

FUNDAMENTALS OF BIOLOGY LABORATORY MANUAL



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Fundamentals of Biology Laboratory Manual

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Licensing

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CHAPTER OVERVIEW

1: Lab 1 Safety, Introductions, Scientific Measurements, and the Metric System

1.1: Lab Intro, Safety, and Procedures

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1.1: Lab Intro, Safety, and Procedures

Learning Objectives

- Explain the layout of the lab classroom and the equipment we'll be using this semester.
- Identify safe ways to use lab equipment and chemicals.
- Locate safety equipment such as the eye wash, safety shower, and broken glass container.
- Maintain safe and responsible behavior in the lab.
- Follow appropriate procedures to maintain sensitive lab equipment.

Introduction: Laboratory Safety

It is important to develop safe laboratory (lab) habits when working with reagents, chemicals, fluids, preserved specimens, and equipment which can be potentially hazardous. We can avoid most issues if we behave responsibly and remain aware of our surroundings. Listed below are some simple procedures that must be followed in a lab setting. Becoming familiar with them now and forming responsible habits will protect us all in the lab from potential injury. Assume that ALL reagents and specimens are a potential hazard. Animal specimens are commonly preserved in a chemical mixture which can be hazardous if it comes in contact with your skin.

Begin by taking a moment to familiarize yourself with your surroundings. Take note of the locations of the safety equipment, waste containers, broken glass containers, chemical spill kits, laboratory instruments, and reagents.

GENERAL LABORATORY SAFETY PROCEDURES

- The Material Safety Data Sheets (MSDS) lists all the chemicals in the lab this semester and any potential hazards associated with them. It is in a yellow binder located in the cabinet next to the front door. It is your right to review the MSDS to be aware of any potential chemical hazards you may be exposed to.
- If your skin comes in contact with a chemical, wash the area thoroughly with soap and water.
- If a chemical comes in contact with your nose or mouth, rinse the affected area with large amounts of water.
- If a chemical comes in contact with your eyes, use the eyewash station (one located next to each sink) for *at least* 10 minutes.
- Contact lens wearers: Contact lenses tend to absorb chemicals. If you use contact lenses it is recommended that you wear glasses instead or use safety glasses or wear disposable lenses.
- No food or drinks should be consumed during labs where chemicals are in use. You may step outside of the lab at an appropriate time to eat or drink.
- Do not drink the tap water or DI water in the lab! The industrial water from the sink and DI water are both dangerous to drink.
- If anything spills (e.g., chemicals, water from your backpack), please alert your instructor right away.
- Wear stable closed-toed shoes (sneakers); no sandals, Crocs, heels, slippers, etc.
- Handle sharp cutting tools carefully.
- Keep your work area clean and free of clutter to avoid spills and breakage of glassware.
- Report any broken glass to your instructor. There is a dustpan and broom to sweep up the broken glass. Dispose of the broken glass in the broken glass container, not in the trash can.
- Do not touch hot plates or heated liquids. Use appropriate tools for working with them.
- Masks and hand sanitizer may be available upon request.
- If you don't know, ask!
- It is recommended to wash your hands before leaving lab.
- Inform your instructor of any unique health factors, such as allergies, sensitivities to particular substances/conditions, pregnancy, or anything else to ensure your safety in the lab.
- We will make any necessary accommodations. Be sure to contact the SRC for official accommodations including test taking, note taking, or necessary breaks during class.

OTHER LABORATORY PROCEDURES

Besides general lab safety, we need to make sure that we use the equipment in our lab properly and with care. Some of the equipment is fragile and/or expensive, especially microscopes, so it's important to follow the procedures below.

Check-out Procedure

Each student sits at a numbered seat. The number includes the letter of the table together with the number of the microscope cabinet; for example, A-1 or E-2. When students wish to check out materials, they must sign out for them on the line that corresponds to their seat number. Corresponding keys to the microscope cabinets are up by the front door. Your same seat number also corresponds to microscope slides. Be sure to return the microscope, key, and slides to their appropriate spots.

Clean-up

Follow the instructions for waste disposal carefully and closely. Make sure you dispose of chemicals in the appropriate waste container, not down the sink! Only water should go down the sink. Once the chemicals have been disposed of properly, rinse the containers (test tubes, beakers, etc) in the sink and place in the bin to dry.

Your lab instructor may direct you to put some items into the trash, but be sure to only put the specific items designated for the trash. Do not throw away anything that is contaminated with chemicals, broken glass, or anything that can be reused (e.g., pipettes).

Before you leave the Lab

All materials (including the microscope cabinet key) that were checked out should be returned to the appropriate place and name crossed out on the checkout sheet. Microscopes should be stored properly and locked in the cabinets. Wet mount slides should be cleaned, and all trash deposited into waste baskets. Your chair should be pushed in at your table and wipe down your desk area to make sure it's clean for the next class. Wash your hands with soap and water before leaving the lab. Thank you for following these instructions explicitly every time you are in the lab.

LABORATORY EXPECTATIONS

Throughout the semester, we will be performing experiments individually, in pairs, in groups, and as benches. Thus, it is important to get to know your classmates and learn to work collaboratively. Here are some activities and ice breaker ideas.

Cultural introductions

This activity is used to get to know one another. Introduce yourself within your group.

- Each person gets to share, uninterrupted, for 1 minute. After that person is finished sharing, the next person begins until everyone has had the opportunity to speak.
- Introduce yourself in a cultural manner. We are defining culture in the broadest context, e.g., ethnicity, race, language, family, spiritual beliefs, religious affiliation, generation, birth order, or any other self-identifying manner.

There are 2 rules:

- Avoid what you do professionally, your job, your area of study, your Degrees or your career path.
- Listen only, while another person is speaking.

Setting Community Norms & Expectations

This activity is to help everyone agree on how we will interact and keep one another safe during the lab. Take a moment to reflect and answer the following questions individually:

- What should our space feel like each time we gather?
- When and how do you learn best? Now, think of the opposite: What impedes your learning?
- What does learning in a lab setting mean to you? What are you excited about? What might you be nervous about?
- How should we help each other remain accountable, present, and committed?
- What are any other thoughts?
- Optional questions:

- We sometimes discuss sensitive subjects. How should we address our differences in opinion and possible conflict as it arises?
- What does hybrid mean to you? What does active learning look like? What do you expect to have to do outside of lab class time?

Now let's open up for a big class discussion, adding our thoughts to the following categories:

- Space feels like...
- Ensuring fun & joy
- How to remain accountable
- Working through disagreements

The instructor will gather the class's thoughts and turn them into a set of class norms for the semester.

Active journaling

Below are some welcome journaling prompts:

- Do you find it easy or hard to believe in yourself as a science student? Why do you feel that way? Are those feelings rooted in past experiences? Can we explore them a little so I can better honor your experiences and support you towards better self-confidence and assuredness when it comes to mastering our subject?
- **Introverts** feel recharged by alone time, while **extroverts** gain energy from social interaction. Most people fall somewhere in between rather than being entirely one or the other. Where do you think you fall on the introvert-extrovert spectrum? Is there one side you favor more? How do you see this connected to our class? How might you be pushed out of your comfort zone in those roles? What can you do to recharge when you feel spent?

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1.2: Lab 1 Background

Learning Objectives

- Measure mass (in grams), volume (in liters), and length (in meters) using proper equipment.
- Convert between metric units and between the metric and Imperial systems.
- Apply these concepts on measurements and conversions to future labs.
- Ask comparative scientific questions that lead to observations and experiments.

Introduction: The Metric System

There are many ways to measure and describe the size of different objects. If we have ever followed a recipe to make dinner, we might have noticed the directions include the amount of each item to add. Take a moment to imagine if we didn't have those measurements, how much salt would we know to add? How would we be able to remake the meal exactly the same a second time? Since we want to be able to repeat successful recipes, we need to be sure to add the correct amount of each ingredient.

The process of science is very similar! In order for different individuals to be able to repeat experiments and get the same results independently, it is important to **standardize** the measurements that are used. So, the scientific community agreed to use the **metric system**. There are three basic units of measurement in the metric system:

- The **meter (m)** measures length
- The **gram (g)** measures mass
- The **liter (L)** measures volume

In the United States, there is a second measurement system called the Imperial system, which includes inches/feet, pounds/ounces, and gallons/ounces/cups. Converting between Imperial units is not very straightforward. For example, there are 12 inches in a foot and 16 ounces in a pound. Unlike the Imperial system, the metric system is based on factors of 10, making conversions much simpler. For example, there are 100 centimeters in a meter and 1,000 grams in a kilogram. Table 1 summarizes the different metric units and how to convert between them.

Table 1. The common metric system prefixes, along with their abbreviations and values relative to the base/standard units (meter, gram, and liter). The solid and dashed arrows have been added to help explain how to convert between the units, described in more detail below.

Prefix	Symbol	Power of 10	Example
Giga	G	$10^9 = 1,000,000,000$	Gigabytes (Gb)
Mega	M	$10^6 = 1,000,000$	Megawatts (Mw)
Kilo	K	$10^3 = 1,000$	Kilometer (Km)
Hecto	H	$10^2 = 100$	Hectogram (Hg)
Deca	Da	$10^1 = 10$	Decaliter (DaL)
Base/Standard unit	m or g or L	$10^0 = 1$	meter, gram, and liter
deci	d	$10^{-1} = 0.1$ (or $1/10$)	deciliter (dL)
centi	c	$10^{-2} = 0.01$ (or $1/100$)	centimeter (cm)
milli	m	$10^{-3} = 0.001$ (or $1/1,000$)	millimeter (mm)
micro	μ	$10^{-6} = 0.000001$ (or $1/1,000,000$)	micrometer (also called a micron) (μ g)
nano	n	$10^{-9} = 0.000000001$ (or $1/1,000,000,000$)	nanogram (ng)

In the table above, the larger units are at the top and the smaller units are at the bottom; this can help us double check that we did the conversion correctly. There are various ways to convert between metric units, such as the **step system** (i.e., moving the decimal) and **dimensional analysis**. No matter the preferred method of conversion, it always involves a change by some factor of 10. Let's practice this skill below!

Metric Conversions: Step System

1. Subtract the exponent in our starting unit and the exponent of the unit we need to convert to.
2. Move the decimal the same number of places from the difference in step 1.
3. If the difference is positive, move the decimal to the right; move it to the left with a negative difference.

- The arrows around the Powers of 10 column in Table 1 also show which direction to move the decimal.
 - If we're converting to a **larger** unit, move the decimal to the **left**.
 - If we're converting to a **smaller** unit, move the decimal to the **right**.

For example:

- Convert 403dm to μm .
 - Decimeters (dm) are 10^{-1} and micrometers (μm) are 10^{-6} . The difference between these two superscripts is 5 ($-1 - (-6) = -1 + 6 = 5$). The difference is positive and we're converting to a smaller unit. So, we move the decimal 5 places to the right:

$$403 \text{ dm} = 40,300,000 \mu\text{m}$$

- Convert 33.8Dag to Kg.
 - Decagrams (Dag) are 10^1 and kilograms (Kg) are 10^3 . The difference between these two superscripts is -2 ($1 - 3 = -2$). The difference is negative and we're converting to a larger unit. So, we move the decimal 2 places to the left.

$$33.8 \text{ Dag} = 0.338 \text{ Kg}$$

Let's try practicing more. Refer to the Answer Sheet to complete the practice metric conversions.

Metric Conversions: Dimensional Analysis

- Determine which unit we are starting with and which we need to convert to.
- Set up the conversion factor in the form of a fraction. How many units that we're converting to are in the units that we're starting with? Always put the unit we're starting with on the bottom of the conversion factor.
- Multiply the starting unit by the conversion factor, cross-canceling units.

For example:

- Convert 1.2 meters to mm.
- There are 1000mm in 1m, so our conversion factor is $\frac{1000 \text{ mm}}{1 \text{ m}}$
- Multiply 1.2m by the conversion factor $\frac{1000 \text{ mm}}{1 \text{ m}}$:

$$1.2 \text{ m} \times \frac{1000 \text{ mm}}{1 \text{ m}} = 1,200 \text{ mm}$$

By putting the starting unit on the bottom of the conversion factor, the units are canceled (meters in this example), and the result is the unit being converted to (millimeters in this example). This can be double checked: millimeters are smaller than meters, so there should be more of them, which there are!

Let's try another example:

- Convert 23mL to L.
 - There are 1000mL in 1L, so the conversion factor is $\frac{1\text{L}}{1000 \text{ mL}}$ (remember that the starting unit goes on the bottom)
 - Multiply 1000mL by the conversion factor $\frac{1\text{L}}{1000 \text{ mL}}$

$$23 \text{ mL} \times \frac{1\text{L}}{1000 \text{ mL}} = 0.023 \text{ L}$$

It can get a little trickier if we don't use the base unit. For example:

- Convert 16Hg to cg.
 - There are 10,000cg in 1Hg, so the conversion factor is $\frac{10,000\text{cg}}{1\text{Hg}}$
 - Multiply 16Hg by the conversion factor $\frac{10,000\text{cg}}{1\text{Hg}}$

$$16\text{Hg} \times \frac{10,000\text{cg}}{1\text{Hg}} = 160,000\text{cg}$$

How do we know there are 10,000cg in 1Hg? Remember to subtract the units' exponents. From Table 1, we can see that Hecto is 10^2 and centi is 10^{-2} , so

$$2 - (-2) = 2 + 2 = 4$$

This means the exponent of the conversion factor is 4 (10^4 in this example); $10^4 = 10,000$.

Note on the Imperial System

The United States does not use the metric system for most measurements. We use inches, feet, yards, and miles for distance, ounces and pounds for weight, and teaspoons/tablespoons, fluid ounces, cups, and gallons for volume. Unfortunately, the conversions between these units are not as straightforward as the conversions between metric units. For conversion factors between Imperial units and how to convert between Imperial and Metric units, simply look them up (e.g., Google).

Introduction: Analyzing Datasets

While designing a study, individuals must select their **variables**, which are the items they predict to *vary*, or change, during the study. There are generally two kinds of variables in each study.

- The **dependent variable**, also called the *outcome variable*, is what they are measuring to determine whether it changes over the course of the study, or is different between the groups. The outcome of the experiment depends on the values of this variable.
- The **independent variable** is what they are purposefully changing. Typically, they want to limit themselves to having just one independent variable, but sometimes it is worthwhile or necessary to have more. The researchers predict that by changing the independent variable, there will be a change to the dependent variable.

We should also describe the **quantitative** (numerical) or **qualitative** (non-numerical) nature of variables. **Continuous** variables are quantitative and include numbers with an infinite range. **Discrete** variables can be quantitative or qualitative and include categories. Knowing the type of variable is especially important when building a graph. **Graphs** allow scientists to quickly show patterns in data and make comparisons between variables or groups. There are many versions of graphs that can be used depending on the type of variable measured. Below are two examples:

Bar graphs show the average differences between groups. For bar graphs, one variable is continuous and the other is discrete.

