General Biology
BioSci 100
Laboratory Manual

Building Knowledge through Experiments

COLLEGE OF THE CANYONS

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Preface

The purpose of creating this custom lab manual for BioSci 100 was to provide our students with a high quality, low cost learning resource. This project would not have been possible without the dedication and commitment of the faculty and staff in the Department of Biological Sciences and the Office of Distance and Accelerated Learning at College of the Canyons.

I am especially grateful to the following individuals for their contributions to the development of the original lab activities and the lab manual chapters: Kelly Burke, Janet Cetrone, Jeannie Chari, Amy Foote, James Glapa-Grossklag, Miriam Golbert, Elizabeth Hernandez, Kim Jesu, Jenna King, Jenny Leadbetter, John Makevich, Patricia Medina, Carri Musser, Gregory Nishiyama, Patricia Palavecino, Christopher Shane Ramey, Kathy Sloan and Jim Wolf.

For this 4th ed. of the lab manual, I am particularly grateful to Dr. Patricia Palavecino and Kelly Burke, for their time, expertise and talent in preparing much of the new content and artwork for the manual. I am also grateful to the Office of Distance and Accelerated Learning for their financial support of this project.

Dilek Sanver-Wang

Note to Students about the Reading Assignments

Each lab activity contains background information that is important for completing the lab activity and questions. However, the background information in the lab manual is not exhaustive and most of the lab activities contain additional required reading assignments from the course textbook. You are expected to complete the textbook reading assignments before coming to class in order to fully understand the theory behind each of the lab activities. You are encouraged to bring your textbook to lab each week so that you can complete the lab assignment and answer all the questions.

Each lab activity also lists some links to websites for optional background information. You are encouraged to look through these additional resources because they provide helpful illustrations, animations and videos to clarify and reinforce the lab material. The URL's are listed for each link; however, to make it easier to access the websites, all these links are also listed on the Biology Department website under "Student Resources" (click on the Bio 100 link at the bottom.) You may want to bookmark the Bio 100 page so that you can access the page easily throughout the semester.

LAB SAFETY GUIDELINES

Each laboratory is a restricted area. Enrolled students may work in a lab only when there are authorized personnel present. Friends of students in lab classes will not be allowed to "visit" inside the laboratory. Students are not permitted into the storage rooms or prep areas unless given specific permission by their instructor or lab personnel. Ensuring safety in the laboratory is the responsibility of everyone working in the lab. Please follow these guidelines carefully.

GENERAL GUIDELINES

1. USE COMMON SENSE WHEN WORKING IN THE LAB.
2. Be prepared for your work in the lab. Read all procedures thoroughly before entering the lab. Follow all written and verbal instructions carefully. If you do not understand a direction or part of a procedure, ask the instructor before proceeding.
3. Do not eat, drink, or smoke in the lab. Do not use laboratory glassware as containers for food or beverages.
4. Always wear close-toed shoes in the lab.
5. Wear safety goggles whenever working with chemicals or when there is an impact risk.
6. Long hair should be tied back when working with flames, chemicals or dissections.
7. Observe good housekeeping practices. Work areas should be kept clean and tidy at all times. Keep aisles clear. Push your chair under the desk when not in use.
8. No open flames are permitted in the laboratory unless specifically indicated by the instructor. When burners or hot plates are being used, caution should be exercised to avoid thermal burns. If you sustain a thermal burn immediately flush the area with cold water and notify the instructor.
9. If there is a blood spill, immediately notify the instructor.
10. ANY ACCIDENTS OR INJURIES THAT OCCUR IN THE LAB MUST BE REPORTED TO THE INSTRUCTOR AT ONCE.
11. Familiarize yourself with the location of the Fire Extinguisher. There is a telephone in each lab for EMERGENCY USE ONLY. In case of emergency dial 7 (OR 77 at the Canyon Country Campus).
12. Broken glass is to be disposed of in the broken glass (sharps) container and reported to the instructor.
13. Keep hands away from face, eyes, mouth and body while using chemicals or preserved specimens. Wash your hands with soap and water after performing all experiments. Clean, rinse and wipe dry all work surfaces and apparatus at the end of the lab activity. Return all equipment to the proper area.
14. Handle all living organisms used in a lab activity in a humane manner.
15. Never use mouth suction to fill a pipette. Use a rubber bulb or pipette pump.
16. When removing an electrical plug from its socket, grasp the plug not the electrical cord. Hands must be completely dry before touching an electrical switch, plug or outlet.

HANDLING CHEMICALS

1. Wear safety goggles whenever working with chemicals.
2. Chemicals and biological stains should be used with caution. Follow specific instructions regarding all chemicals used during lab. Check the label on chemical bottles twice before removing any of the contents. Take only as much chemical as you need. Do not carry a chemical stock bottle to your work station or remove it from the chemical station.
3. If any chemical comes into contact with your skin, immediately flush the area with water for several minutes and notify the instructor.
4. Dispose of all chemical waste properly. Do not pour chemicals down the sink unless told to do so by your instructor. Check the label of all waste containers twice before adding your chemical waste to the container.

DISSECTIONS – Special Precautions

1. Students should consult with the instructor regarding the pros and cons of wearing contact lenses during dissections.
2. Safety glasses or other protective eyewear is recommended for all students performing dissections.
3. Protective gloves should be worn during dissections. If your skin comes in contact with a chemical preservative, immediately run water over the area and notify the instructor.
4. Do not remove preserved specimens from the laboratory.
5. Preserved biological materials are to be treated with respect.
6. When using scalpels and other sharp instruments, always carry them with the tips and points pointing down and away. Notify your instructor of any cuts or other injuries.

Lab 1: Lab Safety and the Scientific Method

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify and apply the correct lab safety procedures to follow for a variety of scenarios.
2. List and explain the steps of the Scientific Method.
3. Identify and provide examples of questions that can be answered scientifically.
4. Explain what distinguishes a good scientific hypothesis.
5. Define, give examples of, and identify dependent, independent and standardized variables.
6. Explain the importance of control treatments and replication.
7. Explain the difference between quantitative and qualitative data.
8. Conduct a simple experiment using the Scientific Method.

II. Background Information:

A. The Scientific Method:

Science is a search for an understanding about the way that things work in the natural world. Scientific inquiry is based on falsifiable **hypotheses**. This means that there is room for any assumption about the natural world to be shown false. Science is fluid and dynamic and changes as new information is introduced, examined and the best explanations are accepted. If a hypothesis cannot be shown to be potentially false, then that hypothesis cannot be investigated using science. A scientific investigation depends on a set of procedures. These procedures or steps are known as the **scientific method**.

1. Observation: An initial observation is made about a phenomenon in the natural world.
2. Question: A question is asked about the phenomenon.
3. Hypothesis: A possible explanation of the phenomenon, or answer to the question is proposed. Oftentimes, the scientist will make a more specific **prediction** based on the more general hypothesis that has been proposed.

It is very important that the hypothesis that is proposed is scientifically testable and potentially falsifiable. For example, the hypothesis: “Picasso is the greatest painter of all time” is not scientifically testable because it is a subjective statement. However, the hypothesis could be changed to make it testable, for example: “Picasso’s paintings are the most valued based on auction prices.”

4. Experiment: An experiment is designed to test the hypothesis.
5. Results: Data are collected in an objective manner.
6. Conclusion: The results are analyzed and the alternate hypothesis is accepted or rejected.

Using the rules of the scientific method ensures that an investigation will be designed so that results can be reviewed in an objective manner and the experiment replicated by others. The ability to repeat an experiment is essential to the validity of its results. If a tested hypothesis can be shown true in repeated testing, it may be that the information will be added to the general body of knowledge that is science. By the way, negating a hypothesis is often just as valuable as is accepting one to be true.

A good test isolates a single factor or variable for examination. Sometimes this is very difficult to do. A crucial step in designing experiments is to identify the variables and treatment groups.

B. Elements of an Experiment:

1. Independent Variable:
This is the variable that is changed by the investigator. This variable is chosen because the investigator predicts that changing it will impact the dependent variables and a functional relationship can be established.

2. Dependent Variable:
This is the variable that is measured, counted or recorded by the investigator. It is the factor that varies in response to conditions manipulated with respect to the independent variable.

3. Standardized Variables:
These are the variables that are kept equal in all treatments so that any changes in the dependent variable can be attributed solely to changes in the independent variable.

4. Experimental Treatment:
The experimental treatment is the one where the independent variable is manipulated.

5. Control Treatment:
A control treatment is one where the independent variable is either eliminated or set at a standard value. The results of the control treatment are compared to the results of the experimental treatment to determine if manipulating the independent variable had a measurable effect on the dependent variable.

6. Replication:
It is important for an experiment to be repeatable; this increases our confidence that that the observed results are due to changes in the independent variable. All biological systems contain natural variability and will therefore respond in slightly different ways each time an experiment is performed. By repeating the experiment multiple times, or by increasing the number of organisms that are experimented on, the investigator can obtain an average value that more accurately reflects the true result.

In certain situations, it is not possible to directly manipulate the independent variable or control all the variables, such as with natural disasters (e.g. an oil spill), naturally occurring phenomena (e.g. the changing of the seasons, phases of the moon), etc. In those cases, the researcher will try to gather as much data as possible before and after the phenomenon and

try to determine which variables were standardized and which variables need to be accounted for in the analysis of the data.

The results of an experiment are the recorded change in the dependent variable. Data are reported in objective terms that allow for independent interpretation by anyone reading the report. The preferred method of reporting data is the presentation of results in tables and graphs that provide a quick and clear overview of any observed effects. There are two types of data: qualitative and quantitative.

D. Types of Data:

1. Qualitative Data:

These kinds of data include qualities such as color, smell and taste. These are subjectively perceived and can be difficult to express in an objective manner. While everyone conducting the experiment may agree that the solution changed color, there may be variation in what individuals identify as blue, light blue, etc.

2. Quantitative Data:

These kinds of data include qualities that can be measured objectively such as weight, volume, length and temperature. Quantitative data have a number associated with them and can be reported in universally accepted metric units. This makes it easy for others to interpret the results.

Both types of data are valid and important. In some instances an experiment may result in just one or the other type of information. Oftentimes, the investigator will collect and report both types of data.

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7^{th} ed.): pp.4-10 (The Process of Science)

B. Recommended background reading (optional):

1. UC Berkeley - Understanding Science
http://undsci.berkeley.edu

2. Baruch's Biology Lab Safety Tutorial
http://www.baruch.cuny.edu/tutorials/weissman/biolab/

IV. Pre-Lab Questions:

1. You have been asked to conduct an experiment with different flavors of ice cream.
a. List one example of a scientifically testable hypothesis about ice cream.

b. List one example of a hypothesis about ice cream that cannot be tested scientifically and explain why it cannot be tested scientifically.

2. Consider the following experiment:
A researcher wants to find out if spraying apples with pesticide affects the vitamin levels in those apples. For this experiment, identify:

a) the independent variable: _____

b) the dependent variable: _____

c) **two** standardized variables: _____

d) the control treatment: _____

e) the experimental treatment(s): _____

3. In each of the following lab safety scenarios listed below, briefly identify how proper lab safety procedures were not followed and provide a safer alternative.

a) Cindy broke a test tube. Carefully she picked up the broken pieces with one hand and placed them in her other hand. Then she dumped the glass pieces into the wastebasket.

b) Frankie was unsure how to get a chemical from a large flask into a small test tube. He poured the liquid as best he could, but still spilled some on his hand and the table. He quickly washed his hands and wiped the table so the teacher didn't find out. Later, he noticed that his hand felt itchy and he worried that the chemical he spilled on himself might be dangerous.

c) Heather walked into lab late and missed the instructor's explanation about the day's activity. She had not read the lab beforehand, so she skimmed it quickly and began the experiment on her own. She did not understand parts of the lab but did not ask any questions to avoid getting into trouble with the instructor. Her experimental results looked very different from her classmates'.

d) James is cleaning up his workbench and finds a beaker containing an unlabeled clear liquid that his partner left behind. Not sure what it is, he carefully pours it down the sink and washes the beaker.

V. Lab Exercise:

Materials

Per Group:	Per Room:
• 1 sealed box containing four items • 1 container with an array of possible content items • 1 unsealed, empty box	• Triple beam balances • Magnets

Procedure

A. Black Box Experiment

The "black box" can be used as a model for scientific inquiry. Frequently, scientists can see what elements go into a process and they can likewise identify the results of the process, but they are unable to see the process itself; that is the "black box." Scientists must devise methods of figuring out what occurs in the "black box."

In the following activity, you will use the scientific method to answer the following question: "Which items are located in the sealed box?"

1. Each group of students should select a sealed box. (Do NOT open the box at any point during this investigation!)

2. Note the assortment of possible items located in the container provided. There are FOUR of the possible items in your sealed box.

3. As a group, you must devise a way to determine what is in the sealed box. Each time you make a guess as to what might be in the box and test it to determine if you are correct, you are conducting a "mini" experiment. Complete the following table and questions as you proceed (an example has been provided for clarification). You may need fewer or more rows to reach a conclusion about the items in your sealed box.

Hypothesis / Prediction	**Experimental Procedure**	**Results/Data**	**Data qualitative or quantitative?**	**Hypothesis supported or refuted?**
1.There is a marble, compass, paperclip and magnet in the sealed box	*Place all four items in the empty box, weigh box and test it with a magnet.*	*The two boxes don't weigh the same and the items are not attracted to the magnet.*	*Qualitative (magnetic) and Quantitative (weight)*	*Hypothesis is refuted. Need to try different combination of items.*
2.				
3.				
4.				
5.				
6.				

If your hypothesis and your result are the same, then your original hypothesis has been **supported**. If your result is different from your hypothesis, then your hypothesis has been **refuted**.

In any scientific inquiry, investigators use all the tools available at their disposal to collect the most accurate data they can and analyze their results to reach a conclusion. They then report their results, along with the statistical significance of their results, which indicates the likelihood that the observed results were in fact due to the experimental manipulations.

When you have determined the four items that are likely to be in your sealed box (**without opening the box**), answer the following questions. Your instructor may ask you to share your results with the rest of the class when all the groups have finished their experiment.

a. What other tools could you use (that were not available today) that would help you in answering your scientific question?

b. Based on your experiment, how confident are you that your results are accurate?

c. How could you increase your confidence and/or the accuracy in your results with the experimental tools that are provided?

B. Designing an Experiment

Background information:

Peanut is an annual herbaceous plant that can grow between 30cm to 50cm (1-1.6 ft) tall. The botanical definition of a "nut" is a fruit whose ovary wall becomes very hard at maturity. Using this criterion, the peanut is not a true nut, but rather a legume, and so it belongs to the same family as bean and peas. Like mot other legumes, peanuts harbor symbiotic nitrogen-fixing bacteria in their root nodules. Peanut pods develop underground, an unusual feature known as ***geocarpy***, where the plant flowers grow above ground but the fruits develop below ground.

After fertilization, a short stalk at the base of the ovary (termed a pedicel) elongates to form a thread-like structure known as a "peg". This peg grows down into the soil, and the tip, which contains the ovary, develops into a mature peanut pod (Fig. 1.1).

Since peanuts are able to fix nitrogen, they do not require fertilizers with nitrogen, and they can also improve soil fertility. Therefore, they are valuable in crop rotations. They grow well in light, sandy, and loamy soil.

Peanuts need warm weather throughout the growing season to develop well. They can grow with as little as 350 mm (14 in) of water, but for best yields need at least 500 mm (20 in) of water.

Fig. 1.1 Diagram illustrating how a peanut plant grows.[1]

[1] Wikimedia Commons: http://commons.wikimedia.org

1. You want to investigate in what type of pH soil, peanuts will grow better. Therefore, you will design a hypothetical experiment to investigate the following question: Do peanuts grow better in acidic, neutral or basic soil? In the space below write a summary of your experiment starting with the statement of your **hypothesis**.

2. Identify the following elements of your peanut growth experiment:

a. independent variable: _____

b. dependent variable: _____

c. control treatment: _____

d. experimental treatment(s): _____

e. standardized variables: _____

3. Indicate the kind of data will you collect and if it will be quantitative or qualitative. (Hint: you need to decide how you would specifically measure peanut growth to answer this question.)

4. Determine what kind of graph you will use to present your data clearly, and what variables they will include. Draw a sample graph with data that **support your proposed hypothesis**.

VI. Post-Lab Questions

1. Consider the following question: *Does eating a sweet snack (e.g. candy) cause more weight gain than eating an oily snack (e.g. potato chips)?*

a) State a scientifically testable hypothesis to answer the question.

2. Identify the following elements of your snack experiment:

a. independent variable: _____

b. dependent variable: _____

c. control treatment(s): _____

d. experimental treatment(s): _____

e. standardized variables: _____

3. How would the results of your experiment be affected if these variables were not standardized?

4. Indicate the kind of data will you collect and if it will be quantitative or qualitative.

Lab 2: Scientific Measurements

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the standard metric units of weight, length, volume and temperature.
2. Make measurements using the metric system.
3. Convert values between different metric units as well as between the imperial and metric units of measurement.
4. Identify the names and functions of laboratory equipment.
5. Make measurements using laboratory equipment.
6. Present data in appropriate and correctly labeled graphs.
7. Use graphical data to predict experimental results.

II. Background Information[1]:

Systems of measurement have a long and diverse history. In the United States, we typically use the United States Customary System, which evolved from a common ancestor to the imperial units traditionally used in Great Britain. This system merged components of the Anglo-Saxon and Roman systems of measurement. The Anglo-Saxon system had an agrarian foundation of barleycorns. Legally, an inch was defined as three barleycorns. Needless to say, the system is a bit confusing and the barleycorn standard led to some measurement differences. While the imperial system is now standardized, conversion between units can still be a bit awkward (e.g., 12in=1ft and 3ft=1yd).

Although the United States continues to hold on to the US Customary System, most nations (including Great Britain) have converted to the International System of Units (SI), which is based in the metric system. Some imperial measurements remain in limited use in Canada, India, Malaysia, Sri Lanka, South Africa and Hong Kong.

Scientific measurements are made using the metric system, which allows scientists to communicate their results in a standardized manner. Quantitative measurements are important in science because they allow for greater precision and improve our ability to communicate information. For example, the terms “tall” and “taller” mean little; however, “29.6 meters tall” and “5.6 centimeters taller” convey more precise information. The purpose of this lab exercise is to familiarize you with the system of quantitative measurement that you will be using throughout this and future science courses.

A. The Metric System

The metric system is based on units of 10. The standard units of measurement in the metric system are meter (m) for length, liter (L) for volume, gram (g) for mass and Kelvin (K) for temperature. The more commonly used standard unit for temperature, however, is degrees Celsius (^{o}C). A gram was originally defined as the mass of 1mL (or ml) of water at 4^{o}C.

[1] Washington State Open Course Library: http://opencourselibrary.org/

Furthermore, 1mL = 1 cm^3.
Whether measuring length, volume, or mass, the prefixes listed below are used to designate the relationship of a unit of measure to the base unit (i.e., m, L, or g).

Prefix	Prefix Abbreviation	Decimal equivalent	Exponential equivalent
Kilo	k	1000	10^3
Centi	c	0.01	10^{-2}
Milli	m	0.001	10^{-3}
Micro	μ	0.000001	10^{-6}

Converting between metric units of measure:
Many students try to jump into unit conversions by moving the decimal place to the left or right. If you are familiar with the metric system, this may work for you. However, common mistakes include moving the decimal in the wrong direction. A more reliable method for unit conversion is the use of conversion factors and unit cancellation. For example, if you are asked to convert 2km to cm, begin by considering the conversion factors that you know.

1. If starting with 2km, we need to multiply by a conversion factor to eliminate the *km*. We need to divide *km* by *km* to eliminate this unit (*km* divided by *km* equals 1). Therefore, the multiplier is the conversion factor between *km* and *m* in fraction form:

$$\frac{1000m}{1\ km}$$

with *km* in the denominator. So, we can multiply our original measure by the conversion factor:

$$2km \times \frac{1000m}{1km} = 2000m$$

which cancels out the *km* and leaves us with *m* in the numerator.

2. But we're not done! We wanted to convert to *cm*, not *m*; therefore, we need another conversion factor. We now have *m* and need *cm*. One meter is equal to 100cm. This conversion factor needs to be arranged to eliminate *m*. In fraction form, we need to divide by *m*, so our conversion factor becomes:

$$\frac{100cm}{1m}$$

Multiplying our new measure by this conversion factor leaves us with *cm*.

$$2000m \times \frac{100cm}{1m} = 200{,}000cm$$

3. Steps 1 and 2 could be combined as follows:

$$2km \times \frac{1000m}{1km} \times \frac{100cm}{1m} = 200{,}000cm$$

While perhaps tedious, using conversion factors for unit cancellation can help prevent mistakes when converting between units of measure.

Sample conversion factors for the metric system:

1kg = 1000g	1km = 1000m	1m = 1000000µm	1L = 1000ml
1g = 1000mg	1cm = 10mm	1km = 1,000,000mm	
1g = 1000000µg	1m = 100cm	1mm = 1000µm	

B. The Imperial System vs. the Metric System

The sample conversion factors listed below should illustrate that converting between imperial units, or imperial and metric units can be harder to do without memorizing a list of conversion factors and having a calculator handy. In this course, we will make all measurements in metric units and focus mainly on conversions between metric units.

Sample conversion factors for the imperial system:

1 foot = 12 inches
1 gallon = 128 fluid oz
1 mile = 5280 feet

Example conversion factors between the imperial and the metric system:

1 meter = 1.094 yards	1 inch = 2.54 centimeters
1 liter = 1.057 quarts	1 ounce = 28.35 grams
1 kilogram = 2.205 pounds	$^{o}F = (^{o}C \times 1.8) + 32$

C. Graphical Representation of Data

Scientists need to collect many types of data when conducting experiments and it is important to record those data as soon as the measurements are made, to avoid errors. A properly labeled data table is an essential tool in any investigation. However, it is often difficult to visualize any patterns in the data by simply looking at list of numbers, which is why it is important to graph the results. This will help you interpret your results more easily and help you determine whether or not your hypothesis was supported or refuted.

Line graphs are used to track changes over short and long periods of time (i.e. the rate at which a beaker of boiling water cools). Line graphs can also be used to compare changes over the same period of time for more than one treatment group (Fig. 2.1). Bar graphs, on the other hand, are used to compare outcomes between different categorical groups, for example the effectiveness of different toothpaste brands at preventing cavities (Fig. 2.2)

As you measure many things you might start to recognize that particular patterns of the **dispersion** of data exist. Often, when the data are put into categories, and graphed, the data points will form a normal or bell curve. Many types of data form this pattern, including the heights and weights of a group of people, as the sample size, or the number of people you measure, increases.

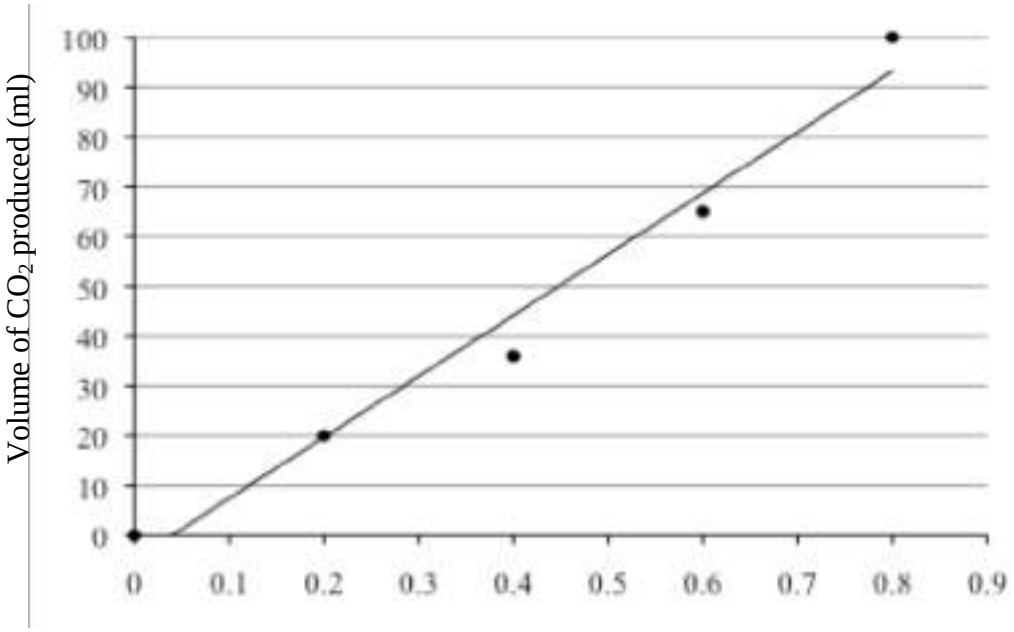


Fig. 2.1 Line graphs can be used to graph variables that are continuously changing (i.e. time or amount of baking soda).

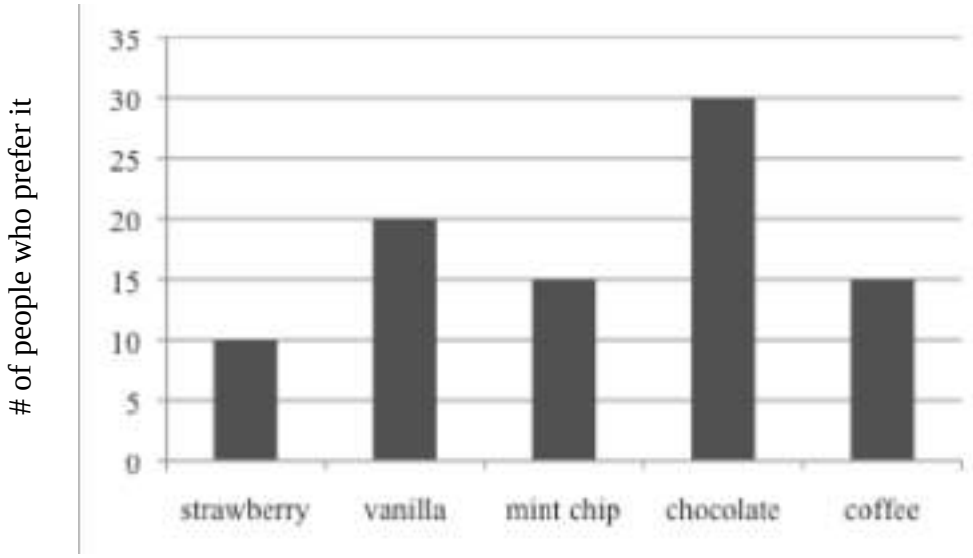


Fig. 2.2 Bar graphs can be used for comparing different groups of categorical data.

How to graph data and draw a best-fit line:

1. Collect data for the graph in a table format first. A graph has two sets of data: independent and dependent. The independent variable is what the investigator manipulates (or a variable that changes constantly, such as time). The dependent variable changes based on the independent variable.

2. Draw the area of your graph with the vertical and horizontal axes. The horizontal axis (x-axis) represents the independent variable. The vertical axis (y-axis) represents the dependent variable. Label each axis with appropriate units based on the data.

3. Determine the range of data for each axis by looking at the maximum and minimum values in your data set and dividing this range into equal sections (e.g. if you are measuring temperature and your values range from 5 to 55 ^{o}C, then your axis range can be 0-60 ^{o}C in increments of 5.)

4. Plot your data on the graph and using a ruler, draw a straight or curved line that appears to "fit" the data. One way to draw a best-fit line through your data points by hand (assuming that your data points do, in fact, show a linear pattern) is to adjust the ruler through your data points such that the distance between each of the data points and the line you are about to draw is minimized and that the number and distance of the points that lie above the line are equal to those below the line. Thus, depending on your data, you may end up with a best fit-line that passes through the majority of your actual data points.

III. Reading Assignments:

A. Required background reading
1. National Center for Education Statistics – Graphing Tutorial
https://nces.ed.gov/nceskids/help/user_guide/graph/whentouse.asp

B. Recommended background reading (optional):
1. The Metric System – Units, Definition and History
http://www.sciencemadesimple.com/metric_system.html

IV. Pre-Lab Questions:

1. Calculate the following using the conversion factors provided in the background reading.

a. 2 meters = _____ centimeters

b. 24.3grams = _____ micrograms

c. 24.3 milliliters = _____ liters

d. 6738 meters = _____ kilometers

2. Write the standard metric unit for:

a. temperature: _____

b. length: _____

c. volume: _____

d. weight: _____

3. Identify the numerical value of the following prefixes:

a. Kilo (k): _____

b. Centi (c): _____

c. Milli (m): _____

d. Micro (μ): _____

4. Which system (imperial or metric) is easier to use when doing conversions? Why?

5. You are interesting in testing the following hypothesis: “Students who drink coffee before an exam will score higher on the exam than students who don’t drink coffee.”

a. For this experiment, identify the following:

Independent variable: _____

Dependent variable: _____

Two standardized variables: _____

Control treatment: _____

b. The independent variable should be plotted on the _____ axis and the dependent variable should be plotted on the _____ axis.

c. Would this experiment require the collection of quantitative or qualitative data? Justify your answer.

d. What kind of graph (line or bar graph) would be more appropriate in graphing the data? Why?

V. Lab Exercise:

Materials

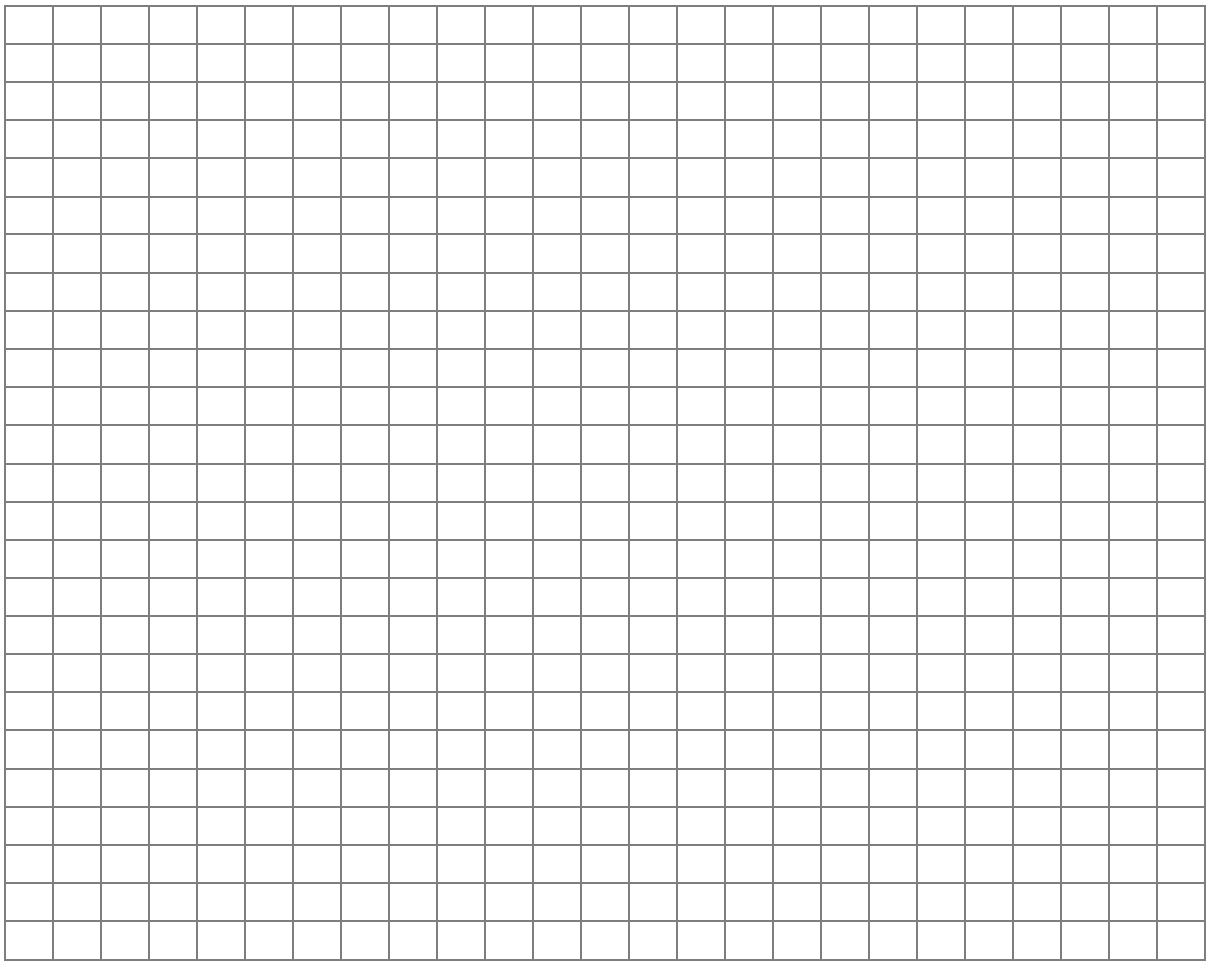
Per Group:	Per Room:
• Rulers • Graduated cylinders • 600mL beaker • Plastic pipettes • Container of water • 1 empty can of soda • Thermometer	• Triple beam balance • Digital scale • Salt • Vinegar • Baking soda • Spatula • Weigh boat

Procedure

A. Temperature Measurement

1. Fill a 600mL beaker with 500mL of tap water and heat it in the microwave for 2 min.
2. Use caution when removing the hot beaker from the microwave. Use the heat gloves provided. Your instructor may decide to complete steps 1-2 for each group.
3. Using the thermometer provided, immediately measure the temperature of the water in ^{o}C and record it under time 0 min.
4. Continue to record the temperature of the water in the beaker every 3 minutes for 33 min (or until the temperature no longer changes) in the data table below. Draw a graph of time (x-axis) vs. temperature (y-axis) and draw a best-fit line through your data points. Refer to the background information on how to draw a best-fit line.

Time (min)	Temperature (^{o}C)	Time (min)	Temperature (^{o}C)
0		18	
3		21	
6		24	
9		27	
12		30	
15		33	



B. Units of Length

1. Measure the length of this page in centimeters: _____ cm

2. Convert the length of the page into the following units:

   millimeters (mm): _____

   meters (m): _____

3. Measure the height of the 600mL beaker in millimeters: _____ mm

4. Convert the height of the beaker into the following units:

   centimeters (cm): _____

   meters (m): _____

   kilometers (km): _____

C. Units of Volume

1. Using the 1L graduated cylinder and an empty soda can, determine the volume of the soda can by filling the can with tap water and emptying it into the graduated cylinder.

   Volume = _____ mL

2. Calculate the volume of the soda can using measurements of length. To calculate the volume of a cylinder, measure the height (h) and radius (r) of the soda can in centimeters (cm). You will need to decide whether to use the inner diameter or outer diameter of the soda can, which will affect the magnitude of your calculated volume.
3. Use the following formula to calculate the volume: $V = \pi r^2 h$. ($\pi = 3.14$)
   Because the measurements were taken in centimeters, the volume will be in cubic centimeters (cm^3).

   Measurements:

   r = _____ cm; h _____= cm

   Calculations:

   Volume = _____ cm^3

4. Compare the measured volume (mL) with the calculated volume (cm^3).
   (Remember that for water at 4^oC, 1g = 1mL = $1cm^3$.)

   Is the measured volume (mL) nearly the same as your calculated volume (cm^3) (Hint: think about whether you used the inner or outer diameter, and how that might have affected your result)?

   If not, what explanations can you provide for the observed difference?

D. Units of Mass

1. Measure the mass of water that a soda can holds by completing these steps:
   Place the empty soda can on the triple-beam balance and record its mass. Then fill the can with water and record the mass of the full can. Calculate the mass of the water by subtracting the mass of the empty can from the mass of the full can.

   Mass of empty soda can = _____ g

   Mass of soda can + water = _____ g

   Mass of water = _____ g

2. Compare the mass of water you measured (g) with your measured volume (mL).

   Are these values similar or different? What might be the reason for any differences?

Note: The term **weight** was avoided in the above discussion because **mass** is a quantity of matter, while weight depends on the gravitational field in which the matter is located. Thus, if you were on the moon you would weigh less, but your mass would be the same as on earth. Although it is technically incorrect, mass and weight are often used interchangeably.

3. Using the container of salt provided and a weigh boat, weigh out 5 grams of salt using the *triple beam balance* (the 5 g will include the mass of the weigh boat.) Be as precise as possible in your measurement.
4. Take the weigh boat with the salt you just measured and place it on the digital scale. Be sure to zero the scale before you begin. Record the mass that registers on the digital scale.

Is the value registered on the digital scale exactly 5g?

What are the benefits and limitations of using a triple-beam balance versus a digital scale to measure mass?

E. Graphing and Predicting the Rate of a Simple Reaction

1. Add 10mL of vinegar into a 100mL graduated cylinder. Weigh out 0.1g of baking soda using the weigh boat provided.
2. Add the baking soda to the vinegar and ***quickly*** observe and record the maximum volume of bubbles that is produced from the reaction in the data table below.

Vinegar (mL)	Baking Soda (g)	Volume of Bubbles Produced (mL)
10	0.1	
10	0.3	
10	0.5	
10	0.7	

3. Pour the completed reaction solution down the sink, and using a fresh batch of vinegar, repeat the experiment for the new amount of baking soda.
4. Do NOT put away your lab equipment yet. You need to conduct further experiments AFTER graphing your data.
5. Draw a graph of the amount of baking soda (x-axis) vs. volume of bubbles (y-axis) and draw a best-fit line through your data points.

6. Using the best-fit line on your graph, predict the volume of bubbles that will be produced if you add 0.2g and 0.6g of baking soda to the reaction.

