the DO probe.

11. When the DO level has stabilized on the digits display, note the DO concentration.

12. Record your data in Table 12.1. Then stop data recording.

Set Up: Cold water

13. Obtain 200 mL of ice cold water (approximately 0 °C) in a 250-mL beaker. There should be no ice in the water.

14. Place the probe into the water.

Collect Data: Cold water

15. Begin data recording.

16. Record the temperature of the room temperature water in Table 12.1.

17. Observe the [DO] of the room temperature water while continuously stirring the DO probe.

18. When the DO level has stabilized on the digits display, note the DO concentration.

19. Record your data in Table 12.1. Then stop data recording.

Set Up: Warm water

20. Obtain 200 mL of warm water (approximately 30 °C) in a 250-mL beaker. The temperature of the water should not exceed 45 °C.

21. Place the temperature probe into the water.

Collect Data: Warm water

22. Begin data recording.

23. Record the temperature of the room temperature water in Table 12.1.

24. Observe the [DO] of the room temperature water while continuously stirring the DO probe.

25. When the DO level has stabilized on the digits display, note the DO concentration.

26. Record your data in Table 12.1. Then stop data recording.

Part 2 – Measuring the effects of light intensity on [DO] and primary productivity

In Part 2, you will be measuring the effects of varying light levels on the dissolved oxygen concentration of algae cultures. You will use the Aquatic Productivity Bottles to simulate the decrease in light that accompanies an increase in depth. To create this model of aquatic depth, you will expose bottles of algae culture to five different light levels: 100%, 75%, 50%, 25% and 0%. These percentages are based on the amount of light that reaches the 100% bottle.

Set up

27. Obtain approximately 2 L of algae culture.

28. Carefully place the optical dissolved oxygen probe into the culture. The silver circle on the side of the probe should be immersed in the culture, but the probe should not be touching the bottom of the beaker.

29. Measure the concentration of DO of the algae culture while gently swirling the probe in the culture.

30. When the DO levels have stabilized on the digits display, note the DO concentration.

31. Record the data in Table 12.2.

32. Rinse the DO probe with the wash bottle and return it to the probe cover.

33. You are going to expose the algae to differing amounts of light over the next 24 hours. The measurement that you just took is the initial concentration of DO. In what part of Table 12.2 do you think this measurement belongs? Is it the initial concentration of DO in the 100% bottle? The 50% bottle? All of the bottles?

The initial concentration of DO should be placed in the column titled "Group Data Initial [DO]" and should be filled in for all bottles.

34. Why is it important to take this measurement before you expose the algae culture to the varying amounts of light?

The only way to determine the change in concentration of DO over time is know how much was present at the start. Measuring the initial DO concentration will allow us determine if light intensity has any effect on the amount of DO produced in each bottle.

35. If your instructor has not already done so, label the bottoms of the five Aquatic Productivity Bottles with the percentages of light.

36. Fill the bottles, one by one, by completely immersing them in the large vessel of algae culture. While each bottle is submerged, shake it to ensure that all air bubbles have left the bottle and then place the cap on the bottle while it is still submerged.

37. Remove the capped bottle from the culture and dry it with a paper towel.

38. When all of the bottles are filled, place them into the cradle chambers, secure the lid on top. Place the bottles in the incubation area under a fluorescent light and allow them to sit undisturbed for 24 hours.

39. List the independent and dependent variables in this part of the experiment.

Independent variable: light intensity

Dependent variable: dissolved oxygen concentration

Collect Data

40. After 24 hours, find your bottles, bring them back to your lab station, and remove the lid.

41. Display Dissolved Oxygen (mg/L) in a digits display.

42. Lay several paper towels onto the lab table. Starting with the 100% bottle, take the bottle out of the apparatus, place it onto the paper towels and carefully remove the cap.

43. Start data recording.

44. Remove the DO probe from the probe cover and carefully insert it into the culture. The silver circle on the side of the probe should be immersed in culture, but the probe should not be touching the bottom of the beaker.

45. Measure the concentration of DO of the algae culture while gently swirling the probe in the culture.

46. When the DO levels have stabilized on the digits display, stop data recording.

Note: Try not to introduce any air bubbles into the solution during data collection.