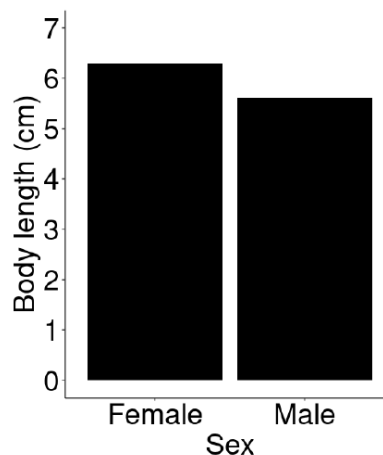


Figure 1. The average body length of female and male Madagascar hissing cockroaches.

Line graphs show a trend between two variables. One common use of line graphs shows how a variable (such as length) **changes** over time. Generally, both variables are continuous for a line graph.

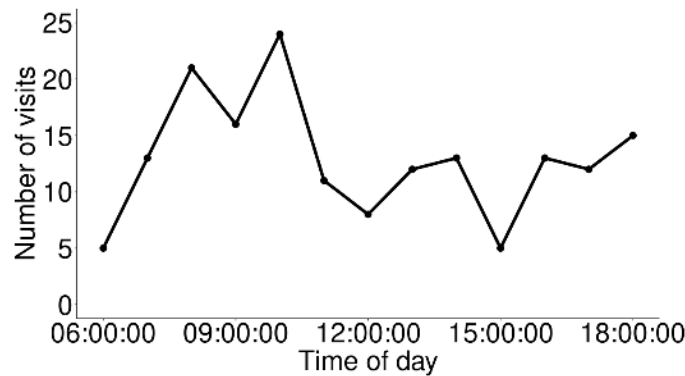


Figure 2. The number of times a pollinator (including bees and butterflies) visited a flower over the course of a day.

Whichever kind of graph we make, we need to make sure that we always label the axes! The *dependent* variable always goes on the **y-axis** (vertical) because that is the variable we are measuring. The *independent* variable goes on the **x-axis** (horizontal). We may also need to use a **legend** to label independent variables if we use more than one. Below the graph, we also add a **caption** to describe what the graph is showing the reader. For example, “Figure 1. The average body length of female and male Madagascar hissing cockroaches.”

In addition, when communicating results and conclusions from studies, scientists generally want to include whether the findings are important or significant. Thus, proper analysis involves various kinds of **statistics**, which are usually included in the graph. Two common ways of determining significance are by comparing the means (averages) of two or more groups (for example, by using a t-test) or calculating a correlation to see if two sets of data are related.

Most statistical tests generate a **p-value**. The “p” stands for probability. We can think of this as the probability/likelihood that something happened by chance. For example, if $p = 0.10$, we can interpret this as there being a 10% chance that the results could happen randomly, and not because of the independent variable. Generally, we consider the results to be **statistically significant** if the p-value is less than 0.05 ($p < 0.05$), which explains that there is a measurable or meaningful difference between the datasets. However, if the p-value is greater than 0.05 ($p > 0.05$), we conclude that there is no difference or that the difference is likely due to random chance.

It is extremely important to remember our biases here. In particular, we want to be aware of **confirmation bias**. This happens when we sift through the results to find something that we agreed with before the test took place. For example, if we think male cockroaches are longer than female cockroaches, we might look for the one or two samples that confirms this for us, ignoring the rest of the **variation** (differences in the data points), even if our statistical test shows that female cockroaches are actually longer! Whenever we make conclusions, we have to support our claims with **evidence**, the objective results of the tests.

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1.3: Lab 1 Procedures (Grasshoppers Version)

PROCEDURE 1: VOLUME AND MASS

Equipment we'll need:

- 2 graduated cylinders
 - Tape and pen
 - Dropper bottle of water
 - Electronic balance (scale)
1. Obtain two graduated cylinders. Place tape towards the top of each and use a Sharpie or pen to label one A and the other B.
 2. Turn on the electronic balance, place graduated cylinder A on the silver disc, and press "Zero" to calibrate the scale to start at 0.
 3. While standing, with your point of view over the top of graduated cylinder A, add 5mL of water into the graduated cylinder.
 4. Place graduated cylinder A with 5mL of water on the scale and record the mass in Table 1.
 5. Place graduated cylinder B on the electronic balance and Zero it out.
 6. This time, crouch down so the graduated cylinder on the table is at eye level. Notice how the water in graduated cylinder A curves into a meniscus. Add water to graduated cylinder B until the bottom of the meniscus reaches the 5mL mark.
 7. For example, the image to the right shows 21.7mL of water.
 8. If you add too much water, use a long pipette to draw water out.
 9. Place graduated cylinder B with 5mL of water on the scale and record the mass in Table 1.
 10. Work through the questions in the Answer Sheet to calculate the percent error for each graduated cylinder.



Clean up/Disposal

1. Pour water into the sink.
2. Remove tape from graduated cylinders and throw the tape in the trash.
3. Place the graduated cylinders back in the containers on the counter.
4. Place the dropper bottles of water back in the boxes.

PROCEDURE 2: BIOLOGICAL MEASUREMENTS

Equipment we'll need:

- 3 grasshoppers per group
- Electronic scale
- Plastic trays
- Ruler (with metric measurements)
- Gloves and paper towels

Now we can apply what we learned about making measurements to living things. We're going to measure the length and mass of male and female lubber grasshoppers (*Romalea microptera*). Each group will have the chance to make measurements on a few individuals, and then the class will combine each group's data to compare males and females.

These grasshoppers have been preserved in various chemicals. Therefore, we should wear gloves while handling them. If we do touch any grasshoppers, don't worry, we'll be fine! Just wash whatever comes in contact with them using soap and water.



Figure 1. Male lubber grasshoppers (bottom) have more of a rounded end to their abdomen. Females (top) have two pointed structures at the end of their abdomen (this is called the ovipositor, from which they lay eggs). They can be hard to distinguish, so ask the instructor for help! (Image credit: Shawn McEachin)

1. Use a ruler to measure the length, to the nearest 0.1cm, of the grasshoppers. For example, the leaf in the image below is 4.1cm. Record the data in Table 2 on the Answer Sheet.



2. Place a 600mL beaker on the electronic scale and press “Zero” to calibrate the scale. Then place each grasshopper, one at a time, in the beaker to measure its mass. Record the data in Table 5 on the Answer Sheet.
 - Make sure that we match each individual’s length with that same individual’s mass.
3. Write the group’s data on the board (or report it to the instructor). After all groups report their data, we will calculate the class averages to complete Table 6 in the Answer Sheet.
4. The instructor will run a [t-test](#), allowing us to determine whether body length or body mass are different between male and female grasshoppers.
5. Answer the rest of the questions on the Answer Sheet.

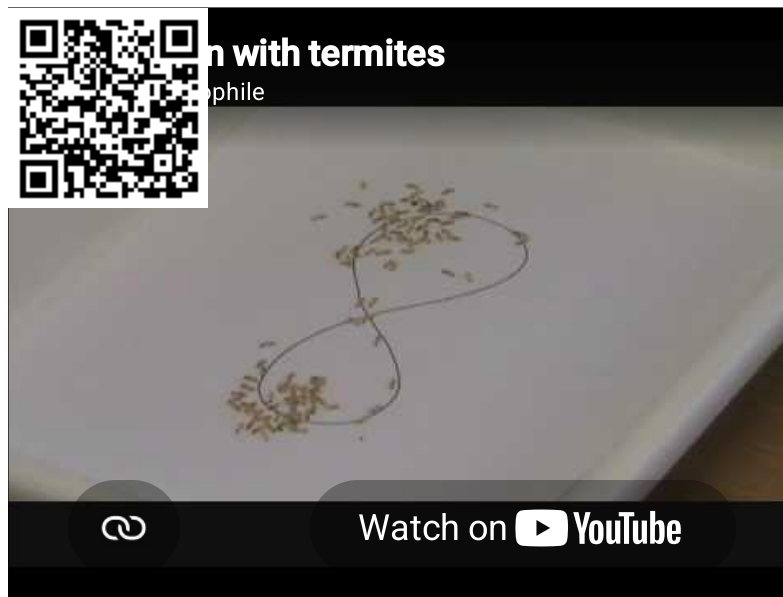
Clean up/Disposal

1. Return all measuring devices to their original locations.
2. Return the used grasshoppers to the correct piles.

PROCEDURE 3: ASKING SCIENTIFIC QUESTIONS

From our experience measuring samples today, we might have a few questions about why there are differences between our samples. There are many types of questions we can ask about the world around us. Some of these questions are answerable by performing experiments, while other questions cannot be answered through scientific experiments. Figuring out the difference between these types of questions and how to come up with answerable questions is our next task.

1. Watch the video one time without writing anything down: [Fun with termites](#).



2. Re-watch the video a few more times. In the Answer Sheet, write down all of the things we observe happening.
3. In the Answer Sheet, write down as many questions as we can think of about what we just witnessed.

Generally, scientific questions need to focus on measurable differences as we've discussed in this lab so far. One type of question that builds this in is called a **comparative question**. These types of questions include the dependent variable and a comparison between two groups or situations within the question. For today's lab, our comparative question was, "Are the leaves on black sage plants longer and/or heavier than the leaves on white sage plants?" Within this question, we included our dependent variable of length and mass. This question also clearly included the comparison we were making.

4. Review the questions from the Answer Sheet. Choose three of the questions and turn them into a comparative question. Bring this for next class!

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1.4: Lab 1 Procedures (Leaves Version)

PROCEDURE 1: MAKING MEASUREMENTS

Equipment we'll need:

- 2 graduated cylinders
- Tape and pen
- Dropper bottle of water
- Electronic balance (scale)

1. Obtain two graduated cylinders. Place tape towards the top of each and use a Sharpie or pen to label one A and the other B.
2. Turn on the electronic balance, place graduated cylinder A on the silver disc, and press "Zero" to calibrate the scale to start at 0.
3. While standing, with your point of view over the top of graduated cylinder A, add 5mL of water into the graduated cylinder.
4. Place graduated cylinder A with 5mL of water on the scale and record the mass in Table 1.
5. Place graduated cylinder B on the electronic balance and Zero it out.
6. This time, crouch down so the graduated cylinder on the table is at eye level. Notice how the water in graduated cylinder A curves into a meniscus. Add water to graduated cylinder B until the bottom of the meniscus reaches the 5mL mark.
7. For example, the image to the right shows 21.7mL of water.
8. If you add too much water, use a long pipette to draw water out.
9. Place graduated cylinder B with 5mL of water on the scale and record the mass in Table 1.
10. Work through the questions in the Answer Sheet to calculate the percent error for each graduated cylinder.



Clean up/Disposal

1. Pour water into the sink.
2. Remove tape from graduated cylinders and throw the tape in the trash.
3. Place the graduated cylinders back in the containers on the counter.
4. Place the dropper bottles of water back in the boxes.

PROCEDURE 2: BIOLOGICAL MEASUREMENTS

Equipment we'll need:

- 3 leaves of each plant species per group
- Electronic balance
- Ruler (with metric measurements)

Now let's apply what we learned about making measurements to living things. We're going to measure the length and mass of the leaves of two species of California native plants, black sage (*Salvia mellifera*) and white sage (*Salvia apiana*) [depending on availability, we may use related species in class, such as Mexican bush sage (*Salvia leucantha*)]. Each group will have the chance to make measurements on a few individuals, and then the class will combine all our data to compare the species of plants.



Figure 3. Representative leaves of black sage (*Salvia mellifera*; left), white sage (*Salvia apiana*; right), and Mexican bush sage (*Salvia leucantha*; bottom). Image credit: Shawn McEachin and Polly Parks.

1. Use a ruler to measure the length of each leaf from tip to stem, to the nearest 0.1cm. For example, the leaf in the image below is 4.1cm. Record the data in Table 2 on the Answer Sheet.



2. Use an electronic scale to measure each leaf's mass, one at a time. Record the data in Table 2 on the Answer Sheet.
 - Make sure that we match each individual's length with that same individual's mass.
3. Record the group's data on the board or in the Lab 1 Class Data Google Sheet (provided by the instructor). After all groups report their data, we will calculate the class averages to complete Table 3 in the Answer Sheet.
4. The instructor will run a [t-test](#), allowing us to determine whether leaf length or leaf mass are different between these two species of plant.
5. Answer the rest of the questions on the Answer Sheet.

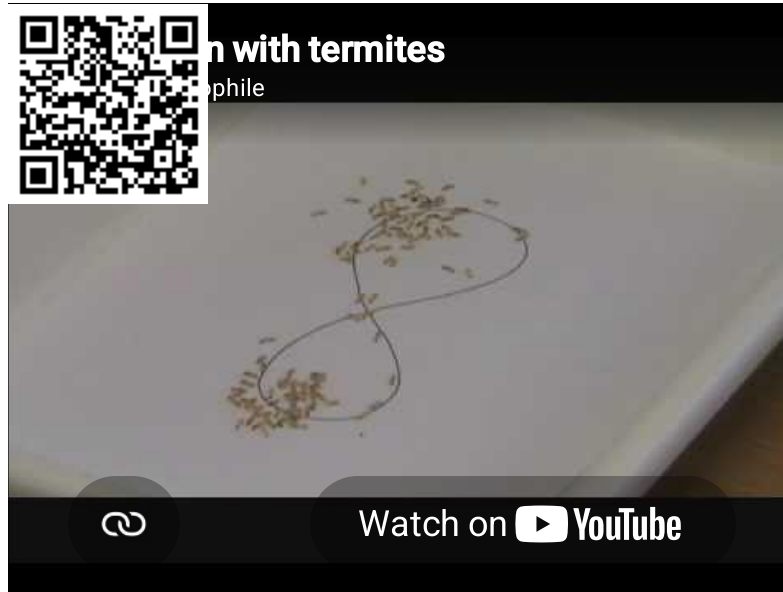
Clean up/Disposal

1. Return all measuring devices to their original locations.
2. Return the used leaves to the correct piles.

PROCEDURE 3: ASKING SCIENTIFIC QUESTIONS

From our experience measuring samples today, we might have a few questions about why there are differences between our samples. There are many types of questions we can ask about the world around us. Some of these questions are answerable by performing experiments, while other questions cannot be answered through scientific experiments. Figuring out the difference between these types of questions and how to come up with answerable questions is our next task.

1. Watch the video one time without writing anything down: [Fun with termites](#).



2. Re-watch the video a few more times. In the Answer Sheet, write down all of the things we observe happening.
3. In the Answer Sheet, write down as many questions as we can think of about what we just witnessed.