   Predicted volume of bubbles that will be produced for:

   0.2g of baking soda: _____ mL

   0.6g of baking soda: _____ mL

7. Repeat steps 1-3 of the experiment using 0.2g and 0.6g of baking soda and record your results below.

   Observed volume of bubbles that were produced for:

   0.2g of baking soda: _____ mL

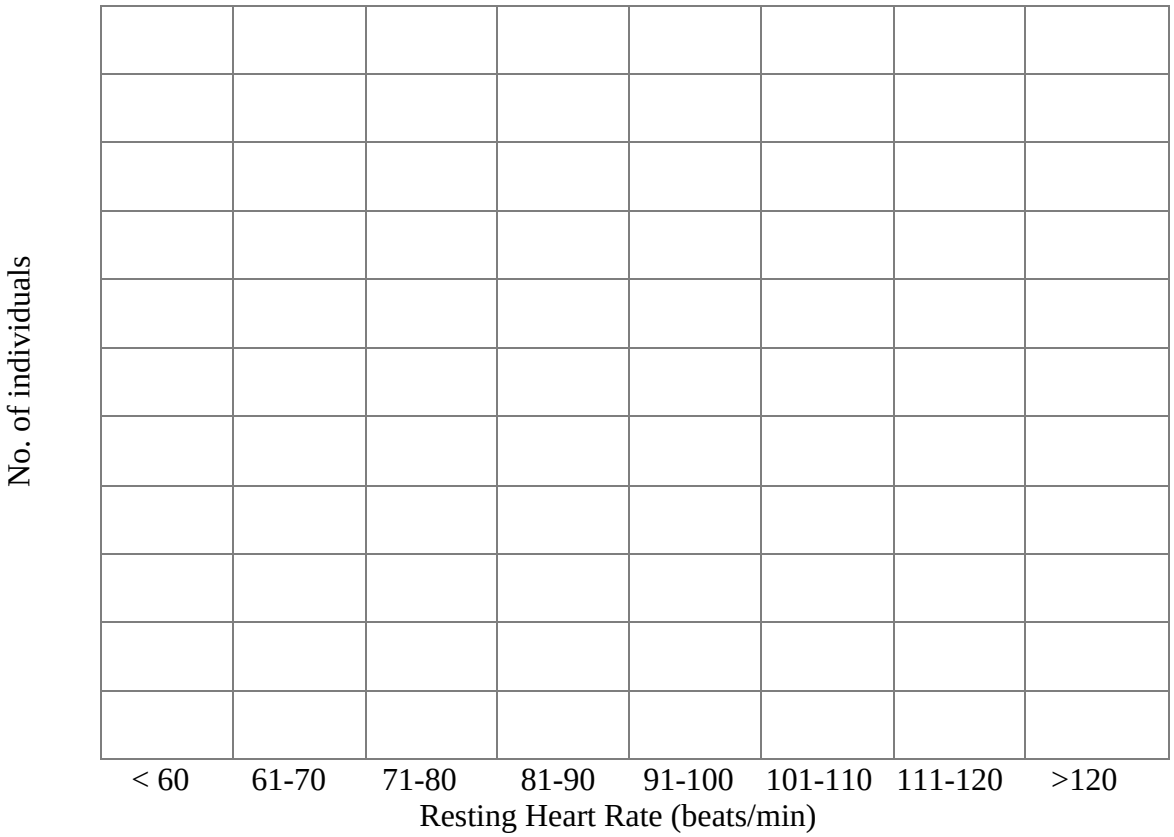
   0.6g of baking soda: _____ mL

Were your predictions correct? If not, how could you explain the discrepancy between your observed and expected results?

F. Dispersion of Data

1. For this activity, you will be determining the dispersion of data using the heart rate of your classmates.
2. Find a quiet spot to sit down and relax. Place two fingers on your carotid artery on your neck or on your vein in your wrist and count how many beats you feel in a minute. You can take a measurement for 15 seconds and multiply this number by 4.
3. Record your heart rate on the table or graph that your instructor has drawn on the board. Note how the dispersion of the data changes as more students record their results.
4. Draw a graph below showing the class data after everyone has recorded their heart rate on the board.

Graph of **Resting** Heart Rate for the class:



Does the graph show a normal distribution or is it skewed to one side?

How could the sample size affect the shape of the graph and the dispersion of data?

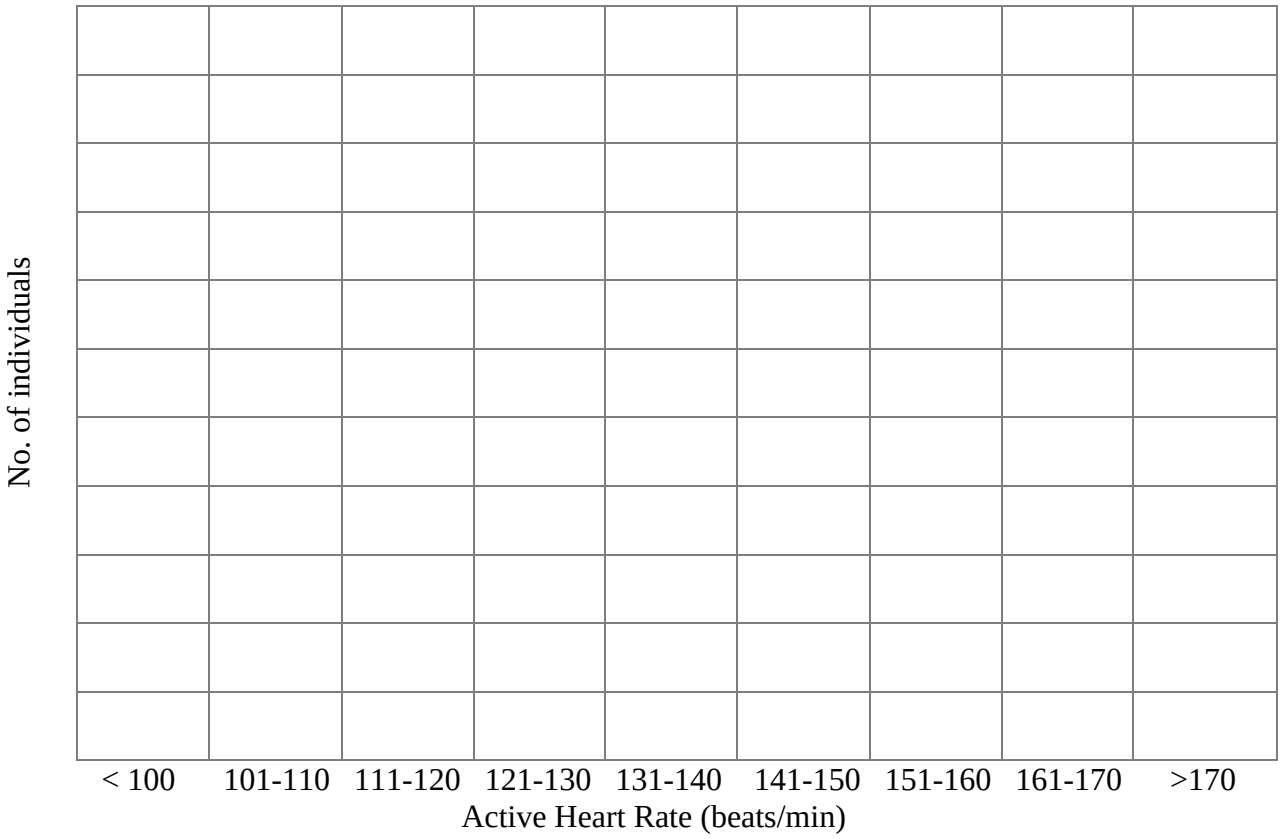
5. A healthy resting heart rate ranges between 60-100 beats per minute. Calculate the average heart rate for the class.

*Average heart rate for class:*_____

How does your heart rate compare with the class average?

6. Spend 30 sec. doing moderately rigorous activity outside (running in place, walking up and down the stairs quickly, jumping jacks, etc.) Your instructor may specify and activity for you to complete to standardize the procedure.
7. Measure your heart rate AFTER you have completed your activity and then repeat steps 3-4.

Graph of **Active** Heart Rate for the class:



Does the graph show a normal distribution or is it skewed to one side?

8. A healthy active heart rate ranges between 100-170 beats per minute depending on age. Calculate the average heart rate for the class.

*Average heart rate for class:*_____

How does your heart rate compare with the class average?

VI. Post-Lab Questions

1. Calculate the following using the conversion factors provided in the background reading.

a. 2 meters = _____ centimeters

b. 2 kilograms = _____ milligrams

c. 5000 micrometers = _____ meters

d. 1 millimeter = _____ micrometers

e. 30 liter = _____ milliliter

2. Calculate the following using the conversion factors provided in the background reading.

a. 3.5 yards = _____ meters

b. 4 pounds = _____ kg

c. 9 inches = _____ cm

d. 20 ^{o}C = _____ ^{o}F

3. Describe how you would draw a best-fit line through sample data points.

4. Explain what kinds of data are best presented in a line graph vs. a bar graph.

<u>Important Note:</u>
In preparation for Lab 3 (Macromolecules & Nutrition), you will need to record what you ate and drank for one of your meals. Refer to the pre-lab questions for Lab 3 for more information about the assignment.

Lab 3: Macromolecules and Nutrition

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Choose the appropriate tests to determine the presence of carbohydrates, protein and lipids in sample foods and analyze the results.
2. Calculate total and % Calories from carbohydrates, protein and fat in sample foods.
3. Analyze the nutritional value of various foods compared to nutritional guidelines.

II. Background Information[1]:

In an age where we have unlimited access to a wealth of information at our fingertips, the challenge we are faced with is making sense of that information and evaluating the accuracy of it. Nutritional information is a good example of this. Every day we are bombarded by news organizations, health magazines, self-help books and food company advertisements, all trying to tell us what to eat and what to avoid, what is the latest fad for losing weight, what is part of "a healthy breakfast", etc. In order to evaluate these claims objectively, it is essential to have an understanding of the basic chemistry of biological macromolecules and human nutritional requirements.

Fig. 3.1. In 2011, the USDA released the federal government's new food icon, MyPlate, to serve as a reminder to help consumers make healthier food choices.[2]

A. Macromolecules:

Macromolecules are very large molecules, formed of smaller subunits. In this lab, we will focus on the three macromolecules that are important energy sources for biological organisms: carbohydrates, proteins and fats.

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[1] Nutrition.gov: http://www.nutrition.gov/smart-nutrition-101

[2] United States Dept. of Agriculture: www.choosemyplate.gov

Carbohydrates:

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen. Your body uses carbohydrates (carbs) to make glucose, which is the fuel that gives you energy and helps keep everything going. Your body can use glucose immediately or store it in your liver and muscles for when it is needed.

In general, the smaller carbohydrate compounds are commonly referred to as sugars, which are found naturally in foods such as fruits, vegetables, milk, and milk products. Simple carbohydrates also include sugars added during food processing and refining.

Starch and dietary fiber are the two types of complex carbohydrates. Starch must be broken down through digestion before your body can use it as a glucose source. Quite a few foods contain starch and dietary fiber such as breads, cereals, and vegetables.

Proteins:

Proteins are part of every cell, tissue, and organ in our bodies. These body proteins are constantly being broken down and replaced. The protein in the foods we eat is digested into amino acids that are later used to replace these proteins in our bodies.

Protein is mainly found in the following foods: meats, poultry, and fish, legumes (dry beans and peas), tofu, eggs, nuts and seeds, milk and milk products, and grains.
Most adults in the United States get more than enough protein to meet their needs. It is rare for someone who is healthy and eating a varied diet to not get enough protein.

There are 20 different amino acids that join together to make all types of protein. Our bodies cannot make some of these amino acids so these are known as ***essential*** amino acids – it is essential that our diet provide these. A complete protein source is one that provides all of the essential amino acids, for example, meat, poultry, fish, milk, eggs, and cheese are considered complete protein sources. An incomplete protein source is one that is low in one or more of the essential amino acids. Complementary proteins are two or more incomplete protein sources that together provide adequate amounts of all the essential amino acids (e.g. rice and beans).

Fats:

Although the term ***lipid*** is sometimes used as a synonym for ***fats***, fats are actually a subgroup of lipids composed of triglycerides. Moreover, triglycerides that are solid at room temperature are often called ***fats*** while those that remain liquid at room temperature are called "***oils***." Triglycerides are also composed of carbon, hydrogen and oxygen atoms, but in different ratios than in carbohydrates. Triglycerides have long chains of carbon and hydrogen bonds, which creates the hydrophobic tail. This property prevents triglycerides from mixing readily with water and causes them to separate relatively easily in solution, a property that we will utilize in our experiment today.

Unsaturated Fats, Saturated Fats and Trans Fats:

Most of the fat that you eat should come from unsaturated sources: polyunsaturated fats and monounsaturated fats. In general, nuts, vegetable oils, and fish are sources of unsaturated fats. Polyunsaturated fats can also be broken down into two types:
Omega-6 polyunsaturated fats: these fats provide an essential fatty acid that our bodies need, but can't make (e.g. soybean oil, corn oil and safflower oil).
***Omega-3 polyunsaturated fats*:** these fats also provide an essential fatty acid that our bodies need (e.g. soybean oil, canola oil, walnuts, flaxseed and fish). In addition, omega-3 fatty acids, particularly from fish sources, may have potential health benefits.

Saturated fats are oftentimes the "solid" fats, but other saturated fats can be more difficult to see in the foods we consume. In general, saturated fat can be found in the following foods: high-fat cheeses, high-fat cuts of meat, whole-fat milk and cream, butter, ice cream and palm and coconut oils. Although animal fats are the primary source of saturated fat, palm and coconut oils, and cocoa butter are also important sources of saturate fat, and these are often added to commercially-prepared foods, such as cookies, cakes, doughnuts, and pies. Solid vegetable shortening often contains palm oils and some whipped dessert toppings contain coconut oil.

Trans fats have received a lot of media attention recently. Consuming trans fat can increase the amount of “bad" cholesterol in the body and may also have other adverse health effects like decreasing the amount of "good" cholesterol. The most common types of trans fats are found in foods that contain partially hydrogenated oil. Food manufacturers use artificial trans fat in food products because it is inexpensive and it increases the food’s shelf life, stability, and texture. Major contributors to artificial trans fat intake include fried items, savory snacks (like microwave popcorn), frozen pizzas, cake, cookies, pie, margarines and spreads, ready-to-use frosting, and coffee creamers.

Sodium:

Sodium occurs naturally in most foods. The most common form of sodium is sodium chloride, which is table salt. Milk, beets, and celery also naturally contain sodium. Sodium is also added to many food products. Some of these added forms are monosodium glutamate (MSG), sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate. These are in items such as Worcestershire sauce, soy sauce, onion salt, garlic salt, and bouillon cubes. Processed meats like bacon, sausage, and ham, and canned soups and vegetables also contain added sodium. Fast foods are generally very high in sodium.

Table salt is 40% sodium; 1 teaspoon of table salt contains 2,300 mg of sodium.
Healthy adults should limit sodium intake to 2,300 mg per day. Adults with high blood pressure should have no more than 1,500 mg per day.

B. Food Calories

When speaking of the energy content of a macromolecule we often refer to calories or Calories. There is a big difference between discussing "calories" and "Calories." A calorie is defined as the amount of heat needed to raise the temperature of 1g of water by 1°C. Notice that the word "calorie" is spelled with a lower case letter "c." This is how a physicist would describe a calorie. Nutritionists measure the amount of energy in food in Calories (with a capital letter "C'), which is equivalent to 1 kcal or 1000 calories (lower case "c"). The amount of Calories per gram of each macromolecule is listed below.

1 gram of carbohydrate = 4 Calories
1 gram of protein = 4 Calories
1 gram of fat = 9 Calories

You can see from the values above that one gram of fat contains more than double the amount of energy as a gram of carbohydrate or protein. By analyzing nutrition labels, it is possible to calculate the amount of Calories that each macromolecule contributes by multiplying the number of grams by the Caloric value. You can also calculate the % Calories by dividing the Calories from each macromolecule by the total Calories.

> Example: A candy bar contains 5g of carbohydrate, 4g of Protein and 2g of Fat.
> The Calories contributed by each macromolecule:
>
> Carbohydrate: 5g x 4 Cal = 20 Calories
> Protein: 4g x 4 Cal = 16 Calories
> Fat: 2g x 9 Cal = 18 Calories
>
> Total: 20 + 16 + 18 = 54 Calories
>
> % Calories from Carbohydrate: (20/54) x 100 = 37%
> % Calories from Protein: (16/54) x 100 = 30%
> % Calories from Fat: (18/54) x 100 = 33%

The *Nutrition Facts* label is designed to give consumers important nutritional information about a product and allow comparisons with other food. The serving size indicates the amount of food that the nutrition information applies to. However, a serving size according to a package may bear little resemblance to the amount of the food that most people eat at a time.

When doing Calorie calculations using the Nutrition Facts label, it is important to also look at the serving size. For example, a bag of chips may be 2 servings, so to calculate the total and % calories for the entire bag (if that is what is being asked in the question), you will need to multiply the values by 2.

C. Chemical Analysis of Food

The presence of various biological macromolecules can be determined using the following tests:

Test Name	Macromolecule it tests for:	If macromolecule is present:	If macromolecule is absent:
Benedict's	Simple sugars (except sucrose)	Color turns yellow, red-orange or green	Color stays clear blue
Iodine	Starch	Color turns black	Color stays yellow/orange
Biuret	Protein	Color turns violet	Color stays light blue
Sudan	Fat (Lipid)	Food stains red-orange	Food does not stain red-orange
Paper	Fat (Lipid)	Paper turns translucent (semi-transparent)	Paper stays opaque (not transparent)

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7^{th} ed.): pp.40-47 (Macromolecules), and pg. 78 (Food Calories).

B. Recommended background reading (optional):

1. 2015–2020 Dietary Guidelines for Americans
http://health.gov/dietaryguidelines/2015/

2. FDA: How to Understand and Use the Nutrition Facts Label
http://www.fda.gov/food/ingredientspackaginglabeling/labelingnutrition/ucm274593.htm

IV. Pre-Lab Questions:

1. List the three types of macromolecules that provide energy for biological organisms and the number of Calories provided by 1g of each macromolecule.

2. A package of peanut butter crackers contains 7g of fat, 16g of carbohydrates and 4g of protein.

a) Calculate the Calories from each macromolecule.

b) Calculate the % Calories for each macromolecule.

3. An unknown food item is tested using Iodine, Biuret, Sudan, Benedict's and paper. The results are listed below. Fill in the table with the required information.

Test	Observed result	What macromolecule does it test for?	Is the macromolecule present or absent?
Biuret	Violet		
Paper	Translucent		
Iodine	Yellow		
Benedict's	Blue		
Sudan	Orange		

4. During the week prior to this lab, you will choose a meal and record what you ate and how much. If you choose a restaurant meal or packaged food, you should record the nutritional information that is posted. If you choose a home-cooked meal, you should record the approximate amount of each food and drink item you consumed. During the lab, you will be asked to calculate the total and % Calories of the meal you have recorded.
Note: If possible, bring in the empty, cleaned food containers (or photos of them) and/or restaurant nutritional information pamphlets with you to lab.

You may also use the following website to help keep track of your meals.
USDA SuperTracker: https://www.supertracker.usda.gov/default.aspx

Circle: Breakfast / Lunch / Dinner
Note: You may need fewer or more rows than those listed; use only as many rows as you need to complete your meal. Attach a separate sheet if you need more space.

Food / Drink Item	Weight of carbohydrate, protein & fat contained in each item.

V. Lab Exercise:

Materials

Per Group:	Per Room:
• 150ml beaker • 2 plastic pipettes • glass stir rod • bottle of DI water • china marker • test tube clamp • test tube rack • 6 test tubes • 1 watch glass • spatula • filter paper	• 100^{o}C water bath • Iodine • Biuret • Sudan • Benedict's • paper • goggles • rubber gloves • experimental foods • mortar and pestle • sample nutritional information sheets

Procedure

A. Chemical Analysis of Sample Foods

1. Fill approx. 1/3 of one of your test tubes with a sample food item that is available. If the food is in solid form (i.e. chips), you will need to grind it to a very fine powder using a mortar and pestle, add enough water to turn it into a liquid, it and mix it well.
2. Label the test tube with your group name and place it in the 100^{o}C water bath for 10 min.
3. Prior to conducting your experiment, formulate a hypothesis about which macromolecules you would expect to find in your chosen food substance. Record your hypothesis in the table below.
4. Remove the test tube and observe the layers. There may be a distinct top, yellowish layer and a bottom layer, or there may be just a single layer. The bottom layer may contain solid precipitate (i.e. chip crumbs). One of the layers may be a liquid and the other one a solid. Write or draw your observation of the layers in the data section of the table below.
5. **Paper test:** Remove 1 drop from the top of your sample food solution using a pipette, place it onto a piece of paper and rub it in. Wait for the paper to completely dry. Observe and record whether the paper turns translucent (+) or remains opaque (-).
6. **Iodine test:** Place 5 drops of your sample food solution into a clean test tube. Add 5 drops of iodine to it and mix well. Immediately observe and record whether the solution turns black (+) or remains yellow/orange (-) (the color will fade after a few minutes).
7. **Biuret test:** Place 5 drops of your sample food solution on to a watch glass. Add 5 drops of Biuret to it and mix well. Wait 5 min. and then observe and record whether the solution turns violet (+) or remains light blue (-).
   Note: the color change may be subtle; for best results observe the solution against a white background.

8. **Sudan test:** Add 2 drops of your sample food solution onto a small piece of filter paper. If your food sample has a solid precipitate at the bottom, be sure to include it onto the filter paper. Soak the paper for 3 min in the Sudan solution. Rinse the filter paper in the room temperature water bath for 1 min. Observe and record whether the sample food stained red-orange (+) or did not change color (-).
9. **Benedict's test:** Place 5 drops of your sample food solution into a clean test tube. Add 5 drops of Benedict's solution to the test tube. Heat your test tube in the 100^{o}C water bath for **exactly 2min**. Remove your test tube and immediately observe and record whether the solution turns red/orange (+) or remains blue (-).

Sample Food 1:

Food substance tested:	
Question:	*Which macromolecules (carbohydrate, protein, fat) does the food contain?*
Hypothesis (indicate which macromolecules you expect your sample to contain):	
Results: **Visual observation of layers (describe or draw):**	
Paper test result (transparency):	
Sudan test result (color):	
Biuret test result (color):	
Iodine test result (color):	
Benedict's test result (color):	
Conclusion: (Which macromolecules are present in this food item?)	

Were there any results that surprised you? If yes, what might be the reason(s) behind your observed result?

10. When you finish analyzing your sample food 1, wash all your materials and repeat steps 1-9 for your second sample food. You may be asked to share your results with the rest of the class.

Sample Food 2:

Food substance tested:	
Question:	*Which macromolecules (carbohydrate, protein, fat) does the food contain?*
Hypothesis (indicate which macromolecules you expect your sample to contain):	
Results: **Visual observation of layers (describe or draw):**	
Paper test result (transparency):	
Sudan test result (color):	
Biuret test result (color):	
Iodine test result (color):	
Benedict's test result (color):	
Conclusion: (Which macromolecules are present in this food item?)	

Were there any results that surprised you? If yes, what might be the reason(s) behind your observed result?

B. Analysis of a Diet

1. Using your food log from the pre-lab questions section, complete the following table for your meal. You may not need to use all the rows provided.

2. For each food/drink item, enter the amount of carbohydrate, protein and fat in grams in cells a-c, then calculate the calories for each macromolecule in cells d-f.

3. Add all the cells labeled (d) and enter the value into cell (g) for the total Calories from carbohydrates. Repeat for cells labeled (e) and enter the value into cell (h) for the total Calories from protein. Repeat for cells labeled (f) and enter the value into cell (i) for the total Calories from protein.

4. Add cells g-I and enter the value into cell j to get the total Calories for your meal. Finally, to calculate the % Calories from carbohydrates, calculate: (g/j)x100. Repeat the same procedure for protein (h/j)x100 and fat (i/j)x100.

Meal Calorie and % Calorie calculations:

Food / Drink Item		Carbohydrate	Protein	Fat
1.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
2.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
3.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
4.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
5.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
Total calories from each macromolecule:		g)	h)	i)
Grand total calories from all macromolecules combined:		j)		
Percentage calories from each macromolecule:		k)	l)	m)

CDC recommended nutritional guidelines:
Calories from carbohydrates: 45-65%
Calories from protein: 10-35%
Calories from fats: 20-35%

Compare the % Calories for your two sample meals with the CDC recommended values (listed above). In what ways are they similar and/or different?

Do the meals in your food log accurately represent the proportion of carbohydrate, protein and fat you consume in the rest of your meals? If not, how are they different?

C. Analysis of Sodium (Salt) and Sugar in Sample Foods

1. Observe the sample food containers on display and match the pre-measured amounts of sodium and sugar to each of the sample foods.
2. The nutrition labels have been purposefully covered. You will have to rely on your own judgment to complete the activity as accurately as possible.
3. Enter your guesses in the "predicted amount" columns in the data tables below and then check your answers with the key provided and make any necessary corrections in the "actual amount" columns below.

Sodium (Salt):

Food/drink item	Predicted sodium amount (mg)	Actual sodium amount (mg)

Sugar:

Food/drink item	Predicted sugar amount (g)	Actual sugar amount (g)

How accurate were you in your predictions? Did any of the answers surprise you?

VI. Post-Lab Questions

1. Use the nutrition label below to answer the following questions.

PLAIN YOGURT		
Nutrition Facts		
Serving Size		1 cup (227g)
Amount per Serving		% Daily Value
Total Fat	9g	14%
Saturated Fat	5g	25%
Trans Fat	0g	
Cholesterol	30mg	10%
Sodium	115mg	5%
Total Carbohydrates	12g	4%
Dietary Fiber	0g	0%
Sugars	11g	
Protein	8g	16%

a) Complete the following table for one serving of yogurt.

Nutrient	**Total Calories**	**% Calories**
Carbohydrate		
Protein		
Fat		

b) Compare the % Calories for carbohydrate, protein and fat in the plain yogurt with the CDC recommended values (listed on pg. 44). Do they fall within or outside the recommended range?

c) Based on these results, is this serving of yogurt a healthy snack choice? Why or why not?

d) Percent Daily Value (DV) on the Nutrition Facts label is a guide to the nutrients in one serving of food. For example, if the label lists 5% for sodium, it means that one serving provides 5% of the sodium you need each day.
Why do you think there are no % Daily values listed for **Trans Fat** and **Sugar**?

Lab 4: Enzymes

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Define the following terms: substrate, product, active site and catalyst.
2. Explain how enzymes function as catalysts.
3. Explain how environmental factors such as temperature, pH and salt concentration can affect the functioning of enzymes.
4. Design an experiment to test the effect of environmental factors on enzyme function and analyze the results of your experiment.
5. Explain the purpose of using a spectrophotometer.

II. Background Information:

A. Enzymes[1]:

Enzymes speed the rate of chemical reactions. A **catalyst** is a substance involved in, but not consumed in, a chemical reaction. Enzymes are proteins that catalyze biochemical reactions by lowering the activation energy necessary to break the chemical bonds in reactants and form new chemical bonds in the products (Fig. 4.1). Catalysts bring reactants closer together in the appropriate orientation and weaken bonds, increasing the reaction rate. Without enzymes, chemical reactions would occur too slowly to sustain life.

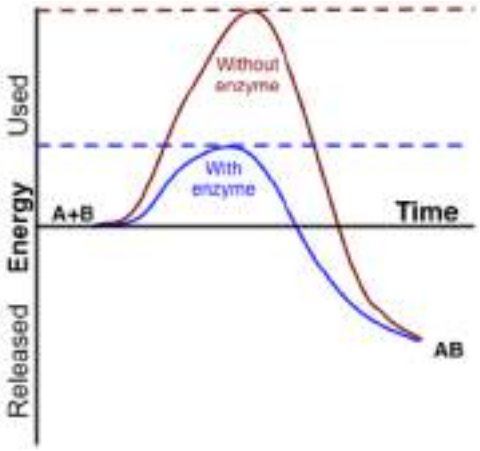

Fig. 4.1. Enzymes catalyze chemical reactions by lowering the activation energy necessary for a chemical reaction to proceed.[2]

The functionality of an enzyme is determined by the shape of the enzyme. The area in which bonds of the reactant(s) are broken is known as **the active site**. The reactants of enzyme

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[1] Washington State Open Course Library: http://opencourselibrary.org/

[2] Wikimedia Commons: http://commons.wikimedia.org

catalyzed reactions are called **substrates**. The active site of an enzyme recognizes, confines, and orients the substrate in a particular direction (Fig. 4.2). Enzymes are substrate specific, meaning that they catalyze only specific reactions. For example, proteases (enzymes that break peptide bonds in proteins) will not work on starch (which is broken down by the enzyme amylase). Notice that both of these enzymes end in the suffix -ase. This suffix indicates that a molecule is an enzyme.

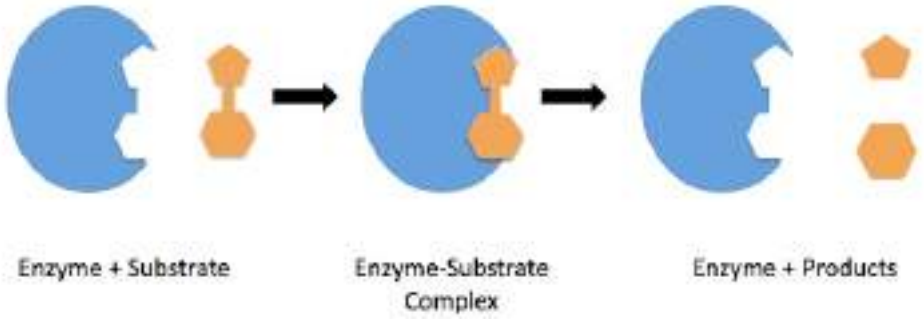


Fig. 4.2. A diagram illustrating a substrate binding to the active site of an enzyme. After the product is formed and released from the active site, the enzyme can accept a new substrate molecule.[3]

B. Environmental Effects on Enzyme Activity:

Environmental factors may affect the ability of enzymes to function. Enzymes function best when they are operating within optimal environmental conditions such as within a specific range of temperatures and pH (Fig. 4.3). It is important to note that enzymes in different organisms, or even in different parts of the same organism function optimally under different conditions. For example, the enzymes in your muscle cells may function better under close to neutral pH whereas the enzymes that break down the food in your stomach function optimally under acidic conditions (Fig. 4.4).

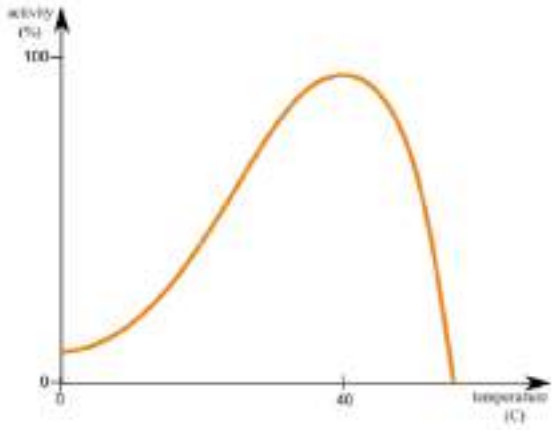


Fig. 4.3. The relationship between temperature and enzyme activity. Different enzymes will have peak activity at different temperatures.[4]

[3] Wikimedia Commons: http://commons.wikimedia.org

[4] Wikimedia Commons: http://commons.wikimedia.org

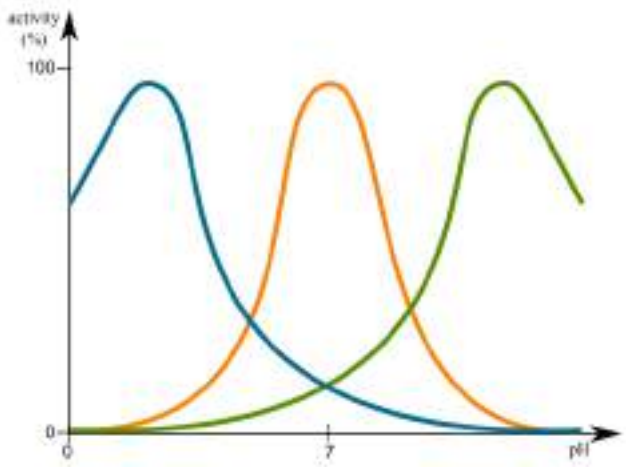


Fig. 4.4. The relationship between pH and enzyme activity for three different enzymes. Note that each enzyme demonstrates peak activity at a different pH.[5]

C. Catecholase and Catechol

Small amounts of the substrate **catechol** occur naturally in fruits and vegetables, along with the enzyme **catecholase**. Upon mixing the enzyme with the substrate and exposure to oxygen (e.g. when a potato or apple is cut and left out), the colorless catechol oxidizes to a reddish-brown product called **quinone**. Quinone has antimicrobial properties, which slows the spoilage of wounded fruits and other plant parts.

Reaction:	Catechol + (substrate)	Catecholase → (enzyme)	Quinone (product)
Color or source:	colorless	potato extract	brown

The enzyme can be inactivated by adding an acid, such as lemon juice, and slowed with cooling. Cooking denatures the enzyme because high heat causes the shape of the enzyme, particularly the active site, to change and no longer be functional. Covering cut fruit in an airtight seal also prevents the browning reaction because oxygen is necessary for the enzyme to break down the substrate.

D. Catalase and Hydrogen Peroxide

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of **hydrogen peroxide** to **water and oxygen**. It is a very important enzyme in protecting the cell from oxidative damage.

Catalase catalyzes the following reaction: $2H_2O_2$ (hydrogen peroxide) $\rightarrow$ 2 H_2O + O_2

[5] Wikimedia Commons: http://commons.wikimedia.org

Adding hydrogen peroxide and observing the reaction can demonstrate the presence of catalase in a microbial or tissue sample. The production of oxygen can be seen by the formation of bubbles. This easy test, which can be seen with the naked eye, without the aid of instruments, is possible because catalase has a very high specific activity, which produces a detectable response, as well as the fact that one of the products is a gas.

E. Amylase and Starch

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into simple sugars. The pancreas and salivary gland make amylase hydrolyze dietary starch into disaccharides and trisaccharides, which are converted by other enzymes to glucose to supply the body with energy. One way to observe the production of simple sugars like maltose and glucose is by using a Benedict's test (refer to Lab 3).

Amylase catalyzes the following reaction:
starch (polysaccharide of glucose molecules) → maltose (disaccharide of glucose molecules)

F. Quantifying Enzyme Activity using the Spectrophotometer:

A **spectrophotometer** is an instrument that is used to measure the amount of light that is transmitted or absorbed as it passes through a solution (Fig. 4.5). According to Beer's law, the amount of light absorbed by the solution is proportional to the concentration of the absorbing material. Thus, spectrophotometry is one method of quantifying reaction rate when the substrate is colorless and the product has color; the darker the product color, the greater the absorbance will be, which would indicate that the reaction magnitude is greater.

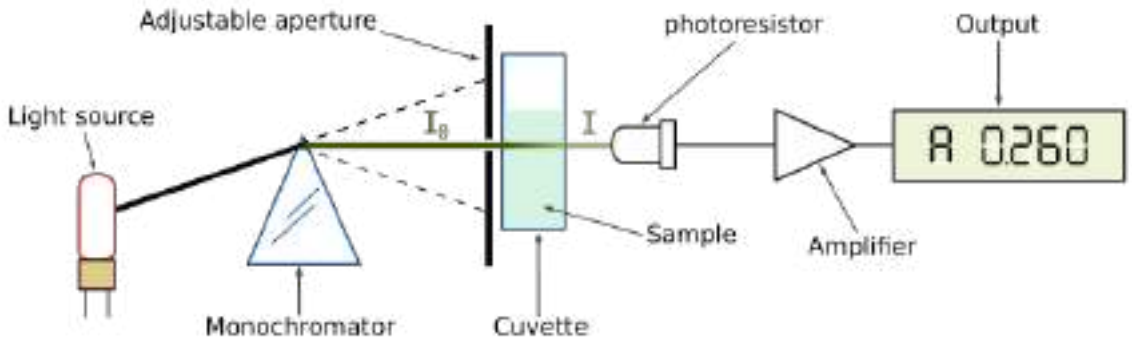


Fig. 4.5. A diagram of how a spectrophotometer works by passing a beam of light through a sample solution located inside a cuvette. The amount of light absorbance is a quantifiable measure of reaction rate in our experiment.[6]

[6] Wikimedia Commons: http://commons.wikimedia.org

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7^{th} ed.): pp.80-82 (Enzymes)

B. Recommended background reading (optional):
1. McGraw Hill Online Learning Center: How Enzymes Work
http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

2. Properties of Enzymes and Use in Industries
https://www.bbc.com/education/guides/zdt4jxs/revision/1

3. Enzymes and Digestion
http://www.bbc.co.uk/schools/gcsebitesize/science/add_aqa_pre_2011/enzymes/enzymes_and_digestion1.shtml

IV. Pre-Lab Questions:

1. a) Enzymes are an example of which macromolecule? _____

b) What are the subunits that make up enzymes? _____

2. a) The location that a substrate binds to an enzyme is called: _____

b) List <u>two</u> environmental variables that may cause an enzyme to dentaure:

c) Explain how an enzyme functions as a catalyst of chemical reactions.

d) Provide one explanation as to why denatured enzymes cannot catalyze chemical reactions.

3. For the **catecholase** experiment that we will be conducting today, identify the following:

a) What is the substrate that catecholase breaks down? _____

b) What is the product that is formed? _____

c) How can we observe enzyme activity with this reaction?

4. For the **catalase** experiment that we will be conducting today, identify the following:

a) What is the substrate that catalase breaks down? _____

b) What is the product that is formed? _____

c) How can we detect enzyme activity with this reaction?