47. Record the data in Table 12.2.

48. Rinse the DO probe with the wash bottle in between each bottle.

49. Repeat this procedure with each of the remaining four bottles (75%, 50%, 25%, and 0%). Return the probe to the probe cover when finished.

50. Gather class data and record in Table 12.2.

51. Net productivity is the amount of light energy converted to chemical energy minus the energy used for respiration. Which bottles would you use to calculate net productivity?

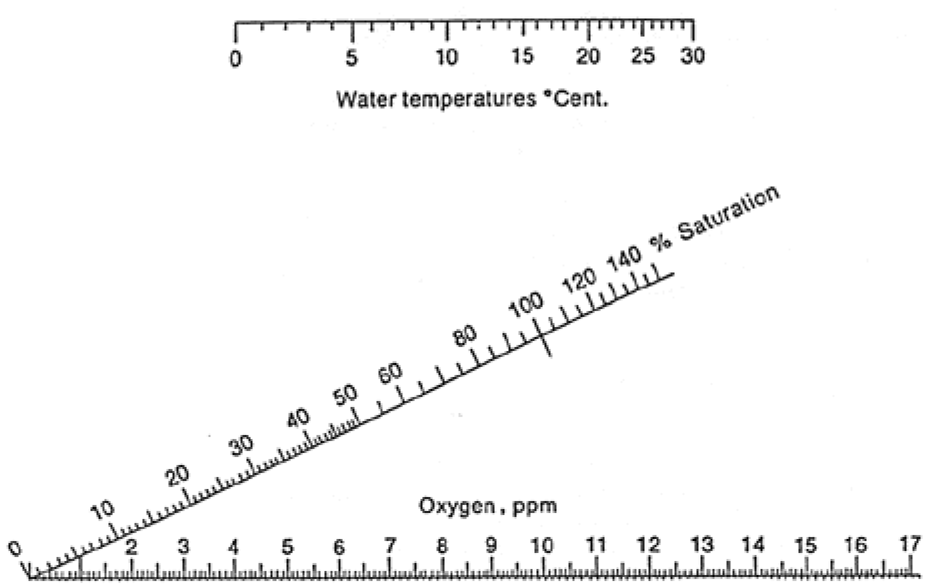
To calculate NPP, you would calculate the change in DO concentration in all of the bottles except the 0% bottle: NPP = final DO concentration – initial DO concentration

52. Gross productivity is the total amount of light energy is converted into chemical energy. Which bottles would you use to calculate gross productivity?

To calculate GPP, use the formula *GPP* = *R* + *NPP*. You need *R*, the change in DO concentration in the dark bottle (0% light), as well as the change in DO of the bottle in question (*NPP*), and then you add these together to yield the *GPP*.

Data Analysis

1. Use the nomogram in the figure below to estimate the percent oxygen saturation of the water sample at each of the three temperatures. To do this, line up the edge of a straight edge or ruler to the temperature of the water on the top scale and the [DO] that you measured at that temperature on the bottom scale. The ruler will pass over the middle scale at the estimated percent saturation. Record this value in Table 12.1.



Nomogram of oxygen saturation

2. Gather class data and enter in Table 12.1.

3. Calculate the respiration rate (*R*) for your group's algae solution. *R* equals the total amount of dissolved oxygen consumed in the bottle that received no light (0% bottle), since there was no photosynthetic activity in that bottle. So:

R = Initial DO concentration – Final DO concentration (0% bottle).