Generally, scientific questions need to focus on measurable differences as we've discussed in this lab so far. One type of question that builds this in is called a **comparative question**. These types of questions include the dependent variable and a comparison between two groups or situations within the question. For today's lab, our comparative question was, "Are the leaves on black sage plants longer and/or heavier than the leaves on white sage plants?" Within this question, we included our dependent variable of length and mass. This question also clearly included the comparison we were making.

4. Review the questions from the Answer Sheet. Choose three of the questions and turn them into a comparative question. Bring this for next class!

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CHAPTER OVERVIEW

2: Lab 2 Scientific Method

[2.1: Lab 2 Background](#)

[2.2: Lab 2 Procedures](#)

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2.1: Lab 2 Background

Learning Objectives

- Apply the scientific process.
- Analyze and interpret scientific data and communicate findings.
- Graph original experimental data.

Introduction: The Scientific Process

Science is not a list of facts, but rather a process for learning something new about the natural world. In its ideal form, science is the objective pursuit of discovery. However, it is important to keep in mind that science is done by people, and we all have inherent and implicit biases. As a scientist, we must try to become aware of our biases so our collection and interpretation of evidence is as objective as possible. Luckily, there's a solution!

The **scientific process** is an effective and efficient means of inquiry. It is also called the scientific method, but the word process better implies its *iterative* nature. Figure 1 demonstrates how the scientific process is iterative by depicting it as a cycle.

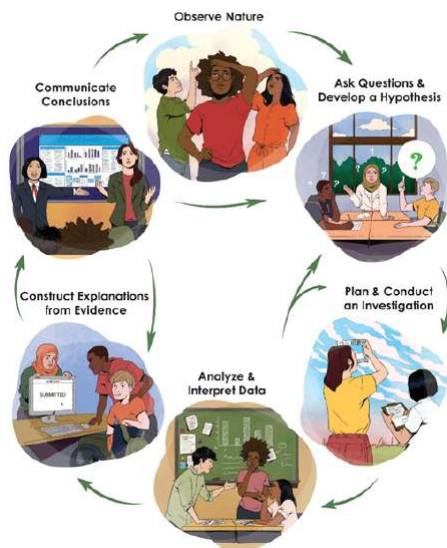


Figure 1. The steps of the scientific process (illustration from [The Globe Program](#)).

An **observation** is a *reproducible description, measurement, or record* of an object or phenomenon. No observation is too “obvious” or simple to make. We never know what could lead to an interesting study later on!

A scientific **question** asks something about one or more of our observations. We can start broad, but at some point, we want to hone in on what exactly we want to know about. It may seem simple, but this will actually dictate the rest of our study! We practiced writing comparative questions during lab 1 as part of the scientific process.

A **hypothesis** is what we think the answer to our question is. A hypothesis is often called an educated guess. In this context, *educated* means it is based on well-accepted *facts* (repeatedly confirmed observations) and *natural laws* (generalizations about how nature works). Like an observation, there are a few important properties of a hypothesis:

- **Testable.** There must be some kind of test (see below) someone can design to determine whether the hypothesis is true.
- **Refutable** (also called *falsifiable*). It must be possible to conclude that the hypothesis is false.
- **Tentative.** A hypothesis is *never proven*! The word “prove” is effectively never used in biological publications. Instead, we can *support or refute* a hypothesis. This is because hypotheses change as more information is gained.

There are two kinds of hypotheses:

- A **null hypothesis (H₀)** guesses that there is no difference or relationship between variables. This is really the hypothesis that we are testing. We want to know if we can refute (or reject) H₀.

- An **alternative hypothesis (H_A)** is just the opposite – it guesses that there is a difference or relationship between variables.

These two hypotheses go hand-in-hand. If we reject H₀, then, by definition, we support H_A. But if we reject H_A, then we support H₀ (or fail to support H_A). The section on analysis and statistics below explains how to know whether we can support or refute H₀.

A **prediction** is a statement that tells us what to measure in order to test our hypothesis. These often come in the form of an “If... then...” statement. We can use the template, “If [our hypothesis is true], then [we expect to find ____].” The part following “then” is what we are measuring.

A **test** is a way to collect **data** (measurements) that will support or refute our hypothesis. There are generally two ways to carry out a test.

Observational studies are ways to collect data, such as counting organisms or measuring sizes, to make comparisons without changing anything for the subjects. We simply compare two or more variables (a specific quantity, circumstance, or factor) that may or may not be different. These can be useful when experiments are impractical or unethical. But the conclusions that can be determined are limited in that we cannot conclude *causation*.

- “*Correlation does not mean causation.*” A **correlation** describes a relationship between two variables. For example, ice cream sales increase during the same time of year that shark attacks increase. Do ice cream sales *cause* shark attacks? Of course not! This is called a *spurious correlation*. There is another variable that is causing ice cream sales and shark attacks to be correlated – can you think of what that might be?

Controlled experiments are considered the “*gold standard*” of scientific tests. We purposefully change one variable, keep everything else the same, then measure the effect of that changed variable. There are a few variables to consider:

- The **dependent variable**, also called the outcome variable, is what we are measuring to determine whether it changes over the course of the study, or is different between the groups. The *outcome* of our experiment *depends* on the values of this variable.
- The **independent variable** is what we are purposefully changing. Typically, we want to limit ourselves to having just one independent variable, but sometimes it is worthwhile or necessary to have more. The researchers predict that by changing the independent variable, there will be a change to the dependent variable measurements.
 - We should also consider **variation** (natural differences in samples). Whether an observation study or controlled experiment, the dependent variable is always prone to variation. My results may be a little different from your results. Comparing the averages from many different samples takes variation into consideration.
- **Control variables/groups** are kept “*normal.*” That means that there is no treatment done to the control variable, i.e., there is nothing done to it. This acts as a variable against which to compare the results of the dependent variable. In some labs throughout the semester, we will set up different controls for comparison.
- **Standardized variables** are conditions that are kept *constant* and *the same* for all groups or situations throughout the experiment.

We should also describe the **quantitative** (numerical) or **qualitative** (non-numerical) nature of variables. **Continuous** variables are quantitative and include numbers with an infinite range. **Discrete** variables can be quantitative or qualitative and include categories.

If we set up our experiment properly, we can determine **causation**! By keeping various conditions standardized and comparing the results, we can determine whether the independent variable caused a change in the dependent variable.

In addition, when communicating results and conclusions from studies, scientists generally want to include whether the findings are important or significant. Thus, proper analysis involves various kinds of **statistics**, which are usually included in the graph. Two common ways of determining significance are by comparing the means (averages) of two or more groups (for example, by using a t-test) or calculating a correlation to see if two sets of data are related.

Most statistical tests generate a **p-value**. The “p” stands for probability. We can think of this as the probability/likelihood that something happened by chance. For example, if $p = 0.10$, we can interpret this as there being a 10% chance that the results could happen randomly, and not because of the independent variable. Generally, we consider the results to be **statistically significant** if the p-value is less than 0.05 ($p < 0.05$), which explains that there is a measurable or meaningful difference between the datasets.

However, if the p-value is greater than 0.05 ($p > 0.05$), we conclude that there is no difference or that the difference is likely due to random chance.

It is extremely important to remember our biases here. In particular, we want to be aware of **confirmation bias**. This happens when we sift through the results to find something that we agreed with before the test took place. For example, if we think male termites are longer than female termites, we might look for the one or two samples that confirms this for us, ignoring the rest of the **variation** (differences in the data points), even if your statistical test shows that female termites are actually longer! Whenever we make conclusions, we have to support our claims with **evidence**, the objective results of the tests.

Graphs allow scientists to quickly show patterns in data and make comparisons between variables or groups. There are many versions of graphs that can be used depending on the type of variable measured. Below are two examples:

Bar graphs show the average differences between groups. For bar graphs, one variable is continuous and the other is discrete.

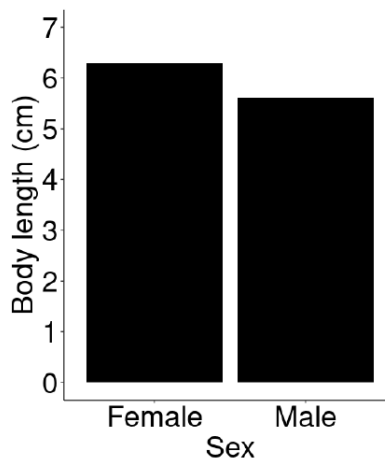


Figure 1. The average body length of female and male termites.

Line graphs show a trend between two variables. One common use of line graphs shows how a variable (such as length) **changes** over time. Generally, both variables are continuous for a line graph.

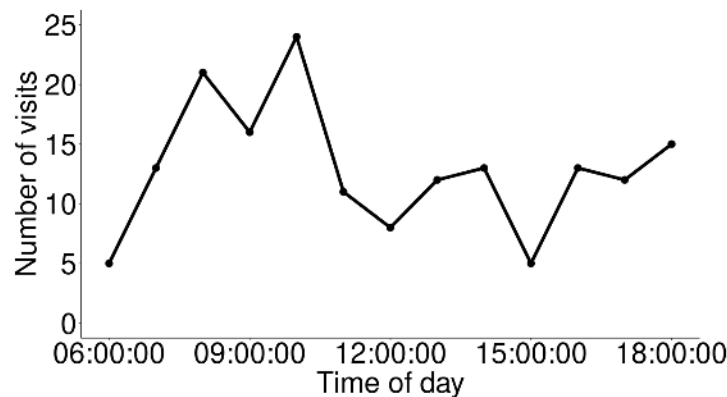


Figure 2. The number of times a pollinator (including bees and butterflies) visited a flower over the course of a day.

Whichever kind of graph we make, we need to make sure that we always label the axes! The *dependent* variable always goes on the **y-axis** (vertical) because that is the variable we are measuring. The *independent* variable goes on the **x-axis** (horizontal). We may also need to use a **legend** to label independent variables if we use more than one. Below the graph, we also add a **caption** to describe what the graph is showing the reader. For example, “Figure 1. The average body length of female and male termites.”

Always keep in mind that the scientific process is **iterative**. Once we get our results, we can ask *more questions*. Why didn’t our independent variable cause a change in our dependent variable? Does another independent variable cause the same or different

change in our dependent variable? So, just as we learn something new, we find ways to start over and make other new discoveries!

Introduction: Termites

The eastern subterranean termite (*Reticulitermes flavipes*) is common throughout North America. They play an important ecological role by feeding on wood, but are also considered a major pest in the United States. Have you ever seen a tented house? Chances are, the house was being fumigated to eradicate a colony of these termites.

Termites have a complex social structure composed of worker, soldier, and reproductive castes. All workers and soldiers in a colony are actually sisters! Worker termites are the only ones to eat wood. When workers find a food source, they will lay down a chemical trail of pheromones to indicate its location to fellow workers. Termites will follow this trail of pheromones very closely, enabling them to reach the food source efficiently.

As it turns out, some pens closely mimic these pheromones! Today, we will be designing experiments to test whether *R. flavipes* worker termites follow trails made by certain pens.

Introduction: Experimental Design

While designing our experiments, we need to keep a few things in mind:

- What is the dependent and independent variable?
 - Remember the difference between continuous and discrete variables. We may want to consider measuring one of each.
 - Amount of time, number of times, types of pens, ink color, etc.
- How will we measure the dependent variable?
- How can we stay organized?
 - Create a table to record the data.
 - Assign roles to the group members. Time keepers, counters, recorders, etc.
- Are we controlling for/standardizing all variables except the independent variable?
- Are we testing one individual at a time? If so, is each termite exposed to alternative treatments in **random** order? Are we controlling for fatigue, habituation, and prior experience?
 - How can we design the experiment to make sure termites in later trials are responding to the ink and not to pheromones laid down by earlier termites?

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2.2: Lab 2 Procedures

PROCEDURE 1: DESIGNING A CONTROLLED EXPERIMENT

Equipment we'll needed:

- 3 termites per group
- Brushes for handling termites
- One small cup for keeping termites at the table
- 2-3 pieces of paper
- Pens (different types, brands, colors, etc; each group need at least one BIC)
- Timer (you can use your smartphone or computer)

Last week, we watched a video of an experiment testing whether termites follow lines drawn by a pen. We recorded observations and built some comparative questions surrounding the behavior of the termites. Now that we have live termites, let's take a closer look! Follow the procedure below and answer the questions on the Answer Sheet.

1. Revisit the comparative questions. These will guide the rest of the experiment we are about to design. Each group can either pick the same questions or revise them if they have thought about different questions they would like to answer today. We're planning on designing two experiments today, so we'll need two comparative questions.
2. Write both a null and alternative hypothesis that attempts to answer each comparative question. Remember, don't worry about trying to make the hypothesis correct!
3. List the dependent and independent variables of both experiments and whether each one is continuous or discrete.
4. Make predictions using the independent and dependent variables and the hypotheses. Remember that predictions tell us what to measure and may take the form of an "If... then..." statement.
5. One of the most crucial parts of the scientific process is getting feedback. Before writing the methods, walk around to a few other groups and share your ideas. Maybe another group thought of something you didn't initially consider! Feel free to change a variable or method based on the feedback.
6. Now we're ready to describe the methods we will use to test our hypotheses. These should be complete and precise enough so that another person can read the instructions and carry out the *exact same* experiment. Some things to include in our procedures:
 - The equipment needed (both amount and type).
 - The number of termites to be used throughout the experiment or the number of times each termite will be used.
 - For today's experiments, each group should include 5 trials.
 - How we will measure the dependent variable.
7. Before we carry out the experiments, we need to devise an organized way to collect data. Build a data table that includes the dependent and independent variables and the number of trials we will have. It may be helpful referring to last week's lab for some examples of how to organize data tables.
8. Now carry out the experiments! Follow the methods exactly. Define roles for each group member so data collection is efficient and accurate. Group members may want to switch roles between trials or experiments.

Termite Handling Instructions

These little termites are fragile! Please be careful while handling them, and handle them as little as possible. Note: we are using the term "handling" broadly, but we should not be using our hands to hold, pick up, or move the termites. Follow the instructions below to avoid harming the termites during our experiments.

- Use the small brushes to handle the termites.
 - To pick up a termite, gently press the bristles of the brush down in front of it to allow it to walk onto the brush. Alternatively, pick up the paper towel or piece of wood with termite(s) and gently tap the termite(s) into the small cup.
- Tip the small cup over to gently drop the termites into the "arena" (paper with drawn paths/shapes).
- Once the termites are on the paper, let them be for the duration of the trial! **Do not continuously move them back toward the path/shape drawn on the paper.** Doing so would bias the results of the experiment and tire the termites or increase the risk of harming them.

- Caveat: termites may venture off the paper altogether (though this is rare). We do not want to have loose termites escaping into the classroom, so gently put these termites back on the paper or into the small cup.
- If a termite is unresponsive or not moving, feel free to swap it out for a more active one.
- Limit using a given termite for two or three trials to prevent fatigue.
- Return the termites to the large bowl they were collected from.