5. For the **amylase** experiment that we will be conducting today, identify the following:

a) What is the substrate that amylase breaks down? _____

b) What is the product that is formed? _____

c) How can we detect enzyme activity with this reaction?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 10 test tubes • 6 cuvettes • 10ml graduated cylinder • Three 1ml plastic pipettes • 150 ml beaker • glass stir rod • squirt bottle with DI water • china marker • test tube clamp	• Potato • Blender • Digital scale and weigh boat • Cutting board, knife and vegetable peeler • 500ml flask, cheesecloth and funnel • 250ml graduated cylinder • Catechol solution • Spectrophotometer and "blank" cuvette • 100^{o} 37^{o} and room temperature water baths • ice bath • pH 4, 8 and 12 solutions • 0%, 10% and 25% salt solutions • Benedict's, starch, amylase and catalse solutions • liver

B. Procedure

1. Isolation of the Catecholase Enzyme:

One group should conduct the following procedure for the whole class:

1. Peel a potato and dice it into small pieces (approx. $2cm^3$).
2. Weigh out 100g of the diced potato and place it in a blender along with 150ml of tap water. Blend for about 1min until the mixture is smooth.
3. Place the extra, diced potato in a beaker and cover it with water to prevent it from browning.
4. Filter the slurry in the blender through several layers of cheesecloth held within a funnel into a flask and keep the flask covered.
5. This is the stock solution of the **catecholase enzyme** that all the groups will use. If more enzyme solution is needed, the above steps should be repeated with the remaining diced potato.
6. Wash all the equipment used to prepare the enzyme solution immediately.

Important Note: The stock solution of potato extract should be used as quickly as possible since the solution will start to oxidize and turn brown when exposed to air.

2. Catecholase Control Experiments:

The purpose of the following control experiments is to observe the color of the solution in the i) absence and ii) presence of the enzyme. A **negative control** ensures that there is no effect when there should be no effect. A **positive control** ensures that there is an effect when there should be an effect. If the positive control does not produce the expected result, there may be something wrong with the experimental procedure and the experiment should be repeated.

Negative Control Experiment:

1. Add 1ml of water to a clean test tube.
2. Add 10 drops of catechol to the tube.
3. Gently mix the solution by holding it upright and gently tapping the bottom.
4. Wait 1 min and observe the color of the solution.

Positive Control Experiment:

1. Add 1ml of potato extract (containing catecholase) to a clean test tube.
2. Add 10 drops of catechol to the tube.
3. Gently mix the solution by holding it upright and gently tapping the bottom.
4. Wait 1 min and observe the color of the solution.

Complete the data table and answer the questions below. Be sure to answer steps 1-3 before conducting each control experiment. Complete rows 4-6 in the data table after the experiment is completed.

	Negative Control	Positive Control
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form and what the final color will be):		
4. Color of solution after **2 min.**		
5. Was the product formed?		
6. Was your hypothesis supported?		

If you observed the solution in the negative control turning reddish-brown, what would this suggest?

If you observed the solution in the positive control remaining colorless, what would this suggest?

3. Quantifying Catecholase Enzyme Activity using the Spectrophotometer:

1. For the second part of today's lab, you will quantify the color change in the experiment using a spectrophotometer (review the background information to understand how a spectrophotometer works).
2. You will need to calibrate the spectrophotometer before you begin. You can then record all your data for one experiment without having to the repeat the calibration every time.
3. Make sure the wavelength control is set to 480nm. Set % transmittance to "0" using the ZERO knob (left side) on the front of the machine – this is zero transmittance.
4. Remove the cork and aluminum foil from the BLANK cuvette, insert it into the specimen holder on the machine and close the lid.
5. Set the knob on the front of the spectrophotometer (right side) to 100% transmittance.
6. Remove the BLANK cuvette, cover it and return it to its original location.
7. Place your solution in the specimen holder, close the lid and ***quickly*** record the value for ***absorbance*** value on your data sheet (the value may not stabilize since more product will continue to form and the solution will continue to get darker over time). Repeat this last step with all your experimental samples.

4. Testing the Effect of Environmental Factors on Catecholase Enzyme Activity

1. You may be asked to complete ALL or SOME of the following experiments (check with your instructor before you begin).
2. Be sure to check your experimental protocol with your instructor ***before*** you proceed and fill in the data table for each experiment that you conduct.
3. Remember to **record your hypothesis before you begin each experiment.**
4. You may need to complete one experiment, record your results, discuss your results with your instructor and wash your glassware ***before*** you begin the next experiment.

A. Temperature:

1. Add 5ml of water to each of four, clean test tubes.
2. Add 1ml of potato extract (containing catecholase) to each test tube.
3. Label the tubes near the top with your group name and the temperature of the water bath using a china marker.
4. Place each tube in its corresponding water bath for 20min.
5. At the end of 20min, remove ONE of your test tubes from one of the water baths and place it at your table.
6. Add 10 ***drops*** of catechol to the test tube.
7. Gently mix the solution by holding it upright and tapping the side of the test tube.
8. Transfer the contents of the test tube into the small cuvette and record the absorbance value using the spectrophotometer. Do **NOT** mark the cuvette – it will affect the spectrophotometer readings.
9. Pour out the contents of the cuvette into the **catechol waste** container and wash it.
10. Repeat steps 5-9 for each of the other test tubes in the remaining water baths.

Experiment 1: Temperature

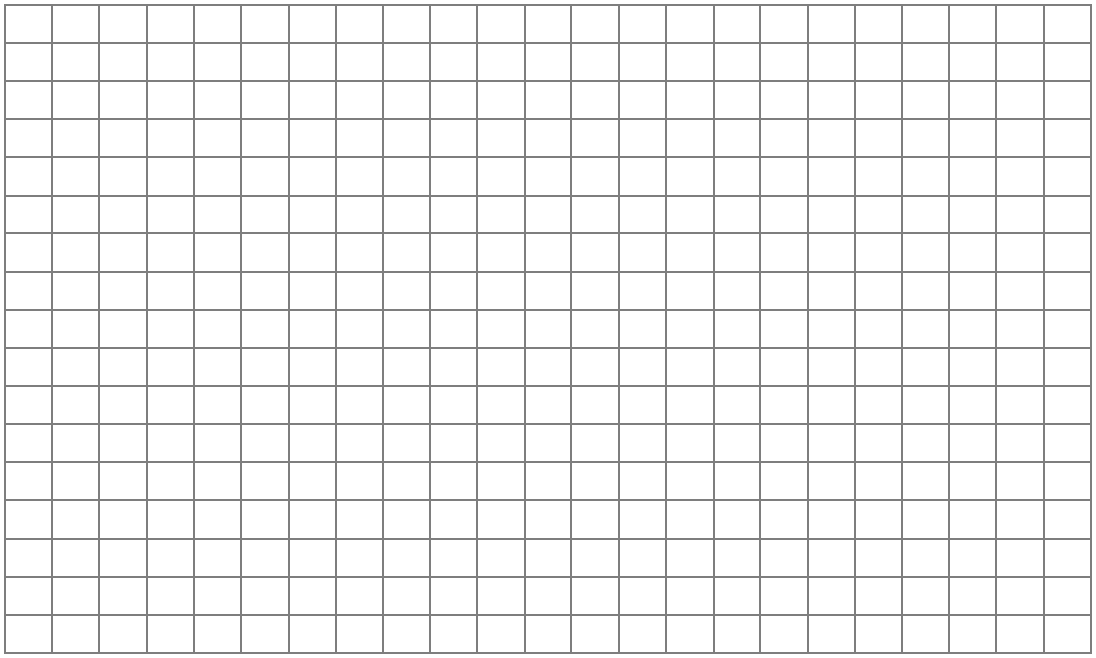
<table>
<tr><td>Hypothesis (Predict how temperature will affect enzyme activity):</td><td colspan="2"></td></tr>
<tr><td>Experimental design changes:
(Explain any changes to the procedure listed above, or any problems you encountered)</td><td colspan="2"></td></tr>
<tr><td rowspan="4">Results:
(Record the absorbance for each temperature tested)</td><td>Temperature (^{o}C)</td><td>Absorbance</td></tr>
<tr><td>20</td><td></td></tr>
<tr><td>37</td><td></td></tr>
<tr><td>100</td><td></td></tr>
</table>

Graphing your results for Experiment 1:

Plot temperature on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary at the different temperatures?

B. pH:

1. Add 5ml of water to each of three, clean test tubes.
2. Add 1ml of potato extract (containing catecholase) to each test tube.
3. Label the tubes with the pH using a china marker.
4. Add 30 ***drops*** of each pH solution to the corresponding test tube.
5. Check the final pH of the solution using pH paper (if available). Be careful not to touch or spill the solution.
6. Add 10 ***drops*** of catechol to each of the test tubes.
7. Gently mix the solution by holding it upright and tapping the side of the test tube.
8. Transfer the contents of the test tubes into small cuvettes and record the absorbance value using the spectrophotometer. Do **NOT** mark the cuvettes – it will affect the spectrophotometer readings.
9. Pour out the contents of the cuvettes into the **catechol waste** container and wash them.

Experiment 2: pH

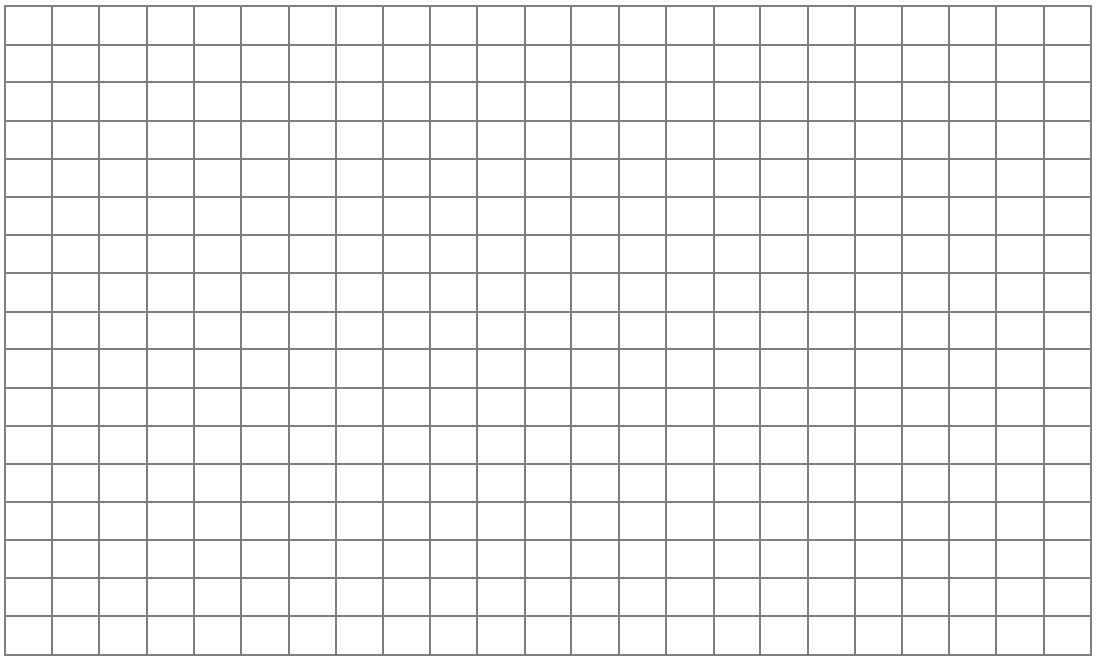
<table>
<tr><td>Hypothesis:</td><td colspan="2"></td></tr>
<tr><td>Experimental design changes:
(Explain any changes to the procedure listed above, or any problems you encountered)</td><td colspan="2"></td></tr>
<tr><td rowspan="4">Results:
(Record the final pH of each solution and the absorbance for each pH tested)</td><td>pH:</td><td>Absorbance:</td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
</table>

Graphing your results for Experiment 2:

Plot pH on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary with the different pH levels?

C. Salt Concentration:
(Time permitting - check with your instructor before proceeding with this experiment.)

1. Add 5ml of water to each of three, clean test tubes.
2. Add 1ml of potato extract (containing catecholase) to each test tube.
3. Label the tubes with your group name and the salt concentration using a china marker.
4. Add 30 ***drops*** of each salt solution to the corresponding test tube. Let the test tubes sit at your counter for 10 min before proceeding with step 5.
5. Add 10 ***drops*** of catechol to each of the test tubes.
6. Gently mix the solution by holding it upright and tapping the side of the test tube.
7. Transfer the contents of the test tubes into small cuvettes and record the absorbance value using the spectrophotometer. Do **NOT** mark the cuvettes – it will affect the spectrophotometer readings.
8. Pour out the contents of the cuvettes into the **catechol waste** container and wash them.

Experiment 3: Salt Concentration

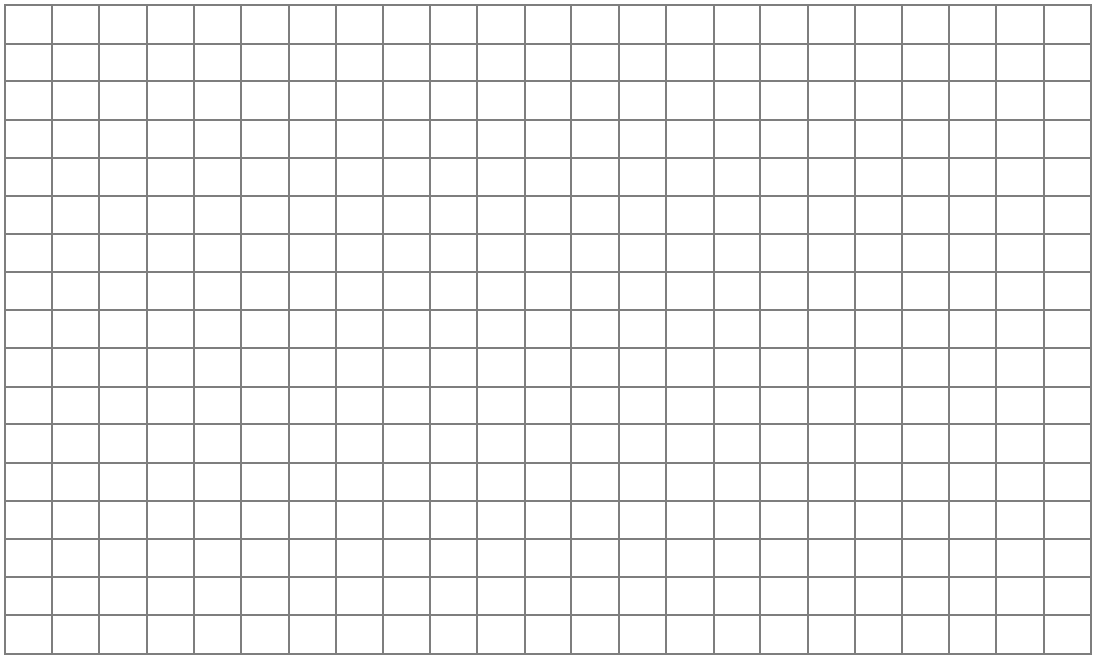
Hypothesis:		
Experimental design changes: (Explain any changes to the procedure listed above, or any problems you encountered)		
Results: (Record the absorbance for each salt concentration tested)	Salt Concentration:	Absorbance:
	0%	
	10%	
	25%	

Graphing your results for Experiment 3:

Plot salt concentration on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary with the different salt concentrations?

5. Observing Amylase Enzyme Activity

1. Add 10 drops of starch solution (substrate) to each of two, clean test tubes.
2. Label one test tube "+ control" and the other one "- control." Mark both tubes with your group name or number.
3. Add 10 drops of amylase (enzyme) to the "+ control" test tube.
4. Add 10 drops water to the "- control" test tube.
5. Add 10 drops Benedict's solution to each tube.
6. Place both tubes in the 100^{o}C water bath for 2min.
7. Remove your test tubes and immediately observe and record whether the solution turns red/orange (+) or remains blue (-).

	Negative Control	Positive Control
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form and what the final color will be):		
4. Color of solution after boiling for **2 min.**		
5. Was the product formed?		
6. Was your hypothesis supported?		

6. Observing Catalase Enzyme Activity

Place a few drops of hydrogen peroxide (substrate) onto the liver sample provided as well as a piece of a potato and complete the table below. The liver and potato may contain different amounts of the catalase enzyme.

	Liver	Potato
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form in each sample):		
4. Observation of the reaction (Did bubbles form?)		
5. Was the product formed?		
6. Was your hypothesis supported?		

VI. Post-Lab Questions

1. Did the results of any of your enzyme experiments refute your hypotheses? If so, propose an explanation for each result that refuted your hypothesis.

2. You conduct an experiment to see how salt concentration affects catecholase enzyme activity. You conduct the experiment with 0%, 5%, 15% and 30% salt and measure the reaction rate at each concentration by recording the absorbance of light using a spectrophotometer. Refer to the data table below when answering the following questions.

Salt Concentration	Absorbance
0%	1.0
5%	0.8
15%	0.5
30%	0.1

a) Catecholase breaks down the substrate _____to produce _____.

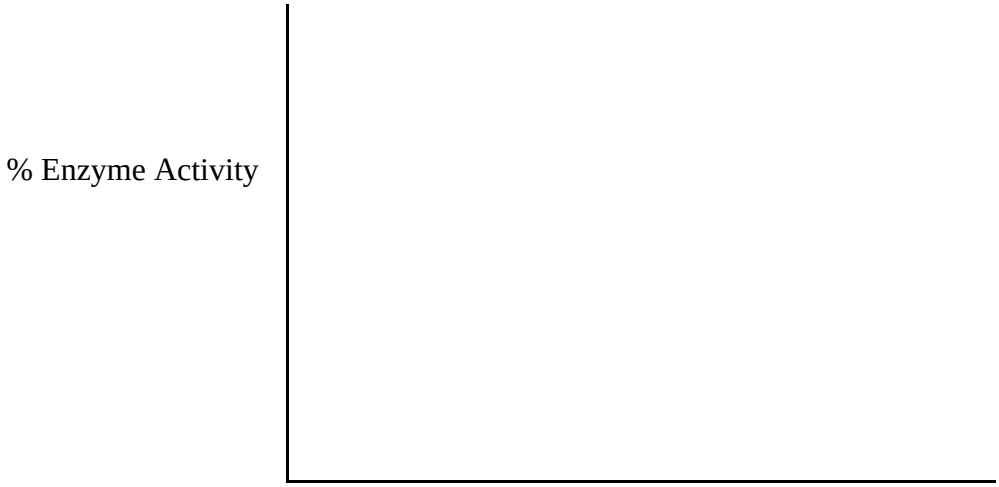
b) At which salt concentration is enzyme activity at an optimal level? _____

c) Provide one biological explanation of how salt concentration affects enzyme activity at 30%.

d) Graph the data in the space below, using appropriately labeled axes.

5. Some animals are **homeothermic**, meaning they maintain a constant body temperature (e.g. mammals) whereas other animals are **poikilothermic**, meaning their body temperature changes with the environmental temperature (e.g. many species of fish).

a) Predict and draw on the graph below what the % enzyme activity curve would look like for a homeothermic and a poikilothermic animal if their body temperature were to change. Be sure to label each curve as homeothermic or poikilothermic.



b) Provide a biological explanation for your prediction.

Lab 5: Photosynthesis and Cellular Respiration

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Write the equation for photosynthesis and cellular respiration.
2. Identify the necessary reactants and products of photosynthesis and cellular respiration.
3. Explain why fluorescence of chlorophyll occurs and describe how it fluoresces.
4. Explain how cresol red can be used as an indicator of photosynthesis and cellular respiration.
5. Explain how paper chromatography works and how to interpret the results.
6. Describe the pigments found in spinach.
7. Identify the elements of the photosynthesis experiments and analyze the results.

II. Background Information:

A. Photosynthesis:

Photosynthesis is a process used by plants algae, and many species of bacteria to convert the light energy captured from the sun into chemical energy that can be used to fuel the organism's activities. Photosynthetic organisms are called photoautotrophs, since they can create their own food. Photosynthesis uses carbon dioxide and water to produce glucose, and releasing oxygen as a waste product.

$$6\ CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2$$

The primary site of photosynthesis in plants is in the leaf, within the chloroplast. **Chlorophyll** is a green pigment found in cyanobacteria and the chloroplasts of algae and plants, and allows the absorption of light energy. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. However, it is a poor absorber of green and near-green portions of the spectrum, hence the green color of chlorophyll-containing tissues.

Photosynthesis consists of two reactions: the **light-dependent reaction** and the **light-independent reaction (or Calvin cycle)**. In the light-dependent reaction, solar energy is captured by chlorophyll and accessory pigments (such as carotenoids), and temporarily stored in ATP and NADPH. The energy in these molecules is then used to power the light-independent reaction. In the Calvin cycle, the enzyme Rubisco captures CO_2 from the atmosphere and forms glucose and other carbohydrate molecules.

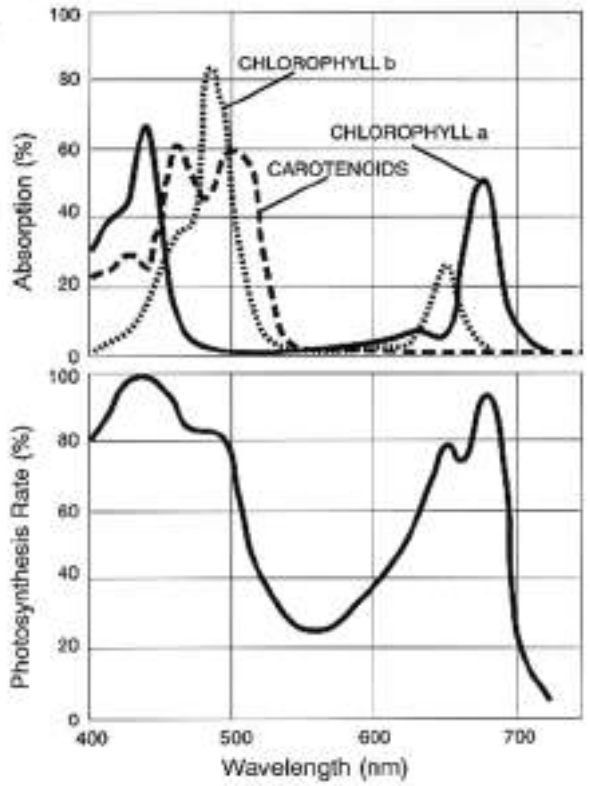


Fig. 5.1. (Top) The absorption spectrum of different light-absorbing pigments in plants. (Bottom) The action spectrum showing the photosynthetic activity of all the light absorbing pigments combined (400nm = violet, 500nm = blue, 550nm = green, 600nm = yellow and 650nm = red light)[1]

B. Cellular Respiration:

Cellular respiration is the set of the reactions that take place in the cells of organisms to convert biochemical energy from food into adenosine triphosphate (ATP), and then release waste products. The products of photosynthesis are the reactants of aerobic cellular respiration:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP$$

Cellular respiration occurs in a series of steps: glycolysis is the first step, and occurs in the cytosol of cells in all living things. The product of glycolysis is pyruvate and is oxidized to acetyl-CoA in the presence of oxygen. If oxygen is present, acetyl-CoA enters the citric acid cycle (Kreb's cycle) in the mitochondria of eukaryotic cells. The final step in the process is oxidative phosphorylation, where the majority of ATP is produced and oxygen acts as the final electron acceptor, forming water as a waste product.

[1] Wikimedia Commons: http://commons.wikimedia.org

C. Measurement of Metabolic Activity

To determine whether a metabolic reaction is occurring, we can measure the reactants being consumed or products being formed (Think back to the enzyme lab where we measured quinone production by measuring the absorbance of light using the spectrophotometer.) Cresol red is a solution that contains a pH indicator. In a basic solution the color will be red and in an acidic solution the color will change to yellow. When carbon dioxide (CO_2) is mixed with water, it forms carbonic acid (H_2CO_3). By adding cresol red to water, then adding CO_2 to it, we can observe a change in color associated with a change in pH. If the addition of CO_2 is due to a metabolic process, such as cellular respiration, then the color change will indicate the occurrence of the metabolic process.

D. Chromatography:

Chromatography is a method commonly used to separate chemicals. With paper chromatography, which is the method we will use in this lab, a nonpolar solvent moves through a polar material (such as paper) that has a chemical sample placed at the starting end. The paper will attract polar molecules more strongly, causing them travel more slowly up the paper. On the other hand, nonpolar molecules will remain in the solvent mixture and travel further up the paper. How the pigment molecules are separated will depend on the chromatography material (paper is just one of many materials that can be used), the solvent, and the properties of the chemical pigments.

As mentioned earlier, plants contain a variety of photosynthetic pigments. We will use paper chromatography to separate the pigments from a spinach leaf and try to identify them using the pigment colors. The main pigments that are usually observed in spinach are chlorophyll (a and b), which are both green, and two types of accessory pigments called carotenoids. One of these accessory pigments is carotene and usually appears yellowish orange and the other is xanthophyll, which appears bright yellow.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7^{th} ed.): pp.92-100 (Cellular Respiration) and pp. 108-115 (Photosynthesis).

B. Recommended background reading (optional):
1. Photosynthesis Video by Paul Andersen:
http://www.youtube.com/watch?v=g78utcLQrJ4

2. Respiration Video by Paul Andersen:
http://www.youtube.com/watch?v=Gh2P5CmCC0M

3. Biology website by Paul Andersen:
http://www.bozemanscience.com/biology-main-page/

IV. Pre-Lab Questions:

1. Write the equation for photosynthesis:

2. a) Which wavelength (color) of light does chlorophyll absorb the ***least***? _____

b) What wavelengths (colors) of light does chlorophyll absorb the ***most***?

_____ and _____

3. Write the equation for cellular respiration.

4. a) What color would cresol become in a solution that contains water and carbon dioxide?

b) What is the reason for this?

5. What are the colors of the following pigments found in spinach?

a) chlorophyll a: _____

b) chlorophyll b: _____

c) carotene: _____

d) xanthophyll: _____

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 2 plastic syringes • 1 hole puncher • 2 plastic cups • power strip • light bulb • aluminum foil • ruler • chromatography tube with solvent • test tube rack	• fresh spinach leaves • cutting board • chromatography paper • scissors • bicarbonate buffer solution • plain buffer solution • colored cups or plastic wrappers

B. Procedure

A. Fluorescence of Chlorophyll extract:

When chlorophyll absorbs light energy, electrons become excited and move to a higher energy state. If the photosystem within the plant does not harness that energy, the electrons will return to their original energy state, and in the process, release the light energy that they had absorbed. This experiment demonstrates the fluorescence of chlorophyll.

Use a solution of chlorophyll extract from spinach and shine a bright light on to it. You may need to turn off the ambient room light to see the results more clearly.

*What color does the chlorophyll extract fluoresce under the bright light?*_____

Provide a reason for this observation.

B. Observing Photosynthesis in *Elodea*:

Observe the two beakers containing the aquatic plant *Elodea* – one has been placed in light while the other has been left in the dark.

	Elodea in the dark	*Elodea* in the light
Indicate which is the + control and which is the - control		
Do you observe gas bubbles?		
What is the gas that is contained in the bubbles you observed?		

C. Measuring Photosynthetic Rate in *Elodea*:

Observe the two test tubes, one containing the aquatic plant *Elodea* and sodium bicarbonate buffer, and the other one containing only sodium bicarbonate buffer (no *Elodea*): Measure the amount of gas produced in each test tube and record your results below.

	Test tube with *Elodea*	Test tube without *Elodea*
Indicate which is the + control and which is the - control		
How much gas is produced?		

D. Use of Cresol Red as an Indicator of Photosynthesis and Cellular Respiration:

Observe and record how cresol red changes color when carbon dioxide is added to water.

Color of cresol red in the absence of CO_2: _____

Color of cresol red in the presence of CO_2: _____

Your instructor will place a sample *Elodea* plant in the cresol red solution that contains CO_2 and water. A similar setup will be prepared with the cresol red solution but without the *Elodea*. Observe and record what happens to the colors of the solutions over time in the data table below.

Is the beaker without the Elodea a positive or negative control? Why?

What color change would you expect to see in the beaker containing the Elodea after 90min? Why?

Time (min)	Color in beaker containing *Elodea*, cresol red and CO_2.	Color in beaker containing only cresol red and CO_2.
0		
45		
90		

Do your observations match your hypothesis? If not, provide an explanation for your observations.

D. Use of Chromatography to Separate Plant Pigments

You will be using paper chromatography today to separate plant pigments.

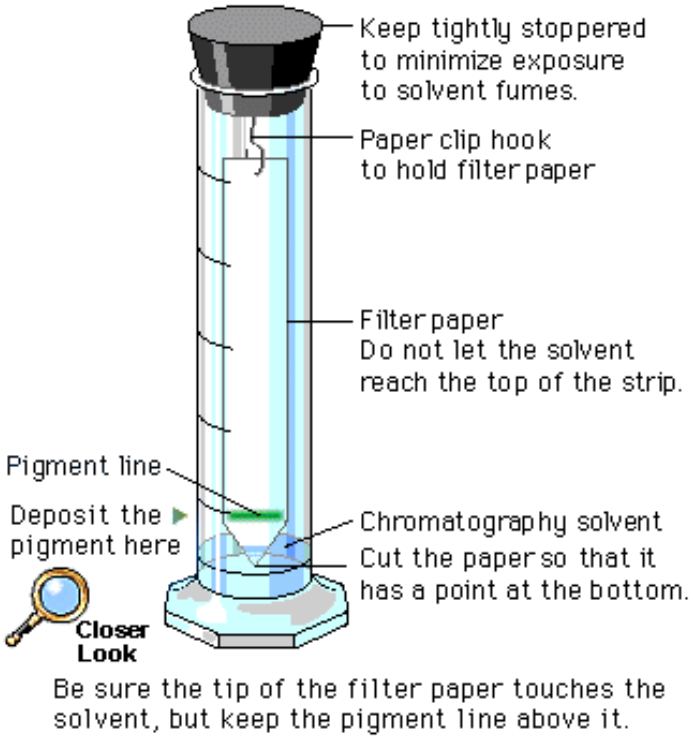


Fig. 5.2. Diagram of the paper chromatography setup.[2]

1. Cut a strip of chromatography paper long enough to hang in the test tube (with cork and hook) such that the bottom of the paper just touches the solvent.
2. Cut a point at the end of the paper. (Refer to the sample drawing or setup provided)
3. Place a spinach leaf near the end of the paper with the point and rub the leaf to transfer some of the pigment onto the paper.

[2] The Biology Place: http://www.phschool.com/science/biology_place/labbench/lab4/design1.html

4. Hang the paper with the pointed end towards the bottom in the test tube with solvent. Make sure you hang the paper such that the leaf rubbing comes close to, but does not touch the solvent.
5. Let the solvent move up the paper until it comes within a few centimeters from the top. Remove the paper and draw a line where the solvent stopped. Lay the chromatography paper on a paper towel and let it air dry for a couple of minutes.
6. Do NOT dump out your solvent, but leave it for the next class. Make sure the cork is on the test tube.
7. Observe your chromatography paper and locate the yellowish orange **carotene** at the top, the blue-green **chlorophyll a**, the yellow-green **chlorophyll b**. The remaining yellow bands are **xanthophylls**.
8. Draw your chromatography paper results below and label the plant pigments.

Chromatography experiment results:

E. Testing the Effect of Environmental Factors on Photosynthetic Rate:
Complete the following experiments. Be sure to check your experimental protocol with your instructor before you proceed and fill in the data sheet for each experiment that you conduct. Remember to record your hypothesis BEFORE you begin each experiment. You will need to complete one experiment, record your results, discuss your results with your instructor and wash your setup ***before*** you begin the next experiment. At the end of all of your experiments, wash and reset your workstation.
Note: If the weather permits, you should conduct your photosynthesis experiments outside (after preparing the syringes inside) and use natural sunlight rather than the lamps in the lab.

1. Presence of Light:

1. Using a hole-puncher, punch out 20 discs from a spinach leaf.
2. Pull the plungers out of two 35 ml syringes and place 10 discs in each syringe.
3. Replace the plungers making sure not to crush the discs.
4. Fill the two syringes with 20 ml of bicarbonate buffer solution.
5. To remove any trapped air from the leaf discs, firmly cover the open tip of the syringe with your thumb and pull on the plunger to produce a slight vacuum. Shake the syringe gently while pulling on the plunger to shake out any gas trapped within the discs. Because gas expands at a negative pressure, gases within the discs should escape.
6. Let go of the plunger while still keeping your finger firmly on the syringe tip. Push on the plunger to fill the vacated air spaces with the bicarbonate buffer solution. The discs should start to sink.

7. Remove your finger from the syringe tip and observe how many of the discs sink to the bottom. Repeat the procedure until most of the discs sink to the bottom.
8. Invert the syringes (syringe tip pointing upwards) and cover one syringe completely with foil and place both the covered and uncovered syringes standing on their plungers the same distance from the light source (approx. 3 cm).
9. Record the # of sunken discs at the beginning of the experiment in the data table below (you need to exclude any discs that don't sink from your calculations).
10. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any discs that were floating before the experiment began).
11. Calculate the percentage of discs that are floating in each syringe:
(# new floating disks / total # sunken in the beginning) x 100.

2. Presence of CO_2:

1. Repeat the procedure listed in (1) but use the bicarbonate buffer (containing CO_2**)** in one syringe and the plain buffer (not containing CO_2**)** in the other syringe.
2. Place both syringes, uncovered, the same distance from the light source.
3. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any disks that were floating before the experiment began).
4. Calculate the percentage of discs that are floating in each syringe.

3. Distance from Light Source (if conducting the experiments outside, skip this activity)

1. Repeat the procedure listed in (1) but place the two syringes at different distances from the light source (3cm and 20cm).
2. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any disks that were floating before the experiment began).
3. Calculate the percentage of discs that are floating in each syringe.

4. Additional Experiments (time permitting):
Color of Light:

1. Repeat the procedure listed in (1) but use a different colored cup or plastic wrap to cover each syringe and change the color of light that reaches the leaf disks inside the syringe.
2. Place both syringes the same distance from the light source.
3. Record your experimental results in the data table below.

Type of Leaf

1. Repeat the procedure listed in (1) but use a different type of leaf for each syringe (old vs. young leaves, leaves from two different plants, etc.)
2. Record your experimental results in the data table below.

F. Experimental Results and Analyses:

Experiment 1: Presence of Light

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Light**	**Dark**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment 2: Presence of CO_2

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Bicarbonate buffer (with CO_2)**	**Plain buffer (without CO_2)**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment 3: Distance from Light Source

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Near (3cm)**	**Far (20cm)**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment 4: Color of Light

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Light Color:**	**Light Color:**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment 5: Type of Leaf

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Leaf Type:**	**Leaf Type:**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

VI. Post-Lab Questions

1. Explain the purpose of each of the following steps in the spinach photosynthesis experiment:

a) Hole punching the leaves: _____

b) Adding bicarbonate solution to the syringe: _____

c) Exposing the disks to a vacuum in the syringe: _____

2. Why was the percentage of floating leaf disks a reasonable measure of photosynthetic activity in this experiment?

3. You conduct an experiment to see how color of light affects photosynthesis in spinach. Refer to the data table below when answering the following questions.

Color of light	% Floating disks
White	100%
Red	70%
Blue	80%
Green	10%

a) Which light is best for promoting photosynthesis in spinach? _____

b) Provide one biological explanation for why % floating disks is low under green light.

c) Graph the data in the space below, using appropriately labeled axes.

4. According to your chromatography experiment, how many different pigments does a spinach leaf contain? What are their names and colors?

5. If you placed an *Elodea* plant in a solution of cresol that was yellow due to the presence of carbon dioxide in water, and you placed this setup in the dark, would you expect the solution to turn red over time? Why or why not?

Lab 6: Microscopes and Cells

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Compare and contrast the characteristics of the compound light microscope and the dissecting microscope.
2. Identify the parts of the compound and dissecting microscopes.
3. Calculate the total magnification obtained by the compound and dissecting microscopes.
4. Operate and care for the microscopes and prepare wet mount slides of samples.
5. Define the following terms and briefly explain how they relate to microscopy: field of view, depth of focus and total magnification.
6. Describe the main characteristics of Domain Bacteria, Archaea and Eukarya.
7. Visually differentiate, and explain the similarities and differences between: *Anabaena, Euglena, Paramecium, Amoeba,* animal cell, and plant cell.

II. Background Information:

In this lab you will be studying the different characteristics of prokaryotic and eukaryotic cells, however, before you begin looking at individual slides, you must learn how to use the dissecting and compound light microscopes to ensure the microscopes are not damaged during the lab activities.

1. Microscopy is the technical field of using a **microscope** to observe samples and objects that are too small to see with the human eye. A microscope magnifies the image of a specimen through the use of lenses and light. There are many different types of microscopes, and the properties of the images produced and the types of specimens that can be studied with each kind of microscope are different. In this lab we will focus on the dissecting microscope and the compound light microscope.

2. Dissecting Microscope

The dissecting microscope provides a three-dimensional view of a sample. As the name implies, this type of microscope can be used to conduct close work such as dissections, microsurgery, etc. and to look at objects that are too big to fit flat under a cover slip on a glass slide. Dual light sources allow for visualization of the specimen using both reflected and transmitted light, and can be adjusted depending on the specimen and on the features you want to observe. The path of light goes through two lenses: the **objective lens** (close to the object) and the **ocular lens** (close to the eye) located on the eyepiece (Fig. 6.1).

3. Compound Light Microscope

The compound light microscope contains a compound set of lenses that provides a much larger range of total magnification than the dissecting microscope (4X-1000X). The ocular lens typically has a magnification of 10X, and there are three or four objective lenses located on a revolving nosepiece depending on the microscope model. The smallest objective lens is also called the **scanning lens** because it allows you to scan or search for the specimen you

want to observe. This lens provides the broadest **field of view** (total area observed). As you increase the magnification, the field of view decreases. Increasing the magnification requires more light or **illumination** in order to better observe the specimen. The compound light microscope depends on transmitted light to illuminate a thin section of a specimen. Consequently, only small organisms can be observed whole; larger specimens need to be sectioned into thin slices to allow enough light to pass through it.