For this example: *R* = 8.9 – 8.8 = 0.1 mg/L of DO (group data) and
R = 8.6 – 8.5 = 0.1 mg/L of DO (class average)

4. Record your group's respiration rate in Table 12.3.

5. Collect class data and record class average of respiration in Table 12.3.

6. Calculate the net primary productivity (NPP) of the algae in each of your group's bottles.

NPP = Final [DO] of bottle – Initial [DO] of bottle

100% NPP = 9.7 – 8.9 = 0.8 mg/L
75% NPP = 9.5 – 8.9 = 0.6 mg/L
50% NPP = 9.2 – 8.9 = 0.3 mg/L
25% NPP = 9.0 – 8.9 = 0.1 mg/L
0% NPP = 8.8 – 8.9 = -0.1 mg/L

7. Record your group's net primary productivity for each of the bottles in Table 12.4.

8. Collect class data, calculate the average, and record this data in Table 12.4.

9. Calculate the gross productivity of the algae in each of your group's bottles.

GPP = *NPP* of bottle + *R*

100% GPP = 0.8 + 0.1 = 0.9 mg/L
75% GPP = 0.6 + 0.1 = 0.7 mg/L
50% GPP = 0.3 + 0.1 = 0.4 mg/L
25% GPP = 0.1 + 0.1 = 0.2 mg/L
0% GPP = -0.1 + 0.1 = 0.0 mg/L

10. Record your group's gross primary productivity for each of the bottles in Table 12.4.

11. Collect class data, calculate the average, and record in Table 12.4.

Table 12.1: Dissolved oxygen at varying temperature

	Temperature (°C)	**Group Data: [DO] (mg/L)**	**Class Average: [DO] (mg/L)**	**Group Data: [DO] from nomogram (% saturation)**	**Class Average: [DO] from nomogram (% saturation)**
Room Temp.	22.1	4.80	4.90	53	54
Cold	5.96	5.10	5.20	40	41
Warm	29.3	4.40	4.30	56	57

Table 12.2: Dissolved oxygen at varying light intensity

Bottle	Group Data: Initial [DO] (mg/L)	Class Average Initial [DO] (mg/L)	Group Data: Final [DO] (mg/L)	Class Average Final [DO] (mg/L)
100%	8.9	8.6	9.7	9.8
75%	8.9	8.6	9.5	9.7
50%	8.9	8.6	9.2	9.1
25%	8.9	8.6	9.0	8.9
0%	8.9	8.6	8.8	8.5

Table 12.3: Total amount of aerobic cellular respiration (R)

	Group data	Class Average
Initial [DO] (mg/L)	8.9	8.6
Final[DO] 0% Bottle (mg/L)	8.8	8.5
Respiration Rate (*R*) (mg/L)	0.1	0.1

Table 12.4: Net primary production (NPP) and gross primary production (GPP)

Bottle	Group Data: GPP (mg/L)	Group Data: NPP (mg/L)	Class Average: GPP (mg/L)	Class Average: NPP (mg/L)
100%	0.9	0.8	1.3	1.2
75%	0.7	0.6	1.2	1.1
50%	0.4	0.3	0.6	0.5
25%	0.2	0.1	0.4	0.3
0%	0.0	-0.1	0.0	–0.1

Analysis Questions

1. The change in DO concentration in the 0% bottle during the incubation period is a measure of respiration. Why is this particular bottle used to calculate respiration rate?

The 0% bottle is used to calculate the respiration rate because it has been kept in complete darkness during the incubation period. Since photosynthesis will not occur in the absence of light, no oxygen will be produced through photosynthesis in the 0% bottle. However, the algae in this bottle will not die overnight, they will continue to live and undergo cell respiration. The dissolved oxygen in the bottle will be the source for the oxygen that the yeast will use to undergo aerobic cell respiration during the night. Therefore, we will be able to calculate the amount of cellular respiration by measuring the decrease in DO concentration in the 0% bottle.

2. What is the relationship between temperature and the solubility of gases like oxygen in solution? Use evidence from part 1 to support your claim.

The data from part 1 suggests that as the temperature of a solution increased, the solubility of gases in solution decreases.

3. What is the relationship between temperature and the % saturation of a solution? Use evidence from part 1 to support your claim.

The data from part 1 suggest that as temperature increases, the % saturation of the solution increases.

4. Compare the NPP and GPP of the five bottles and make a statement about the relationship between light intensity and photosynthetic activity. Use evidence from your experiment to support your claim.

Students should use their data to explain that as light intensity increases, photosynthetic activity, NPP and GGP increase. As light intensity increases, more energy is available for photosynthesis and the ecosystem becomes more productive.

Synthesis Questions

Use available resources to help you answer the following questions.