Clean up/Disposal

1. Gently return the used and tired termites to the labeled bin.
2. Return all writing utensils to their matching locations.
3. Dispose of paper in the trash.
4. Return tray to the original location.

PROCEDURE 2: ANALYZING QUESTIONS & GRAPHS

After running the experiments and collecting data, the next step is to analyze the data to see whether there are differences between the independent variables. One of the more common statistical tests is a t-test. There are many online t-test calculators, but the [t-test calculator by GraphPad](#) is a great tool (there is also statistical software like Excel or R).

1. Use a t-test to test for differences between the independent variables for both experiments and report the results in the Answer Sheet.
 - If you're using the GraphPad website, keep boxes 1 and 2 on the default settings, enter the data into box 3, and click Calculate Now in box 4.
 - Refer to the Introduction to determine where the results are statistically significant.
2. Now interpret and graph the results. Make two graphs, one for each experiment. Remember to include all the necessary components of a graph. Refer to the Introduction for instructions.

Remember that science is iterative. Whether or not your results are statistically significant or support your hypothesis, there are always more questions you could ask! After doing two experiments, what else are you curious about with the termites?

Oftentimes in science, things do not go as planned. This is especially true in biology where we collect data on live organisms, including humans! We may think we designed the perfect experiment, but then when we carry it out, maybe the organisms do not “cooperate” or behave expectedly, equipment does not work as planned, we run out of time, or the results do not give us the answer we had hoped for. Think through these options to answer the remaining questions in the Answer Sheet.

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CHAPTER OVERVIEW

3: Lab 3 Biomolecules

[3.1: Lab 3 Background](#)

[3.2: Lab 3 Procedures](#)

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3.1: Lab 3 Background

Learning Objectives

- Explain the functions of the four main groups of biomolecules.
- Identify the monomers of biomolecules.
- Explain how enzymes catalyze chemical reactions between specific substrates.
- Design experiments to test for the presence of biomolecules and enzymatic reactions.
- Describe and graph the results of experiments.

Introduction: Biomolecules

The large molecules necessary for life that are built from smaller molecules are called **biological macromolecules**, or **biomolecules** for short. The four major classes of biomolecules are carbohydrates, lipids, proteins, and nucleic acids. Each is an important component of the cell and performs a wide array of functions. They typically contain carbon, hydrogen, oxygen, nitrogen, as well as phosphorus, sulfur, and other minor elements. Most biomolecules are called **polymers** and are built from repeating segments called **monomers** (the prefix mono means one and the prefix poly means many). We can think of monomers as legos and polymers as the structure built from legos, like a castle or spaceship. Today, we will explore the four classes of biomolecules and figure out how to determine whether the biomolecules are in a solution.

Carbohydrates

Carbohydrates are a diverse class of macromolecules with which we are probably rather familiar. One of the most common examples of a carbohydrate is **glucose**, which can be found in just about every cell in every organism. Glucose is a simple **sugar** that can be broken down easily to provide energy for the organism. There are many other examples of energy-providing carbohydrates, as well as others with different functions.

Carbohydrates consist of carbon, hydrogen, and oxygen in a 1:2:1 ratio, meaning that for every one carbon atom, a carbohydrate has two hydrogen and one oxygen atoms. It is helpful that the name carbohydrate itself reminds us of this ratio. Think of carbo- as referring to a carbon atom and hydrate as in water, or H₂O (two hydrogens and one oxygen).

Broadly, carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides (mono = one; sacchar = sweet) are simple sugars whose names end with the suffix “-ose”, the most common of which is glucose. The chemical formula for many monosaccharides, including glucose, is C₆H₁₂O₆ (which matches the 1:2:1 ratio described above). In most living species, glucose is an important source of energy. During cellular respiration, energy is released from glucose and that energy is used to help make adenosine triphosphate (ATP). During photosynthesis, energy from sunlight converts carbon dioxide and water into glucose. Other example monosaccharides include fructose (as in high fructose corn syrup) and galactose.

Disaccharides (di = two) form when two monosaccharides are covalently bonded together. Common disaccharides include lactose, maltose, and sucrose. **Lactose**, the sugar found in milk, is a disaccharide consisting of the monomers glucose and galactose. The most common disaccharide is **sucrose**, or table sugar, which is composed of the monomers glucose and fructose.

Polysaccharides (poly = many) are long chains of monosaccharides linked by covalent bonds. Polysaccharides may be very large molecules, including starch, glycogen, cellulose, and chitin. Plants store sugar in the form of **starch**, which is made up of many molecules of glucose monomers. Similarly, animals, including humans, store sugar in the form of **glycogen**, which is also made up of many glucose monomers. Glycogen is a highly branched molecule usually stored in liver and muscle cells. Whenever glucose levels decrease, glycogen is broken down to release glucose. Also, when animals consume plants or other animals, the starch or glycogen gets broken down into the glucose building blocks. **Cellulose** is one of the most abundant natural **biopolymers**, built from many glucose monomers. The cell walls of plants are mostly made of cellulose, which provides structural support to the cell. In fact, wood and paper are mostly cellulose.

Lipids

Lipids are a diverse group of biomolecules that include fats, oils, waxes, phospholipids, and steroids. Lipids are not true polymers because there is not one kind of monomer that bonds together to make all lipids (unlike how all polysaccharides are made up of many monosaccharides). Lipids are nonpolar molecules, which makes them hydrophobic (“water-fearing”) and insoluble in water. Lipids perform many different functions in a cell.

For example, **fats** are a common lipid that store energy for long-term use. In fact, fats are far more efficient at storing energy than sugars, containing about twice the energy in only one-sixth the volume of equal weight of carbohydrate! A fat molecule, such as a triglyceride, consists of two main components—glycerol and **fatty acids**. Fatty acids are commonly mistaken as a monomer because they are found in many different kinds of lipids. All fatty acids have a carbon “backbone” with several carbon atoms bonded together along the middle of the molecule. Depending on the chemical structure of the fatty acid, it is classified as either saturated or unsaturated.

Saturated fatty acids tend to have a *straight* chain of carbon atoms, each of which only have *single bonds* between neighboring carbons. They are saturated with hydrogen; in other words, the molecule has the most hydrogen atoms it could possibly hold. Because of this, saturated fatty acids are tightly packed and tend to be *solid* at room temperature, such as *animal fats* contained in meat and the fat contained in *butter*.

Unsaturated fatty acids contain at least one *double bond* between carbons. The double bond causes a *bend* or a “kink” that prevents the fatty acids from packing tightly, keeping them *liquid* at room temperature. *Fish oil* is one example, but most unsaturated fatty acids are plant-based, including *vegetable oils* such as olive oil, corn oil, and canola oil.

Phospholipids are the main component of **cell membranes**. One property that allows them to make an effective plasma membrane is that they have both **hydrophobic** and **hydrophilic** regions. They have fatty acid chains that are hydrophobic and a phosphate group that is hydrophilic. Cells are surrounded by a phospholipid **bilayer**, where the fatty acids face each other, away from water, whereas the phosphate groups can face either the outside environment or the inside of the cell, which are both aqueous.

Unlike the phospholipids and fats discussed earlier, **sterols**, such as cholesterol, steroids, and hormones, have a ring structure. This ring structure separates them from the other lipids, though they are still hydrophobic. **Cholesterol** is mainly synthesized in the liver and is the precursor of many **steroid hormones**, such as testosterone and estradiol. It is also the precursor of vitamins E and K.

Waxes are made up of a hydrocarbon chain with an alcohol (–OH) group and a fatty acid. Examples of animal waxes include *beeswax* and cerumen (*earwax*). Plants also have waxes, such as the coating on their leaves that helps prevent them from drying out.

Proteins

Proteins are one of the most abundant molecules in living systems and have the most diverse range of functions of all macromolecules. Proteins may be structural, regulatory, contractile, or protective; they may serve in transport, storage, or membranes; or they may be toxins or enzymes. Enzymes are a common kind of protein, which we will explore later on in this lab. Each cell in a living system may contain thousands of different proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, polymers of **amino acids** (which are the monomers).

While there are many tens of thousands of proteins that exist, there are just 20 different amino acid monomers! We can think of the amino acids like the 26 letters of the English alphabet and proteins like the many thousands of words the letters (or amino acids) make. Each amino acid has the same fundamental structure, which consists of a central carbon atom bonded to an **amino group** (–NH₂), a **carboxyl group** (–COOH), and a hydrogen atom. However, what sets them apart from each other is a variable group bonded to the central carbon atom known as the **R group**. The R group is the only difference between the 20 amino acids; otherwise, the amino acids are identical.

The sequence and number of amino acids ultimately determine a protein’s shape, size, and function (like the sequence and number of letters differentiating words). Each amino acid is attached to another amino acid by a covalent bond, known as a **peptide bond**. Thus, the polymer is called a **polypeptide chain**. Before a protein becomes fully functional, the polypeptide chain needs to fold into a unique shape. To understand how the protein gets its final shape, or conformation, we need to understand the four levels of protein **structure**: primary, secondary, tertiary, and quaternary.

The **primary structure** is the unique sequence and number of amino acids in a polypeptide chain. The unique sequence for every protein is ultimately determined by the gene that encodes the protein. The **secondary structure** describes local folding, including the β -sheet (β = “beta”), and the α -helix (α = “alpha”). The **tertiary structure** describes global folding, where distant sides of the polypeptide chain interact to produce a unique three dimensional shape. Some proteins are fully functional after the tertiary structure takes shape, but others have a **quaternary structure** where multiple polypeptide chains bond together. When proteins are exposed to high temperatures, high salt concentrations, or extreme pH, their three dimensional shape breaks down, or **denatures**. Proteins cannot recover from being denatured and will no longer function.

Nucleic acids

Nucleic acids are key macromolecules in the continuity of life. They carry the genetic blueprint of a cell and instructions for the functioning of the cell. **Nucleic acids** are made up of monomers called **nucleotides**. Each nucleotide is made up of three components: a nitrogenous base attached to a sugar molecule, which is attached to a phosphate group. The nucleotides bond to each other to form the polynucleotides deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). DNA is the genetic material found in all living organisms, from single-celled bacteria to multicellular mammals. RNA is mostly involved in protein synthesis. The DNA molecules never leave the nucleus, but instead use an RNA intermediary to communicate with the rest of the cell.

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3.2: Lab 3 Procedures

PROCEDURE 1: PRACTICE WITH BIOMOLECULES

1. Before moving on to the experiments for today, complete Table 1 on the Answer Sheet to review the differences between the four classes of biomolecules.

PROCEDURE 2: POSITIVE AND NEGATIVE CONTROLS

Today we will be designing an experiment to test for the presence of proteins (in a solution of albumin, a.k.a. egg whites), carbohydrates (glucose & starch), and lipids in a mystery food item. To test for the presence of these biomolecules, we will be using reagents and solutions called indicators. Indicators produce a physical change, often a change in color, in a solution when mixed with a particular molecule. We will be using **iodine potassium iodide (IKI; iodine for short) solution**, **Biuret's reagent**, and **Benedict's solution**. But in order to determine which molecule they test for (protein, glucose, or starch), we have to set up an experiment. Then, we can use the indicators to test for the presence of these molecules in the mystery food item.

For certain experiments, there are two types of control groups - positive and negative. The **positive control** is a solution where we expect to see a positive result (in this case color changing). The **negative control** is a solution where we expect to see a negative result (in this case no color change or no difference at all). Follow the procedures below to create positive and negative controls for each of the biomolecules and determine the biomolecules that each indicator tests for.

Equipment we'll need:

- 12 test tubes and a test tube clamp
- Two test tube racks
- Grease pencil
- Iodine potassium iodide (IKI) solution, Benedict's reagent, and Biuret's reagent
- Flasks of water, glucose, starch, egg whites, dropper bottles with lipids, and the mystery food
- Two small paper towel squares
- Two 400 mL or 600 mL beakers
- One hot plate with beaker of water

Before we start this experiment: Fill a 600 mL beaker with about 400 mL of tap water. Put the beaker on the hotplate and set the heat to about level 5. We will need this water on the hotplate for the duration of the lab today, so if the beaker gets low on water, fill it back up.

- **Do not let the water in the beaker completely evaporate!** An empty beaker on a hotplate will break and may send shards of glass shooting across our desk!

Lipids

The following experiment will set up a positive and negative control for lipids.

1. Obtain two small paper towel squares. With a pen, label each square "L" or "W".
2. On the "L" square, put a single drop from the lipid dropper bottle.
3. On the "W" square, put a single drop of water.
 - We may need to gently dab the drop of lipid or water into the paper using a finger.
4. Set them aside. In 30 minutes, hold them up to the light and see if there is a stain.

Iodine solution

The following experiment will determine which biomolecule the iodine solution tests for.

1. Obtain four test tubes. Use a grease pencil and ruler to mark lines 1 cm and 2 cm from the bottom of each test tube. Label the test tubes 1-4, where the numbers refer to:
 - 1 = Albumin
 - 2 = Glucose

- 3 = Starch
 - 4 = Water
2. Fill each tube with the **iodine solution** to the 1 cm line.
 3. Fill each with its corresponding solution to the 2 cm line.
 - For example, fill tube 1 with albumin to the 2 cm line, tube 2 with glucose to the 2 cm line, etc.
 4. Swirl each tube to mix the contents.
 5. Record the color of each tube in Table 2 on the Answer Sheet.

Biuret's reagent

The following experiment will determine which biomolecule the Biuret's reagent tests for. We will repeat the same steps as above.

1. Obtain four test tubes. Use a grease pencil and ruler to mark lines 1cm and 2cm from the bottom of each test tube. Label the test tubes 1-4, where the numbers refer to:
 - 1 = Albumin
 - 2 = Glucose
 - 3 = Starch
 - 4 = Water
2. Fill each tube with the **Biuret's reagent** to the 1cm line.
3. Fill each with its corresponding solution to the 2cm line.
4. Swirl each tube to mix the contents.
5. Record the color of each tube in Table 2 on the Answer Sheet.

Benedict's reagent

The following experiment will determine which biomolecule the Benedict's reagent tests for. **Note the difference in step 4!**

1. Obtain four test tubes. Use a grease pencil and ruler to mark lines 1cm and 2cm from the bottom of each test tube. Label the test tubes 1-4, where the numbers refer to:
 - 1 = Albumin
 - 2 = Glucose
 - 3 = Starch
 - 4 = Water
2. Fill each tube with the **Benedict's reagent** to the 1 cm line.
3. Fill each with its corresponding solution to the 2 cm line.
4. Put each test tube into the beaker with hot water on our hot plate for 5 minutes.
 - Make sure there is enough water in our beaker.
5. After 5 minutes in the hot water bath, record the color of each tube in Table 2 on our Answer Sheet.
 - The test tubes will be very hot! Use the test tube clamp to move the test tubes from the hot water bath to the test tube racks.