The main parts of a microscope include the **base**, which rests on the table and the **arm**, which rises from the base and supports the **stage,** where the slide is placed and secured in place with the **stage clips**. The slide can then be moved using the **stage control knobs** located on the side of the stage. Most microscopes have a **condenser** located below the stage. It concentrates the light on the object and may be raised or lowered using the **condenser control knob**. Usually, the condenser should be raised to its highest position. There are two focusing knobs. The **coarse focus knob** is the larger knob and is used to bring objects into rough focus when using the smallest objective lens. The **fine-focus knob** is the smaller knob and is used to fine-tune the focus after the specimen has been located. **The fine-focus knob is the ONLY focus that should be used with the higher objective lenses.**

Fig. 6.1. A compound light microscope (left) and a dissecting microscope (right).[1]

[1] Wikimedia Commons: http://commons.wikimedia.org

4. Calculating total magnification:
When calculating the total magnification of the specimen being observes, use the following formula:

Total magnification = (ocular lens magnification) x (objective lens magnification)

The ocular lens magnification on the compound microscope is 10x.

Example: What is the total magnification when using the objective lens (4x)?
Total magnification = (10x) x (4x) = 40x

5. Cells[2]
Cells are the fundamental unit of life; all living things are composed of cells. While there are several characteristics that are common to all cells, such as the presence of a cell membrane, cytoplasm, DNA and ribosomes, not all cells are the same. There are two general types of cells: **prokaryotic** and **eukaryotic**. These two words have their root in the Greek word karyon (nut), which refers to a cell's nucleus. The prefix pro- means "before" or "prior to", thus prokaryotic means "before having a nucleus." Prokaryotic cells do not have a membrane-bounded nucleus and their genetic material (DNA) is only loosely confined to a nuclear area within the cell. The prefix “eu”- means "true." The cells of eukaryotes have true, membrane-bounded nuclei containing their genetic material. Prokaryotic cells are generally smaller than eukaryotic cells (about ten times smaller) and lack membrane-bound organelles, whereas eukaryotic cells are compartmentalized by membrane-bound organelles with specialized functions.

Organisms belonging to the Domains **Bacteria** and **Archaea** are prokaryotic, whereas organisms belonging to the Domain **Eukarya** are eukaryotic. Organisms such as protists, fungi, plants and animals belong to Domain Eukarya.

a) Bacteria:
Present-day bacteria are found everywhere: in soil, in water, in ice, in boiling hot pools of water, even kilometers underground! Many bacteria are heterotrophic (consume organic compounds) while others are autotrophic (make their own food through photosynthetic). Morphologically, bacteria are either spherical (**cocci**), rod-shaped (**bacilli**), or spiral-shaped (**spirillum**). Furthermore, bacteria are often found in clusters or in chains. Some have one or more flagella. They are extremely small (approximately 1 to 2 μm in diameter). To view them with the light microscope, one must usually use an oil-immersion lens (100X). Even then, not much more than their basic shapes will be visible. Some of the photosynthetic bacteria, called **cyanobacteria**, are large enough to be seen at a total magnification of 400X.

b) Plant and Animal cells:
The cells of plants are eukaryotic and contain a large **central vacuole** used for storing water, pigments, and wastes. Within the cytoplasm are various types of plastids. These include the green-colored chloroplast, which is responsible for photosynthesis and others for storing starch or pigments. A **cell wall** composed of cellulose surrounds the plant cell and provides structural support. Animal cells are also eukayotic and contain many of the same organelles

[2] Washington State Open Course Library: http://opencourselibrary.org/

as plant cells, although they lack a large, central vacuole and plastids. Both plant and animal cells contain **mitochondria**, the organelles responsible for converting organic compounds into energy in the form of ATP to power cellular processes.

c) Protists

Protists are a large and diverse group of eukaryotic organisms that include unicellular, colonial and multicellular members that are not closely related through evolution. Protists have diverse life cycles, trophic levels, modes of locomotion and cellular structures. Protists live in almost any environment that contains liquid water. Many protists, such as algae, are photosynthetic and are vital primary producers in aquatic ecosystems. Other protists include pathogenic (disease-causing) organisms such as species of *Plasmodium*, which cause malaria. Members of the genus *Amoeba* move using extended cytoplasmic projections called **pseudopodia**. These structures can also be used to engulf food in a process called **phagocytosis**. *Euglena* is a genus characterized by having a **flagellum** (*pl.* **flagella**); a long, thin, whip-like projection that is used for locomotion. Some members of this genus are both autotrophic and heterotrophic; the cells contain chloroplasts for photosynthesis and they may also engulf food by phagocytosis when light is not available. *Paramecia* contain hair-like structures for movement called **cilia** and they are heterotrophic.

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7^{th} ed.): pp.56-57 (Microscopes), pp. 58-70 (Cells), pp.300 (Bacterial shapes) and pp.307-310 (Protists).

B. Recommended background reading (optional):

1. Bacteria:

    Bozeman Science:
    https://www.youtube.com/watch?v=h-z9-9OOWC4

    Frank Gregorio – Introduction to the Bacteria
    https://www.youtube.com/watch?v=qCn92mbWxd4

2. Protists:

    Bozeman Science:
    https://www.youtube.com/watch?v=8deF3Rw4ti4

    Frank Gregorio – Introduction to the Protists:
    https://www.youtube.com/watch?v=0-6dzU4gOJo

IV. Pre-Lab Questions:

1. Calculate the total magnification for the following:

Ocular lens magnification	Objective lens magnification	Total magnification
	10x	
	40x	
	100x	

2. Circle the objective lens that would show the ***smallest field of view:*** ***4x*** **/** ***10x*** **/** ***40x***

3. List <u>two</u> similarities and <u>three</u> differences between prokaryotic and eukaryotic cells.

Similarities:

Differences:

4. Which two Domains contain organisms composed of prokaryotic cells?

5. a) Which Domain contains organisms composed of eukaryotic cells? _____

b) What organisms belong to the Domain you listed in part 5a?

6. Number the following parts of the compound light microscope using the diagram below.

	Number		Number
Ocular lens		Stage	
Objective lens		Coarse focus knob	
Revolving nosepiece		Fine focus knob	
Light		Condenser	
Stage clips			

Fig. 6.2. The compound microscope.[3]

[3] Wikimedia Commons: http://commons.wikimedia.org

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• Compound light microscope • Dissecting microscope • One glass slide • One cover slip • Bottle of DI water • Plastic pipettes • 10ml graduated cylinder	• Prepared slides: letter "e", colored threads, *Euglena, Paramecium, Amoeba, Anabaena,* human cheek cell and bacterial shapes. • Live *Paramecium, Euglena, Anabaena* and mixed protist cultures • Sample objects for viewing • Photos of bacterial shapes • Yeast solution • Elodea plant • Scissors • Plastic pipette

B. Procedure

1. Caring for the Microscope

1. Obtain a compound light microscope from the cabinet and carry it using both hands: one on the arm and the other one under the base.
2. Carefully, place the microscope on the table with the arm away from you. Do NOT drag the microscope on the table because the lenses may come loose and fall off.
3. If the lens is dirty, **Use ONLY lens cleaning solution and lens paper** (located on your lab bench) to clean it.
4. **NEVER** use a paper towel or other paper for cleaning the microscope lenses because it may scratch them.
5. **Do NOT touch the lenses;** body oils may also damage them.
6. For the safety of your eyesight, **keep both eyes open** when working with the microscopes (both monocular and binocular microscopes.) This will take some practice, but you will soon master the technique.
7. When you are finished with using your microscope refer to the following instructions before putting away your microscope:
    1) Remove the slide from the stage and return it to its proper place.
    2) Make sure the stage is clean and dry.
    3) Reposition the slide mount arm so that it does not extend out past the stage.
    4) Turn the revolving nosepiece to the lowest objective.
    5) Bring the stage all the way down (but don't force the coarse focus knob past its lowest point.)
    6) Turn off the light switch and unplug the electrical cord.
    7) Wrap the cord and secure it with the Velcro attachment.
    8) Cover the microscope and tuck the cord **ON THE SIDE** of the microscope. **NEVER** tuck the cord in between the base and the stage as this damages the control knobs.
    9) Return the microscope to the appropriate cabinet and numbered space with the microscope arm facing you.

2. Wet Mount Slide Preparation

Most specimens must be killed, fixed, sectioned, and stained for microscopy. These prepared slides contain a label on the side indicating the name and orientation of the specimen (i.e. "w.m." is whole mount, "c.s." is cross-section and "l.s." is longitudinal section). In addition to viewing prepared slides in this lab, you will also be viewing live organisms. If both prepared slides and live specimens are available for an organism, you may want to look at the prepared slide first so that you can more easily locate and observe the specimen before making a wet mount slide of the live organism. Oftentimes the prepared slide contains artificial staining to make the organism easier to find but masks the actual coloration of the organism.

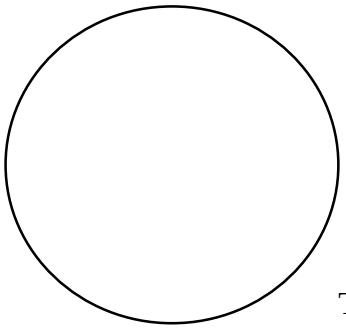
Follow the steps below to prepare a wet mount slide when asked to do so in the lab procedure.

1. Obtain a clean, blank glass slide.
2. Place a drop of the sample in the center of the microscope slide. If the specimen is not in water, place a small, thin section of the specimen on the slide and add a drop of water on top of it.
3. Place a plastic coverslip over the drop of sample by positioning its edge onto the slide to one side of the drop and then lowering the coverslip slowly over the specimen. Do this slowly to avoid excess bubbles; however, if you get a couple of bubbles, be aware not to confuse them with your cells, a bubble looks like a perfect circle with a dark circumference.
4. When you are finished with your wet-mount, wash your slide and coverslip and reuse it for the next activity. At the end of the lab, wash your slide and coverslip and place it on the counter to dry.

3. Dissecting Microscope

a) Orientation: Letter "e" slide

1. Plug in the microscope and turn the light switch on. There are two light sources: the small knobs on either side of the arm control the two different lights.
2. Place the slide with the letter "e" on the stage and center it over the light source.
3. Use the focus knob on the side of the arm and focus on the object.
4. Practice using both lights to determine which light source provides the clearest view.
5. Turn the magnification knob (located at the top of the eyepiece) to the lowest magnification. The number that is pointing towards you indicates the total magnification.
6. Draw the orientation of letter "e" below as you observe it under the microscope.

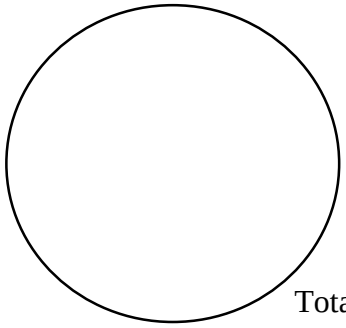


Total magnification: _____

4. Compound Light Microscope

a) Orientation: Letter "e" slide

1. Plug in the microscope and turn the light switch on.
2. Place the slide with the letter "e" on the stage and secure the slide by using the stage clips.
3. Position the letter "e" over the circular opening in the stage by using the stage control knobs.
4. By rotating the revolving nosepiece, allow the smallest objective to click into position for viewing.
5. Using the condenser height adjustment knob, make sure the condenser is all the way up under the stage.
6. While looking at the stage from the side, carefully turn the coarse adjustment knob to bring the stage up until it stops.
7. Look through the ocular lens and slowly turn the coarse focus knob away from you to lower the stage until you see the letter "e" clearly.
8. Fine focus your image by until the image is clear to your eyes.
9. Draw the orientation of the letter "e" as you observe it under the microscope.



Total magnification: _____

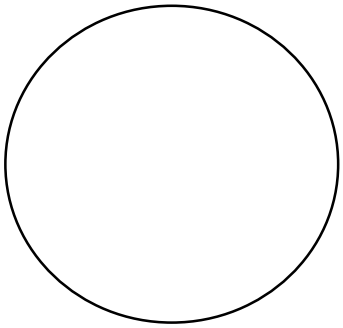
b) Cells

1. When looking at each of the specimens listed below, always start your examination using the smallest objective lens (scanning lens).
2. After you have located the specimen, switch to the medium objective to draw a detailed diagram of 1-2 representative organisms in the space provided and label as many of the cell structures as you can (plasma membrane, nucleus, etc.)
3. If prepared slides are provided for any of the listed organisms, observe and draw them first.
4. Prepare a wet mount slide of all the live specimens provided and make additional sketches and notes to compare the preserved specimens with the live ones.
5. If you cannot find any live specimens on the wet mount slide that you have prepared, clean your slide, get another sample and try again.
6. Many of the live specimens will be swimming around rapidly and may move out of view quickly so be prepared to go on a treasure hunt!
7. If you are having trouble finding the organisms, be sure to ask your instructor. You are responsible for being able to identify the main characteristics of each of the organisms that you observe in the lab.

Bacteria: *Anabaena*

Anabaena is the genus name of a group of cyanobacteria (photosynthetic bacteria). It is a colonial organism as grows in a filamentous strand composed of many cells that look like a string of pearls. Since *Anabaena* ore photosynthetic, they contain chlorophyll, although the preserved specimens are stained various colors to make them more visible. There are occasional cells in the filament that are larger than the rest. These cells are called **heterocysts** and allow the bacteria to fix nitrogen from the environment, which is necessary for photosynthesis.

1. Observe the prepared slide of *Anabaena*. Draw several colonies below and **label the cell membrane and the heterocysts**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.



Anabaena (total magnification: _____)

Would you expect to see nuclei in the cells of Anabaena? Explain why or why not.

_____

In what ways are the live and preserved specimens different?

_____

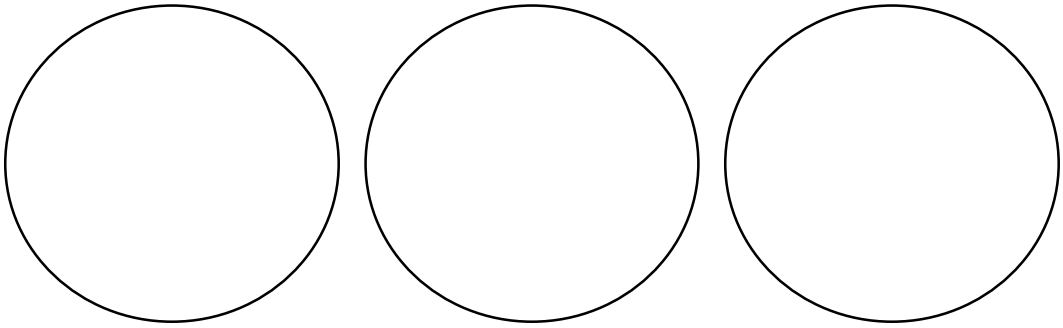
What is the color of the live colonies of Anabaena? _____

What does this suggest about whether these bacteria are autotrophic or heterotrophic? Why?

_____

Bacteria: Cell Morphology

1. Observe the three types of bacterial cell morphology (slides may be set up on display microscopes or you may need to obtain a slide with all three bacterial shapes and set it up yourself.) If you want, place a piece of hair on the slide to give you a sense of size of the bacteria. Most bacteria are less than 10um long, which is generally 10 times smaller than the width of a human hair!
2. Refer to the micrographs (photographs) of the different bacterial shapes. These were taken using an electron microscope, which has a much higher magnification than a light microscope.
3. Draw below the three bacterial shapes you observe on the compound scopes and record the total magnification for each drawing.



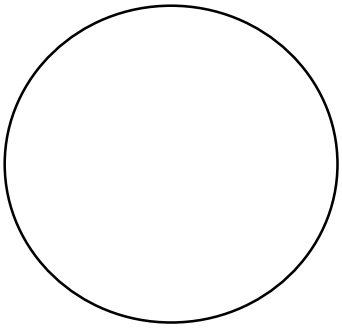
Spherical (Cocci) Rod-shaped (Bacilli) Spiral

Total magnification: _____ _____ _____

Protist: *Euglena*

Euglena is the genus name of single-celled protists that use **flagella** for locomotion. Some species in this genus are photosynthetic; others are heterotrophic and some species are both.

1. Observe the prepared slide of *Euglena*. Draw several individuals below and **label the cell membrane, nucleus and flagella (if visible)**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.



Total magnification: _____

What is the color of the live Euglena? _____

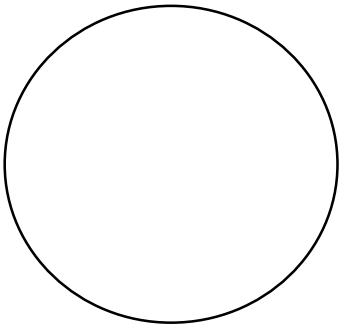
What does this suggest about whether these protists are autotrophic or heterotrophic? Why?

_____

Protist: *Amoeba*

Amoeba is the genus name of single-celled protists that use **pseudopodia** for locomotion. All members of this genus are heterotrophic.

1. Observe the prepared slide of *Amoeba*. Draw a sample individual below and **label the cell membrane, nucleus and pseudopods**.

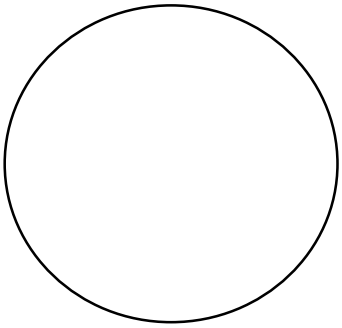


Total magnification: _____

Protist: *Paramecium*

Paramecium is the genus name of single-celled protists that use **cilia** for locomotion. In some species these cilia are grouped near one end of the organism and are used for feeding or "walking". All members of this genus are heterotrophic.

1. Observe the prepared slide of *Paramecium*. Draw a sample individual below and **label the cell membrane, nucleus and cilia (if visible)**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.
5. If you cannot find any Paramecia on your slide, clean your slide and obtain another drop of sample. Be sure to scan your entire sample.
6. If the Paramecia are swimming too fast for you to observe them, you can add a drop of Protoslo or Detain solution to slow their movement.



Total magnification: _____

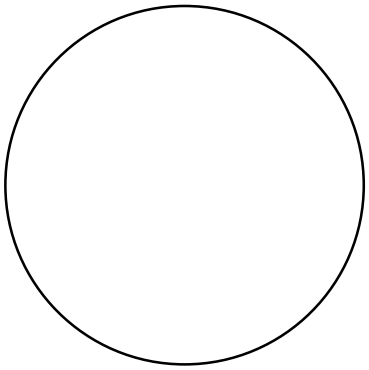
What is the color of the live Paramecia? _____

What does this suggest about whether these protists are autotrophic or heterotrophic? Why?

_____

Protist: Mixed Protist Culture
(Time permitting – check with your instructor)

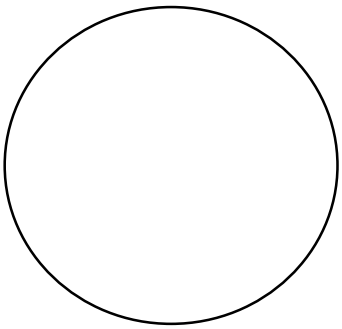
1. Make a wet mount slide of the **mixed protist culture** by taking a drop of sample near the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
2. Place a clean plastic coverslip over your sample.
3. Observe the various organisms in your sample. Some organisms may be single-celled, having different shapes, while others will be in clumps of cells called colonies.
4. Draw as many different kinds of organisms as you can see.
5. **BEFORE** you clean your mixed protist sample, complete the activity in the next section.



Total magnification: _____

Animal: Human cheek cell (squamous epithelial cell)
Animals are eukaryotic, multicellular, heterotrophic organisms that obtain their energy from feeding on other organisms or organic materials. We will use preserved, stained human cheek cells as a representative of animal cells.

1. Obtain the prepared slide labeled "squamous epithelial cell".
2. Locate and observe the cells under low magnification. Carefully switch to medium magnification to observe more detail of the cells.
3. Draw a couple of representative cells below and label the following structures: **cell membrane, cytoplasm and nucleus.**

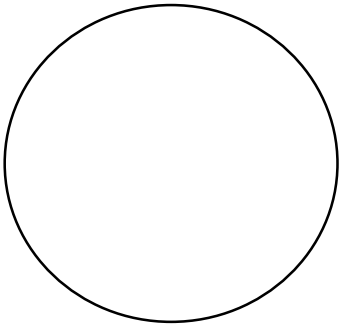


Total magnification: _____

*Can you see any organelles inside the cells?*_____

Plant: *Elodea*
Elodea is a photosynthetic aquatic plant. Plants are multicellular, autotrophic organisms and their cells contain three main structures that animal cells lack: a **cell wall** which provides the cell with structural support and gives the cell a rigid shape such as a cube or rectangle, **chloroplasts** which perform photosynthesis, and a large **central vacuole**, which stores water, pigments and toxins.

1. Obtain a **small** piece of a healthy (green) *Elodea* leaf and add a drop of water onto the leaf sample.
2. Add a coverslip over the sample. If the coverslip does not lay flat on the slide, then your sample is too thick, in which case remove the coverslip and place a smaller sample on the slide.
3. Using the lowest magnification, scan your specimen for the thinnest green section of the leaf.
4. Carefully switch to the medium magnification. Make sure the objective lens does not touch the slide.
5. You may need to adjust the condenser or diaphragm settings to get a clearer image with good contrast.
6. Draw several sample cells below and label the following structures: **cell wall, cell membrane, cytoplasm, chloroplasts, central vacuole and nucleus (if visible).**



Total magnification: _____

What color are the cells? _____

Which structures in the cell contain the green chlorophyll pigment? _____

Where are the chloroplasts located in the cell? _____

*Do you see any movement of the chloroplasts in the cell due to **cytoplasmic streaming**)?*

_____

Can you determine where the central vacuole is located? (Think about the location of the other organelles if a central vacuole is present)

_____

*List **three** ways in which you can differentiate between a plant and animal cell as viewed under the microscope.*

_____

5. Identifying Unknown Samples

1. Once you have completed all of the drawings and answered the questions in the previous sections, you will need to ask your instructor for three different unknown specimens that you will need to identify under the compound microscope.

2. Complete the table below and have your work checked when you are finished.

Note: This is an individual assignment – you must practice the proper technique for using the microscope and locating a specimen on the slide in order to be successful.

Unknown specimen #	Organism identification	Total magnification

6. Storage of the microscope

1. Remove the slide from the stage and return it to its proper place.
2. Make sure the stage is clean and dry.
3. Reposition the slide mount arm so that it does not extend out past the stage.
4. Turn the revolving nosepiece to the lowest objective.
5. Bring the stage all the way down (but don't force the coarse focus knob past its lowest point.)
6. Turn off the light switch and unplug the electrical cord.
7. Wrap the cord and secure it with the Velcro attachment.
8. Cover the microscope and tuck the cord **ON THE SIDE** of the microscope. **NEVER** tuck the cord in between the base and the stage as this damages the control knobs.
9. Return the microscope to the appropriate cabinet and numbered space with the microscope arm facing out.

VI. Post-Lab Questions

1. Complete the summary table below.

Organism	Identify group: bacteria, protist, animal or plant	prokaryotic or eukaryotic	single-celled, colonial, or multicellular	autotrophic or heterotrophic	Domain name
Anabaena					
Euglena					
Amoeba					
Paramecium					
Human					
Elodea					

2. Calculate the total magnification for the following:

Ocular lens magnification	Objective lens magnification	Total magnification
	4X	
	20X	
	50X	

3. When using the compound light microscope, it is important to use the _____ objective lens when locating a specimen on the slide for the first time because:

_____

4. Name three organelles or structures found in plant cells but not in animal cells.

5. Complete the following table to contrast prokaryotic and eukaryotic cells:

Characteristics	**Prokaryotic**	**Eukaryotic**
Membrane-bound nucleus present or absent?		
Membrane-bound organelles present or absent?		
Cell size		
Sample organisms		

Lab 7: Microbes

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Compare and contrast the characteristics of different types of microbes.
2. Identify the different Domains and Kingdoms that contain microorganisms.
3. Describe various microbes in your environment.
4. Compare the effectiveness of disinfectants and antiseptics on bacteria.
5. Identify and describe the characteristics of yeast.
6. Describe the characteristics and ecological importance of Fungi and lichen.
7. Conduct an experiment to determine which sugars best promote fermentation in yeast.
8. Identify the elements of the fermentation experiment and analyze the results.

II. Background Information:

Microorganisms (or microbes) are organisms that are microscopic (or too small to be seen with the eye without the use of a microscope). Microorganisms are very diverse; they include bacteria, fungi, protists and even animals.

A. Bacteria:

Bacteria are microscopic, single celled or colonial prokaryotic cells. Some cause disease, but most are actually helpful, if not essential to our daily lives. They help us digest our food and produce vitamins and important nutrients. The help out-compete pathogens and they run the biogeochemical cycles on earth. Bacterial cells come in a variety of shapes (even shaped like stars), but the most common are cocci (spheres), bacilli (rods), and spirilli (corkscrews). You observed these three cell shapes in the previous lab. In this lab, you will observe colonies of bacteria rather than individual cells.

B. Fungi:

Most fungi are multicellular and can be observed without the need for a microscope. The common pizza mushroom, *Agaricus*, is familiar to most of us, but the part that we eat is the reproductive body. This mushroom reproduces by producing sexual spores on the lamellae inside the button cap. When the spores mature they are released as the cap opens. The pattern of spores, called a **spore print**, can be observed by placing a closed mushroom cap on a piece of filter paper, wrapping it in foil, and placing it in the dark for several weeks. The cap will open and release the spores on the filter paper.

Yeast are an important type of fungi that are single-celled. Some of them cause disease, but many are important in food (and beverage) production, such as bread, beer and wine, kimchi, sauerkraut, yogurt, etc. Yeast ferment sugars (such as glucose) to produce ATP for energy, and produce alcohol and carbon dioxide (CO_2) as byproducts in this fermentation process.

$$C_6H_{12}O_6 + \text{yeast} \rightarrow 2\ CO_2 + 2\ C_2H_5OH + 2\ ATP$$

Because yeast perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. In order to carry out fermentation however, the yeast need to possess the right enzymes to break down the sugars. Today you will test different sugars to determine which one(s) yeast can use for fermentation by observing the amount of CO_2 gas produced.

Fig. 7.1. The underside of a mushroom cap, showing the lamellae.[1]

C. Lichen:

Lichens are actually two organisms living together in a **mutualistic** symbiotic relationship between fungi and an algae (which is a photosynthetic protist) or cyanobacteria (photosynthetic bacteria). This relationship is mutually beneficial because the fungus provides structure, habitat, moisture retention and protection, while the algae or cyanobacteria provide nutrients via photosynthesis. Consequently, lichens are able to survive in extremely harsh environments such as the arctic tundra, hot deserts and rocky coasts. They are also abundant on leaves and branches in tropical rainforests and temperate woodlands as well as on bare rock, including walls and gravestones.

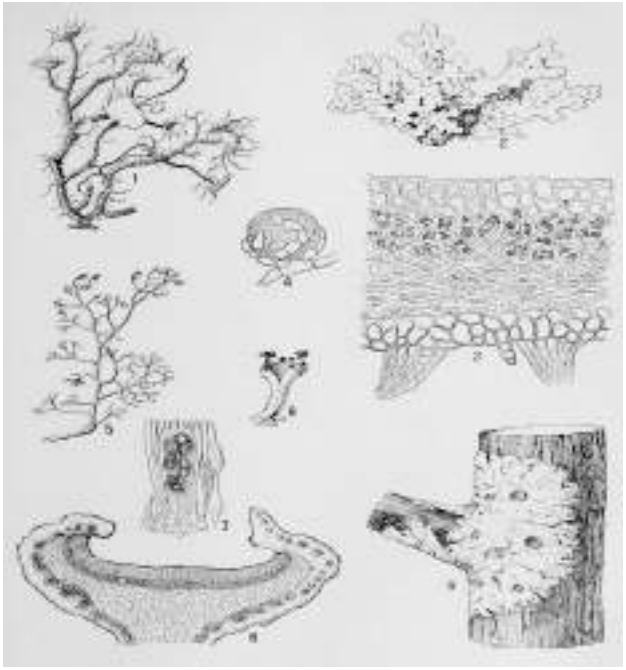


Fig. 7.2. Illustration of various lichen forms.[2]

[1] Wikimedia Commons: http://commons.wikimedia.org

[2] *The New International Encyclopædia*, v. 11, 1905, between pp. 210 and 211

Lichens are widespread and may be long-lived; however, many are also vulnerable to environmental disturbance, and are useful to scientists in assessing the effects of air pollution, ozone depletion, and metal contamination in soils. The three lichen growth forms that you will observe in lab are **crustose** (small and flat), **fruticose** (branched) and **foliose** (leafy). Watch the following video and see and hear more about this unusual partnertship: https://youtu.be/d167NrioW7c

D. Microbes in our Food and Environment:
Bacteria and fungi are everywhere in our environment. They are in the air, on surfaces and yes, all over our bodies - our "normal flora". Although many microbes cause serious diseases to plants and animals, most are not harmful and are actually necessary to the environment and to our health and well-being. Many places in your home provide the ideal conditions for bacterial and mold growth. Placing food products in the refrigerator might slow down the growth of bacteria and mold but it does not stop them altogether. Many processed foods contain preservatives to slow down or prevent bacterial and fungal growth. However, some people prefer to buy foods with as little chemical additives as possible. When buying and consuming preservative-free foods, it is important to use other methods (such as refrigeration, addition of salt, etc.) to retard microbial growth. Preservative-free foods usually have a much shorter shelf life than traditionally processed foods.

III. Reading Assignments:

A. Required background reading:
Campbell Essential Biology (7^{th} ed.): pp.58-59 (Cells), pp. 101-103 (Fermentation) and pp. 303-304 (Ecology of Prokaryotes).

B. Recommended background reading (optional):
1. What are Microbes?
https://www.youtube.com/watch?v=_Vj0cIgwpQI

2. The Microbes We're Made of:
https://www.smithsonianmag.com/videos/category/smithsonian-channel/the-microbes-were-made-of/

3. A lichen ménage à trois
https://youtu.be/d167NrioW7c

IV. Pre-Lab Questions:

1. What is a microorganism?

2. List three different groups that contain microorganisms. Identify whether each of these groups is eukaryotic or prokaryotic.

i)

ii)

iii)

3. What Kingdom do yeast belong to? _____

4. a) Write the equation for fermentation in yeast.

b) Name the substrate, the main product and the by-products of fermentation.

5. In a lichen:

a) Which organism provides structure and protection? _____.

b) Which organism provides nutrients? _____.

c) Describe the three growth forms of lichen that you will be observing in lab.

6. Watch the following video: https://youtu.be/d167NrioW7c

a) Lichen are oftentimes a mutualism between how many different organisms? _____

b) In what way does this type of mutualism affect lichen diversity?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 5 large test tubes • test tube rack • compound microscope • DI water • plastic pipettes • agar plates (TSA) • sterile swabs in tubes • glass stir rod • ruler • small beaker Per Room: • agar plates (SDA) • swab disposal biohazard bucket • demo plates of bactericides	• hand cleansers (soap, alcohol wipes, hand sanitizer) • 45^{o}C water bath • dry yeast • sample sugars solutions (glucose, lactose, sucrose and starch) • medium beaker • glass stir rods • weigh boats • spatula • fresh mushroom for dissections • razor blades • glass slides and coverslips • fungi and lichen specimens for display • prepared lichen slides

B. Procedure

1. Testing for Microbes in the Environment:
You will label plates as shown by your instructor and then seal them tightly immediately after exposure. DO NOT OPEN THEM AGAIN. Plates will be incubated and stored until next week for observations.

a) Air demo:

1. ONE group in the class will expose an agar plate to the air for 15 min.
2. The group may choose to place the plate inside or outside but should AVOID the bathrooms. Choose a location where the plate will not be disturbed, remove the cover and place the plate facing up.
3. At the end of 15min, place the cover back on the plate, seal it closed and label the location of where the plate was exposed.
4. Place the plate in the appropriate area to be incubated (check with your instructor).

b) Soil demo:

1. ONE group in the class will sprinkle a small amount of soil onto an agar plate. The soil will likely contain many kinds of fungal spores that should grow on the agar medium.
2. After placing the soil on the agar, cover the plate, seal it closed and label the location of where the soil was obtained.
3. Place the plate in the appropriate area to be incubated (check with your instructor).

c) Surfaces:

1. Each group will conduct this experiment.
2. Choose 4 surfaces to test for microorganisms. These surfaces may be part of the lab room (door handle, table, floor, etc.) or your personal belongings (cell phone, bag, book, etc.) **Do NOT choose any surfaces on your body and do NOT swab any surfaces in the bathrooms.**
3. Obtain a Petri plate of TSA agar and divide it into 4 quadrants with a marking pen on the bottom of the plate.
4. Label the outer edge of each quadrant with the location name you wish to test.
5. Obtain a sterile swab and rub it on the surface you wish to test.
6. Streak the matching quadrant with the exposed swab by rubbing it gently across the surface of the agar. Do **NOT** puncture the agar with the swab.
7. Seal the plate closed and place it in the appropriate area to be incubated (check with your instructor).

On which of the surfaces you are testing would you expect to find the greatest <u>quantity</u> of microbial growth? Why?

On which surface would you expect to find the least microbial growth? Why?

On which surface would you expect to find the greatest <u>diversity</u> of microbial growth? Why?

d) Hand cleansers:

1. Each group will conduct this experiment.
2. Obtain a Petri plate of TSA agar and divide it into 4 quadrants with a marking pen on the bottom of the plate.
3. You are provided three different types of chemicals (alcohol wipes, soap and hand sanitizer) to test the effectiveness of the cleansers/antiseptics in removing microorganisms from fingers.
4. Label the outer edge of each quadrant with the hand cleanser type. The 4^{th} quadrant should be labeled "control." For the control treatment, you can either use water or not apply any substance to the finger.
5. One person in the group should clean each finger with a different cleanser and gently rub the cleaned finger across the surface of the agar in the appropriate quadrant. Do **NOT** puncture the agar.
6. Seal the plate closed and place it in the appropriate area to be incubated (check with your instructor).

In which quadrant would you expect to see the most microbial growth? Why?

In which quadrant would you expect to see the least microbial growth? Why?

e) Household cleaners:
Observe the demo plate(s) of bacteria and chemicals that kill or inhibit bacteria (disinfectants). There may be multiple plates divided into quadrants, each with a different species of bacteria. Each quadrant has a different chemical applied to it on a circular paper. If the chemical killed the bacteria growing on the plate, there will be a clear ring around the paper. This is called the **zone of inhibition.** Measure the **radius** of the zone of inhibition for each quadrant on the demo plate(s) to determine the effectiveness of each disinfectant.

Bacteria used: *Staphylococcus saprophyticus*

Chemical	Zone of inhibition (mm)	Chemical	Zone of inhibition (mm)
Water		Hydrogen Peroxide	
Formaldehyde		Isopropanol	
Lysol		Listerine	
Bactine		Antibacterial Soap	

Which household cleaner is most effective in killing/inhibiting this bacteria according to these results?

Explain how the quadrant containing water is a negative control.

Explain how the quadrant containing formaldehyde is a positive control.

2. Fungi:
a) Fermentation by Yeast:

In this experiment, you will compare the fermentation rate of yeast in the presence of different sugars. The ability of the yeast to metabolize a particular sugar will depend on whether it has the appropriate enzyme to break down the sugar molecule. The sugars that you will test are **glucose**, **lactose**, **sucrose** and **starch**.

Glucose, **fructose** and **galactose** are simple 6-carbon sugars (monosaccharides).
Lactose is a disaccharide made up of a **glucose** joined to a **galactose** molecule.
Sucrose is a disaccharide made up of a **glucose** joined to a **fructose** molecule.
Starch is a very large molecule (polysaccharide) made up of many **glucose** molecules.
Complete the following table before proceeding with your experiment.

Question:	
Hypothesis:	
Independent variable:	
Dependent variable:	
Control Treatment:	
Experimental Treatment(s):	

Preparing the Yeast Solution

1. One group should prepare the stock solution of yeast for the whole class.
2. Add 200ml of warm water to the large empty beaker provided (You may use warm tap water or heat up the water in the microwave for 30-60 seconds). The water temperature should be close to 45^{o}C.
3. Weigh out 14g of yeast and add it to the beaker of warm water. If using individual yeast packets, use two packets.
4. Using a clean glass stir rod, thoroughly mix the yeast until it dissolves.

Fermentation with Different Sugars:

1. Obtain 5 clean test tubes and label them with your group name and also in the following order: 1-Water, 2-Glucose, 3-Sucrose, 4-Lactose, 5-Starch
2. Add 5ml of DI water to test tube 1.
3. Add 5ml of the labeled sugar to each of the other 4 test tubes.
4. Gently mix the stock solution of yeast on the counter, pour 25ml of the solution into a small, clean beaker and bring it to your lab bench.
5. Add 5ml of the yeast solution to each of the 5 test tubes. Be sure to gently mix the yeast solution in the beaker just before transferring it to each tube.
6. Mix the sugar and yeast solution in each tube by gently swirling the test tube or using a clean stirring rod.
7. Place your test tubes in the 45^{o}C water bath making sure that they are clearly labeled.
8. Record the experiment start time below and check on your test tubes every 2 min. Record the time when bubbles first form in each of your test tubes. If bubbles do not form in a test tube by the end of the experiment, write N/A.
9. End your experiment after 30min, when the solution in one or more of your test tubes starts to froth up to the top of the test tube or when the gas bubbles have peaked. Do NOT let the solution in your test tubes overflow.
10. Before you clean up the experiment, measure the amount of gas bubbles formed by using a ruler and recording the height of the gas bubbles in millimeters. This a relative measure of the amount of carbon dioxide formed.
11. Wash, dry and return all your experiment materials to their appropriate places.

Fermentation Experiment Results:

Complete the following table with class data after all the groups have completed their experiment so that you can compare the results.