1. Design an experiment that would test the effects of varying pH on dissolved oxygen concentration and the productivity of an aquatic ecosystem.

Students should identify the problem or question, create a hypothesis, set up a controlled experiment, identify controls, independent and dependent variables, indicate how they will measure the independent variable, indicate how they will organize their data, indicate how they will analyze their data, make predictions about the results of the experiment, and indicate that the experiment must be repeatable and validated by the scientific community.

2. What are some adaptations that aquatic organisms have that allow them to adapt to the very low dissolved oxygen concentration in aquatic ecosystems?

Answers will vary. Some examples include fish gills, countercurrent exchange, stomata on the upper surface of leaves.

3. When this experiment is conducted with pond water instead of pure algae solutions, results can vary greatly. Explain why pond water might respond differently to varying light intensity than pure algae solution.

Pure algae solution consists of only one type of organism, photosynthetic algae. These organisms respond to light by increasing photosynthetic activity and DO production. Pond water usually contains heterotophic organisms in addition to algae. These organisms consume DO during aerobic cellular respiration and are generally unaffected by light intensity. This creates a situation where light intensity is the not the only factor determining the DO concentration of the solution.

4. At 22°C a solution contains 4.8 mg/L of DO and is at 53% saturated. The same solution at 29°C contains 4.4 mg/L of DO and is 56% saturated. Explain why increased temperature decreases the solubility of a gas in solution but increases the percent saturation of a solution.

At constant pressure and volume, increased temperature decreases the solubility of a gas in solution and increases its percent saturation. This is because solutions at increased temperature have a decreased capacity to hold gas in solution, so they become saturated at lower concentrations of dissolved gases. Since 100% saturation is relative, solutions at increased temperature reach 100% saturation at lower concentrations of dissolved gases than those at lower temperatures.

Multiple Choice Questions

1. Which of the following is the best definition of net primary productivity?

- **A.** The amount of oxygen produced by plants and algae.
- **B.** The amount of oxygen used by plants and algae.
- **C.** The total primary production in an ecosystem.
- **D.** The amount of light energy converted to chemical energy by autotrophs during a given time period.
- **E. The amount of light energy converted to chemical energy by autotrophs during a given time period minus the amount used by the autotrophs in respiration.**

2. When comparing photosynthesis and respiration, which of the following statements is true?

- **A.** Carbohydrates are produced in respiration, but not in photosynthesis.
- **B. Oxygen is produced in photosynthesis, but not in respiration.**
- **C.** Oxygen is produced in respiration, but not in photosynthesis.
- **D.** Water is produced in photosynthesis, but not in respiration.
- **E.** Carbon dioxide is produced in photosynthesis, but not in respiration.

3. A scientist studying the oxygen concentration in sealed chambers containing cultured plant cells finds that when the chambers are illuminated, the concentration of oxygen increases. However, when the chambers are kept in the dark, the concentration of oxygen decreases. Why does the oxygen concentration decrease when the chamber is kept in the dark?

- **A. Plant cell mitochondria consume oxygen by aerobic respiration.**
- **B.** Plant cell chloroplasts run the photosynthetic pathways backwards to consume oxygen.
- **C.** Plant cell chloroplasts switch their structure and function and become mitochondria.
- **D.** The chambers are not properly sealed and oxygen is leaking out.
- **E.** The cultures in the chambers must be contaminated with some animal cells, since only animal cells consume oxygen.

Extended Inquiry Suggestions

Use the EcoZone System to conduct an experiment comparing primary productivity in aquatic and terrestrial ecosystems. Fill one EcoChamber with soil, plants, and small insects. Fill another with distilled water, algae, aquatic plants, and fish. In the terrestrial EcoChamber, measure CO_2 gas and O_2 gas. In the aquatic EcoChamber, measure DO and pH. Although pH is not a direct indicator of CO_2 concentration, it will give an indirect measure of CO_2 because CO_2 is converted to carbonic acid. Use these measurements to calculate primary productivity and respiration rates.

Allow the students to design their own controlled experiment testing the effects of a variable other than light on the productivity of an aquatic ecosystem.