Clean up/Disposal

1. Pour the solutions from the test tubes into an empty 400 mL beaker.
2. Pour the beaker into the hazardous waste container next to the sink.
3. Rinse the test tubes and beaker thoroughly in the sink.
4. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
5. Put the clean test tubes in the metal box next to the sink and take the beaker back to the desk.

PROCEDURE 3: MYSTERY FOOD ITEM

Based on the results from Procedure 3, we should now know which biomolecules each indicator tests for and what the positive and negative controls look like. Now, we are going to design an experiment to find out which biomolecules are in a mystery food item.

Equipment we'll need:

- 3 test tubes and a test tube clamp
- Test tube rack
- Iodine solution (IKI), Benedict's and Biuret's reagents, and a paper towel square
- One hot plate with beaker of water

Before we start: Make sure there is enough water in the beaker on the hotplate.

1. Answer questions in the Answer Sheet to build this experiment.
2. Obtain a paper towel square. Place a single drop of the mystery food solution on the square. Set aside and review in about 30 minutes.
3. Obtain three new test tubes. Use a grease pencil and ruler to mark lines 1 cm and 2 cm from the bottom of each test tube. Label the test tubes 1-3, where the numbers refer to:
 - 1 = Iodine solution
 - 2 = Biuret's reagent
 - 3 = Benedict's reagent
4. Fill each tube with the appropriate chemical indicator to the 1 cm line.
5. Fill each test tube with the mystery food solution to the 2 cm line.
6. Swirl each tube to mix the contents. Place test tube #3 with Benedict's solution into the hot water bath.
 - Do not put test tubes #1 or #2 in the hot water bath.
7. Record color changes and answer questions in the Answer Sheet.

Clean up/Disposal

1. Pour the solutions from the test tubes into an empty 400 mL beaker.
2. Pour the beaker into the hazardous waste container next to the sink.
3. Rinse the test tubes and beaker thoroughly in the sink. Using a wire brush remove any mystery food remnants from the bottom of the test tubes.
4. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
5. Put the clean test tubes in the metal box next to the sink and take the beaker back to the desk.

Introduction: Enzymes

Many chemical reactions can occur in a beaker or test tube. Generally, these reactions either need a large amount of time to occur or need to be heated to high temperatures. When thinking about biology, life does not have large amounts of time to digest its food for energy and life cannot be heated to high temperatures. So for cells to be able to perform chemical reactions at a reasonable rate, life needed to evolve something to help. The result was enzymes. **Enzymes** are a type of protein that speed up chemical reactions. Their three dimensional shape is essential to their ability to speed up reactions.

Enzymes have a special pocket called the **active site** where the reactant(s), called **substrate(s)**, connect or bind. Due to the specifically shaped active site, only certain substrates can fit. Once the substrate binds to the active site, the enzyme will either help build or break a chemical bond, but the enzyme itself is unchanged. Then, when the new molecule(s) are released, another substrate enters the enzyme and the process continues. When we write chemical equations, we put the name of the enzyme on top of the arrow to show that it is involved but it is not changing like the substrates are changing into products.

Today we will be performing an experiment on the enzyme catalase. **Catalase** breaks down the molecule hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen gas (O_2). When oxygen gas is present in liquid, it forms bubbles. Catalase is found in nearly every single organism, such as in the human liver or in potatoes. We will use blended potatoes as a source of catalase. During the experiment, the oxygen gas produced by this chemical reaction will be visible as bubbling (and large amounts of it). We will be designing an experiment to answer the question "how does temperature affect the function of an enzyme?" Hint: recall that shape is essential to the enzyme functioning properly and that various environments could cause the folding of the enzyme protein to fall apart thus making the enzyme non-functional. We will be measuring and comparing the amount of bubbling to determine whether the enzyme catalase is still working.

PROCEDURE 4: ENZYME EXPERIMENT

Part 1: Positive and negative controls

Just as we did with the biomolecules experiments, we will set up positive and negative controls for the enzyme catalase. Positive and negative controls are important to not only compare future, unknown results to, but also to verify that the materials are working correctly. Here, we will test that the enzyme, substrate, and indicator do what they should and we will have positive and negative results to compare our experiment to.

Equipment we'll need:

- 2 test tubes
 - Test tube rack
 - Potato juice (as a supply of catalase), hydrogen peroxide, and water
1. Answer the questions in the Answer Sheet prior to setting up this experiment.
 2. Label 2 glass tubes with a grease pencil. Label one with "+" and the other with "-" at the top.
 3. Measure 2cm from the bottom of each tube and draw a line. Mark another line on each tube at 5cm.
 4. Fill both test tubes up to the 2cm line with the Catalase enzyme solution (potato juice).
 5. Fill the "+" tube to the 5cm line with hydrogen peroxide.
 6. Fill the "-" tube to the 5cm line with pure water.
 7. Swirl both tubes carefully to mix for 10 seconds.
 8. After 3 minutes, measure the height of the "bubble column" in centimeters (cm)- from the top of the liquid to the top of the bubbles.
 9. Record the height of the bubble columns in Table 4 on the Answer Sheet.

Part 2: Perform the experiment

Equipment we'll need:

- 3 test tubes
 - Test tube rack
 - Potato juice and hydrogen peroxide
 - One hot plate with beaker of water
1. Answer the design questions in the Answer Sheet to build an experiment looking at temperature's effect on enzymes.
 2. Obtain three test tubes. Number these tubes 1-3 using a grease pencil. Measure and mark lines for 2cm and 5cm from the bottom of each tube.
 3. Fill all three tubes with potato juice up to the 2 cm line. Apply the independent variable at this time. Allow the independent variable to affect the enzymes in the potato juice for at least *10 minutes*.
 4. Retrieve the test tubes and put them into the test tube rack. IMMEDIATELY add hydrogen peroxide up to the 5 cm line and swirl for 10 seconds.
 - It is important to add the hydrogen peroxide right away before the different temperature treatments do not return to room temperature.
 5. After 3 minutes, measure the amount of bubbles in the tube.
 6. Record the height of the bubble columns in Table 5 and answer the questions in the Answer Sheet.

Clean up/Disposal

1. Pour the solutions from the test tubes down the sink and rinse the test tubes thoroughly. Use a wire brush to remove any potato remnants from the bottom of the test tubes.
2. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
3. Put the clean test tubes in the metal box next to the sink and place the beaker on the towel to dry.

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CHAPTER OVERVIEW

4: Lab 4 Microscopes and Cells

4.1: Lab 4 Background

4.2: Lab 4 Procedures

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4.1: Lab 4 Background

Learning Objectives

- Identify the name and function of the microscope parts.
- Focus the microscope in low and high power without damaging the microscope or the slide.
- Adjust the contrast of a specimen.
- Calculate total magnification of a specimen.

Introduction: Microscope Safe Usage & Handling

In biology we make many observations of the world around us using our eyes. In the seventeenth century, the invention of a more powerful microscope by Anton van Leeuwenhoek allowed scientists to see that the living world is smaller than the eye can perceive. Van Leeuwenhoek could see single celled organisms such as bacteria and protists. Using his microscope, he discovered red blood cells and studied insects and almost anything else that could be placed under his lens. Since then, biologists have improved upon this tool and continue to use it to answer questions about the smallest aspects of life.

The microscopes in our lab are very sensitive (and expensive) and need to be handled with care. Please follow the guidelines below every time we use a microscope.

Always hold compound microscopes with **two hands**! One hand holding the side arm, the other hand should be under the base/bottom. **Never tilt** the microscope while working with wet mounts (this may cause major damage to the microscope). If the microscope needs to be moved or adjusted on the table, **pick it up with two hands and move it**; do not try to drag it.

Microscope lenses should be cleaned every time the microscope is used. **The only proper way to clean the lenses is with lens paper and the Windex solution.** Never use water, paper towels, clothing, or anything else, as these may damage and scratch the lenses.

Avoid smudging the lenses by keeping our eyes a half inch away from the ocular lens, and by keeping fingers away from the objective lenses.

The compound microscopes are stored with the **scanning objective in position**, the power switch off, the electrical cord loosely wrapped around the base, and the plastic **dust cover** placed over the microscope. Each time we use a microscope, we need to write our name on the checkout sheet and indicate which cabinet our microscope was in. Match the letter/number on the desk with the corresponding cabinet. Use the key to open the cabinet to obtain the microscope, but then return the key to the wall during lab so as not to lose it. After lab, return the microscope (after cleaning it according to the instructions above) to the same cabinet it was removed from and cross off your name from the checkout sheet.

Slide Cleaning

Prepared slides must be cleaned with lens paper and be fingerprint free. Wet mount slides should be rinsed with water, dried with a paper towel, and returned to the container. Coverslips should be removed from the slide and thrown in the trash. Do not leave any slides or coverslips in or around the sink area.

Prepared slides are issued from the slide boxes at the front of the lab room. They have a label on the left side and an attached coverslip in the middle. These slides must be checked for breakage before starting the lab exercise. Report slides that have breaks in the glass slide or the coverslip over the specimen to the instructor. Do not report slides that have a crack in the coverslip if there are initials on the label. This indicates that the slide is still usable and that the crack has been reported.

Compound Microscope

There are two types of microscopes we have in class. Microscopes are pieces of equipment that allow us to see incredibly small things. A microscope will contain a **lens**, which is a small piece of glass that reflects light and our eye interprets the image as larger than it really is (i.e. **magnified**). For a **compound microscope**, the image of the specimen is magnified by two lenses by the time it reaches our eye. There are the objective lenses in the center of the microscope and the ocular lenses at the top that we look into. To

calculate how much the image has been magnified, the objective magnification is multiplied by the ocular magnification using the formula below.

$$\text{Total Magnification} = \text{Objective Magnification} \times \text{Ocular Magnification}$$

It is important to become familiar with the microscopes used in this course. Below is a list of microscope parts and their functions.

- The **base** of the microscope is flat and rests on the table.
- A **light source** is at the base of the microscope and directs light up through the specimen into our eyes.
- The **power switch** is on the rear of the microscope to turn the light source on and off.
- A **brightness control dial** changes the brightness of the light source.
- Above the light source is the **stage** with **stage clips** that hold the microscope slides in place.
- On the right of the stage are **stage control knobs**; one knob moves the slide forwards or backwards, and the other moves the slide right or left.
 - Always use the stage control knobs to adjust the position of the slide on the stage. Never use your hands to adjust the slide on the stage.
- Under the stage are the condenser, which focuses light onto the specimen, and condenser/iris diaphragm, which slides left and right to control the contrast and depth of field of the specimen.
 - *Opening* the condenser/iris diaphragm (sliding it to the left) creates a *low contrast* image with shallow depth of focus.
 - It may be difficult to see small details, and we will be focused on only a thin section.
 - The image will become very bright, so *turn down the brightness* control.
 - *Closing* the condenser/iris diaphragm (sliding it to the right) creates a *high contrast* image with a deep depth of focus.
 - Small details will be more visible, and a thick layer of the specimen will be in focus.
 - The image will become very dark, so *turn up the brightness* control.
 - Closing the condenser/iris diaphragm is the best way to find the specimen when we begin: the high contrast and deep depth of focus make it easier to see small objects.
- The **eyepiece** or **ocular lens**, which magnifies an image 16 times (16x), is found on the viewing tube.
 - When we look through the ocular lens, we will see a circular area with light called the **field of view** (see Fig. 1).
- Above the stage are 4 **objective lenses** on the **revolving nosepiece**.
 - The **scanning objective** with the red stripe is the shortest and magnifies 4 times (4x).
 - This is the objective we will *use first* to focus our specimen before switching to the other objectives.
 - This is also the objective that should be in place when we take out and store the microscope.
 - The **low power objective** with the yellow stripe magnifies 10 times (10x).
 - The **high power objective** with the blue stripe magnifies 40 times (40x).
 - The **highest power objective** with the green stripe magnifies 60 times (60x).
 - We should hear a click when we turn the nosepiece to switch from one objective to the next.
 - Examine Figure 1 and notice the distance between the end of the objective lens and the stage. This is the **working distance**.
- The **arm** connects the base to the rest of the microscope.
 - To carry the microscope, grasp the arm with one hand and place the other hand under the base.
- The focus knobs are on the arm. The **coarse focus knob** is larger and moves the stage up and down by large amounts, which is used to bring the specimen into approximate focus.
 - This knob is *only used with the scanning objective!*
 - We should *NEVER use the coarse focus knob while the low, high, or highest power* (10x, 40x, or 60x) objectives are in place. This could damage the lens and break the slide.
- Extending from the coarse focus knob is the smaller **fine focus knob** which is used to bring the specimen into fine focus and make minor adjustments.
 - Use this knob *only* when using the low power, high power, and highest power objectives.
- The **power switch** is on the side of the arm next to the power cord.

- Always turn the microscope off with the power switch before unplugging it
- **Tip:** If you wear glasses, remove them before looking into the microscope! It may seem counterintuitive, but the point of the focus knobs is to get the specimen into focus. So, no matter what your prescription is, you can focus the specimen without your glasses.

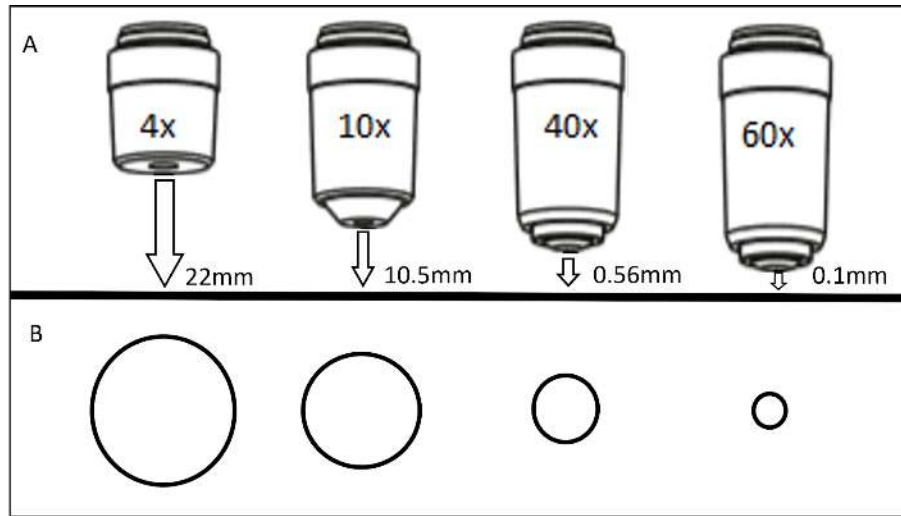


Figure 1. A.) The **working distance** between the objective lenses and the stage. B.) Representation of the diameter of the **field of view** for each objective lens.