	Volume of gas bubbles formed (mm)				
Group	Water	Glucose	Sucrose	Lactose	Starch

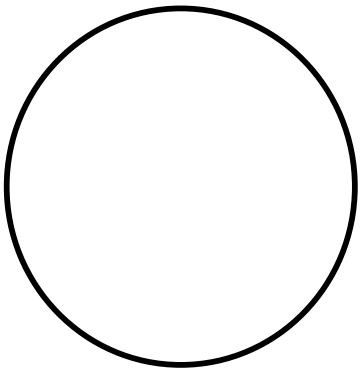
Were there any discrepancies in the experimental data among the different groups?

Were there any unexpected or surprising results? If so, how might you explain them?

Fermentation Experiment Conclusion:
Was your hypothesis supported by the experimental data? Provide a biological explanation for the results that you observed.

b) Mushroom Dissection:

1. Dissect the mushroom cap by removing the stem and cutting the cap in half using a razor blade (this may already be done for you). **Use caution with the sharp razor blade.**
2. Carefully remove one single "gill" (lamella) from under the cap. The spores will be on the lamellae.
3. Place the gill on a slide with a drop of water and add a coverslip over your sample.
4. Observe the sample under the microscope under low magnification. Look for small dark dots, which are the spores on the surface of the lamella.
5. Draw the lamella and spores of the mushroom in the space below. Include the total magnification of your drawing.

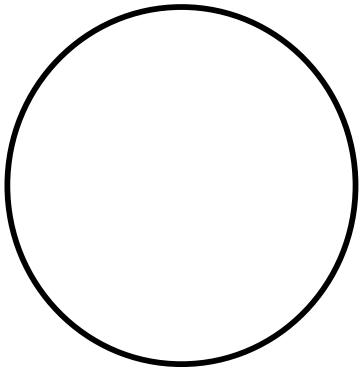


Mushroom spores (total magnification: _____)

c) Spore Print Demo Drawing:
Observe the spore print and draw it below.

d) Lichen observations:

1. Observe the prepared lichen slide and draw your observations below.
2. Label the fungus (which are stained green) and the algae cells (stained red).



Lichen (total magnification: _____)

3. Observe the lichen specimens on display and draw the three growth forms below.

Crustose (small and flat)	Foliose (leafy)	Fruticose (branched)

VI. Post-Lab Questions

1. Draw a bar graph below that illustrates the results of your fermentation experiment. Include the results for your control experiment as well as all your experimental treatments. Be sure to label the axes.

2. a) When baking bread, what product of fermentation causes the dough to rise?

3. a) Based on your experiment, which sugars (glucose, sucrose, lactose or starch) could you successfully use to make the dough rise when baking bread? Why?

b) Provide a biological reason for why yeast cannot use certain sugars for fermentation.

4. Lichens can often survive in very harsh environments that most other organisms cannot tolerate. What characteristics of lichens allow them to survive in such environments?

Lab 8: Microbe Analysis

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the presence and type of bacterial and fungal colonies on exposure plates.
2. Identify which exposure plates contain a greater diversity of microorganisms.
3. Analyze the amount of bacterial growth for different hand cleansers.
4. Explain the various ways in which bacteria communicate with each other and how to develop new antibiotics.
5. Discuss the ecological implications of antibiotic resistance.

II. Background Information:

A. Environmental Sampling Plates:

Remember that last week your class did environmental sampling to see what kinds of microbes (fungi and bacteria) exist in the soil, in the air and on surfaces. This week we want you to take a close look at your plates and observe the characteristics of the organisms on them.

B. Colony Morphology:

Bacteria have distinctive characteristic growth patterns on solid media. A colony essentially starts with one bacterium, which then multiplies logarithmically via binary fission. Within hours a colony becomes visible on the surface of the media. Colony morphology (shape, size, pigmentation, etc.) is used as starting point in the identification of bacteria. While these characteristics can't completely identify bacteria, (additional biochemical tests or genetic analysis are necessary), today you will use colony morphology to describe the growth on your plates and take a few guesses as to what some of the bacteria could be. Here are the characteristics most often used in describing the colony morphology of bacteria:

- Overall shape
- Elevation-height of the colony up from the plate
- Margin –outline of the edge of the colony
- Surface- is the surface dull or glossy? Smooth or wrinkled?
- Optical properties - transparent (clear), opaque, translucent (almost clear)
- Pigmentation—is the colony white, cream, golden, yellow, pink, red, purple, etc.

Colony Morphology Characteristics:

1. Overall shape:

Punctiform Circular Irregular Rhizoid

2. Elevation:

Flat Raised Convex Umbonate

3. Margin:

Entire Undulate Lobate Erose Curled

C. Organism Identification:

The following microorganisms are commonly found on skin, soil, and surfaces (remember that on surfaces like desks most organisms will not be actively growing, but there may be spores and cells carried on dust, aerosols, etc.).

Staphylococcus epidermidis (skin): circular, entire, convex, pinpoint, smooth, shiny, white, opaque

Staphylococcus aureus (skin): circular, entire, convex, moderate, smooth, shiny, cream colored with yellow or golden tint, opaque

Micrococcus spp. (skin and soil): circular, entire, convex, small, smooth, shiny, yellow, pink, or red, opaque

Actinomycetes spp. (soil): white powdery, moderate, threadlike filaments (look fungal like, but filaments are much smaller than hyphae, and colonies are not a robust in growth)

Bacillus spp. (soil, surfaces): Irregular, undulate, raised, moderate-spreading, smooth, dull, cream, sometimes gray-white, opaque

Yeasts: Often large colonies, creamy, pinkish, pasty, smooth, sometimes slightly glossy.

Fungi: Typically fuzzy colonies that might be white, gray, black, yellow, red, green, etc. The fuzzy structures are microscopic hyphae that the fungi extend out in order to absorb nutrients from the surrounding medium.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7^{th} ed.): pg. 265 (Antibiotic Resistance), pp.58-59 (Cells) and pp. 303-304 (Ecology of Prokaryotes).

IV. Pre-Lab Questions:

1. What is a bacterial colony?

2. Describe one method of determining whether a colony growing on your exposure plate is a fungus or bacteria.

3. a) Last week your group used different hand cleansers to determine whether it would inhibit growth of microorganisms. What was the control treatment for that experiment?

b) Briefly describe your experimental design (which finger(s) did you sample, was there more than one participant's fingers being sampled in the experiment, etc.). How might your experimental design have affected the outcome of your experiment in unexpected ways?

4. TED Conference Video of Dr. Bonnie Bassler: How Bacteria "Talk" (April 2009) http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate.html

Watch Dr. Bonnie Bassler's TED presentation and answer the following questions:

a. In terms of the number of cells and amount of genetic information, approximately what percent of your body is "bacterial" vs. human?

b. What is special about *Vibrio fischeri* bacteria?

c. What benefit does the Hawaiian Bobtail squid get from the bacteria?

d. How does the squid prevent bacteria build-up in its body?

e. What is "Bacterial Quorum Sensing" and how does it work?

f. Which bacteria use quorum sensing to communicate?

g. How can bacteria control their pathogenicity (virulence)?

h. How can bacteria distinguish between members of their own species and other species?

i. What kinds of antibiotics is Dr. Bassler proposing developing and how would these new kinds of antibiotics be better than the kinds currently being used?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• Incubated hand cleanser and surface exposure plates	• Dissecting microscope • Incubated air and soil exposure plates

B. Procedure

Do NOT open any of the exposure plates at any point.

1. Observe your group's exposure plate for **surfaces**. Sketch your exposure plate below. Label which colonies are most likely fungi and which are bacterial. Be sure to label the locations that were swabbed for each quadrant.

In which quadrant do you see the most microbial growth?

In which quadrant do you see the least microbial growth?

Which quadrant contains the greatest diversity of microbial growth?

Do these results match your hypotheses from last week? If not, what might be the reason for the discrepancy?

2. Observe your group's exposure plate for the **hand cleansers**. Sketch your exposure plate below and label the treatment applied in each quadrant.

In which quadrant do you see the most microbial growth?

In which quadrant do you see the least microbial growth?

Do these results match your hypotheses from last week? If not, what might be the reason for the discrepancy?

3. Using your group's exposure plate for **surfaces and/or hand cleansers**, complete the following data table for four of the colonies on your plate. Use a dissecting microscope to see more detail if possible.

Origin of Sample (location)	Shape	Elevation	Margin	Surface	Opaque?	Pigmentation (color)	Possible Organism
1.							
2.							
3.							
4.							

4. Compare your plate with other groups in the class.
Did you sample similar things? If so, do you have similar looking colonies growing? Why might that be?

5. Observe the exposure plates for the soil and air demo.

What location did the organisms on the soil and air demo plates come from? Which plate has more microorganisms?

What similarities and differences are there between the colonies growing on the soil and air exposure plates?

VI. Additional Activity (time permitting) & Post-Lab Questions

TED Talk by Tal Danino:
Programming Bacteria to Detect Cancer (and Maybe Treat it)

https://www.ted.com/talks/tal_danino_we_can_use_bacteria_to_detect_cancer_and_maybe_treat_it#t-383825

Watch Tal Danino's TED talk and answer the following questions. (You should have already watched Bonnie Bassler's video from the pre-lab activity to provide you with the necessary background information.) You may be asked to discuss your answers with your classmates.

1. What are the main ideas that Tal Danino presents in his talk regarding bacterial communication?

2. What are the beneficial medical applications that Tal Danino is proposing using bacterial quorum sensing?

Lab 9: Analysis of DNA

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Describe the general structure and composition of DNA.
2. Describe the relationship between nucleotides, DNA, genes and chromosomes.
3. Compare and contrast STR and VNTR analysis.
4. Describe the process of RFLP analysis.
5. Use a micropipette to load DNA samples into an agarose gel.
6. Perform DNA gel electrophoresis to produce a DNA fingerprint.
7. Analyze DNA fingerprints to identify a suspect.
8. Identify the various phases of the cell cycle in a eukaryotic cell.

II. Background Information:

1. DNA Structure & Composition:

The basic structure of DNA can be described as a double-helix composed of the four **nucleotides**: adenine (A), thymine (T), guanine (G), and cytosine (C) (Fig. 9.1). The nucleotides themselves are molecules that contain three main components: 1) a **base** that is unique to the specific nucleotide, 2) a **deoxyribose sugar**, and 3) a **phosphate group**, with the latter two components being identical in all four nucleotides (Fig. 9.2).

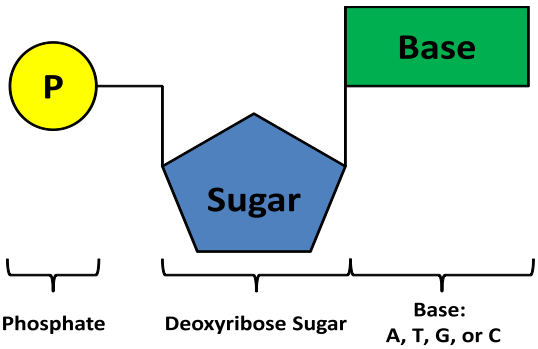


Fig. 9.2. The basic structure of a nucleotide. *Figure by Dr. Ramey.*

The basic structure of DNA. *Courtesy: National Human Genome Research Institute*

Each nucleotide pairs with a specific partner through **hydrogen bonding**, commonly referred to as **complementary base pairing**. *A* always pairs with *T*, and *G* always pairs with *C*; two hydrogen bonded nucleotides constitute a **base pair** (**bp**). The nucleotides of each strand are covalently linked by the sugar phosphate groups, creating a **sugar-phosphate backbone**.

Cells of **eukaryotic** organisms contain multiple linear chromosomes. **Prokaryotic** organisms, such as single-celled bacteria like *Escherichia coli* (*E. coli*), contain a single circular chromosome. However, despite the differences between the chromosomes of eukaryotes and prokaryotes, the primary component of all chromosomes, the DNA, functions in exactly the same way.

The nucleotides within chromosomal DNA store information in the form of **genes**. However, gene sequences do not account for all of the DNA contained within chromosomes; there are other, **non-coding**, regions in-between genes. These 'other, non-coding,' DNA segments are sometimes referred to as "**junk DNA**," but this is a misnomer because numerous studies have determined that at least 80% of 'junk DNA' serves important functions regarding gene regulation.

2. DNA Polymorphisms:

Individuals of the same species have the same chromosomes and the same genes. For example, all humans have 23 pairs of chromosomes, each of which contain the same genes. Why then, do we all look different and possess characteristics (hair, eye and skin color, height, build, etc.,) that make each one of us unique in a world of more than seven billion humans? The truth is that our unique characteristics are a result of very small differences (≤0.1%) within the nucleotide sequence of our genome, called **polymorphisms**. In this lab, we will focus on two types of polymorphisms:

a. Variable numbers of tandem repeats (VNTRs) are DNA sequences within chromosomes, usually from ~20 to 100bp in length, that are repeated multiple times, with different individuals having different numbers of repeats. VNTRs are found in multiple chromosomes and can be highly variable from one person to another (Fig. 9.3).

b. Short tandem repeats (STRs) are similar to VNTRs with one primary difference—repeated sequences of STRs are much smaller, usually between 3 to 10bp. An advantage of using STRs for DNA fingerprinting instead of VNTRs is that, since STRs are typically much smaller than

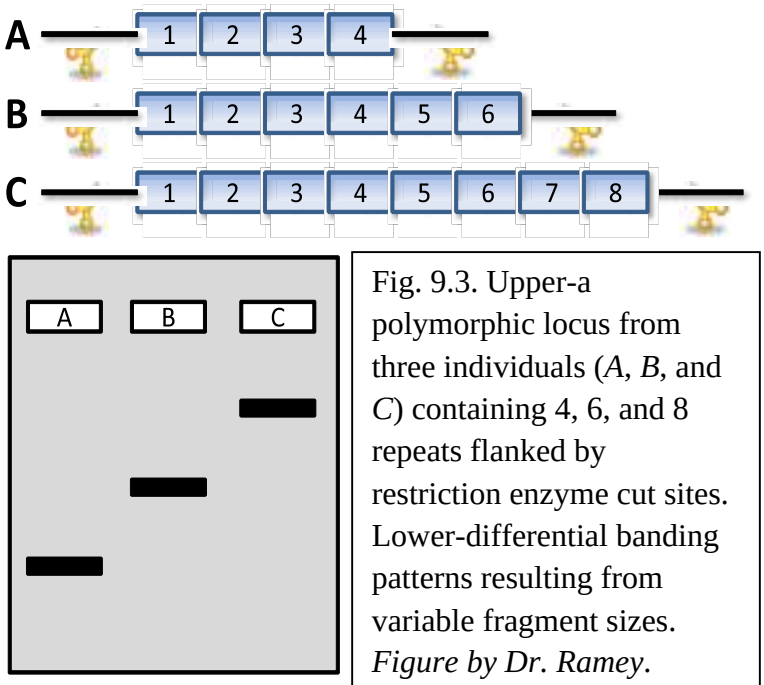


Fig. 9.3. Upper-a polymorphic locus from three individuals (*A*, *B*, and *C*) containing 4, 6, and 8 repeats flanked by restriction enzyme cut sites. Lower-differential banding patterns resulting from variable fragment sizes. *Figure by Dr. Ramey.*

VNTRs, STR analysis allows the use of DNA that has been degraded more significantly, since even small DNA fragments may contain suitable STR loci.

3. Restriction Enzyme Digestion:
One class of proteins, called **restriction enzymes** specialize in cutting DNA molecules. They typically have odd-sounding five-letter names based on the bacteria that produce them, like *EcoRI* isolated from *E. coli*, and *BamHI* isolated from *Bacillus amyloliquefaciens*. A key characteristic of almost all restriction enzymes is that they have cut-site specificity, meaning that each restriction enzyme will make a cut only at a particular DNA sequence, called a **recognition sequence** (Fig. 9.4).

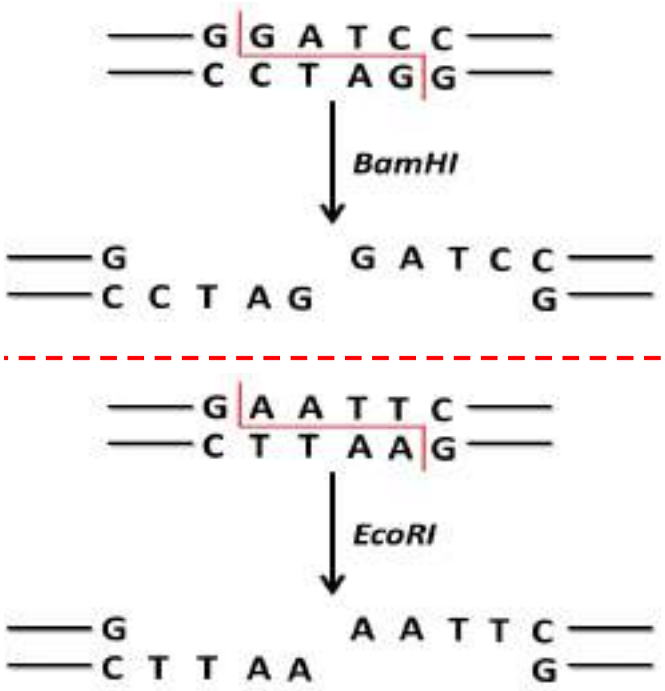


Fig. 9.4. Restriction enzyme recognition sequences (cut sites) for *BamHI* (upper) and *EcoRI* (lower). *Figure by Dr. Ramey*.

Cut-site specificity of restriction enzymes is the basis for producing a DNA fingerprint using **restriction fragment length polymorphism (RFLP)** analysis. Since we know the recognition sequence for any given restriction enzyme, along with the sequence of a DNA molecule that has been sequenced (like the chromosomes of the human genome), we know exactly where they will make a cut, and, consequently, the number and sizes of the DNA fragments produced. However, regions of the genome that contain VNTRs and STRs will be variable in length from one person to another, thus producing fragments of different sizes from DNA that has been cut with a specific restriction enzyme.

4. DNA Gel Electrophoresis:
DNA gel electrophoresis is a technique used to separate DNA fragments of different lengths resulting from a restriction enzyme digestion. It takes advantage of the fact that a DNA molecule, or fragment, has an overall negative charge, and is thus attracted to a positive electric field. In addition to a digested DNA sample, DNA gel electrophoresis requires a **gel electrophoresis chamber**, which consists of a water-tight apparatus, with a positive electrode on one end and a negative electrode on the other, containing an **agarose gel** (Fig.9.5).

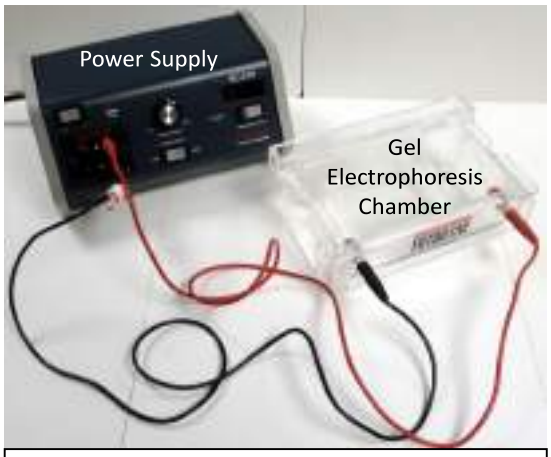


Fig. 9.5. Gel electrophoresis chamber and power supply. *Photo by Dr. Ramey and Jenny Leadbetter.*Fig. 9.1. The basic structure of DNA.

The agarose gel, which is typically the shape of a thin slab, is a semi-solid gelatin matrix that has tiny holes that DNA fragments can move through (think of gelo-gelatin without the food coloring). The gel is placed into the chamber with the wells closest to the negative electrode. The chamber is then partially filled with a clear **electrophoresis buffer**, which contains ions that allow it to carry electrical current. The gel has several empty spaces on one end called **loading wells** into which the DNA samples are loaded. Once the DNA samples are loaded into the wells, the chamber is then connected to a power supply and voltage is applied. Because of DNA's negative charge, fragments will migrate through the gel toward the positive end of the chamber. The smaller the DNA fragment, the faster it will migrate (run) through the gel matrix, resulting in the separation of the fragments based on size. Once electrophoresis is complete, the smallest DNA fragment in each well will be closest to the positive electrode, and the largest DNA fragment will be closest to the loading well (the negative electrode). DNA fragments of the same size will collect at the same location in their lane, creating a **band** that can be seen (Fig. 9.6). Any given band within a gel may have millions of DNA fragments of equal length.

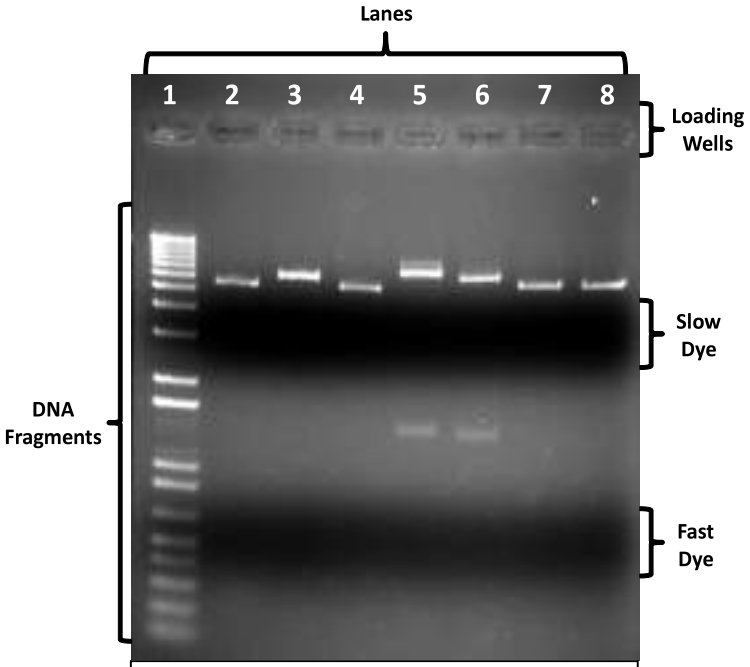


Fig. 9.6. Agarose gel showing bands of DNA fragments after gel electrophoresis. *Photo by Dr. Ramey.*

5. DNA Fingerprinting:

Nucleotide polymorphisms can be used to create what is commonly referred to as a **DNA fingerprint**, the unique banding pattern observed from a DNA sample based on the size and the number of DNA fragments in the sample (Fig. 9.7). DNA analysis technology has improved significantly over the past few decades, to the extent that very little DNA needs be recovered at a crime scene (just a few cells will usually suffice) to produce a DNA fingerprint using STRs.

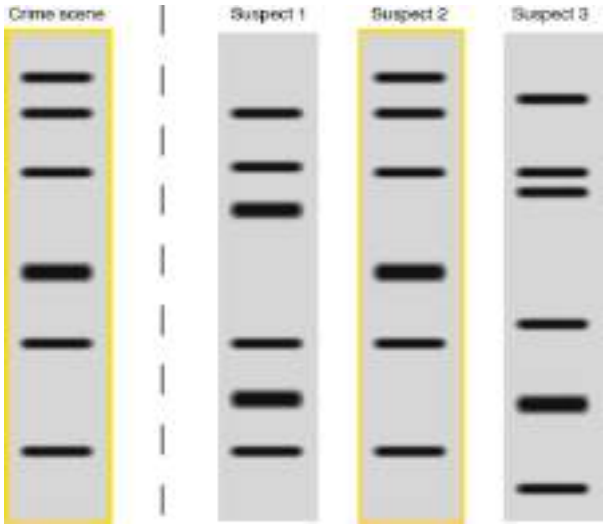


Fig. 9.7. DNA fingerprints constructed from evidence collected at the crime scene and three suspects. *Courtesy: National Human Genome Research Institute*

Sequencing different regions of the human genome has allowed us to identify a large number of STRs that can potentially be used to distinguish individuals. When more STR regions are used to produce a DNA profile, there is a greater likelihood that every individual will have a unique DNA fingerprint. Currently, the FBI has identified 13 STR regions (loci) on 12 different chromosomes used for DNA fingerprinting, making the likelihood that two people will have the same DNA fingerprint at least 1 in 1 billion! These 13 STR loci are used to produce the **Combined DNA Index System (CODIS)** database, which contains the DNA fingerprints of those arrested, prosecuted or convicted of a crime. DNA fingerprints produced from crime scene samples are now compared to profiles in CODIS the same way that actual crime scene fingerprints are compared to fingerprint databases to search for matches, which in turn can identify the individuals who were present at a crime scene.

6. The Cell Cycle[1]:

In eukaryotic cells, the stages of the cell cycle are divided into two major phases: **interphase** and the **mitotic (M) phase**. During interphase, the cell grows and makes a copy of its DNA. Chromosomes are not clearly visible in the nucleus during this phase, although a dark spot called the nucleolus may be visible. During the mitotic (M) phase, the cell separates its DNA into two sets and divides its cytoplasm, forming two new cells. Mitosis consists of four phases: **prophase** (chromatin in the nucleus begins to condense, becoming visible under the light microscope as chromosomes, and the nucleolus disappears.), **metaphase** (chromosomes line up along the metaphase plate), **anaphase** (sister chromatids move to opposite ends of the cell), and **telophase** (nuclear membrane reforms and chromosomes unwind into chromatin).

Some textbooks list five phases, breaking prophase into an early phase (simply called prophase) and a late phase (called prometaphase). **Cytokinesis** is the process of dividing the cell contents to make two new cells and starts in anaphase or telophase.

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7th ed.): pg. 172-173 (DNA Structure), pg. 218-224 (Genetic Engineering and pg. 225-229 (DNA Profiling and Forensic Science).

B. Recommended background reading (optional):

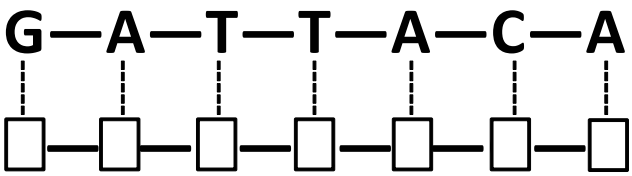
1. National Institute of Justice: DNA Evidence Basics
http://www.nij.gov/nij/topics/forensics/evidence/dna/basics/welcome.htm

2. DNA Forensics: News and Information about DNA Databases
http://www.dnaforensics.com/

[1] Khan Academy: www.khanacademy.org

IV. Pre-Lab Questions:

Use the following DNA fragment to answer questions 1- 4:



1. Fill in the correct nucleotides on the complementary strand.

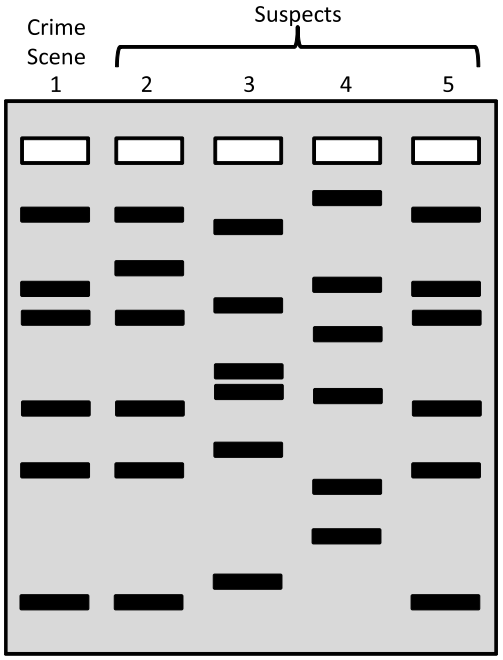
2. How many base-pairs long is this DNA fragment? _____

3. What do the solid lines in between nucleotides on the same strand represent?

4. What do the dotted lines in between nucleotides on different strands represent?

Use the DNA fingerprints provided to answer questions 5-10:

5. The DNA fragments were separated using what technique?



6. What do the white rectangles represent?

7. What do the black rectangles represent?

8. Which suspect or suspects are most likely innocent?

9. Which suspect most likely committed the crime?

10. Which suspect may be related to the person who committed the crime?

Use the following DNA sequence to answer question 10-11 (complementary strand not shown):

C-G-A-A-G-G-A-T-C-C-A-G-A-A-T-G-T-A-T-T-C-A-T-G-G-C-A-C-T-G-T

10. Write in the complementary strand of DNA above.

11. a) Do either of the restriction enzymes *BamHI* or *EcoRI* cut the DNA fragment? (Hint: Refer to the background information on pg. 125)

b) If so, circle the recognition sequence(s) and mark the actual cut site(s) on the strand above.

c) How many total DNA fragments are produced as a result of the cut(s)?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• Micropipette • Pipette tips • Practice loading gel • Practice loading sample • Agarose gel • Gel electrophoresis chamber • Power supply • Prepared DNA samples (A-F) • 150ml beaker • graduated centrifuge tube with cap • hooked paper clip or tweezers • Bottle of DI water	• Electrophoresis buffer • Ice container • Meat tenderizer • Dish detergent • 95% Ethanol • Sodium Bicarbonate • Wheat germ and strawberry • Digital scale • Ziploc bag • Salt • Spatulas, funnel and weigh boats • cheesecloth • 2 transfer pipettes • Glass stir rod • Two 150ml beakers • Test tube marker • Prepared slide: onion root tip

B. Procedure

1. Practice Gel Loading:

When working with DNA samples, you are measuring and dispensing volumes in **microliters** (µl). 1µl is one millionth of a liter! In order to accurately measure such tiny volumes requires a special device called a **micropipette**. Prior to working with your actual DNA samples and your actual agarose gel, you will practice with the loading dye and a 'practice gel' (Fig. 9.8). After watching a demonstration of how to use a micropipette and load a gel, perform the following:

1. Cover your practice loading gel with DI water (to simulate gel electrophoresis buffer).
2. Load a tip onto your micropipette.

Note: do **NOT** change pipette tips during gel loading practice.

3. Draw up 10µl of practice loading dye:
   a) With your thumb, depress the micropipette plunger down to the first stop.
   b) Place the micropipette tip into the practice loading dye.

Fig. 9.8. Example of correct loading procedure. *Photo by Dr. Ramey and Jenny Leadbetter.*

c) Draw up practice loading dye by slowly releasing the plunger.
d) Pull the tip out of the practice loading dye—you now have exactly 10µl of dye in the micropipette tip.

4. Carefully load the practice loading dye into a well of the practice loading gel:
   a) Place the tip of the micropipette into the well, being careful not to pierce the bottom of the gel with the tip (this would cause your sample to 'leak' out of the bottom of the gel) (Fig. 10).
   b) Dispense the dye into the well by slowly depressing the plunger to the second stop.
   c) Without releasing the plunger, pull the tip out of the well (if you release the plunger too soon, you will suck your sample back up).
   d) After the plunger is out of the well, slowly release the plunger.
5. Each group member should repeat steps 3-4 several times so that everyone is comfortable using a micropipette and loading a gel.
6. When finished, carefully remove the tip from the micropipette and dispose of it in the **biohazard container**.
7. Rinse your practice gel with cold tap water to remove the dye and return it to your table.

2. DNA Fingerprinting:

1. Take the agar gel that has been set and solidified in the plastic rectangular casing.
2. Gently loosen the plastic screws and lower the plastic gates on both sides. Retighten the screws **gently** (don't over-tighten or you will strip the screws).
3. Remove the cover of the chamber by sliding it off while holding the chamber firmly.
4. Place the gel in the center of the electrophoresis chamber, oriented such that the wells are closest to the negative (black) electrode of the chamber.
5. If necessary, add more electrophoresis buffer to the chamber so that the top of the gel is covered by approximately 1cm of buffer.
6. Obtain crime scene DNA samples *A-F* (Table 1). These samples have already been digested and mixed with loading dye and are ready for gel electrophoresis.
7. Carefully load 10µl of each sample into the wells of the gel in sequential order as shown in Table 1.

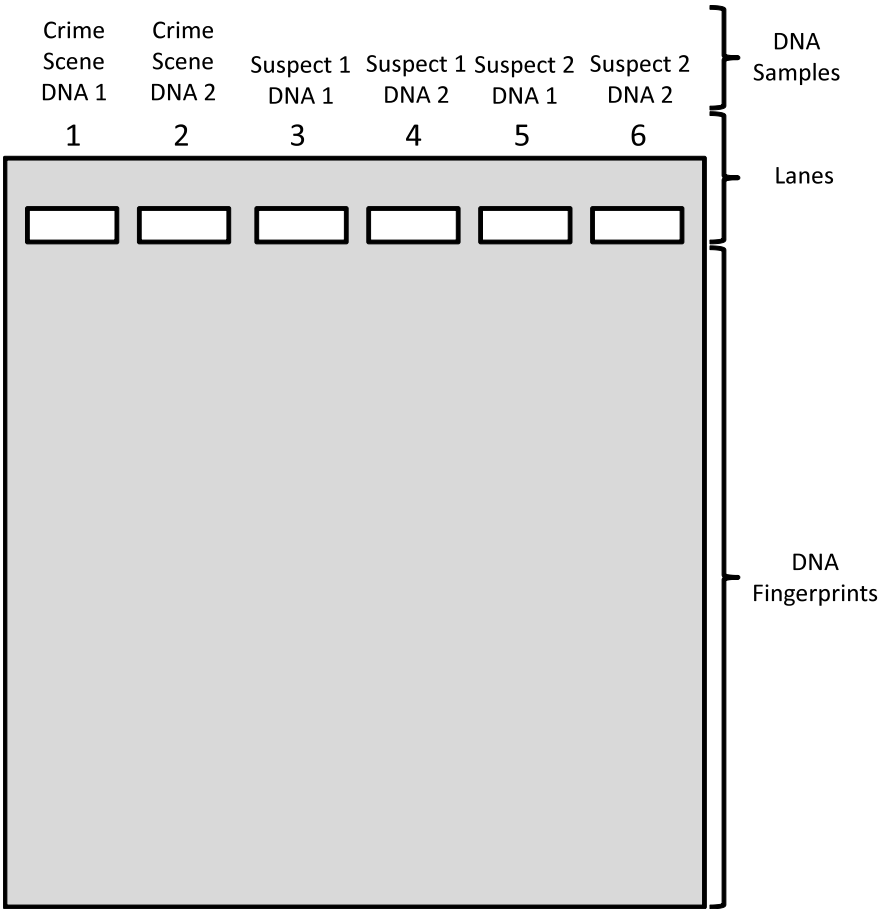
Table 1

Lane	Tube	Sample
1	A	Crime Scene DNA 1 (cut with *BamHI*)
2	B	Crime Scene DNA 2 (cut with *EcoRI*)
3	C	Suspect 1 DNA 1 (cut with *BamHI*)
4	D	Suspect 1 DNA 2 (cut with *EcoRI*)
5	E	Suspect 2 DNA 1 (cut with *BamHI*)
6	F	Suspect 2 DNA 2 (cut with *EcoRI*)

8. Place the cover on the gel electrophoresis chamber and connect the electrode terminals to the power source.
   a) Make sure that the negative (black) and positive (red) color-coded indicators on the cover and chamber are properly oriented.

b) Likewise, make sure that the electrodes are plugged into the correct electrode terminals on the power source. Before proceeding to the next step, ask the instructor to inspect your setup.

9. Plug in and turn on the power source and set it to ~100V. You should be able to see bubbles forming at each end of the chamber.
10. Run your gel for approximately 45 minutes.
    a) Caution: do NOT allow your gel to run too long, or else the DNA fragments will literally run out of the end of the gel!
    b) Carefully monitor the progress of your DNA samples every 5 minutes.
    c) Typically, the gel is done and should be turned off when the blue 'fast dye' has run approximately 2/3rds down the gel.
    d) When you think the gel has finished running, let your instructor know.
11. After electrophoresis is complete, turn off and unplug the power source, then carefully remove the cover from the chamber.
12. Using the figure below, draw a picture of your results and answer the post-lab questions.
13. Follow your instructor's instructions on how to clean and reset your gel electrophoresis experiment. Do **NOT** dump the buffer solution out; the next lab section will reuse it.



According to your results, which suspect's DNA matches the crime scene DNA? _____

3. DNA Isolation from Biological Material:
Check with your instructor to find out which protocol you will be using for this section. You may be asked to work with another group to observe the results of both experiments.

To simulate a biological sample recovered from a crime scene, you will be using wheat germ and/or a strawberry. Wheat germ is the embryo (sprouting) section of the wheat kernel and is an excellent source of DNA. The strawberry is an aggregate fruit because it contains seeds from multiple ovaries of a single flower and thus, is also an excellent source of DNA.

The wheat species you will be using is likely a polyploidy species with 6 copies of each chromosome (**hexaploid**). The strawberry is most likely **octoploid** (8 copies of each chromosome).

Based on this information, what is your hypothesis about which plant (wheat or strawberry) would yield a greater amount of extracted DNA?

A. Wheat Germ DNA Extraction

1. Measure 6.75ml of DI water into your graduated vial and place in the 55° water bath for five minutes.
2. Weigh out 0.3g of wheat germ.
3. Add wheat germ and 0.5ml of detergent to the graduated vial with water and shake well.
4. Place the graduated vial at your table and let it sit for 5 min.
5. After the five minutes, add 0.3g meat tenderizer and 0.75ml of sodium bicarbonate solution and stir well.
6. Incubate this mixture for an additional 15-20 minutes in the 55° water bath.
7. Place the vial containing the wheat germ mixture into an ice bath for two minutes to quickly cool it to room temperature. Stir gently during this time.
8. Using a pipette, carefully layer 2ml of ice cold 95% alcohol over the wheat germ solution in the vial. Allow the alcohol to flow from the tip of the pipette along the wall of the vial just above the liquid layer.
9. There should be a visible interface between the alcohol layer and the wheat germ mixture layer and a fibrous white precipitate should accumulate at the interface; this is DNA, consisting of thousands of DNA strands.
10. Dip the paper clip hook or tweezers into the white precipitate and try to separate it from the solution. The clump of DNA will break apart easily so this step must be done carefully and without stirring.
11. After you have observed the DNA and answered the questions below, dispose of the waste in the designated containers, wash all of your materials and return them to where you obtained them.