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4.2: Lab 4 Procedures

PROCEDURE 1: PARTS OF THE COMPOUND MICROSCOPE

Equipment we'll need:

- Compound microscope (one per person)
1. On the Answer Sheet, label the diagram of the microscope. Use the list of keywords as a guide.

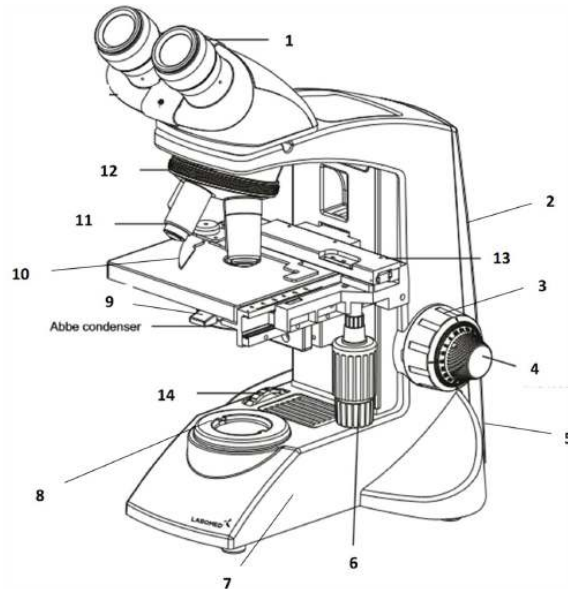


Figure 2. Labomed CxL, Compound microscope.

2. Use the formula above to complete Table 1 in the Answer Sheet.

PROCEDURE 2: FOCUSING THE COMPOUND MICROSCOPE

Equipment we'll need:

- Compound microscope
- Prepared slide of the letter “e”

Examining specimens with the microscope can take some practice. Follow the procedures below to become familiar with how to properly focus on specimens under the microscope.

Low Power

1. Obtain a “letter e” slide. Observe the orientation of the “e” on the slide before putting it on the microscope stage and record it in Answer Sheet.
2. Obtain the microscope. Use lens paper to gently wipe clean the **ocular lens** and the **objective lenses**.
3. If necessary, rotate the **nosepiece** until the **scanning objective** is in position. Use the coarse focus knob to lower the stage all the way.
4. Pull the stage clip lever handle to open the bow shaped **stage clip**, place the prepared slide of the letter “e” on the **stage**, move the slide toward the back of the stage until it sits snug, and release the stage clip.
 - The slide should be flat on the stage and the stage clip should be resting against the corner of the slide (not on top of it).
5. Use the **stage control knobs** to center the letter “e” over the circular opening above the condenser.
6. Use the **coarse focus knob** to raise the **stage** all the way up.
7. While looking through the **ocular lens**, slowly turn the coarse focus knob to lower the **stage**. Stop turning the coarse focus knob when the specimen comes into view. Slightly adjust the focus until the specimen is clear.

8. Adjust for best illumination by adjusting the **brightness control dial** and/or the **condenser diaphragm**. See tips above.
9. Using the **stage control knobs**, move the stage to the left. What direction does the letter e move while looking through the oculars?
 - Move the stage away from the arm. What direction does it move while looking through the oculars?
10. Center the specimen directly in the middle of the **field of view**.
11. Once the specimen is in focus, centered, and the illumination is right, rotate the nosepiece until the **low power objective** is in position. We should hear a click.
12. Use the **fine focus knob** to adjust for clarity.
 - Remember - do not use the coarse focus knob with objectives more powerful than the scanning objective!
13. Answer questions on the Answer Sheet.

High Power

In order to see the specimen under higher magnification, be sure that the specimen is in the very center of the field of view and adjusted for best clarity.

1. Rotate the nosepiece to the **high power objective**. We should hear a click.
2. Adjust for clarity and sharp focus using the **fine focus knob** only.
3. Adjust for proper illumination.

Tip: If we ever lose the specimen (i.e., cannot find it) while viewing under high power magnification, rotate the nosepiece back to the scanning objective to find it again. Then, re-focus the specimen and return to high power magnification.

4. Answer questions on the Answer Sheet.

PROCEDURE 3: MAKING A WET MOUNT SLIDE

The slide we just observed was prepared for us. However, we will need to prepare many of our own slides of specimens throughout the course. Care should be taken to avoid getting any liquids on the objectives or other areas of the microscope.

Equipment we'll need:

- Compound microscope
 - Clean slide and cover slip
 - Pond water
 - Sterile plastic pipette
1. Obtain a clean slide and cover slip. (Cover slips are the plastic squares; disregard the packing tissues.)
 2. Add just 1 drop of the pond water to the slide.
 - A little goes a long way! Adding more liquid runs the risk of having water drip off the slide and onto the microscope.
 3. Place one edge of the cover slip next to the water drop and slowly lower the cover slip on top of the sample. Be sure there is no water on top of the cover slip or leaking out from around the edges. Water will damage the objective lenses. Be sure the bottom of the slide is dry to avoid making a mess on the stage.
 - If there is any water outside the coverslip (especially if it is on the bottom of the slide), use a paper towel to gently wick away the water.
 4. Use the same procedure as with the "letter e" to gradually focus the specimens. Start with the scanning objective, then after the focus and illumination are right, move on to the stronger magnifications.
 - Here, it is especially important to get the illumination right. We will likely have to play with the brightness and contrast (using the brightness control dial and condenser diaphragm) until we see some organisms come into view.
 - If we don't see anything right away, and we're confident that we're focusing on the right area, use the stage control knobs to move the slide to see different spots in the water.
 5. Answer the questions in the Answer Sheet.
 6. Rinse the glass slide in the sink and set it on the towel next to the sink to dry. Throw the coverslip away in the trash.

Common cells/organisms we may see

Here are some examples of cells or microscopic organisms we may see in the pond water.

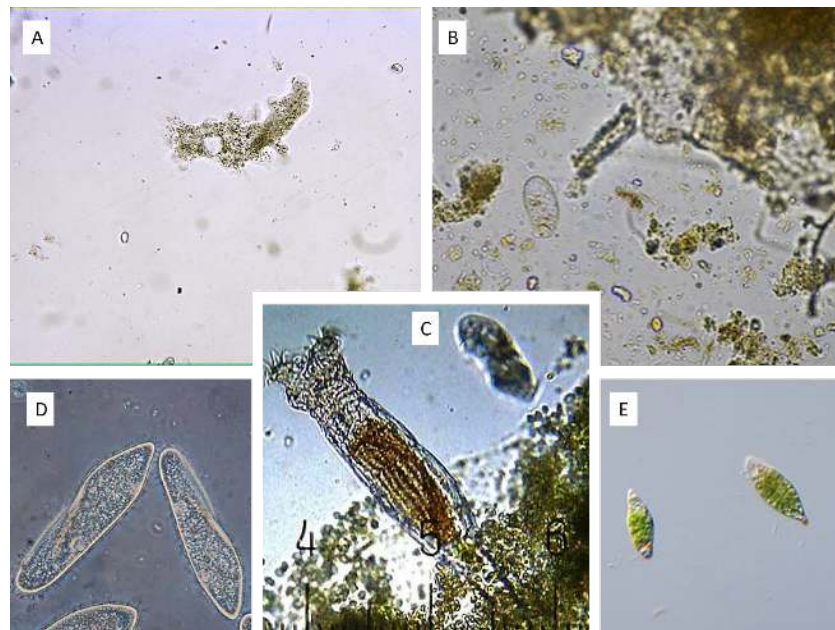


Figure 3. Examples of organisms we may see in pond water, including A.) *Amoeba* ([image source](#)), B.) assorted microorganisms ([image source](#)), C.) rotifer ([image source](#)), D.) *Paramecium* ([image source](#)), and E.) *Euglena* ([image source](#)).

Things often mistaken for cells

It is common for students learning to use the microscope to see various structures and mistake them for cells or microorganisms. Below are some examples. If we see these, keep looking! **These are not cells!**

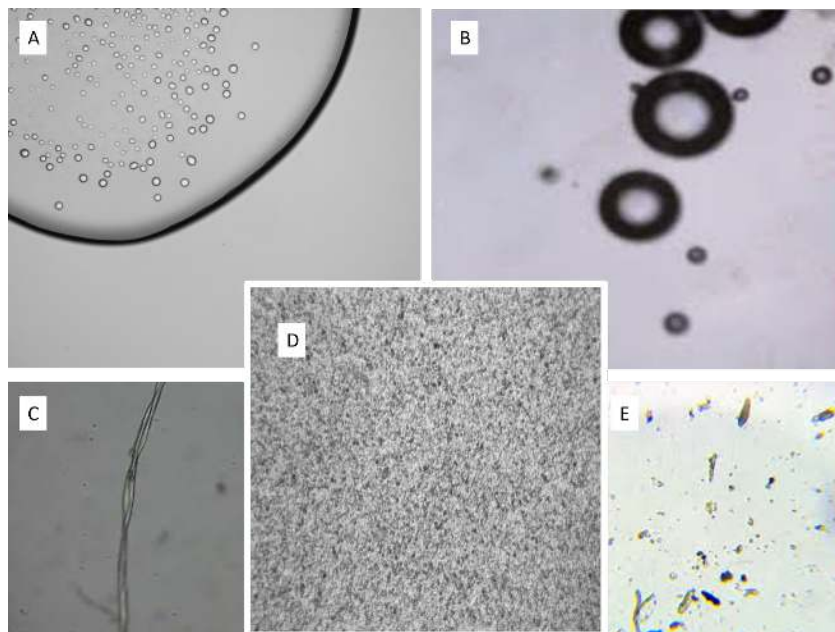


Figure 4. Examples of things mistaken for cells under the microscope. These include A-B.) air bubbles, C.) lint or a scratch in the slide, D.) the glass slide, and E.) dust (dry, dead skin cells). Image credit: Ernie Kwok.

PROCEDURE 4: HUMAN CHEEK EPITHELIAL CELLS

Now we get to look at some of our own human cells (and not so human cells) under the microscope! We are going to look at some cells from our cheeks, and along with them some bacteria that call our mouths their home. But our cheek cells are rather difficult to

see because there is no color to them, so first we have to stain them. The stain, called **methyl blue**, is very strong and can easily stain clothes, so be careful not to spill!

Equipment we'll need

- Clean glass slide and coverslip
 - Toothpick
 - Methylene blue
1. Place a small drop of methylene blue stain in the center of a clean glass slide.
 - Remember to be careful with the stain! Avoid spilling it, especially on any clothing. If any gets on our skin, wash with soap and water immediately.
 2. Use a clean toothpick to gently scrape the inside of your cheek.
 3. Carefully rub the toothpick in the stain on the slide to stain and deposit your cheek cells on the slide. Dispose of the toothpick in the trash can.
 4. Slowly lower a clear plastic coverslip on top of the sample.
 5. Be sure there is no stain on top of the cover slip or leaking out from around the edges. Any liquid will damage the objectives. Use a paper towel to dab away any excess stain.
 6. Focus the microscope in the same way as the previous procedures. Use either the low power (10x) or high power (40x) objective.
 7. Draw or take a picture of a few of the cheek cells and bacterial cells on the Answer Sheet.
 8. Rinse the glass slide in the sink and set it on the towel next to the sink to dry. Throw the coverslip away in the trash.

PROCEDURE 5: PLANT CELLS

Elodea is an aquatic, multicellular, eukaryotic plant that is commonly used in freshwater aquariums. Interestingly, the leaves are just one cell layer thick, making it very easy for us to observe individual cells. We will also observe the organelles that make these cells green.

Equipment we'll need:

- Clean glass slide and coverslip
 - *Elodea*
 - Forceps
1. Use forceps to remove a leaf from a sprig of *Elodea* and place it on a clean glass slide.
 2. Add a single drop of water over the leaf.
 3. Gently add a plastic cover slip. Be sure that the top and bottom of the slide are dry.
 4. Focus the microscope in the same way as the previous procedures. Use either the low power (10x) or high power (40x) objective.
 5. Draw or take a picture of a few of the plant cells on the Answer Sheet.

Clean up/Disposal

Microscope

1. Put the 4x objective lens in place over the stage.
2. Lower the microscope stage all the way down.
3. Remove slide from microscope.
4. Turn off the microscope.
5. Unplug the microscope and wrap the cord neatly around the base.
6. Cover the microscope and return to its appropriate cabinet. Then lock the cabinet and return the key.

Slides

1. Wipe down any prepared slides and return to the wooden slide holder by the front of the room.
2. Remove the coverslip from the wet mounts. Dispose of the coverslip in the trash.

3. Dispose of any large sample materials (such as the *Elodea* leaf) in the trash. Do not rinse this down the drain.
4. Rinse the slide clean. Scrub off any residue or stain left behind.
5. Set slides out to air dry on the towels.

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CHAPTER OVERVIEW

5: Lab 5 Physical Transport

5.1: Lab 5 Background

5.2: Lab 5 Procedures

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5.1: Lab 5 Background

Learning Objectives

- Explain how molecules move across a concentration gradient.
- Describe the process of diffusion and osmosis across a membrane.
- Predict the movement of molecules in hypotonic, hypertonic, and isotonic solutions.

Introduction: Diffusion and Osmosis

Each atom and molecule is always moving, whether in air, in a liquid, in a solid like the classroom desks, or within living cells. This movement is largely random, but sometimes we can predict the direction that molecules will move. A **concentration gradient** describes a situation in which one area has more molecules (high concentration) than another area (low concentration). For example, if we spray an air freshener at the front of the classroom, we create a concentration gradient where there is a high concentration of scent molecules at the front and a low concentration of scent molecules at the back of the classroom. **Diffusion** explains that the scent molecules will move with the concentration gradient, from *high* concentration to *low* concentration. In this case, molecules would diffuse from the front to the back of the classroom. Diffusion is a *passive* process because it does not use energy - molecules diffuse on their own. It is similar to watching a ball roll downhill - we don't need to push the ball, it just rolls on its own.

This same kind of movement occurs across cell membranes. However, membranes are considered **selectively permeable** because only certain substances can cross them. Small molecules, like O₂ and CO₂, can freely cross the cell membrane on their own in a process called **simple diffusion**. Similarly, molecules with a similar hydrophobic and nonpolar chemical structure to the membrane's phospholipid bilayer can also cross by simple diffusion. However, polar or charged molecules can only cross the membrane via **facilitated diffusion**, which requires different kinds of transport proteins.