B. Strawberry DNA Extraction

Steps 1-8 should be carried out by one group for the whole class.

1. Prepare the extraction buffer by adding 50ml of water into a small beaker.
2. Add 5ml of dish soap to the water.
3. Add approx. ¼ tsp salt to the water and soap and mix until the salt dissolves.
4. Place a strawberry in a Ziploc bag.
5. Gently smash the strawberry using your fist and fingers. **Be careful not to break the bag!**
6. Add the soapy extraction buffer to the Ziploc bag and gently kneed/mush the strawberry again until no large pieces remain.
7. Place the cheesecloth inside the funnel and place the funnel over a clean beaker.
8. Pour the strawberry mixture through the cheesecloth into a clean beaker.
9. Each group should pour 5 ml of the strawberry extract into a clean vial and complete the remaining steps.
10. Using a pipette, carefully layer 2ml of ice cold 95% alcohol over the strawberry solution in the vial. Allow the alcohol to flow from the tip of the pipette along the wall of the vial just above the liquid layer.
11. There should be a visible interface between the alcohol layer and the wheat germ mixture layer and a fibrous white precipitate should accumulate at the interface; this is DNA, consisting of thousands of DNA strands.
12. Dip the paper clip hook or tweezers into the white precipitate and try to separate it from the solution. The clump of DNA will break apart easily so this step must be done carefully and without stirring.
13. After you have observed the DNA and answered the questions below, dispose of the waste in the designated containers, wash all of your materials and return them to where you obtained them.

Did your results support your hypothesis regarding the amount of DNA extracted from wheat germ and strawberry?

Was the same procedure used in both the wheat germ and strawberry experiments? If not, how could these differences affect the outcome of the experiments?

What was the purpose of using the meat tenderizer and the detergent to extract DNA? (Hint: think about where the DNA is located in a cell)

4. Observation of Chromosomes in an Onion Cell

1. Observe the prepared slide of the onion root tip, which contains many cells in different stages of the cell cycle. Focus on the root tip, where mitosis rates are highest.
2. Identify and draw 1-2 cells in EACH of the following stages of the cell cycle: interphase, prophase, metaphase, anaphase, telophase and cytokinesis.
3. You may need to scan the entire slide and multiple root tips to find all the listed stages.

Total magnification: _____

Interphase	Prophase	Metaphase
Anaphase	Telophase	Cytokinesis

5. Further DNA Gel Analysis: Fragment Length Determination:
Check with your instructor before proceeding with this section.

As you know, VNTRs and STRs cause certain chromosomal loci to be variable in length. One method for determining DNA fragment length is by creating a **standard curve** using a **DNA ladder** which contains DNA fragments of known lengths (Fig. 9.9). For example, when you load the DNA samples that you prepared above in an agarose gel, you would also load a sample of DNA ladder in a separate lane on the gel (see Fig. 9.9, lane 1). After gel electrophoresis is complete, you would then be able to create a '*DNA fragment length standard curve'* and use it to determine the fragment lengths of the VNTR loci.

1. Using a ruler, measure the migration distance (in cm) of each DNA fragment in the **DNA ladder** lane from the center of the well to the center of each DNA band and record the lengths in Table 2.
2. Using the semi-log graph paper provided, construct a standard curve by plotting the fragment migration distance (in cm; on the x-axis) vs. the DNA ladder fragment size (in bp; on the y-axis).
3. Using a ruler, draw a 'best fit' line to complete the standard curve. **Note that a 'best fit' line does NOT mean connect the dots.**
4. As in step #1, measure the migration distance of each VNTR fragment in the **crime scene DNA** lane and record the lengths in data table.
5. Also, estimate how long these three fragments are by making careful observations of their location on the gel in reference to the DNA ladder bands of known lengths.
6. Using the **DNA fragment length standard curve** completed in steps #1- 3 and the VNTR fragment migration distances measured in step #4, determine the actual VNTR fragment size (in bp) and record it in the data table.

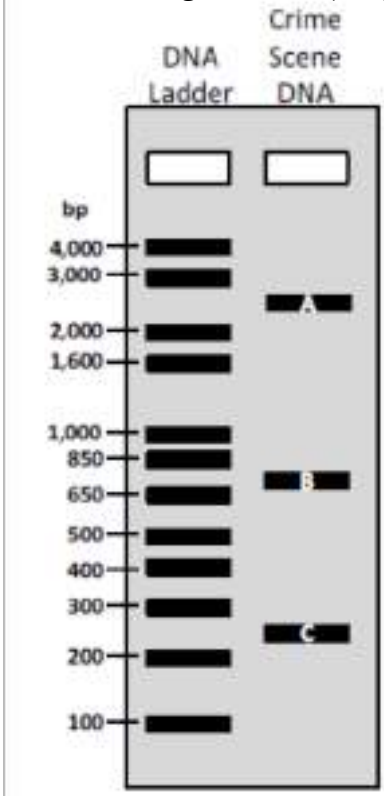
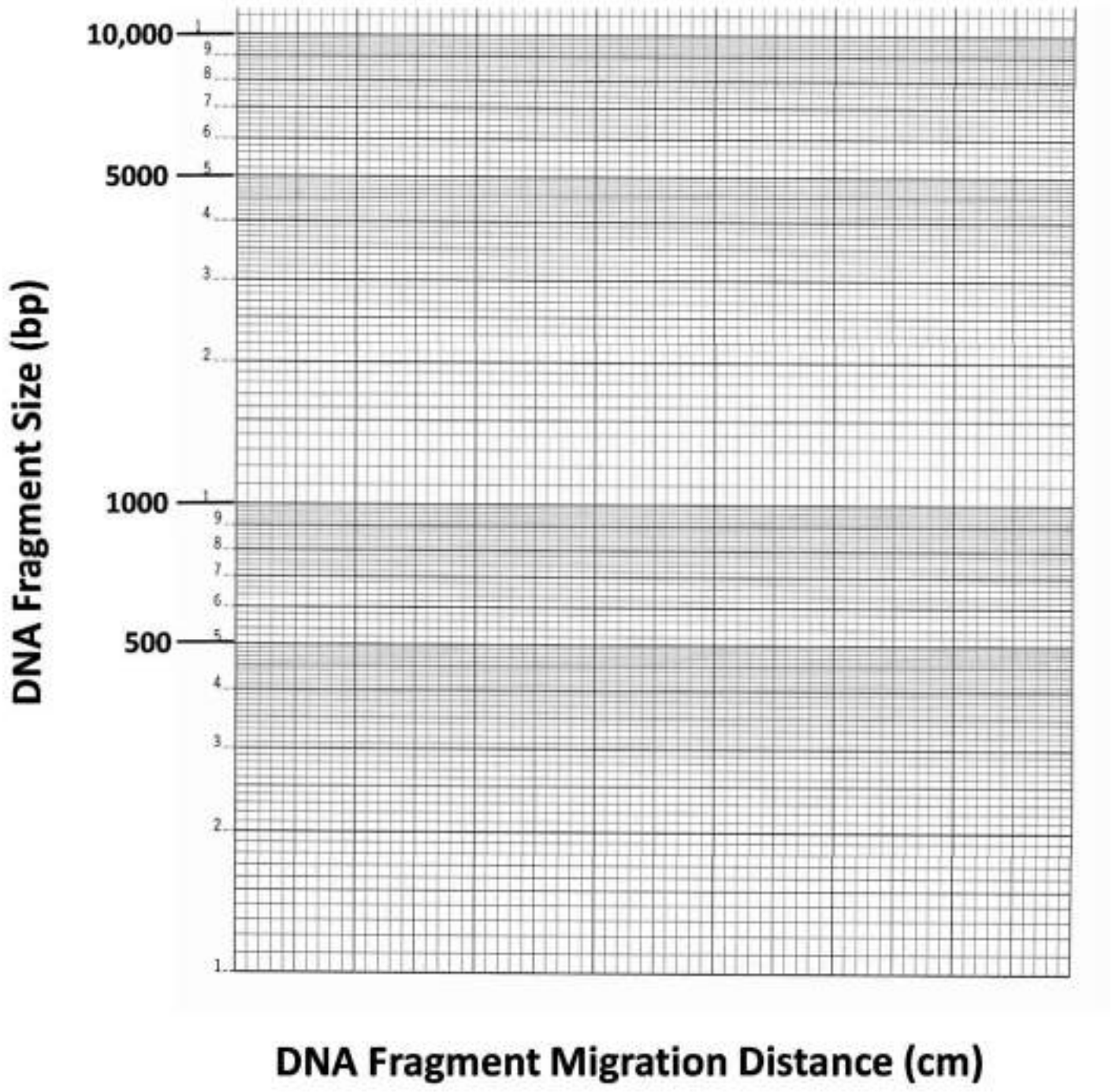


Table 2

DNA Ladder		Crime Scene DNA			
Fragment Length (bp)	Migration Distance (cm)	VNTR DNA Fragment	Distance Migrated (cm)	Estimated Fragment Length (bp)	Actual Fragment Length (bp)
100		A			
200		B			
300		C			
400					
500					
650					
850					
1000					
1600					
2000					
3000					
4000					

Fig. 9.9. RFLP Analysis. *Figure by Dr. Ramey.*

Standard Curve:
DNA Fragment Length vs. Migration Distance



VI. Post-Lab Questions

1. Based on the evidence obtained from the analysis of the DNA fingerprints in your gel electrophoresis experiment, which suspect is a match for the crime scene DNA?

2. Based on the DNA fingerprints alone, can you conclude without a doubt that this suspect indeed committed the crime? Why or why not?

3. What kind of evidence would you look for at a crime scene to obtain a DNA sample?

4. Why is it important that DNA fingerprints are also produced for the crime victim, and perhaps even the crime scene investigator who collected the evidence and the technician who analyzes the DNA?

5. What is the benefit of using more than one restriction enzyme to produce the DNA fingerprints? (Hint: Review your experimental results for the BamHI enzyme – columns 1,3,5)

6. Is it possible for two people to have identical DNA fingerprints? Why or why not?

7. Describe one advantage of using STR analysis compared to VNTR analysis.

8. What is the purpose of positioning the sample wells on the negative electrode end of the electrophoresis chamber?

9. What would happen if you positioned the agarose gel backwards within the chamber, with the sample wells on the positive electrode end of the electrophoresis chamber?

10. Describe why larger (longer) DNA fragments move through an agarose gel more slowly than smaller (shorter) fragments?

Lab 10: Plant Diversity

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the characteristics that unite all plants
2. Identify the main characteristics that are used to distinguish and classify plant groups
3. Recognize the haploid and diploid generations in the plant life cycle
4. Identify reproductive structures and the dominant stage of each of the plant groups.
5. Describe the function of the parts of a flower.
6. Identify and describe the differences between monocot and dicot angiosperms.
7. Identify the types of stem and leaf modifications exhibited by different plants.

II. Background Information:

Plant cells differ from animal cells in the following ways: plant cells are surrounded by cell walls, there is a central vacuole that stores water, and pigment or toxins, and there are chloroplasts which are the site of photosynthesis. Molecular and physiological evidence suggests that land plants evolved from a group of aquatic green algae approximately 475 million years ago.

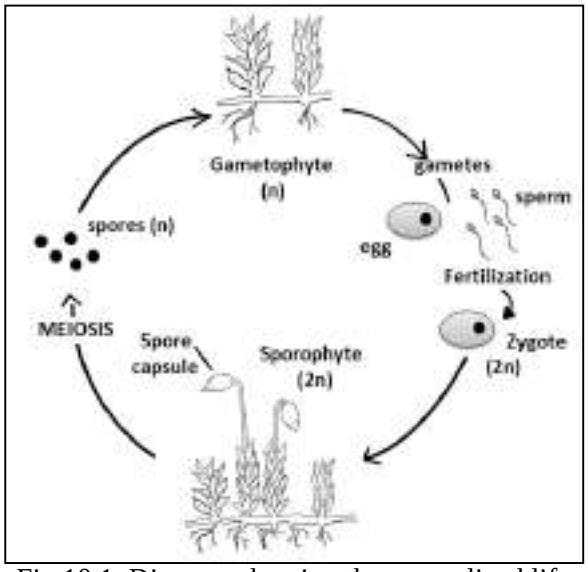


Fig.10.1. Diagram showing the generalized life cycle of alternation of generations in a moss (Drawn by Dr. Palavecino)

All land plants exhibit an **alternation of generations** life cycle, which can be defined as having multicellular haploid and diploid stages (Fig. 10.1). Compare this with the animal life cycle where only the diploid stage is multicellular and the haploid stage (the sperm and the egg) are single-celled. The dominant stage of a plant's life cycle is defined as the stage in which the plant spends the majority of its life. With the earliest land plants, the dominant stage was haploid (**gametophyte**), whereas in later groups, the dominant stage became diploid (**sporophyte**). One of the benefits of having a dominant diploid stage is that it provides protection against environmental mutagens since the plant possesses two copies of every gene.

While life on land certainly has advantages over life in the water (e.g. better access to light and carbon dioxide) there were also numerous challenges that the earliest land plants faced and had to overcome, such as preventing desiccation and overcoming the force of gravity.

In the following section there are detailed descriptions of each of the major plant groups that we will study in this lab. As you read through the information, identify the similarities and differences between the different groups of plants (Fig. 10.2).

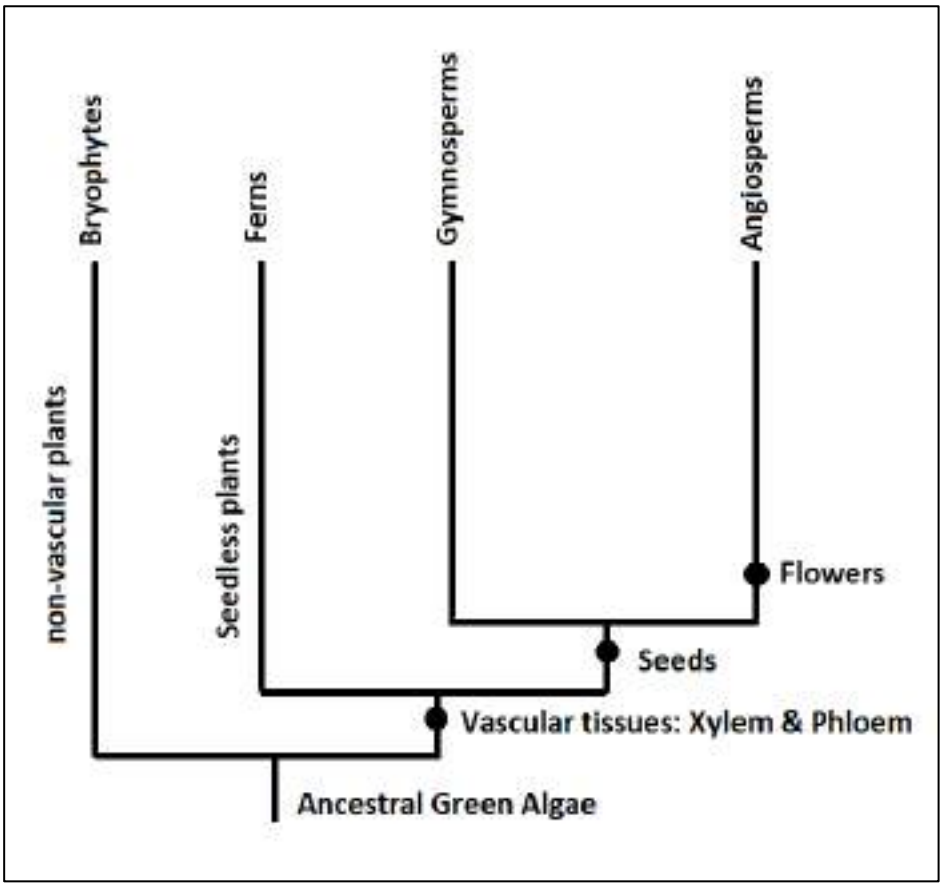


Fig.10.2. Phylogenetic tree showing the evolution of land plants (Drawn by Dr. Palavecino)

Nonvascular plants:

Nonvascular plants are the earliest land plants and include the mosses, liverworts and hornworts, which are called **Bryophytes**. These plants lack specialized tissues for conducting water and nutrients, and rely on diffusion from the environment into the cells. Therefore, these plants cannot grow to be very large. The dominant stage in this group of plants is the **gametophyte** (haploid structure), and is the green plant that you see most often growing close to the ground in damp areas. Another reason that these plants are often limited to areas that contain enough moisture is that the sperm has to swim to the egg. This distance is not very far, and a drop of rainwater usually provides enough moisture for this to happen, but without it, reproduction cannot occur. Mosses can be found around the world and can be common and diverse in moist coniferous, temperate and tropical forests, as well as wetlands.

Vascular tissues:

After the first non-vascular plants colonized the land approximately 470 million years ago, the second period of plant evolution began with the diversification of plants with **vascular tissues** to transport water and nutrients. The tissue that transports water and dissolved nutrients from the root and also helps to form the woody element in the stem is called the **XYLEM**, whereas the tissue that transports sugars and other products from the leaves is called the **PHLOEM** (Fig. 10.3, 10.4)[1]. The evolution of vascular tissues allowed vascular plants to colonize a greater variety of habitats. Vascular plants include **ferns and its allies** ("seedless plants"), and those that produce seeds; **gymnosperms** ("naked seeds"), and **angiosperms** ("contained seeds").

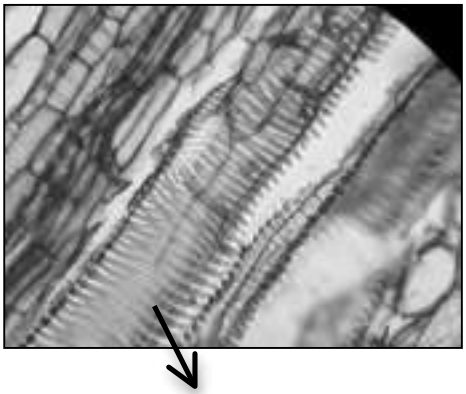
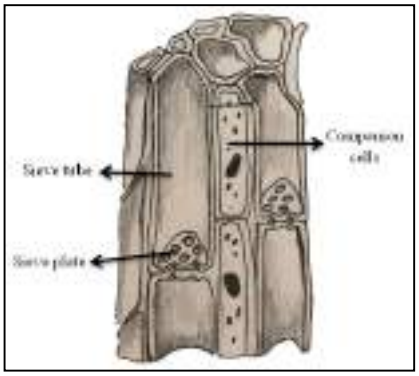


Fig.10.3. **Xylem cells** in stems of winter squash under the scope[1] **(LS)**



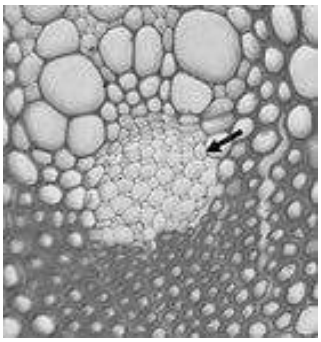


Fig.10.4. (a) **Phloem** elements diagram (**LS**) by Dr. Palavecino (b) Phloem in a vascular bundle (**CS**) under the scope [2]

Seedless Plants:

Seedless vascular plants include **ferns** and horsetails and lycophytes. The dominant stage of ferns and horsetails is the **sporophyte** (diploid structure). On the underside of a fern leaf you might see small, dark colored bumps. These are clusters of sporangia called **sori**. The sporangia produce spores, which are haploid and are released into the air. The spores settle and grow into the gametophyte stage, which is small and short-lived. Ferns were one of the dominant plants approximately 350 million years ago (during the Carboniferous period) and extensive fern forests existed. Today they are less abundant; however, they can still be major components of the flora in tropical habitats and to a lesser extent in temperate forests. Reproduction in these plants still requires water because the sperm has to swim to the egg in order to fertilize it.

[1] Wikimedia Commons: http://commons.wikimedia.org

Seeds:

Besides the development of vascular tissues, three additional adaptations allowed plants to better adapt to terrestrial conditions, and become independent of water for reproduction; (a) a further reduction of the gametophyte, (b) pollen, and (c) **seeds**. A seed consists of a plant embryo packaged along with a food supply within a protective coat (Fig. 10.5). Seeds develop from ovules that are the structures that contain the female gametophytes. A **pollen grain**, on the contrary, is the much-reduced male gametophyte in which the sperm will develop. Gymnosperms and Angiosperms produce seeds.

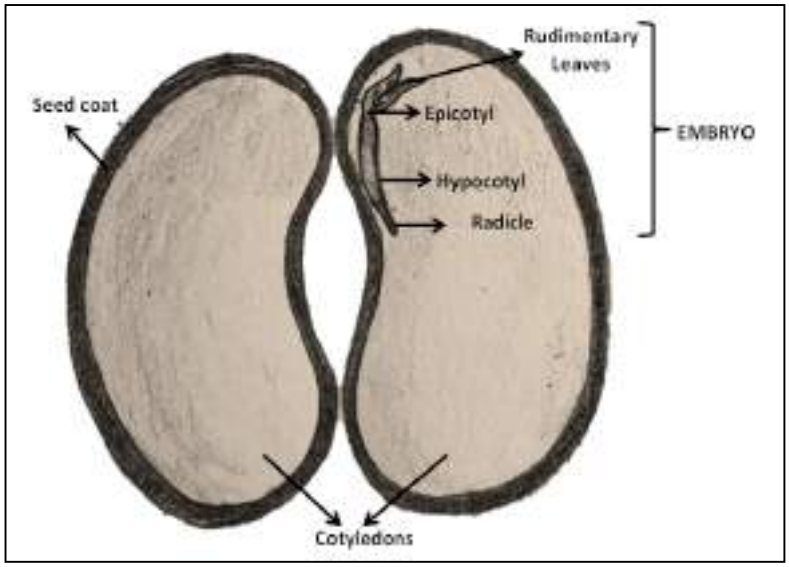


Fig.10.5. Structure of a seed (Drawn by Dr. Palavecino)

Gymnosperms ("naked seed" plants)

Gymnosperms include the conifers (pine trees), cycads, gnetophytes and gingko. Similar to the seedless vascular plants, gymnosperms contain vascular tissue. Gymnosperms were the first group of plants to produce seeds. Another evolutionary innovation of this group was the production of pollen. These were great innovations because these plants could now survive and reproduce in drier and harsher environments and thus expand their territory.

Gymnosperm literally means "naked seed." The seeds are held under the scales of a **cone**, which is the reproductive structure in this group. This offers some protection and support for the developing embryo (although not as much as the next group of plants we will discuss.) In gymnosperms, the dominant stage is the **sporophyte** (the trees that you see growing) and the gametophyte stage is microscopic and contained within the cone. Pollen from small, **male cones**, which are located on the lower branches of the tree, make their way on wind currents to the **female cones** located on the upper branches of another tree. There, the pollen fertilizes the eggs and seeds develop in the female cones. When mature, the seeds fall out of the cones, settle on the ground, and grow into a new tree. Many gymnosperms also have modified needle-like leaves. These leaves are an adaptation to drier conditions because they reduce water loss from the surface of the leaf.

Flowers:

The evolution of **flowers**, the site for reproduction, account for the unparalleled success of the angiosperms; the group of plants that dominate the modern landscape.

A typical flower is composed of sterile, vegetative structures that include the petals and sepals. These structures often function in attracting pollinators and protecting the reproductive structures that they surround. The female reproductive structure is called the **carpel**, and includes the **stigma** (which is sticky to catch pollen), **style** (which ensures proper fertilization of the egg), **ovule** (which develops into the seed) and the **ovary** (which develops into the fruit). The male reproductive structure is called the **stamen**, and includes the **anther** (which produces pollen), and the **filament**, which supports the anther (Fig. 10.6). The pollen and the ovules are the male and female gametophyte stages of angiosperms. They are microscopic compared to the gametophyte stages of earlier land plants. To attract pollinators, angiosperms have evolved a great diversity of flowers. Some flowers have developed such specialized relationships that they can only be pollinated by a single species.

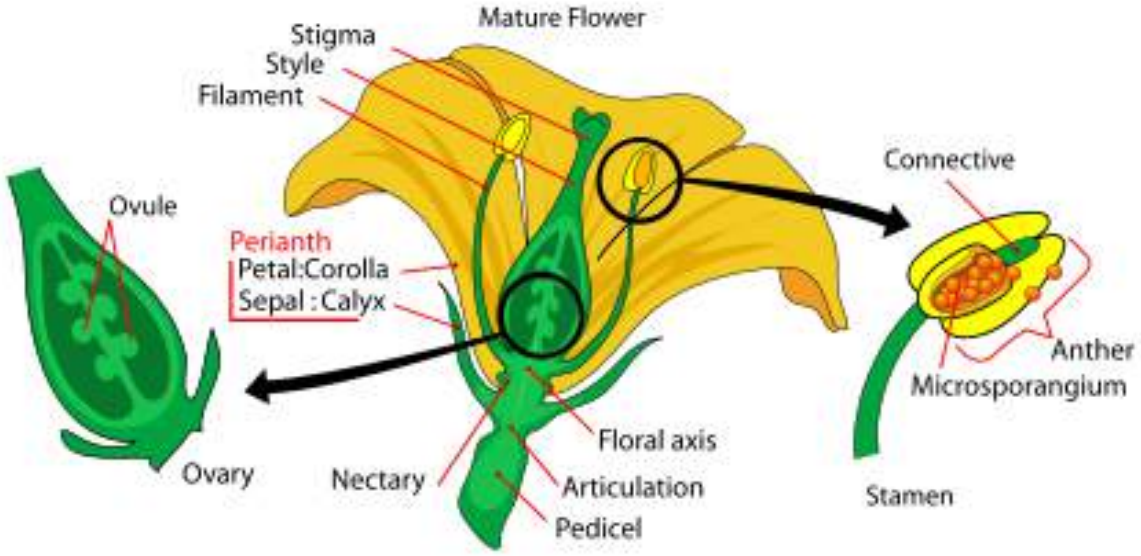


Fig.10.6. Diagram of a generalized flower structure.[2]

Angiosperms (flowering plants):

Angiosperms are the most recently evolved group of land plants, and they are the dominant group among all the land plants with respect to number of species and diversity of forms. As with the gymnosperms, the dominant stage of angiosperms is the sporophyte, and the gametophyte stage has been reduced to microscopic structures located within the flower. In addition to containing vascular tissue and producing seeds, angiosperms contain evolutionary

[2] Wikimedia Commons: http://commons.wikimedia.org

innovations that the other plant groups lack, such as **flowers** and **fruits**, which are responsible for this group's great success and diversity. A few angiosperms are successfully pollinated by wind (i.e. the grasses), but most angiosperms are pollinated by animals (i.e. insects, birds and bats.) Similarly, the seed, which is protected inside the fruit, is transported by a variety of methods, including wind, water and animals. If the seed is to be transported by wind, the ovary will contain specialized structures to catch the wind currents (i.e. the "umbrella" on a dandelion seed). If the seed is to be transported by animals, the ovary may develop into a sweet, fleshy fruit to entice the animals to eat it and later deposit it in another location, along with a pile of fertilizer. If the seed is to be transported on the fur of passing mammals, the ovary will develop spines or projections that will easily get caught on the animals' fur (or the clothes of passing humans).

When pollen grains reach the stigma of a flower, the pollen grain will grow a pollen tube and the sperm inside the pollen will reach and fertilize the egg located inside the ovule. The diploid zygote will then develop into the embryo within the seed.

Angiosperms are divided into two major groups: **Monocots** and **Dicots**. Some of the morphological differences between these two groups are listed in the following table:

Feature	Monocots	Dicots
Number of flower parts (petals, sepals, etc.)	multiples of three	multiples of four or five
Number of cotyledons (leaves in the seed)	one	two
Arrangement of major leaf veins	parallel	branched / net-like
Arrangement of vascular bundles (xylem and phloem) in the stem	scattered randomly	in concentric circles (rings)

Plant Morphology:

Plant morphology studies both the vegetative structures of plants, as well as the reproductive structures. The vegetative structures of vascular plants include two major organ systems: 1) the **shoot system,** which includes the stems, leaves and flowers and 2) the **root system** which includes the taproot and the lateral roots (Fig. 10.7). These two systems are common to nearly all vascular plants, and provide a unifying theme for the study of plant morphology. By contrast, the reproductive structures are varied, and are usually specific to a particular group of plants. For example, structures such as flowers and fruits are only found in the angiosperms, **sori** are only found in ferns, and seed cones are only found in conifers and other gymnosperms. Reproductive structures are therefore regarded as more useful for the classification of plants than vegetative characters.

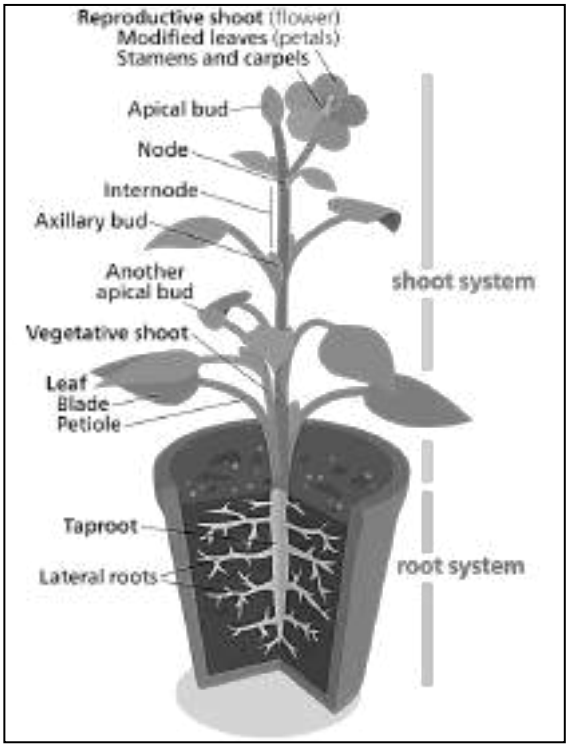


Fig.10.7. Diagram of a plant showing the shoot and root systems.[3]

Root and Shoot Modifications

The shoot and root systems of many plants have been modified for specialized functions such as food storage, protection, attachment and vegetative reproduction. The following are some examples of modified stems and leaves.

a. Modified Roots

Storage roots (i.e. carrots, beets and radishes), are modified for storage of starch and water. **Aerial roots** are found in many different types of plants and can provide support (i.e. banyan tree), and absorption of moisture and nutrients in epiphytic plants (i.e. orchids).

b. Modified Stems

Stolons are horizontal stems that grow above ground and develop new plantlets at the tips wherever the stems touch the ground. New shoots and roots are formed at nodes. Strawberry plants are an example of stolons. **Rhizomes** are horizontal fleshy stems that grow underground. They also produce new shoots and roots at the nodes. They allow storage of food (e.g. ginger) and vegetative propagation of the plant to new areas (e.g. grass and

[3] Wikimedia Commons: http://commons.wikimedia.org

bamboo). **Bulbs** and **tubers** are short, underground stems that allow vegetative propagation of the plant as well as food storage. An example of a bulb is an onion and an example of a tuber is a potato.

c. Modified Leaves

Tendrils are threadlike leaves modified for climbing and are found in pea plants and grapes. **Spines** provide protection and reduce water loss for plants such as cacti. **Scale leaves** are thin, dry structures that surround and protect the axillary bud, such as those found in asparagus. **Floral leaves (bracts)** are large, colored leaves that serve the same function as petals. The large red bracts on poinsettia are actually leaves; the actual flower petals are small and inconspicuous. **Insectivorous leaves** are adapted for catching and trapping insects and are found in plants living in nutrient-poor soils. The leaves of the Venus flytrap and pitcher plants are examples of insectivorous leaves.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7^{th} ed.): pg. 316-327 (Plant Diversity)

B. Recommended background reading (optional):
Plants Video by Paul Andersen:
http://www.bozemanscience.com/plants

IV. Pre-Lab Questions:

1. Complete the following table by placing an X in the boxes that apply to each plant group.

	Nonvascular plants	Seedless vascular plants	Gymnosperms	Angiosperms
Water needed for reproduction				
Vascular tissue present				
Embryo protected inside a seed				
Plant produces pollen				
Plant produces flowers				
Seed is protected inside a fruit				

2. Complete the following statements:

a) "Alternation of generations" is defined as: _____

_____

b) In gymnosperms, the reproductive structure is the: _____

c) In angiosperms, the reproductive structure is the: _____

d) Vascular tissue refers to: _____

3. Answer the following questions.

a) Is the gametophyte diploid or haploid? _____

b) Is the sporophyte diploid or haploid? _____

4. Angiosperms are divided into two major groups: _____ and _____.

5. Explain what the root and shoot systems of plants refer to.

V. Lab Exercise:

A. Materials

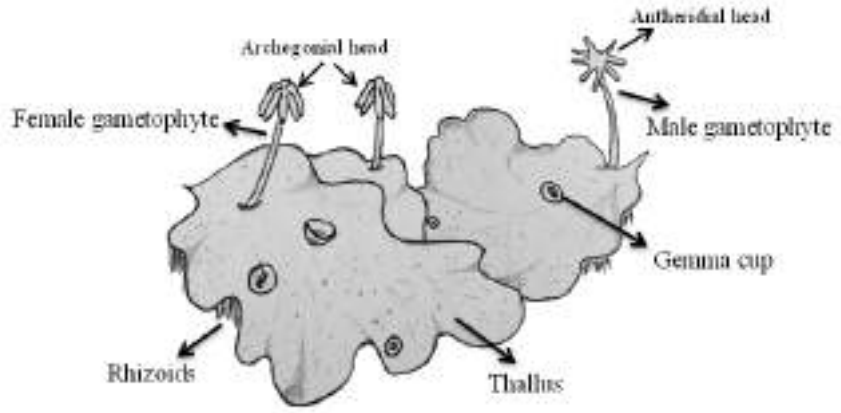
Per Group:	Per Room:
• dissection tools (razor, probe, watch glass) • dissecting microscopes • rulers	• display of representative plants of different plants groups • monocot and dicot plant display • single and composite flowers display • pine, bean/pea seeds for dissection • monocot/dicot/gymnosperm slides

B. Procedure

1. Comparison of Plant Groups:

- Observe the specimens on display for nonvascular plants, seedless vascular plants, gymnosperms and angiosperms and answer the questions that follow.
- You will need to refer to the background information section to answer some of the questions.

a. Nonvascular Plants:
The diagram below shows an example of a nonvascular plant called **liverwort**. Circle the structures that you observe and recognize in the living specimen on display (Drawn by Dr. Palavecino).



What structures are produced in the Archegonial head of a female gametophyte? _____

What structures are produced in the Antheridial head of a male gametophyte? _____

The plant observed represents the haploid or diploid generation? _____

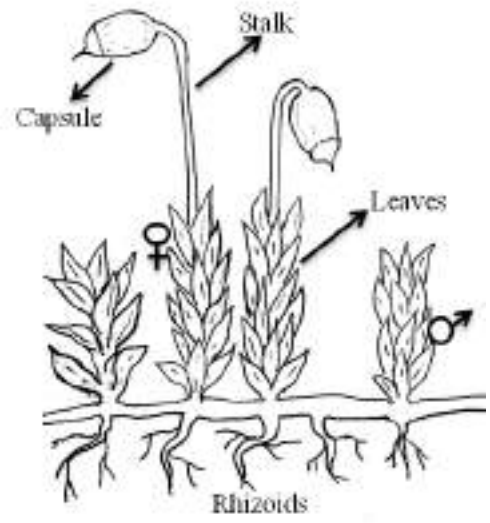
On what structure does the sporophyte generation develop? (Female or male gametophyte)

_____

What is the function of the Gemmae cups? _____

Carefully, using the ruler provided, estimate the size of an individual thallus and a (male or female) gametophyte if present.

The diagram below shows an example of another nonvascular plant called **moss**. Circle the structures that you observe and recognize in the living specimen on display (Drawn by Dr. Palavecino).



Is the green leafy structure of the moss the gametophyte or sporophyte? _____

Where the spores are produce? _____. Are they haploid or diploid? _____

Carefully, using the ruler provided, estimate the size of the gametophyte and the sporophyte (if it is present.) _____

In mosses and liverworts, how does the sperm reach the egg? _____

Do mosses and liverworts contain vascular tissue (xylem and phloem)? _____

VASCULAR TISSUES

The **xylem** and **phloem** that make the vascular tissues are present in all the subsequent plants you will study.

Observe a sagittal section of a stem of a gymnosperm and an angiosperm. Recognize the xylem and phloem cells. Use the information provided in the resource binder to answer the following question:

List the features that you were able to identify as part of the xylem and phloem.

Observe a cross section of a stem of a dicot and a monocot plant. Identify the vascular bundles that contains the xylem and phloem. Draw the arrangements of these vascular bundles for each plant (Use the 4X objective lens). Label the structures observed.

Monocot **Dicot**

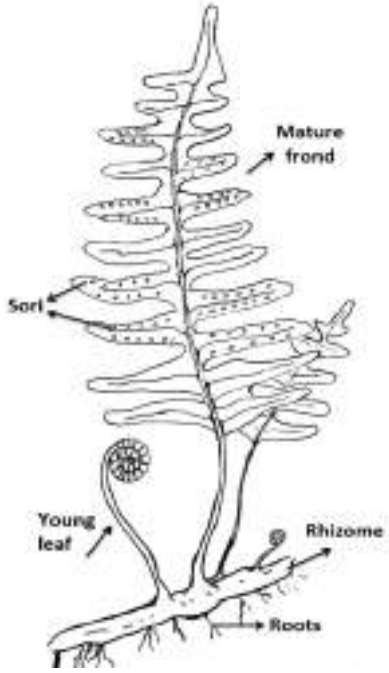
What does the xylem transport? _____ *is the xylem a living tissue?* _____

What does the phloem transport? _____ *is the phloem a living tissue?* _____

What tissue make up the wood? _____ *What tissue makes up the bark?* _____

b. Seedless Plants:

The diagram below shows the diagram of a **fern**. Circle the structures that you observe and recognize in the living specimen on display. Observe the life cycle of a fern to answer the questions below (Drawn by Dr. Palavecino).