Osmosis is the passive diffusion of water across a selectively permeable membrane. It happens in the same way as with diffusion, although we describe the direction of movement in a different way. To predict the direction in which water will move, we need to know the concentration of solutes (molecules dissolved in water, such as salt or sugar) in the water. Osmosis occurs from *low solute* concentration to *high solute* concentration (Figure 1). This may seem counterintuitive at first, but let's consider the relationship between solute and water concentration. If a glass of water is 10% salt, then it's 90% water (100-10=90). If another glass of water is 30% salt, then it's 70% water. Moving from low to high solute concentration is the same thing as moving from high to low water concentration.

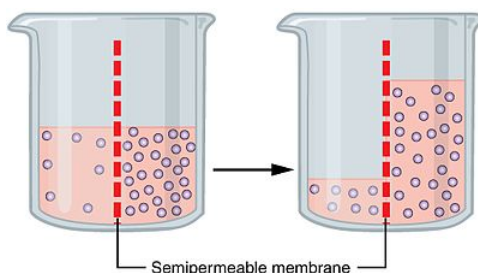


Figure 1. Osmosis across a semipermeable membrane. Water moves from low solute concentration (from left to right in the figure). Source: OpenStax Concepts of Biology.

Let's say we put a 10% salt solution and 30% salt solution on opposite sides of a membrane. In which direction should the water move? It moves from low solute concentration to high solute concentration, so it should move toward the 30% salt solution.

Diffusion and osmosis occur until the system reaches **equilibrium**, meaning that the concentration of substances is equal everywhere (i.e., there is no longer a concentration gradient). Remember that all molecules are always moving, so even though there is *no net movement* at equilibrium, molecules are still moving! They are just moving equally in all directions.

There are three different conditions that help to describe osmosis in and out of a cell. When a cell is in a **hypotonic** solution, there is a higher concentration of solutes inside the cell than outside the cell (hypo = below, like hypothermia). Therefore, water moves into the cell, making it swell up. In fact, animal cells may swell so much with water that they burst! Plant cells are protected by a

cell wall, so they simply stop taking in water when they get too full. When a cell is in a **hypertonic** solution, there is a higher concentration of solutes outside the cell than inside the cell (hyper = too much, like a hyper pet with too much energy). In which direction should water move, and what effect will that have on the cell? The water gets sucked out of the cell, causing it to shrivel up like a raisin. Lastly, when a cell is in an **isotonic** solution, there is no concentration gradient, meaning that the solute concentration inside the cell equals the concentration outside the cell. In other words, cells in an isotonic solution are at equilibrium. Remember that water is still moving in and out of the cell, but it is moving into the cell at the same rate it is moving out of the cell.

Different conditions can affect the rate of diffusion and osmosis. For example, molecules move faster at higher temperatures. If diffusion is based on the movement of molecules, how do we think temperature affects the rate of diffusion (how quickly diffusion can occur)? Other factors that affect the rate of diffusion include the density of the medium through which molecules move and the size of the molecules.

In today's lab, we are going to design experiments to explore the concepts of diffusion and osmosis. We will start by measuring the effects of different conditions on the rate of diffusion. Next, we will simulate diffusion across a membrane using dialysis tubing bags. Finally, we will use a potato to determine the effect of hypotonic, hypertonic, and isotonic solutions on osmosis in plant cells.

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5.2: Lab 5 Procedures

PROCEDURE 1: OSMOSIS IN POTATO CELLS

In this part, we get to test osmosis in real cells by putting pieces of potato in solutions with different concentrations of salt. We are going to measure and weigh potato slices before and after they go into different solutions to see whether there is a change.

Equipment we'll need:

- Three pieces of potato
 - Three test tubes, one each with 10% salt solution, 0.9% salt solution, or DI water
 - Ruler and electronic scale
1. Use a cork borer to make three potato tissue cores. Cut each core to a length of 4cm.
 2. On a paper towel, use a pen to write 10%, 0.9%, and DI water spaced apart. Put a potato core under each label to make sure we keep track of which is which.
 3. Weigh each core and record its starting mass in Table 3 on the Answer Sheet (make sure the electronic scale is tared to 0.0g first).
 4. Place the 10% core in the test tube with the 10% salt solution, the 0.9% core in the test tube with the 0.9% salt solution, and the DI water core in the test tube filled with DI water.
 5. Keep the potatoes fully submerged for at least one hour.
 6. Now that we're familiar with the experimental setup, let's make predictions before seeing the results. Answer the questions on the Answer Sheet.
 7. After at least an hour, remove the potato cores and gently dry them on a paper towel.
 8. Weigh each core and record its final mass in Table 3 (make sure the scale is tared first).
 9. Answer the remaining questions about this experiment on the Answer Sheet.

Clean up/Disposal

1. Throw away the used potato pieces in the trash.
2. Pour any remaining liquids down the drain.
3. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
4. Rinse test tubes and beakers. Place on the towel to dry.

PROCEDURE 2: DIFFUSION AND OSMOSIS ACROSS A MEMBRANE

A dialysis bag is a semipermeable plastic bag. While the plastic is chemically different from a phospholipid bilayer, it can act as a decent representation of a cell's membrane. Thus, the dialysis bag will simulate a cell in this experiment. Our job is to determine which substances (glucose, starch, iodine, and water) the dialysis bag is permeable to. Recall from our experiments last week that the iodine solution (IKI) is a chemical indicator that turns black or dark blue in the presence of starch.

Equipment we'll need:

- 1 dialysis tubing bag
 - 100mL beaker and 250mL beaker
 - DI water, iodine solution (IKI), 5% glucose solution, and 1% starch solution
 - 2 pieces of string
 - Paper towel
 - Electronic scale
 - Benedict's reagent, a test tube, and a hotplate
1. Fill the 250mL beaker with about 150mL of tap water. Place the beaker onto the hotplate and turn the hotplate on to about level 6 to create a hot water bath that is close to boiling. Keep an eye on this and turn down the heat/refill with more water as needed.
 2. Fill the 100mL beaker with 50mL of DI water.
 3. Add about 2mL of iodine solution (the pipette cap in the iodine bottles is about 1mL) to the DI water and gently swirl to mix. The beaker should be a light amber/golden color.

4. Obtain one pre-soaked dialysis bag and cut two pieces of string. Fold over the end of the dialysis bag and use one of the strings to tie a tight knot over the fold to close off that end.
5. Open the other end of the dialysis bag and fill the bag with 15mL of the starch and glucose solution.
6. Fold over the other end of the dialysis bag and use the other string to tie a tight knot across the top of the dialysis bag.
 1. We should now have a dialysis bag filled with starch and glucose solution sealed on both ends with string. Check for leaking and re-tie if necessary.
7. Use DI water to rinse off the outside of the dialysis bag over the sink. We want to make sure there is no starch or glucose on the outside of the bag.
8. Use a paper towel to dry the dialysis bag.
9. Place the dialysis bag on the electronic scale to measure its mass in grams. Record the initial mass on the Answer Sheet.
10. Now that we're familiar with the experimental setup, let's make predictions before seeing the results. Answer the design questions on the Answer Sheet.
11. Set the dialysis bag into the beaker with iodine solution. Let it sit for at least 30 minutes.
12. After 30 minutes or more, remove the dialysis bag from the beaker and pat it dry with paper towels.
13. Once again, place the dialysis bag on the electronic scale to measure its mass. Record the final mass on the Answer Sheet.
14. Record the color of the beaker solution and the solution inside the bag in Table 2 on the Answer Sheet.
15. Use a ruler to mark a 1cm line and 2cm line with a grease pen on the test tube.
16. Pour the solution in the 100mL beaker into the test tube up to the 1cm line.
17. Pour Benedict's solution into the test tube up to the 2cm line.
18. Place the test tube into the hot water bath for 5 minutes. Record the color in Table 2 on the Answer Sheet.
19. Complete Table 2 and answer the follow-up questions in the Answer Sheet.

Clean up/Disposal

1. Throw away the used dialysis bags in the trash.
2. Pour liquids into the hazardous waste container by the sink.
3. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
4. Rinse test tubes and beakers. Place on the towel to dry.

PROCEDURE 3: DIFFUSION IN DIFFERENT CONDITIONS

We are going to test the rate of diffusion through agar, a semisolid jelly-like substance, in different conditions. The agar is semipermeable, allowing some substances to diffuse through it. There are several things that affect the rate of diffusion, including temperature, density of the medium, size of the molecules, and amount of molecules. For example, we have two dyes available for today's lab with very different molecular mass: potassium permanganate (KMnO_4) is 158.03 g/mol and methylene blue is 319.85 g/mol. Our job for this experiment is to find out exactly *how* one of those factors affects diffusion.

Equipment we'll need:

- Two plates with agar per group
- Plastic straw
- Dye (such as potassium permanganate, KMnO_4)

1. Work through the questions on the Answer Sheet to set up this experiment. Each group will choose their own independent variable to test.
2. Obtain 2 agar filled petri dishes. Using a straw, poke and remove one hole in the center of each petri dish.
3. Into the hole, add _____ drops of dye(s), such as potassium permanganate (KMnO_4).
4. Place the dish in the group's specific condition.
5. Allow diffusion to happen for _____ minutes.
6. Record the results in the Answer Sheet.

Clean up/Disposal

1. Throw away the used agar plates into the white and green bucket.
2. Return used straws to the beaker.

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CHAPTER OVERVIEW

6: Lab 6 Photosynthesis

6.1: Lab 6 Background

6.2: Lab 6 Procedures

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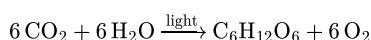
6.1: Lab 6 Background

Learning Objectives

- Explain ways of measuring and looking for evidence of photosynthesis.
- Describe how variation in leaf color is due to differences in photosynthetic pigments.

Introduction: Photosynthesis

Photosynthesis is the process by which some organisms take sunlight energy and convert it into stored chemical energy. We typically think of plants performing photosynthesis, but other microorganisms like bacteria and protists do photosynthesis as well. The overall chemical reaction for photosynthesis takes carbon dioxide (CO₂) and water (H₂O), along with light energy, and turns them into a reduced carbon source like glucose (C₆H₁₂O₆), releasing oxygen gas (O₂) in the process (following the chemical equation below). The light energy is converted into chemical energy that gets stored in the covalent bonds of glucose or a similar molecule.



There are two parts of photosynthesis: the Light Dependent Reactions and the Calvin Cycle. The **Light Dependent Reactions** use water (H₂O) and light to store the energy in intermediate molecules such as ATP and NADPH. The Light Dependent Reactions produce oxygen gas (O₂) as well and do not occur when light is absent (such as during night time). The **Calvin Cycle**, sometimes called the Dark Reactions, uses the other reactant carbon dioxide (CO₂) along with the ATP and NADPH produced by the Light Dependent Reactions. This step takes the energy out of the ATP and NADPH molecules and stores it in new chemical bonds between carbon atoms forming a sugar molecule like glucose (C₆H₁₂O₆). These reactions can happen with or without light present.

For today's lab we will be measuring photosynthesis. First, let's think about some possible ways to see photosynthesis happening. We could look for an increase in products like glucose molecules! Maybe we could use a chemical reagent that changes color if glucose is present (does this sound familiar?). Another option is to look for the presence of oxygen gas. These two measurements could work but end up being pretty challenging. Most plants use the glucose they make to do other things rather than store it, making glucose hard to measure. Oxygen gas molecules are miniscule and immediately mix into the air. Sometimes we can see small gas bubbles forming on leaves under water but that is hard to compare and quantify. Other options include looking at the removal of reactants. Water molecules are hard to track since cells are made mostly of water. But we can track carbon dioxide pretty easily! If the plant is performing photosynthesis, then it would be using carbon dioxide and the levels of CO₂ would decrease.

Today, we will be designing controlled experiments to answer the question: *will photosynthesis happen more under a lamp (direct) or sunlight (indirect)?* To do this experiment, we will be using the aquatic plant *Elodea* which must remain in water at all times to survive. We will be using a chemical indicator called **phenol red** that changes colors in response to pH changes. **pH** is a measure of hydrogen ions in a solution and is reported on a scale of 0-14 (Figure 1). Smaller numbers (less than 7) on the scale are considered **acids**; while larger numbers (greater than 7) are considered **basic/alkaline**. Right in the middle is a pH of 7 which is considered a **neutral** pH. Pure water has a neutral pH of 7. When CO₂ is added to water, the water becomes acidic. Phenol red is red/pink when CO₂ is absent (neutral or basic pH) and orange/yellow when CO₂ is present (acidic pH). We will first add CO₂ to the water by exhaling into the liquid through a straw until we get an orangey color. We will use this liquid for all tests. The process of photosynthesis takes time so let's use multiple pieces of *Elodea* in each test tube and allow at least 30 minutes before collecting results.

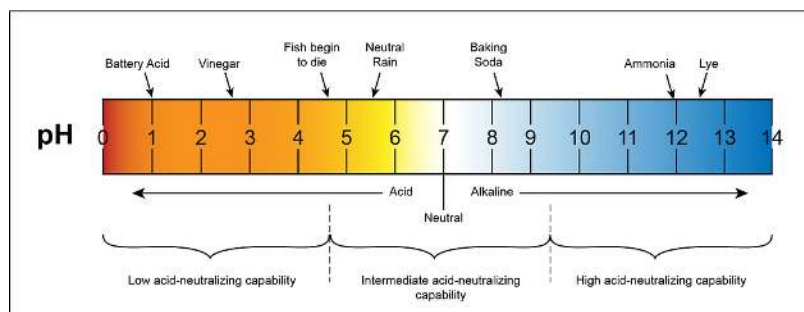


Figure 1. The pH scale. ([Image source](#))

Introduction: Chloroplasts & Pigments

Taking an even closer look, there are small organelles within leaf cells that perform photosynthesis called **chloroplasts**. Some plant cells have higher numbers of these organelles than others depending on their role for the plant. The palisade mesophyll cells in a leaf are filled with lots of chloroplasts since they perform the most photosynthesis for a plant. Plant cells have the ability to move their chloroplasts around within the cell to capture the most light possible. Within the chloroplasts are various pigments that have different light capturing abilities. A **pigment** is a chemical molecule that has the ability to capture light and emit a color that our eye interprets. Plants have evolved the ability to use pigments to capture the light's energy and convert it into stored chemical energy during photosynthesis. The most abundant pigment in plants is called **chlorophyll a**, which reflects a bluish green color. A secondary pigment is **chlorophyll b**, which is a more yellowish green color. Other pigments include **xanthophyll**, which is a dull yellow color, and **carotene**, which is a bright yellow/orange color. Every plant will have evolved various quantities of these pigments and have the ability to change the amount of each pigment made. This helps explain the variation in leaf color across plant species. Today, we will investigate the movement of chloroplasts within cells and the various pigments in leaves.