Is the plant that you observe the gametophyte or sporophyte? _____

*What is the function of **sori**?* _____

In ferns, where are the eggs and sperm are produced? (Check the fern life cycle on display)

_____

How does the sperm reach the egg? _____

Carefully, using the ruler provided, estimate the size (high) of the plant on display _____

Observe the **horsetail** that is on display, which is another example of a seedless plant. Check the horsetail life cycle provided and answer the following questions:

Is the plant that you observe the gametophyte or sporophyte? _____

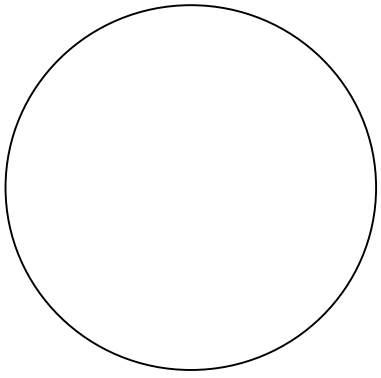
Observe the small dark structures along the stem. How are horsetail leaves different from fern leaves?

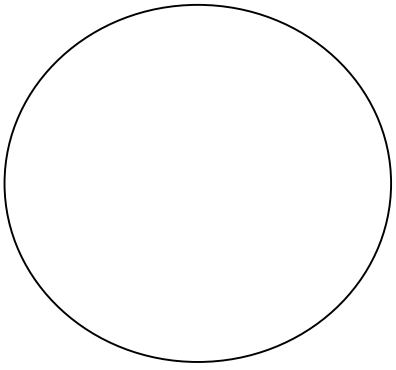
In which part of the horsetail plant does the majority of photosynthesis occur?

*(Hint: which part of the plant is green?)*_____

SEEDS

Gymnosperms were the first group of plants to produce seeds, an important novelty trait in the evolution of plants that help them to become successful and a dominant group by the time of the dinosaurs.
In the space provided below, draw a *pine seed, and an angiosperm seed* that are on display. Label the following structures: ***embryo***, ***food***, *and the* ***seed coat***.





Total magnification_____

c. Gymnosperms

Observe the gymnosperms on display and read the information provided to answer the following questions:

What are the common names of the gymnosperm specimens on display? _____

Are the plants you observe the gametophyte or sporophyte? _____

Are they bearing cones? _____ *If so, where are they located?*_____

What does the male cone produce? _____

What does the female cone produce? _____

How does the sperm (inside the pollen grain) reach the egg? _____

Describe the shape of pine leaves. What is the benefit of this leaf shape?

In the space provided, draw and label the **male and female cones** on display.

FLOWERS

Flowers, a site for reproduction, were an important evolutionary innovation in the evolution of plants and made angiosperms the most successful and diverse group in the world. Flowers having all four floral parts: *sepals, petals, stamens, and carpels* as it is shown in Fig. 10.6 (background information) are said to be *complete*. If one or more of the floral parts are absent, the flower is said to be *incomplete*.

Some flowers are called *composite* flower because they are a cluster of smaller flowers that give the appearance of a single flower. The function of the composite flower is the same as a

single flower – to attract pollinators – but the larger floral display that often results from a composite flower, and the fact that many flowers that can be pollinated by a single visiting animal suggests that this kind of flower may provide a reproductive advantage to the plant.

d. Angiosperms

Observe the **flowering plants** that are on display, choose 4 different plants and complete the chart below (You may need to refer to the background information).

Flower name	Complete/ Incomplete	Single/ Composite	# of sepals and color	# of petals and color	Do the flowers smell?	Type of pollinators attracted

Complete the following questions about Angiosperms.

Are the plants that you observe the gametophyte or sporophyte? _____

In a flower:
i) The male reproductive structure is called: _____

ii) The female reproductive structure is called: _____

iii) The function of the stigma is: _____

v) The function of the petals is: _____

What are the structures produced inside the anther (stamen) and the ovary (carpel)?

_____*&* _____*. Are they the gametophyte or sporophyte?* _____

In what part of the flower do the seeds develop? _____

What part of the flower develops into a fruit? _____

How are seeds dispersed in angiosperms? _____

What is the reproductive advantage of a plant with a composite flower compared to a single flower?

2. Monocot and Dicot Plants:

- Observe the monocot and dicot plant specimens and the additional information on display to complete the chart and the questions below. You may need to refer to the background information for guidance.

Angiosperms	**Seed**	**Root**	**Vascular bundles**	**Veins in leaf**	**Petal #**
Monocot					
Dicot					

- For each of the numbered plants on display, identify whether it is a monocot or a dicot.

1. _____ 3. _____

2. _____ 4. _____

3. Plant Morphology

Use the picture below to label the following structures: **leaf (blade, veins, and petiole), stem, terminal bud, axillary bud, nodes and internodes**. You may need to refer to Fig. 10.7 in the background information section for guidance.[4]

[4] Wikimedia Commons: http://commons.wikimedia.org

VI. Post-Lab Questions

1. Explain why nonvascular plants are much smaller than vascular plants.

2. List two adaptations that gymnosperms have for surviving and reproducing in drier climates.

3. List two evolutionary innovations that are unique to angiosperms and absent in other plant groups.

4. For each of the following, identify which part of the plant it represents (e.g. leaf or stem) and explain the function of this modification.

a) potato tuber: _____ function: _____

b) Venus flytrap: _____ function: _____

c) cactus spine: _____ function: _____

d) onion bulb: _____ function: _____

e) pea tendril: _____ function: _____

5. Many of the “vegetables” that we eat are actually fruit. A vegetable is an edible plant, or part of a plant, such as the leaf, stem or root. The word "fruit" has a precise botanical meaning: a structure that develops from the ovary of a flowering plant and contains seeds.

For each of the following, identify whether it is a fruit or a vegetable according to the botanical definition.

a) celery: _____

b) tomato: _____

c) eggplant: _____

d) carrot: _____

e) bell pepper: _____

f) cucumber: _____

g) lettuce: _____

h) radish: _____

Lab 11: Animal Diversity

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the general characteristics that unite all animals.
2. Identify main characteristics that are used to classify animal groups.
3. Describe the main characteristics of each of the nine animal phyla discussed.
4. Identify examples of microscopic animals and their characteristics.
5. Identify the correct phylum name of different animals based on their main characteristics.

II. Background Information:

Animals are eukaryotic, multicellular, heterotrophic organisms (with internal digestion), mobile at least during some part of their life cycle, and most have muscle and nerve cells.

While we can quickly identify dogs, lizards, fish, spiders, and worms as animals, other animals, such as corals and sponges, might be easily mistaken as plants or some other form of life. Moreover, some animals are so small that we need a microscope to be able to observe them; for instance, the house dust mite shown in figure 11.1. These animals can measure between 200 to 300 micrometers in length and are likely an inhabitant in your house.

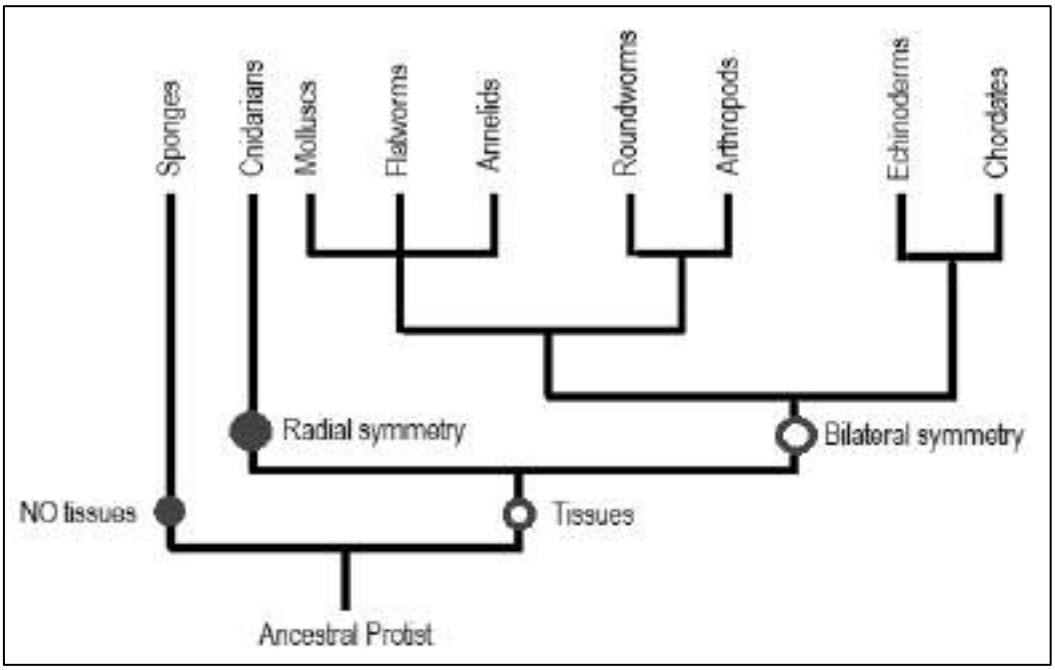
Copepods, rotifers, and tardigrades (water bears) are some other examples of microscopic animals.

Figure 11.1: **House dust mite** (*Dermatophagoides* sp.) Drawn by Dr. Palavecino (Not to scale)

Figure 11.2: **Parasitic mite**. Photo Dr. Palavecino Light microscope, 100X

1. Overview of animal relationships

The figure below shows current hypothesis on the evolutionary relationship among the nine animal phyla represented taking into account body plan, and other characteristics (Re-drawn from Campbell Essential Biology Ed. 6 by Dr. Palavecino).



a. Presence/Absence of Tissues

Tissues are groups of similar cells that together carry out a specific function in the body (e.g. muscle tissue, nerve tissue, etc.) All animals except for sponges (phylum Porifera) produce two or three primary tissue layers (sometimes called primary germ layers). Animals with radial symmetry, like cnidarians, produce two germ layers (the outer **ectoderm** and the inner **endoderm**) making them **diploblastic**. Animals with bilateral symmetry produce a third layer between these two layers (the **mesoderm**) making them **triploblastic**. Germ layers eventually give rise to all of an animal's tissues and organs.

b. Body Symmetry[1]

Animals may be asymmetrical, radial, or bilateral in form (Fig. 11.3). **Asymmetrical** animals have no pattern or symmetry; an example of an asymmetrical animal is a sponge. An organism with **radial symmetry** has a longitudinal (up-and-down) orientation. Any plane cut along this up–down axis produces roughly mirror-image halves. An example of an organism with radial symmetry is a sea anemone. In animals with **bilateral symmetry** a vertical plane cut from front to back separates the animal into roughly mirror-image right and left sides. Animals with bilateral symmetry also have a "head" and "tail" (anterior versus posterior) and a back and underside (dorsal versus ventral).

Organisms such as sea stars and sea urchins (phylum Echinodermata) exhibit radial symmetry as adults, but because they are bilaterally symmetrical during the larval stage, they are classified along with other bilaterally symmetrical animal phyla.

[1] OpenStax College: http://cnx.org/

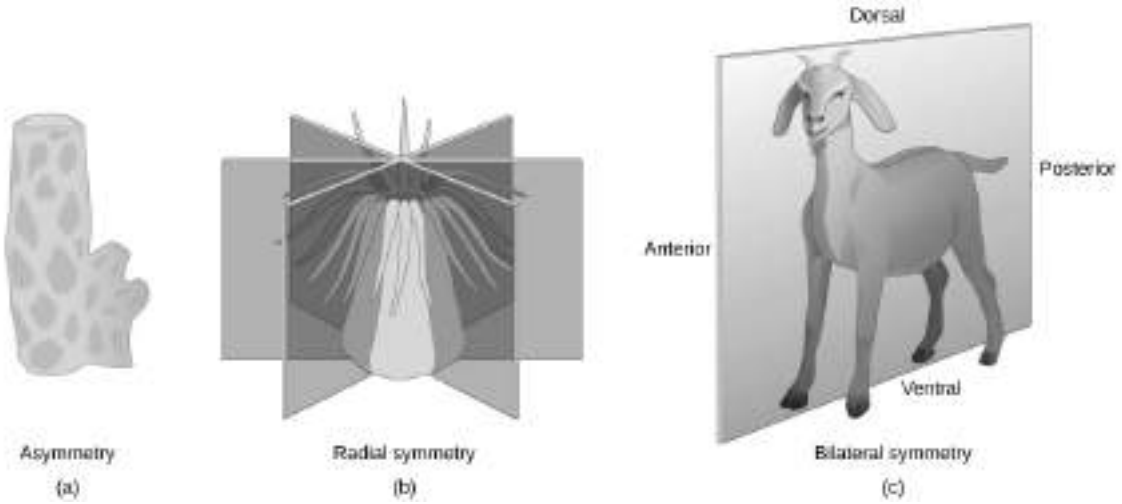


Fig.11.3. Diagram of different types of body symmetry in a (a) sponge, (b) sea anemone and (c) goat[2]

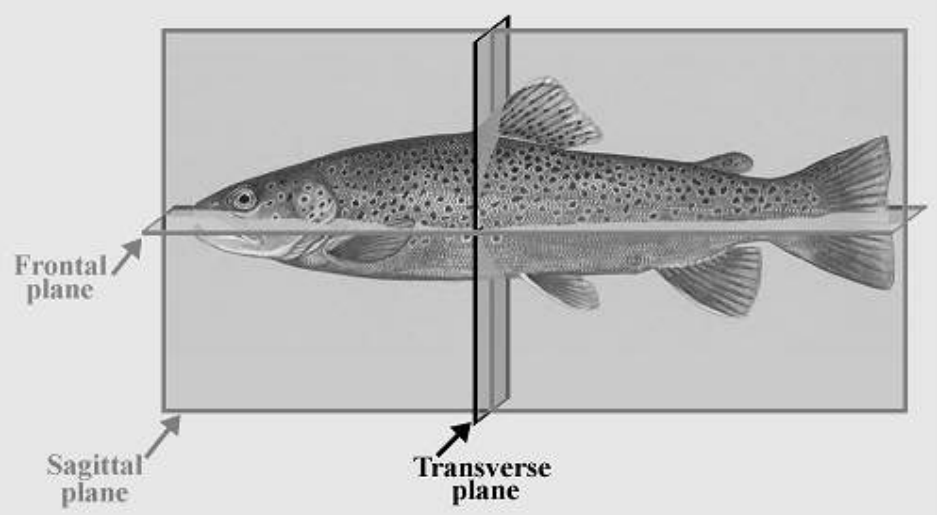


Fig.11.4. Diagram of major anatomical planes of section[3]

2. Overview of Animal Phyla

The following information is a brief overview of the major animal phyla organized following the main distinctions that divide them. You will need to refer to your textbook for more details about each animal phylum.

[2] OpenStax College: http://cnx.org/

[3] Wikimedia Commons: https://commons.wikimedia.org

a. Sponges (Phylum Porifera):
This phylum includes animals known as sponges. They have bodies full of pores and channels that allow water to circulate through them. Sponges lack nervous, digestive or circulatory systems. Instead, most rely on maintaining a constant water flow through their bodies to obtain food, oxygen and remove waste. Water is pumped through the hollow interior of a sponge by the action of many individual cells called collar cells. Most sponges have no body symmetry (asymmetrical), although some have radial symmetry.

b. Cnidarians (Phylum Cnidaria):
This phylum includes corals, sea anemones, jellyfish, and hydras. Most live in salt water with a smaller number living in freshwater. Cnidarians have radial symmetry and develop true tissues. They form two layers of tissues; an external one called **ectoderm**, and an internal one called **endoderm** (also called ***gastrodermis***). There are two body types of Cnidaria: a sessile **polyp** and a free-floating **medusa**. They have a gut with only a single opening (no separate mouth and anus) and a nerve net with no centralized control. Like sponges, Cnidarians capture food from the water as it passes near them. Unlike sponges, cnidarians capture larger prey items by injecting them with toxins. Their stinging cells or *cnidocytes* produce those toxins. In a few species, these toxins can be extremely painful, even fatal, to human beings.

c. Flatworms (Phylum Platyhelminthes):
This phylum includes worms called "flatworms" such as planarians, tapeworms and flukes. Flatworms may be free-living or parasitic, although most species are parasites, particularly of vertebrates. Free-living species are found in both saltwater and freshwater although some can be found in very moist habitats on land. Some small free-living flatworms have cephalization, with a head bearing chemoreceptor organs, two simple eyes and a tiny brain. The digestive tract consists of a single opening into a blind sac. This opening serves as both the "mouth" and the "anus."

d. Segmented worms (Phylum Annelida):
This phylum includes segmented worms such as earthworms, leeches, and polychaetes. Segmentation allows these animals to move different parts of their body independently of one another, giving them much better control of their movement. They develop three layers of tissues, like most of the other phyla: the **ectoderm**, the **endoderm**, and the **mesoderm** in between. They have a complete digestive system (with separate openings for the mouth and anus), and an independent nerve center (called a ganglion) per segment, with a nerve cord that connects and coordinates the function of the ganglia. Leeches, earthworms, and their relatives live in freshwater and on land in moist areas. Polychaeta (meaning "many hairs") live in marine environments.

e. Mollusks (Phylum Mollusca):
This phylum includes clams, snails, squid and their relatives. Mollusks are a very diverse group, both with respect to the numbers of species and the environments they occupy. Mollusks have a muscular foot that in octopi and squid has been modified to form the arms and tentacles. Most of their organs are concentrated in a centralized visceral mass. The mantle is a fold of tissue that secretes the hard, calcareous shell that is typical in many

mollusks. Octopi, squid and their relatives have lost most or all of their shells and are much more mobile than other mollusks. Mollusks exhibit continuous growth; even the species that have shells will exhibit growth rings on the shell.

f. Roundworms (Phylum Nematoda):
This phylum includes worms called "roundworms". Nematodes and their relatives are abundant and diverse and many are microscopic. The largest known nematode, which reaches a length of 9 meters (29.5 ft.!) is a parasite in the placentas of sperm whales. Nematodes are slender animals generally with a body that is round in cross section without many external features and tapered to a point at both ends. A tough cuticle covers the body of a nematode; therefore these animals have to molt in order to grow in size. Nematodes may be parasites, predators or herbivores. Many species are serious plant pests while others play an important role as decomposers in soil.

g. Arthropods (Phylum Arthropoda):
This phylum includes insects, crustaceans, centipedes, millipedes, spiders, scorpions, and ticks. Arthropods are the most diverse group of animals with respect to number of species and they are found in almost every environment on earth. Approximately 75% of the known animal species are arthropods, mostly insects. Arthropod bodies are divided into specialized segments with a rigid exoskeleton composed largely of a substance called chitin. Like nematodes, arthropods have to molt in order to grow in size. Arthropods occupy an enormous variety of Earth's habitats. Most species of crustaceans are aquatic, whereas arachnids and insects are almost entirely terrestrial. Some arthropods transmit diseases while others play an important role in pollination.

h. Echinoderms (Phylum Echinodermata):
This phylum includes sea stars, sea urchins, sea cucumbers, sand dollars, brittle stars, and sea lilies. All members live in marine environments and can be predators, herbivores or filter feeders. Echinoderms have a bilaterally symmetrical larva but during their development they acquire penta-radial (5-point radial) symmetry. Most species move around slowly on numerous, small tube feet, while others are sessile. They have a unique hydraulic system called a water vascular system, which functions in locomotion, food and waste transportation and feeding. Echinoderms have a calcareous endoskeleton and many have the ability to regenerate lost limbs.

i. Chordates (Phylum Chordata):
This phylum includes the well-known vertebrates and a few other small marine organisms, such as lancelets and tunicates. Vertebrates (mammals, birds, fishes, reptiles, amphibians) are familiar to most people. All chordates have a notochord (stiff rod of cartilage that extends the length of the body and forms the spinal column in vertebrates), a dorsal nerve cord (which develops into the spinal cord), a tail that extends beyond the anus, pharyngeal slits (which function in feeding and respiration), and segmented muscles. Chordates occupy a wide variety of aquatic and terrestrial habitats. Chordates may be filter feeders, herbivores, predators, scavengers or parasites.

3. Diversity of Vertebrates

All vertebrates have endoskeletons, which are unique in having a skull and a backbone. The backbone is composed of a series of bones called vertebrae (singular, *vertebra*) that give the group its name.

The first vertebrates were aquatic, and we refer to them as **Fishes**. The term includes different groups that exist today; the jawless fishes: hagfishes and lampreys, and those that have jaws: Cartilaginous fishes and Bony fishes.

Amphibians which mean "living a double life" are another vertebrate group. It includes frogs, salamanders, newts, and a less known group called apoda. Amphibians were the first group of vertebrates to colonize land and most of their species are tied to water for reproduction.

Reptiles, which also include birds, are along with mammals, called amniotes. They have evolved an amniotic egg, a fluid-filled egg with a water-proof shell that encloses the developing embryo. This structure enables amniotes to complete their life cycle on land. Reptiles include animals such as snakes, crocodiles, lizards, and turtles. **Birds** have evolved from a group of dinosaurs. They lay eggs, have feathers, which are modified scales, and have many adaptations related to their ability to fly.

Mammals, the second group of amniotes, are mostly terrestrial. They have two unique characteristics: mammary glands that produce a secretion (milk) to feed the young and hair that helps to insulate the body and maintain a constant temperature. Like birds, mammals are endotherms.

III. Reading Assignments:
A. Required background reading
Campbell Essential Biology (6^{th} ed.): pg. 337-360 (Chapter 17: The evolution of Animals)

B. Optional background reading:
1. Animal Diversity Web – University of Michigan, Museum of Zoology
http://animaldiversity.ummz.umich.edu

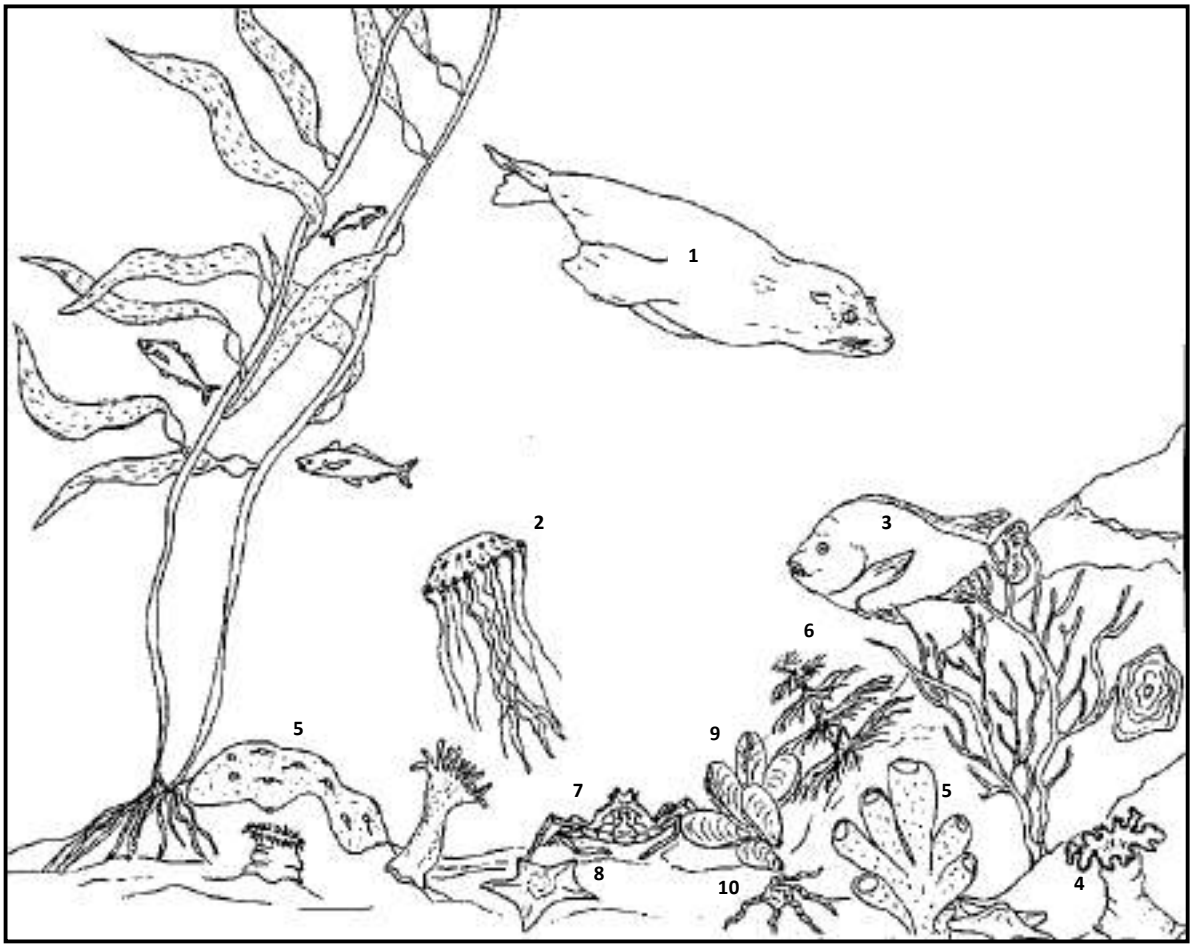
2. Shape of Life: The Story of the Animal Kingdom
http://shapeoflife.org

IV. Pre-Lab Questions

1. List the characteristics that unite all animals.

2. List the characteristics that are used to classify and divide the animal phyla.

3. Observe the diagram below that represents a **Marine Biome** (Drawing by Dr. Palavecino- not to scale)



4. Complete each row in the table below using the animals depicted in the picture. Name them and indicate what kind of food/prey you think the animal may feed on.

# of the Organism	Animal (common name)	Food/prey
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

V. Lab Exercise

A. Materials

Per Group:	Per Room:
• resource binder containing background information on animals • compound microscope	• display of animal phyla • dissecting microscopes • unknown animal specimens • slides of Porifera, Cnidaria , Annelida, & Tardigrada • crayfish exoskeleton and clam shell • live vinegar eels

B. Procedure

1. Presence or Absence of Tissues

Note: *You will need to place the specified slides under your compound microscope to complete this activity and find out the total magnification of the diagrams below.*

1. Observe a prepared slide of a cross (transverse) section of the calcareous sponge *Grantia*. Use the information provided in the lab to recognize and label the structures that can be identified in the diagrams below.

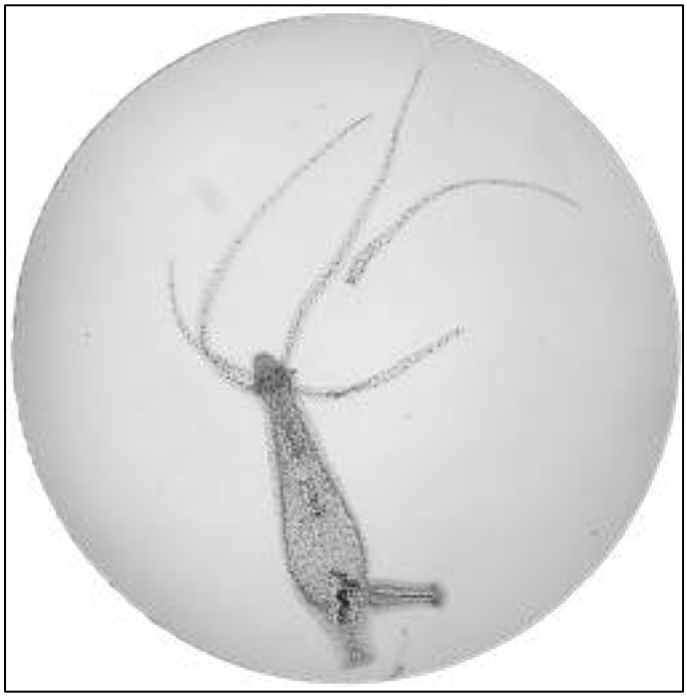
Total Magnification____

Tissues present or absent? Circle the correct option

Total Magnification _____

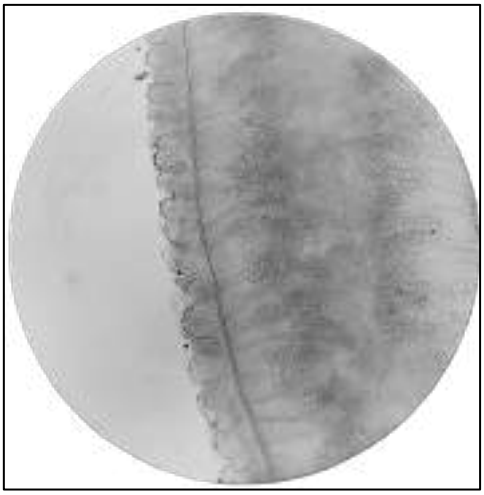
2. Observe a prepared slide of a sagittal (or longitudinal) section (L.S.) of the cnidarian *Hydra*. Use the information provided in the lab to recognize and label the structures that can be identified in the diagrams below.

Hydra (L.S.)



Total Magnification _____

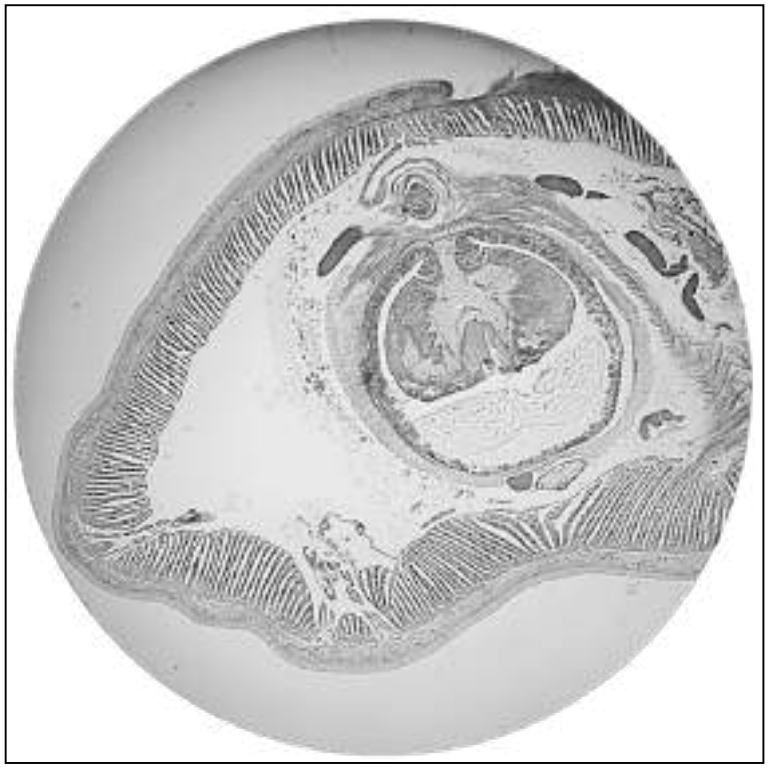
Hydra (detail of the wall in a closer view)



Total Magnification _____

Tissues: present or absent? # of tissues layers_____ Circle Body Type: polyp or medusa

3. Observe a prepared slide of a cross section (C.S.) of the annelid earthworm. Use the information provided in the lab to recognize and label the structures that can be identified in the diagram below. Underline all the structures that belong to the **mesoderm**.



Total magnification_____ Tissues: present or absent? # of tissues layers_____

2. Body Symmetry:
Observe the animals displayed around the room and find representative animals for each type of body symmetry. Be sure to include the name of the animal and the phylum it belongs to.

Body Symmetry	Animal names	Phylum/Phyla
Asymmetry		
Radial symmetry		
Bilateral symmetry		

3. Overview of Animal Phyla

Fill in the required information in the following table. You will need to observe the displays around the room, refer to the background information for this lab activity, and the resource binder at your table to help you complete this activity.

Phylum name	Examples of organisms	Mode of life (terrestrial, aquatic, parasitic)	Distinguishing characteristics of the Phylum (list at least two)
Porifera			
Cnidaria			
Platyhelminthes			
Mollusca			
Annelida			
Nematoda			
Arthropoda			
Echinodermata			
Chordata			

4. Microscopic Animals

Visit the display for Microscopic Animals. Read the information provided and observe the animal under the microscope.

1. *What Phylum does the organism belong to?* _____
2. *Estimate the size of the observed organism* (follow the instructions next to the scope).
3. *Indicate two characteristics or features you find interesting about them*

6. Identifying Animal Phyla

Using the information available in the room and your lab manual, identify the **Phylum** name for each of the numbered animal specimens at your table and record your answers in the space provided. Check your answers with your instructor.

Specimen #	Phylum Name	Specimen #	Phylum Name
1		8	
2		9	
3		10	
4		11	
5		12	
6		13	
7		14	

1. *Indicate which specimens (if any) you identified incorrectly and the reason why it happened.*

2. *Mention two key things that helped you to identify the phyla of two of the organisms above. Were there any animals or characteristics that were hard to identify? If so, what were they?*

VI. Post-Lab Questions

1. Complete the following summary table.

Phylum name	Body Symmetry	Tissues present or absent? (if present, name what they are)
Porifera		
Cnidaria		
Platyhelminthes		
Mollusca		
Annelida		
Nematoda		
Arthropoda		
Echinodermata		
Chordata		

2. Use the hints given below to identify the phylum name and 2 sample organisms for each of them. You may need to refer to the background information and your notes.

Hints	Phylum	Sample organisms
Exoskeleton, body divided into segments (head, thorax, abdomen)		
Aquatic, sessile, body has pores and channels to filter feed, no tissues		
Dorsal nerve cord, notochord, pharyngeal slits		
Pentaradial symmetry, water vascular system		
Segmented, worm-like body, complete digestive system		
Radial symmetry, two tissue layers, stinging cells		
Flat, worm-like body, digestive tract with single opening		
Mantle, muscular foot, visceral mass, hard external shell in most members		
Round, worm-like body, no segmentation, tapered at both ends		

3. Use the diagram of the **Marine Biome** from the Pre-Lab section to identify the phylum name of each numbered organism depicted in the figure. You may need to refer to the background information and your notes.

# of the organism	Animal (common name)	Phylum Name
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Lab 12: Ecology

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. List the requirements for evolution to occur through natural selection.
2. Describe the effect that different selective pressures can have on a population.
3. Describe the characteristic climate patterns and plant and animal species of the different terrestrial biomes.

II. Background Information:

1. Evolution through Natural Selection

Natural selection is one of the cornerstones of modern biology. Charles Darwin introduced the term in his influential 1859 book "*On the Origin of Species*". In order for a trait to evolve by natural selection, three conditions must be met: a) variation in the trait, b) the trait affects the survival and/or reproduction of the individual and c) the trait is heritable.

Natural selection can act on any variable phenotypic trait, however, only traits that are heritable will evolve through natural selection. Variation in such heritable traits occurs among the individuals of any population of organisms. Many of these differences do not affect survival (such as differences in eye color in humans), but some differences may improve the chances of survival of a particular individual. A rabbit that runs faster than others may be more likely to escape from predators, and algae that are more efficient at extracting energy from sunlight will grow faster. Something that increases an animal's survival will often also include its reproductive rate. Ultimately, what matters is total lifetime reproduction of the animal. Thus **fitness**, in an evolutionary context, refers to the lifetime reproductive success of an individual.

2. Ecosystem Ecology

Ecology is the scientific study of the relationships that living organisms have with each other and with their natural environment. An **ecosystem** is a community of living organisms (plants, animals and microbes) in conjunction with the nonliving components of their environment (things like air, water and mineral soil), interacting as a system through nutrient cycles and energy flows. Some parts of the earth have similar biotic and abiotic factors spread over a large area, creating a typical ecosystem over that area. Such major ecosystems are termed **biomes** and are characterized by the average temperature and amount of precipitation, as well as by the degree of seasonality.

Biomes are classified broadly as **terrestrial** and **aquatic** (which includes the freshwater and marine biomes). Freshwater biomes include: lakes and ponds, rivers and streams, and wetlands. Marine biomes include: coral reefs, the intertidal zone, estuaries, the continental shelf, the benthic realm and the pelagic realm (open water). Terrestrial biomes include: tropical forest, desert, savanna, temperate grassland, temperate deciduous (broadleaf) forest, chaparral, coniferous forest, tundra and polar ice. Each biome contains characteristic species,

and the species composition is dictated by the physical and biological constraints of those biomes. The species that live within a particular biome are subject to natural selection due to physical constraints (i.e. climate) and biological interactions (i.e. predator-prey interactions) and evolve adaptations as a result. In this lab, we will explore some of these ideas further with experimentation and comparative observation.

3. Terrestrial Biomes[1,2]

Most maps illustrate the different biomes as having sharp boundaries when in fact they do not. The map below provides a general outline of 6 of the world's major biomes (Fig. 12.1).

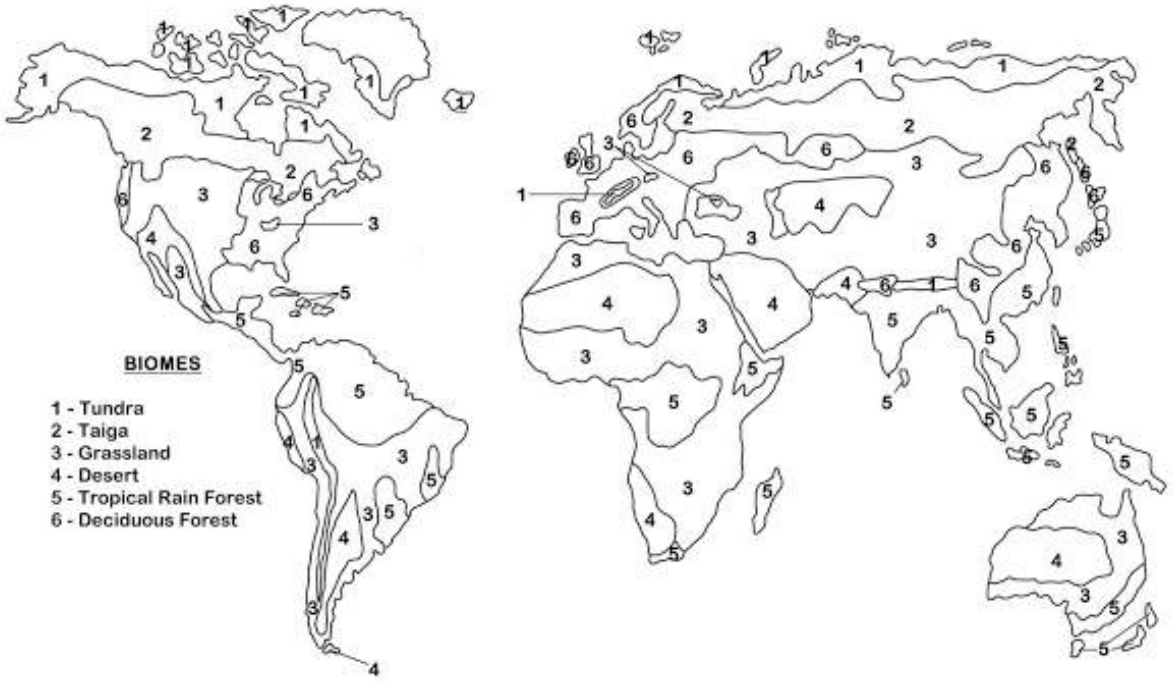


Fig. 12.1. Map of the world's major biomes.[3]

The general characteristics of some of the terrestrial biomes (climate, plants and animals) are listed below.

a. Tropical Forest

Tropical forests occur in equatorial areas. The tropical rainforest is a hot, moist biome where it rains all year long. Precipitation ranges from 200-400cm per year. Average monthly temperatures are above 18°C

The tropical rainforest is known for its dense canopies of vegetation that form different layers. The top layer or canopy contains giant trees that grow to heights of 75 m (about 250ft)

[1] Earth Observatory: http://earthobservatory.nasa.gov/Experiments/Biome/

[2] Radford University: https://php.radford.edu/~swoodwar/biomes/

[3] Worksheet Place: http://worksheetplace.blogspot.com

or more. This layer of vegetation prevents much of the sunlight from reaching the ground. Thick, woody vines are also found in the canopy. They climb trees in the canopy to reach for sunlight. The bottom layer or floor of the rainforest is covered with leaf litter, which decomposes rapidly, sending nutrients back into the soil. Few plants are found on the forest floor due to the lack of sunlight. However, the hot, moist atmosphere and all the dead plant material create the perfect conditions in which bacteria and other microorganisms can thrive.

Animal life is highly diverse. Common characteristics found among mammals, birds, reptiles and amphibians include adaptations to an arboreal life (for example, the prehensile tails of New World monkeys), bright colors and sharp patterns, loud vocalizations, and diets heavy on fruits.

b. Savanna (tropical grassland)

Savannas are warm year-round and have dry and wet seasons. Annual precipitation averages between 30-50 cm. During the dry season, for at least five months of the year, rainfall averages less than 1cm a month.

Savannas are characterized by a continuous cover of perennial grasses, which have underground stems or rhizomes that protect them during a fire. Savannas may also have an open canopy of drought-resistant, fire-resistant, or browse-resistant trees, or they may have an open shrub layer.

The world's greatest diversity (over 40 different species) of large herbivores is found on the savannas of Africa (e.g. impala, gazelle, zebra, rhino, wildebeest, giraffe, and elephant). These species coexist by dividing up the resources spatially and temporally; each having its own food preferences, grazing/browsing height, time of day or year to use a given area, etc. The large populations of herbivores support a diverse group of carnivores, including cats (lions, leopards, cheetahs), dogs (jackals, wild dogs), and hyenas.

Termites are especially abundant in the tropical savannas of the world, and their tall nests are conspicuous elements of the savanna landscape. These detrivores are important in soil-formation, their nests provide shelter for other animals, and they are a crucial food source for animals such as anteaters, aardvarks and pangolins.

c. Desert

Desert biomes are the driest of all the biomes. In fact, the most important characteristic of a desert is that it receives very little rainfall. Most deserts receive less than 30 cm a year. The temperature in the desert can change drastically from day to night because the air is so dry that heat escapes rapidly at night. The daytime temperature averages 38°C while in some deserts it can get down to -4°C at night. The temperature also varies greatly depending on the location of the desert.

Since desert conditions are so severe, the plants that live there need to have adaptations to compensate for the lack of water. Some plants, such as cacti, store water in their stems and use it very slowly, while others like bushes conserve water by growing few leaves or by

having large root systems to gather water. Some desert plant species have a short life cycle of a few weeks that lasts only during periods of rain.

Desert animals have many adaptations for surviving in the desert, such as small body size to prevent over-heating, concentration of urine to reduce water loss, light coloration to reflect sunlight, etc. Many of these desert animals are also nocturnal and live underground. Reptiles are well-adapted to the dry conditions of deserts and are diverse in this biome. Desert mammals include the kangaroo rat, desert shrew, camel, etc. Birds are often able to travel far to find water in deserts and are not as limited by the lack of water.

d. Chaparral

The chaparral biome, also known as the Mediterranean climate, occurs approximately between 30° and 40° latitude on the west coasts of continents, where there are cold ocean currents circulating offshore, for example along coastal California. The climate is characterized by hot, dry summers and mild, rainy winters. The chaparral area receives about 38–100 cm of precipitation a year.

Throughout the world, the Mediterranean biome is characterized by shrubs. In most regions these shrubs are evergreen and have small, leathery leaves with thick cuticles. Sometimes the leaves are so reduced as to appear needle-like. Many typical members of the shrub flora are aromatic (for example, sage, rosemary, thyme, and oregano) and contain highly flammable oils. The chaparral ecosystem as a whole is adapted to recover from periodic wildfires. Many chaparral plant species require some fire cue (heat, smoke, or chemical changes in the soil following fires) for germination.

Animals living in the chaparral do not require much water, have a smaller body size to prevent over-heating, and are usually nocturnal. Characteristic animals of the chaparral biome include mammals such as coyote, bobcat, mountain lion, jackrabbit and mule deer. Birds include hummingbirds, roadrunner, Western scrub jay, etc.

e. Temperate Grassland

Temperate grasslands include the prairies of North America, the steppes of Eurasia and the Pampas in South America. Temperate grasslands receive about 25 to 75 cm of rain per year. Warm to hot summers are experienced, depending on latitude. While temperatures are often extreme in some grasslands, the average temperatures are about -20°C to 30°C.

Temperate grasslands are composed of a rich mix of grasses and herbaceous plants and contain some of the world's most fertile soils. Perennial grasses, with their growth buds at or just below the surface, are well-adapted to drought, fire, and cold. The narrow upright stem reduces heat-gain in the hot summers; the intricate root systems trap moisture and nutrients.

The temperate grassland fauna is relatively low in diversity, especially compared to the savannas of Africa. In North America the dominant herbivores are bison and pronghorn. Rodent herbivores include the pocket gopher, ground squirrels, and the prairie dog.

Carnivores include coyote (actually an omnivore), badger and the black-footed ferret. Birds include hawks, sparrows, quail, etc.

f. Temperate Deciduous (Broadleaf) Forest

Temperate deciduous forests are found between the polar regions and the tropics and are exposed to warm and cold air masses, which cause this biome to have four seasons. The temperature varies widely from season to season with cold winters and hot, wet summers. The average yearly temperature is about 10°C. Average annual precipitation is between 75-150 cm, spread fairly evenly throughout the year.

Many different kinds of trees, shrubs, and herbs grow in deciduous forests. Most of the trees are broadleaf trees such as oak, maple, beech, hickory and chestnut. The trees have adapted to the winter by going into a period of dormancy. They also have thick bark to protect them from the cold weather. Trees flower and grow during the spring and summer growing season. There are also several different kinds of plants like mountain laurel, azaleas and mosses that live on the shady forest floor where only small amounts of sunlight get through.

Characteristic animals living in this biome are birds that feed on nuts and acorns (e.g. woodpecker, chickadee and blue jay), omnivores (e.g. raccoon, opossum, skunk and black bear) and carnivores (e.g. timber wolf, mountain lion, bobcat and coyote). Many of the mammals show adaptations to an arboreal life and some hibernate during the winter months.

g. Coniferous Forest

Between the tundra to the north and the deciduous forest to the south lies the large area of coniferous forest, also known as boreal forest or taiga. Precipitation in coniferous forests varies from 30-90 cm annually, with some temperate coniferous forests receiving up to 200 cm. The amount of precipitation depends on the forest location. In the northern boreal forests, the winters are long, cold and dry, while the short summers are moderately warm and moist. In the lower latitudes, precipitation is more evenly distributed throughout the year.

Coniferous forests consist mostly of conifers, evergreen trees that have needle-like leaves, and cones instead of flowers. Being evergreen allows these trees to take advantage of the short growing season and the leaves have adaptations to reduce water loss during the winter. The conical shape of the trees prevents excessive snow from accumulating on the branches in the winter. Some of the more common conifers are spruces, pines, and firs.

Fur-bearing predators like the lynx and various members of the weasel family (e.g., wolverine, mink, ermine, and sable) are perhaps most characteristic of the coniferous forest. The mammalian herbivores on which they feed include the snowshoe hare, red squirrel, lemmings, and voles. Among birds, insect-eaters like the wood warblers are migratory and leave after the breeding season is over. Seed-eaters (e.g., finches and sparrows) and omnivores (e.g., ravens) tend to be year-round residents.

h. Tundra

Tundra is found in the regions just below the ice caps of the Arctic, extending across North America, to Europe, and Siberia in Asia. Tundra is also found at the tops of very high mountains elsewhere in the world. Tundra winters are long, dark, and cold, with average temperatures below 0°C for six to 10 months of the year. The temperatures are so cold that there is a layer of permanently frozen ground below the surface, called permafrost. This permafrost is a defining characteristic of the tundra biome. In the tundra summers, the top layer of soil thaws only a few inches down, providing a shallow growing surface for plant roots. Precipitation in the tundra is only 15-25 cm a year but it is usually wet because the low temperatures result in slow evaporation of water. Much of the arctic has rain and fog in the summers, and water gathers in bogs and ponds.

Vegetation in the tundra has adapted to the cold and the short growing season. Mosses, sedges, and lichens are common, while few trees grow in the tundra. The trees that do manage to grow stay close to the ground so they are insulated by snow during the cold winters.

The bird and mammal species that live in the tundra year-round (e.g ptarmigan, arctic hare, arctic fox), have adaptations such as thick, insulating cover of feathers or fur, plumage that turns white in winter and brown in summer, and accumulation of thick deposits of fat during the short growing season. Migratory species such as shorebirds and caribou adapt to the tundra by avoiding the most severe conditions of winter. Each year at the end of the short growing season they move southward but return to the tundra to breed.

III. Reading Assignments:

A. Required background reading

1. Campbell Essential Biology (7^{th} ed.): pg. 374-379 (Ecology) and pp. 380-390 (Biomes)

2. Making of the Fittest: Natural Selection and Adaptation (Howard Hughes Medical Institute)
http://www.hhmi.org/biointeractive/shortfilms/index.html#pocketmouse

B. Optional background reading:

1. UC Berkeley Museum of Paleontology: Biomes
http://www.ucmp.berkeley.edu/glossary/gloss5/biome/

2. Radford University – Biomes of the World
https://php.radford.edu/~swoodwar/biomes/

IV. Pre-Lab Questions

1. List the three requirements for a trait to evolve through natural selection.

2. Individual A lives for 8 years and has 4 surviving offspring. Individual B of the same species lives for 4 years and has 6 surviving offspring. Which of these individuals is more "fit" from an evolutionary perspective? Why?

3. Identify the biomes in each of the following categories:

a) Freshwater biomes:

b) Marine biomes:

c) Terrestrial biomes:

4. Color the biome map on pg. 152 (Fig. 12.1). Use a different color for each biome and mark the legend with the corresponding color. In the space below, describe any patterns you notice about the biomes with respect to their latitudinal range (near the tropics, poles, etc.) or their location (inland, coastal, etc.).

V. Lab Exercise

A. Materials

Per Group: • Resource binder with biome information • Cloth habitat (patterned and white) • Beads of different colors and shapes	 • Empty cup • Predator tools (spoon, fork, etc.)

B. Procedure

1. Making of the Fittest: Natural Selection and Adaptation
https://www.hhmi.org/biointeractive/making-fittest-natural-selection-and-adaptation

Watch the video describing the research of Dr. Michael Nachman and colleagues on rock pocket mouse populations and answer the questions below.

a. What is unique about the landscape of New Mexico's Valley of Fire?

b. What is the color of the rock pocket mice are found on the sand? _____

c. What is the color of the rock pocket mice are found on the lava rock? _____

d. What selective pressure do the rock pocket mice experience by their predators?

e. How have the mice changed as a result of this selective pressure?

f. How did dark fur color in pocket mice originate?

g. Explain the following statement: "Mutations are random but natural selection is not."

h. How are pocket mice living on dark lava rock in different regions similar to each other? How are they different?

2. Natural Selection Experiment

In this experiment, you will be testing the influence of predators and prey camouflage on the survival of organisms.

1. Form groups of 3 or 4 and assign 1-2 people to be the ORGANIZERS, one to be the COUNTER, and one to be the PREDATOR.

2. The organizer(s) and counter will set up the materials. It is important that the predator person does not watch. The organizer(s) and counter will spread the **patterned** cloth habitat flat on the table. They will then spread **30 beads of each color** randomly on the cloth "habitat." Each group should have bags of beads of three different colors. Record the bead colors in the 1st column in the table below.

3. The predator will choose a tool (spoon, fork, forceps, etc.) to "catch" the prey (beads). These implements represent various structures used by predators to capture prey. Record the tool used in the table below.

4. At this point, the predator should not have seen the habitat. The counter person will then give the start signal and the predator has **1 minute** to pick up as many of the beads of any color and place it in the cup provided. The predator must use **ONLY** the selected tool to catch the prey and collect the beads **one by one**. The counter will let the predator know when one minute has passed. If the predator manages to collect all the beads of all three colors on the cloth within 1 minute, reset the experiment and allow the predator only 30 seconds to collect the beads.

5. The organizer(s) and counter will count the number of beads of each color still remaining on the cloth ("survivors") and fill in the 2nd column in the table below. For each **surviving** individual, the counter will add one more of that color bead to the cloth. This will simulate the successful reproduction of the surviving individuals, thus passing on their traits to the next generation.

6. The same predator will repeat step 4, using the same tool as before. The organizer(s) and counter will count the number of survivors and record it in the 3rd column in the table below.

7. Fill in the table below and answer the questions.

Background:	**Patterned**	
Predator implement used:		
Prey (bead) color	**# surviving after 1st round**	**# surviving after 2nd round**
1.		
2.		
3.		

Draw a bar graph below that illustrates the results of your predation experiment. Include only the # of surviving beads after the 2nd round. Be sure to label the axes.

Identify the independent, dependent and standardized variables in this experiment.

What color of bead was captured the most? Which color was captured the least? What is a likely explanation for this result?

Did evolution by natural selection take place in your experiment? (Hint: Which of the requirements for evolution by natural selection were met or not met?)

8. Repeat steps 1-7 with a different predator tool. Also, the group members should switch roles to give someone else a chance to be the predator.

Background:	**Patterned**	
Predator implement used:		
Prey (bead) color	**# surviving after 1st round**	**# surviving after 2nd round**
1.		
2.		
3.		

How were the results similar or different with this new predator and tool compared to the previous experiment?

9. Repeat steps 1-7, but this time use the **white cloth**, the colored beads and the same predator tool used for the first or second experiment. The group members should switch roles again.

Background:	**White**	
Predator implement used:		
Prey (bead) color	**# surviving after 1st round**	**# surviving after 2nd round**
1.		
2.		
3.		

Draw a bar graph below that illustrates the results of your predation experiment. Include only the # of surviving beads after the 2nd round. Be sure to label the axes.

Identify the independent, dependent and standardized variables in this experiment.

How were the results similar or different with the white cloth compared to the patterned cloth?

10. Repeat steps 1-7, but this time with the white cloth and the **clear** beads. Use **45 beads** of each shape. The predator can choose a new tool or one that has been used before.

Background:	**White**	
Predator implement used:		
Prey (bead) shape	**# surviving after 1st round**	**# surviving after 2nd round**
Round		
Tripod		

Draw a bar graph below that illustrates the results of your predation experiment. Include only the # of surviving beads after the 2nd round. Be sure to label the axes.

Identify the independent, dependent and standardized variables in this experiment.

What shape of bead was captured the most? What is a likely explanation for this result?

Did evolution by natural selection take place in your experiment? (Hint: Which of the requirements for evolution by natural selection were met or not met?)

3. Biomes

1. You will need to refer to the background information section and the resource binder to complete this section.
2. Read the clues listed below and identify the **biome** that each plant or animal is best adapted to.
3. Describe the important adaptation(s) for each organism that helped you reach your conclusion.
4. Check your answers with your instructor and make corrections to your answers as needed.

Biome Clues:

1. American Bison
The American bison, also known as the American buffalo, once roamed the grasslands of North America in massive herds. Bison are herbivores, grazing on the grasses and sedges of the North American prairies. They are also migratory and have usual daily movements between foraging sites during the summer. A bison has a shaggy, long, dark-brown winter coat, and a lighter-weight, lighter-brown summer coat.

2. Sidewinder
This snake can often be found buried in the sand or in animal burrows. The sidewinder is primarily nocturnal to prevent overheating during the day. Its diet consists of small rodents such as the kangaroo rat and lizards, and it can meet all its water needs from the prey it eats. The coloration of the sidewinder is pale brown with small dark patches, which allows it to blend in with the sand that dominates this biome.

3. Poison Dart Frog
Poison dart frogs live in humid, tropical environments of Central and South America. Adult frogs lay their eggs in moist places such as on leaves, in plants, and among exposed roots. Once the eggs hatch, the adult carries the tadpoles on its back to a suitable location, such as the water that collects in bromeliads or other epiphytic plants that grow on the branches of tall trees. Most species of poison dart frogs are brightly colored to warn off potential predators.

4. Joshua Tree
The Joshua tree is native to southwestern North America and is well adapted to very dry environments. It has a deep and extensive root system that allows it to collect water very efficiently. The dark green leaves are thin, which reduces water loss from the plant. New plants can grow from seed, but in some populations, new stems grow from underground stems that spread out around the parent tree. Joshua trees don't bloom every year because their blooming is dependent on rainfall at the proper time, which is often scarce.

5. Lion
In Africa, lions can be found in grasslands with scattered Acacia trees, which serve as shade. Lions spend much of their time resting and are inactive for about 20 hours per day. Although lions can be active at any time, most of their activity, including socializing, grooming and hunting, takes place at night. Their prey consists mainly of medium-sized herbivorous mammals, such as wildebeest and zebra.

6. Chamise
Chamise is an evergreen shrub that grows in dense stands that cover the dry hills of coastal California. The leaves are small and pointed, and are shiny with flammable oils, especially in warmer weather. It is very drought tolerant and adaptable, with the ability to grow in nutrient-poor soil and on dry, rocky outcrops. It is extremely fire-tolerant; quickly re-sprouting after a fire has passed through the area.

Organism	**Adaptations it possesses**	**Biome it is found in**
1. American Bison		
2. Sidewinder		
3. Poison Dart Frog		
4. Joshua Tree		
5. Lion		
6. Chamise		

The Origin of Species: Lizards in an Evolutionary Tree
https://www.hhmi.org/biointeractive/the-origin-of-species-lizards-in-an-evolutionary-tree

Watch the video describing the research of Dr. Jonathan Losos on anole lizard species in the islands of the Caribbean and answer the questions below. You may be asked to share your answers with your classmates.

What are the characteristics of the different species and body types of lizards?

How do the traits of the different species provide an adaptation to their different environment?

What does the evidence suggest regarding the evolution of body types of anole lizards on the different islands?

VI. Post-Lab Questions

1. Complete the following table for terrestrial biomes.

Climate (temperature & rainfall):	Plant Adaptations:	Animal Adaptations:
Tropical Forest		
Savanna		
Desert		
Chaparral		
Temperate Grassland		

Climate (temperature & rainfall):	Plant Adaptations:	Animal Adaptations:
Temperate Deciduous Forest		
Coniferous Forest		
Tundra		

Lab 13: Senses

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Describe the different senses humans have.
2. Compare human senses with those of other animals.
3. Describe the lab activities that test different senses.
4. Analyze the information gathered from the different senses.
5. Determine which senses are most useful for gathering information in different environments.

II. Background Information:

1. Senses in Humans

A sensory system consists of sensory receptors, neural pathways, and parts of the brain involved in sensory perception. The human sense organs (eyes, ears, tongue, skin and nose) contain receptors that relay information through sensory neurons to the appropriate places within the nervous system (Fig.13.1).

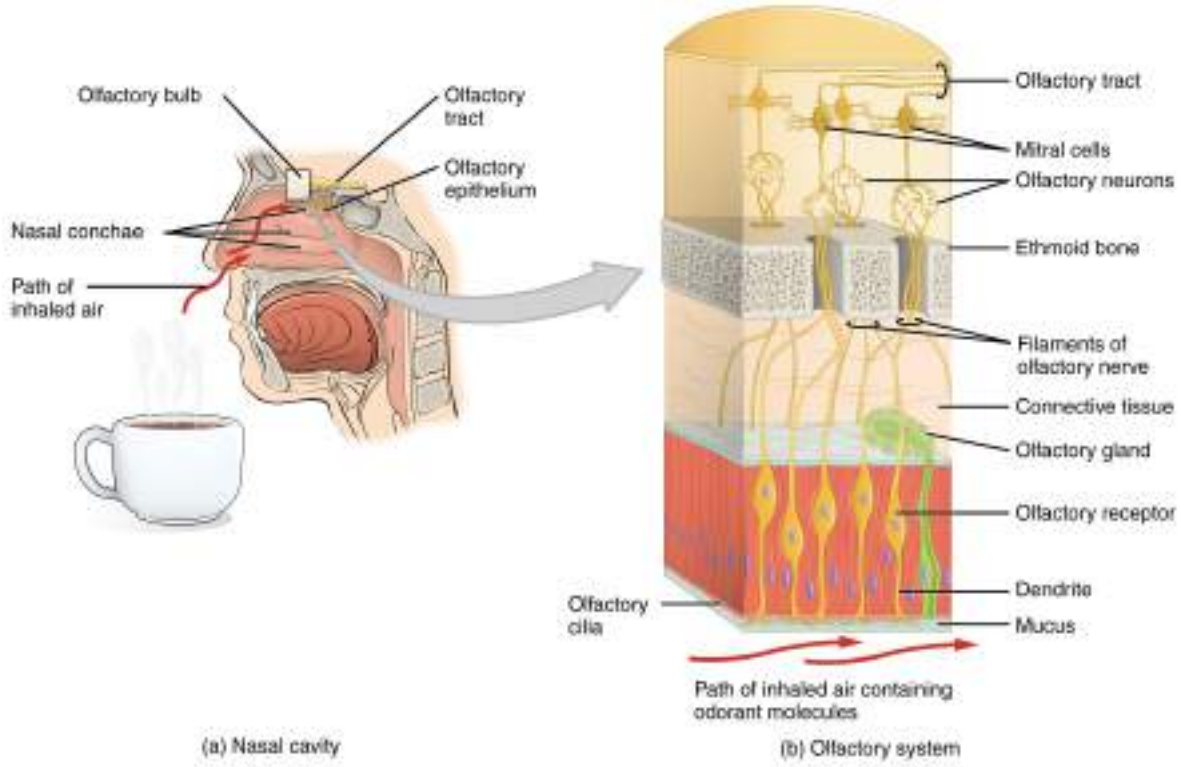


Fig. 13.1. The human olfactory system relays messages from the nasal cavity to the brain.[1]

[1] OpenStax College: http://cnx.org/contents/s3XqfSLV@6/Sensory-Perception

Human beings have a multitude of senses. While sight, hearing, taste, smell and touch are the five traditionally recognized, scientists recognize that humans have additional senses to detect temperature, thirst, hunger, time, and balance, just to name a few.

Sight or **vision** is the capability of the eyes to focus and detect images of visible light on photoreceptors in the retina of each eye. There are two types of photoreceptors: rods and cones. **Rods** are very sensitive to low light, but do not distinguish colors. **Cones** distinguish colors, but are less sensitive to dim light. The eye takes approximately 20–30 minutes to fully adapt from bright sunlight to complete darkness and become ten thousand to one million times more sensitive than at full daylight. In this process, the eye's perception of color changes as well. Conversely, it takes approximately five minutes for the eye to adapt to bright sunlight from darkness.

Hearing is the sense of sound perception and is all about vibration. Mechanoreceptors turn motion into electrical nerve pulses, which are located in the inner ear. Hearing is a mechanical sense because sound vibrations are mechanically conducted from the eardrum to hair-like fibers in the inner ear. Humans can detect a range of about 20 to 20,000 Hertz.

Taste refers to the ability to detect the taste of substances such as food, certain minerals, and poisons, etc. The sense of taste is often confused with the "sense" of flavor, which is a combination of taste and smell perception. Flavor depends on odor, texture, and temperature as well as on taste. Humans perceive taste through sensory organs called taste buds concentrated on the upper surface of the tongue. The sensation of taste can be categorized by five primary taste qualities: sweet, bitter, sour, salty and umami (savory).

Smell or **olfaction** is the other "chemical" sense. Unlike taste, there are hundreds of olfactory receptors, each binding to a particular molecular feature. Odor molecules possess a variety of features and, thus, excite specific receptors more or less strongly. This combination of excitatory signals from different receptors makes up what we perceive as the molecule's smell.

Touch or **mechanoreception**, is a perception resulting from activation of neural receptors, generally in the skin including hair follicles, but also in the tongue and throat.

2. Senses in Other Animals

Other living organisms have receptors to sense the world around them, including many of the senses listed above for humans. However, the mechanisms and capabilities vary widely.

Vision:

Cats have the ability to see in low light due to muscles surrounding their irises to contract and expand pupils as well as having a reflective membrane that optimizes the image. Pit vipers, pythons and some boas have organs that allow them to detect infrared light, such that these snakes are able to sense the body heat of their prey. It has been found that birds and some insects have the ability to see in the ultraviolet range.

Hearing:
Different animals can detect different frequency ranges than humans. Sounds that are lower than human hearing (below 20 Hz) are called infrasound and sounds that are higher than human hearing (above 20,000 Hz) are called ultrasound. The frequency ranges that some animals can hear are listed below.

Animal	**Frequency (Hz)**
Human	20 - 20,000
Elephant	5 - 12,000
Dog	50 - 45,000
Cat	45 - 65,000
Mouse	1,000 - 100,000
Bat	2,000 - 120,000
Dolphin	75 - 150,000

Smell:
Most non-human mammals have a much keener sense of smell than humans, although the mechanism is similar. Sharks combine their keen sense of smell with timing to determine the direction of a smell. They follow the nostril that first detected the smell. Insects have olfactory receptors on their antennae.

Vomeronasal organ:
Many animals (salamanders, reptiles, mammals) have a vomeronasal organ that is connected with the mouth cavity. In mammals it is mainly used to detect pheromones to mark their territory, trails, and sexual state. Reptiles like snakes and monitor lizards make extensive use of it as a smelling organ by transferring scent molecules to the vomeronasal organ with the tips of the forked tongue.

Taste:
Flies and butterflies have taste organs on their feet, allowing them to taste anything they land on. Catfish have taste organs across their entire bodies, and can taste anything they touch, including chemicals in the water.

Echolocation is the ability to determine orientation to other objects through interpretation of reflected sound (like sonar). Certain animals, including bats and cetaceans, have this ability and they use it most often this to navigate through poor lighting conditions or to identify and track prey.

Electroreception is the ability to detect electric fields. Several species of fish, sharks, and rays have the capacity to sense changes in electric fields in their immediate vicinity.

Magnetoreception is the ability to detect the direction one is facing based on the Earth's magnetic field. Directional awareness is most commonly observed in birds. It has also been observed in insects such as bees.

III. Reading Assignments:

Recommended background reading (optional):

1. PTC: Genes and Bitter Taste
http://learn.genetics.utah.edu/content/basics/ptc/

2. Crittervision: The world as animals see (and sniff) it
http://www.newscientist.com/special/crittervision

3. Discover Magazine: 20 Things You Didn't Know About Animal Senses
http://discovermagazine.com/2014/may/26-20-things-animal-senses

4. 10 Unusual Animal Senses
http://listverse.com/2013/04/13/10-unusual-animal-senses/

5. Hearing Test
https://www.youtube.com/watch?v=H-iCZElJ8m0

6. Vision Test
https://enchroma.com/pages/test

IV. Pre-Lab Questions

1. List the five basic, as well as the additional senses that humans possess.

2. List three senses that other animals have and that humans lack. How does each of these senses help these animals find mates, catch prey or avoid predators?

3. a) What is the range of sound frequencies that humans can detect? _____

b) How does this range compare to that in dogs? _____

c) A dog whistle emits a sound in the ultrasonic range, which people cannot hear but dogs can. What frequency range does a dog whistle likely have? _____

4. a) Based on the background information, which animal can detect infrasound?

b) Which animals can detect ultrasound?

V. Lab Exercise

A. Materials

Per Room:	Per Room:
• Colored foam sticks • Taste solutions and papers • Jelly beans • Q-tips • Tuning forks • Meter stick • Smell kit	• Ribbon/fishing line • 2-point discriminator • Paper bag containing unknown objects • Colored pens • Black light • Colored beads

B. Procedure

A. Vision

1. Adaptation to low light

1. To determine how the different photoreceptors in your eyes adjust to changing light conditions, you will be asked the following activity with the help of your instructor.
2. Under regular light, sort the foam pieces in the plastic bag into separate piles of matching color as quickly as you can. This is your ***control experiment***.

How long does it take you to sort the colors? Check with a partner to make sure the colors were sorted correctly.

3. Shuffle the colored shapes to mix up the colors.
4. When your instructor turns off the classroom lights, immediately sort the pieces by color again, working as quickly as you can (don't take longer than you did for your control experiment).
5. Review the results of your experiment when the lights are turned back on.

Are any of the colors sorted incorrectly? If so, which ones?

6. Reset the experiment, and wait for the instructor to turn off the lights again. This time, wait 1 minute before quickly sorting the colors.

How do your results compare with the previous test under low light?

How does it compare with the control experiment?

Under what circumstances would natural selection favor the ability of the eyes to quickly adjust to changing light conditions?

2. Ultraviolet Light

Which animals can see ultraviolet light?

What is the evolutionary advantage of being able to see ultraviolet light?

1. This activity will simulate the visual cues that some pollinators perceive from plants.
2. Observe the colored beads on display on the side or back counter in the room using the ambient white light in the room.

   Which bead color is the most conspicuous under white light? _____

3. Now turn on the black light provided and observe the beads again.

Which bead color is the most conspicuous under black light? _____

What is the selective pressure on plants if their pollinators can see only visible light?

How does the selective pressure on plants change if the pollinators can see both visible light AND UV light?

3. Peripheral Color Blindness

The peripheral regions of your retina have less sensitive color perception because there are fewer cones along the periphery of the retina compared to the center. This phenomenon is called peripheral color blindness.

1. Hold one of the provided colored pens in front of your right eye approximately 30cm in front of your face.
2. Slowly move it to your right in a circular arc (towards your ear) until you can no longer determine the color of the pen but can still see the pen.

Which kind of photoreceptor in our eyes can detect color? _____

Which photoreceptor is very sensitive to low light? _____

Why do you think that there are fewer color-sensitive photoreceptors along the periphery of our eyes? What kind of visual stimuli can we detect "out of the corner of our eyes"?

B. Touch

1. Concentration of Touch Receptors

You have different concentrations of touch receptors on different parts of your body. Places on you body that come in contact with tactile stimuli frequently tend to have many touch receptors and are therefore more sensitive to touch. Also, places on your body where touch is necessary for a particular physiological function may also have many touch receptors.

Where on your body would you expect to have a high concentration of touch receptors (of the five locations listed in the table on the next page)?

1. Choose 1cm^2 areas on the face, neck, shoulder, back of arm and fingertip of your partner.
2. Firmly tap this area with a piece of ribbon **20 times**.
3. Vary the speed of the taps so that your partner cannot predict when the next tap will be.
4. The person being tapped should not watch their partner, but should look away and count the number of times they feel a tap.
5. Both partners should take turns with this activity and record their data below.

	Number of Hits Detected (out of 20)		
Location on Body	Person 1	Person 2	Average of the two partners
Face			
Neck			
Shoulder			
Back of Arm			
Fingertip			

Was your hypothesis regarding the concentration of touch receptors supported by your experimental results?

2. Two-Point Discrimination

Touch receptors can also differ from each other by the total area over which each receptor detects touch stimuli. These areas are called **receptive fields**. Large receptive fields allow the cell to detect changes over a wider area, but lead to less precise perception. For example, fingers have many, densely packed receptors with small receptive fields while the back has fewer receptors, each with large receptive fields.

*Of the five locations listed in the table above, where would you expect to have touch receptors with the **smallest** receptive fields?*

1. Choose the same $1cm^2$ areas on the face, neck, shoulder, back of arm and fingertip of your partner as you did in the previous test.
2. Using the calipers or two-point discriminator tool, firmly touch the chosen location, starting with the smallest distance. Make sure the two points make contact with the partner's skin at the same time.
3. Do NOT tell your partner what distance you are testing.
4. The person being tested should indicate, without looking, whether they feel one point or two separate points for each distance.
5. Both partners should take turns with this activity and record the minimum distance at which they felt two separate points for each location tested.

	Minimum distance at which two points were detected		
Location on Body	Person 1	Person 2	Average of the two partners
Face			
Neck			
Shoulder			
Back of Arm			
Fingertip			

Was your hypothesis regarding receptive fields supported by your experimental results?

3. Mystery Bag

1. Each student should complete this experiment individually and not share the results with group members until everyone in the group has had a chance to complete the activity.
2. Pick up the marked paper bag that contains six items inside it.
3. **Without looking in the bag**, place your hand inside and feel the different objects.
4. **Do NOT take the items out of the bag at any point.**
5. Complete the data table below with the requested information.
6. When you are finished, look inside the bag to check your answers.

Material composition of item (plastic, wood, metal, etc.)	Texture (rough, smooth, etc.)	Item name (your guess)

Were there any items in the bag that were difficult to identify using only your sense of touch?

D. Hearing

1. Place a meter-stick next to your partner's ear and have them hold it in place.
2. Strike one of the tuning forks on a book to make it vibrate.
3. Put the fork close to your partner's ear and move it away from his or her ear along the meter-stick. Move the tuning fork at a rate of 10cm/sec.
4. Stop moving the tuning fork when your partner tells you when they can no longer hear the sound.
5. Your partner should record the pitch of the tuning fork and the max distance at which they could hear it.
6. Repeat the experiment with two other pitches.
7. Switch roles and have your partner conduct the same experiment on you.

Pitch	Maximum distance at which sound could be detected

How did the pitch affect how far you could hear the sound? Which pitch is more easily detectable over longer distances?

Discuss the ways in which this experimental design could be improved to achieve more accurate results.

E. Taste

1. Identifying Tastes

1. Add a few drops of solution A to the end of a clean Q-tip or strip of paper.
2. Gently touch the Q-tip or paper to the surface of your tongue and identify the taste (sweet, salty, bitter or sour).
3. Repeat this procedure with a clean end of the Q-tip or paper for each of the other solutions.
4. Record your results in the data table below then check your answers with the key provided.

Taste Solution	Taste
A.	
B.	
C.	
D.	

How accurate were you in your analysis? Were some tastes easier or harder to identify than others?

2. Taste Paper - PTC

PTC (Phenylthiocarbamide) has the unusual property in that it either tastes very bitter or is virtually tasteless, depending on the genetic makeup of the taster. The ability to taste PTC is dependent on an inherited dominant genetic trait. Although PTC is not found in nature, the ability to taste it correlates strongly with the ability to taste other bitter substances that do occur naturally, many of which are toxins. Ability to taste PTC may be correlated with a dislike of plants in the *Brassica* genus (cabbage, cauliflower, broccoli, Brussels sprout) presumably due to chemical similarities.

Touch one piece of the PTC paper to your tongue.

Are you able to taste PTC? _____

Do any plants in the Brassica genus taste bitter to you? _____

*Under what evolutionary conditions would natural selection favor a **genetically** predetermined tendency as opposed to a **learned** tendency to avoid or seek out a certain taste?*

3. Taste vs. Flavor

Describe the difference between taste and flavor. Which senses do we rely on to determine flavor?

1. Take one jellybean out of the container carefully, using the spatula or spoon provided.
2. **Hold your nose closed tightly** and bite off **half** the jellybean and chew it.

*Can you determine the flavor of the jellybean?*_____

3. Let go of your nose and eat the rest of the jellybean.

*Are you able to determine the flavor this time?*_____

What does this result suggest about how we perceive the flavor of jellybeans and other artificial and complex flavors?

F. Smell

1. Smell each of the numbered scents and record what you think the smell is.
2. Also record the memory the smell invokes (if any).
3. In between each sample, smell the jar of coffee or the jar marked with an asterisk to clear your senses.

Sample #	Name of smell	Memory
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		

VI. Post-Lab Questions

1. For each of the following senses, identify the advantages and the disadvantages of predominantly relying on that sense to find food, escape predators, find mates, etc. To answer the questions, you will need to think of different animals that rely primarily on that sense to gather information. One example has been provided for you.

Sense	Advantages / Benefits	Disadvantages / Limitations
Taste	*Sense of taste can provide detailed information if the animal has sensitive taste receptors.*	*Requires close contact with the stimulus, which can be harmful if the item being tasted is poisonous.*
Smell		
Touch		
Sight		
Hearing		
Echolocation		
Electroreception		