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6.2: Lab 6 Procedures

PROCEDURE 1: MEASURING PHOTOSYNTHESIS

Equipment we'll need per group:

- Flask with Phenol Red
- 4 empty test tubes
- *Elodea aquatic plant species*
- Tape for labels
- Tweezers/forceps
- Straw
- Second location
- 1 test tube rack
- Pipette

1. Answer the questions in the Answer Sheet to build a controlled experiment.
2. Obtain the flask of Phenol Red, four empty test tubes, and one straw.
3. Have one person slowly exhale through a straw into the Phenol Red solution in the flask. Be careful not to create large bubbles or splashing. Cover the top of the beaker with a paper towel to protect the face. Continue exhaling into the liquid until it turns a yellow color.
4. Use the pipette to pour $\frac{1}{4}$ of this yellow solution into each empty test tube. Label these tubes: "Tube 1", "Tube 2", "Tube 3", and "Tube 4". We should now have four test tubes with equal amounts of the yellow phenol red solution.
5. Obtain 4-6 small strands of *Elodea* for each tube. Put half of the *Elodea* upside down into Tube 2 and the other half into Tube 4. Use tweezers/forceps to push the plant fully into the liquid until they are fully submerged.
6. Each group will decide which two light treatments to test. Place Tubes 1 and 2 into one light treatment, and place Tubes 3 and 4 into the other light treatment.
7. Allow photosynthesis to occur for at least 30 minutes.
8. Record results in Table 1 and answer the follow-up questions in the Answer Sheet.

Clean up/Disposal

1. Throw away straws and paper in the trash.
2. Remove all plant segments using tweezers and place in the beaker by sink.
3. Pour liquids into the hazardous waste container by sink.
4. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
5. Rinse test tubes and beakers. Place on the towel to dry.

PROCEDURE 2: CHLOROPLASTS & PIGMENTS

Part 1: Leaf Structures- Chloroplasts

Equipment we'll need:

- Clean microscope slides
- *Elodea aquatic plant*
- Tweezers/forceps
- Cover slips
- Cell phone flashlight

1. Obtain a clean microscope slide and coverslip.
2. Use forceps to remove one leaf from the *Elodea* plant. Place it on the slide with a drop of water. Cover with coverslip.
3. Using the scanning objective lens (4x), get the specimen into focus. Once in focus, move to the low power objective (10x). Draw what is seen in the Answer Sheet. Alternatively, take a picture and paste it in the Answer Sheet.

4. Turn off the microscope light for 10 minutes. Turn the microscope back on with the brightness low and view immediately. Note any changes in the locations of the chloroplasts.
5. Using either a cellphone light or small lamp, direct light on the sample. Watch the chloroplasts. Draw observations in the Answer Sheet.

Clean up/Disposal

Microscope

1. Remove slide from microscope.
2. Lower the microscope stage all the way down.
3. Put the 4x objective lens in place over the stage.
4. Turn off the microscope.
5. Unplug the microscope and wrap the cord neatly around the base.
6. Cover the microscope and return to its appropriate cabinet. Then lock the cabinet and return the key.

Slides

1. Remove the coverslip from the wet mounts. Dispose of the coverslip in the trash.
2. Dispose of the *Elodea* leaf in the trash. Do not rinse this down the drain.
3. Rinse the slide clean. Scrub off any residue or stain left behind.
4. Set slides out to air dry on the towels.

Part 2: Leaf Structures- Pigments

Chromatography is a method used in biology to separate out molecules using a chemical solvent. This allows for various molecules from within cells to be studied and isolated. This procedure uses a toxic solvent, so do not touch or inhale this substance directly. We will transfer pigments from leaf samples onto a paper that gets inserted into the liquid solvent. The solvent carries the pigments up the piece of paper. Depending on the atoms and bonds in the pigment, they will move either faster or slower up the paper strip, and thus be separated along the length of the paper. Figure 2 below provides an overview of this procedure.

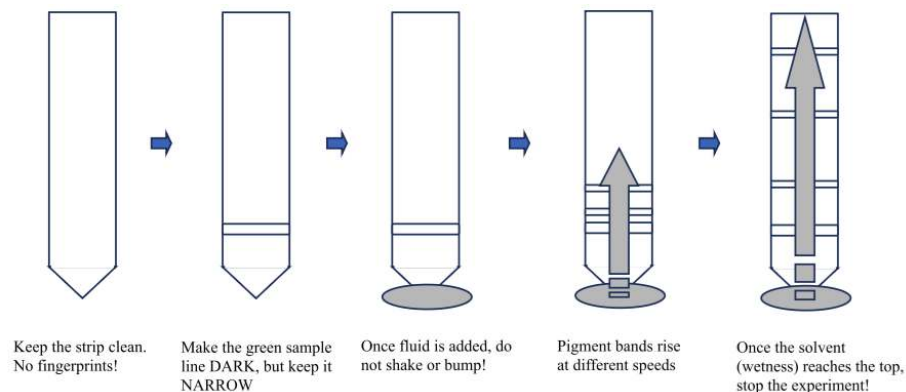


Figure 2. Diagram of chromatography procedure and expected results.

Equipment we'll need per pair:

- Spinach leaves or other leaf sample
- Test tube, pre-filled with solvent by instructor
- Chromatography paper
- Coin/washer
- Cork with hook

1. We will be comparing chromatography results from a spinach leaf to the results from a leaf each group finds outside.
2. Fill in the experimental design portion of the Answer Sheet.
3. Venture outside and obtain a leaf from a plant in the area immediately surrounding our building.
4. Obtain a spinach leaf, chromatography paper, test tube with solvent, and a cork stopper.

5. Keep the test tubes closed at all times.
6. Place the paper on the bench. Stack the leaf sample on top of the paper about 2cm up from the pointed end.
7. Using a coin or washer, roll the coin over the leaf surface on top of the paper horizontally. This will transfer the leaf pigments onto the paper.
8. Attach the paper to the cork stopper. Gently insert the paper into the test tube. The tip of the paper should just touch the solvent at the bottom.
 - Ensure that the pigmented line transferred from the leaf is not submerged in the solvent.
9. Repeat steps 5-8 for both leaf samples.
10. Watch as the solvent rises up the paper separating out the pigments in the leaf. Once it reaches the top, carefully remove the paper from the stopper and place on a paper towel.
 - Try not to directly touch or breathe in the wet chromatography paper.
11. Record the results in the Answer Sheet.

Clean up/Disposal

1. Throw away used leaves in trash.
2. Throw away used chromatography paper in trash.
3. Pour liquid into the hazardous waste container by the sink.
4. Rinse out test tubes and place on rack to dry.

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CHAPTER OVERVIEW

7: Lab 7 Cellular Respiration and Fermentation

7.1: Lab 7 Background

7.2: Lab 7 Procedures

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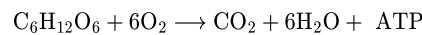
7.1: Lab 7 Background

Learning Objectives

- Explain the connection between exercise and cellular respiration.
- Describe the most beneficial conditions for optimal fermentation.
- Calculate the nutritional components of a meal and determine the percentage of their daily value certain foods cover.

Introduction: Cellular Respiration

Cellular respiration is the process by which some organisms take in food sources and convert them into usable forms of energy. Many different organisms perform the same set of chemical reactions to break down their foods. The overall chemical reaction takes a sugar like glucose ($C_6H_{12}O_6$) and a final electron acceptor like oxygen gas (O_2) and breaks down the sugar molecule releasing the energy stored within its chemical bonds. This energy that is released is stored as ATP, which is more readily used by the cells to do work. As waste products, the oxygen gas is reduced and forms water (H_2O) while the carbons from the glucose are released as carbon dioxide (CO_2).



There are four steps to cellular respiration: (1) Glycolysis, (2) Pyruvate Oxidation (also called Preparatory Reactions), (3) Citric Acid Cycle (also called Krebs Cycle), and (4) Oxidative Phosphorylation. Steps 1 through 3 are a series of chemical reactions that remove the energy from glucose and store it as ATP and on diffusible electron carriers such as NADH and $FADH_2$. These reactions include the removal of carbons from the 6 carbon glucose producing individual CO_2 molecules. The energy stored within the NADH and $FADH_2$ is on electrons rather than stored within chemical bonds. The NADH and $FADH_2$ can diffuse to the Electron Transport Chain (part of Oxidative Phosphorylation), where the energy in the electrons is transferred into more ATP. The low energy electrons are then added onto an O_2 gas, forming H_2O .

The process of breaking down sugars using the above four steps is highly conserved across most living species. In addition to humans, organisms like fungi, plants, protists, bacteria, etc. all perform these chemical reactions to make ATP, though there is some variability with the use of O_2 gas or some other final electron acceptors. Today's lab will include experimenting on what can affect the rate of cell respiration and which species perform these chemical reactions. We will be using the pH indicator Phenol Red again for today's lab. As a reminder, this solution is red/pink when CO_2 is absent (neutral or basic pH) and orange/yellow when CO_2 is present (acidic pH).

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7.2: Lab 7 Procedures

PROCEDURE 1: HUMAN RESPIRATION

In this first experiment, we will test the rate of cellular respiration in a trusty volunteer. A group member will exhale into a test tube of phenol red to turn it yellow, then exercise for 10 minutes and exhale into a second test tube with phenol red. We want to determine whether exercise affects the time it takes to turn the phenol red yellow and why that might be.

Equipment we'll need:

- 4 empty test tubes
 - 1 test tube with Phenol Red or BTB
 - A volunteer student
 - 2 straws
 - Timer on phone
 - Test tube rack
1. Answer the questions in the Answer Sheet to build this experiment.
 2. Divide the Phenol Red liquid evenly between the four test tubes. Label the tubes "1 Before", "1 After", "2 Before" and "2 After".
 3. Choose a volunteer (Person 1) from the group who is willing to elevate their heart rate for 10 consecutive minutes two times. Choose another person (Person 2) to be the time keeper.
 4. Have Person 1 take 5 slow deep calming breaths. Person 2 will set up their timing device.
 5. Using the tube labeled "1 Before", Person 1 will exhale slowly through the straw into the liquid until it changes to a bright yellow color. Person 2 will start the timer when Person 1 begins blowing into the test tube and stop the timer when the color is sufficiently yellow. Record the time in Table 1 on the Answer Sheet.
 - a. The color change might take a few breaths to occur. The key is to continue exhaling consistently.
 - b. The other group members can help with when to stop exhaling at the right color.
 6. Person 1 will now perform some sort of exercise routine that will quickly and consistently elevate their heart rate. Options include: squats, quickly walking up and down the stairs, mountain climbers, going for a slight jog, burpies, etc. Continue performing the exercise for 10 minutes.
 7. When the 10 minutes are over, immediately repeat step 5 with the "1 After" tube. Record results in Table 1.
 - a. Other group members are essential for this. Compare the "1 Before" tube to the "1 After" tube to get nearly the exact same color before stopping the timer. But also Person 1 should continue to exhale at the same rate as they did for the before round.
 8. Rest & Recover! Person 1 will spend at least 20 minutes recovering. While we are waiting, let's set up Procedure 2 (see below).
 9. When Person 1 is done recovering, we will repeat the entire experiment a second time using tubes "2 Before" and "2 After".
 10. Record results in Table 1. Answer the follow-up questions.

Clean up/Disposal

1. Throw away straws and paper in the trash.
2. Pour liquids into the hazardous waste container by sink.
3. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
4. Rinse test tubes and beakers. Place on the towel to dry.

PROCEDURE 2: PLANT RESPIRATION

Equipment we'll need:

- 1 empty test tube
 - 1 test tube with Phenol Red
 - 1-2 sprigs of *Elodea*
 - Tweezers
1. Answer the questions in the Answer Sheet to build this experiment.

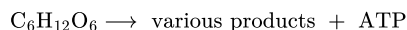
2. Divide the liquid evenly between the two test tubes. Label the tubes “1” and “2”.
3. Add *Elodea* to Tube 1, making sure it is fully submerged in the liquid.
4. Place both Tubes 1 and 2 in the dark cabinet.
5. Allow the tubes to sit for about 60 minutes. Then record results in Table 2 of the Answer Sheet.

Clean up/Disposal

1. Remove all plant segments using tweezers and place in the beaker by sink.
2. Pour liquids into the hazardous waste container by sink.
3. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
4. Rinse test tubes and beakers. Place on the towel to dry.

Introduction: Fermentation

As mentioned above, many species perform the same chemical reactions to break down glucose. The most efficient (i.e., has the highest ATP yield) series of reactions uses oxygen gas as the final electron acceptor and is referred to as **aerobic respiration**. However, there are various environments where oxygen gas may not be available. Some species evolved variations on these reactions so that they could use other molecules as the final electron acceptor, referred to as **anaerobic respiration**. Other species only perform step 1 Glycolysis and are therefore independent of using a final electron acceptor, performing separate reactions called **fermentation**. Fermentation breaks down glucose into various products such as acids, gasses, or alcohols depending on the species.



Today's experiment will use yeast which are microscopic fermenting powerhouses! Yeast are so amazing that we humans have harnessed their powers to make all sorts of foods/beverages- bread, pizza, alcohol, kombucha, etc. When yeast perform fermentation, they typically produce CO₂ and ethanol. We will be testing what starting or fuel molecule yeast can breakdown in fermentation. In addition, we will look at their preferred temperature for doing fermentation. In order for fermentation to occur, the yeast must be in an oxygen free environment. We accomplish this by using fermentation tubes that have a sealed end and bulb that traps the yeast and gasses produced.

PROCEDURE 3: FERMENTATION

Equipment we'll need:

- 4 empty fermentation tubes (labeled)
 - Flasks of each mixture of yeast- sucrose, protein, warm or cold water
 - Rulers
1. Obtain four fermentation tubes. Label them 1 through 4 using a grease pencil. These are FRAGILE.
 2. Fill each tube's bubble opening with the following:
 - a. Tube 1 with yeast and warm water solution
 - b. Tube 2 with yeast, sucrose, and warm water solution
 - c. Tube 3 with yeast, protein, and warm water solution
 - d. Tube 4 with yeast, sucrose, and cold water solution
 3. Place a thumb over the opening and invert then twist to get the liquid to fill the vertical tube fully with no bubbles/air.
 4. Place tubes 1 through 3 at the desk. Place tube 4 in the metal tray for the instructor to move to the refrigerator.
 5. Once set up, answer the questions in the Answer Sheet to make predictions of the results.
 6. Allow the tubes to sit for 30 minutes.
 7. Using a ruler, measure the CO₂ gas produced in centimeters (cm). Record results in Table 4 on the Answer Sheet.

Clean up/Disposal

1. Pour liquids down the drain.
2. Scrub off the grease pencil lines and any other markings. Remove any tape labels.
3. Rinse fermentation tubes. Place on the towel to dry.

PROCEDURE 4: MOLECULES FUELING RESPIRATION

In this part of the lab, we will look at the nutrition values of various foods and think about how what we eat fuels the chemical reactions that occur inside of our cells.

1. Write out a typical day's meals including all of the ingredients/items in the Answer Sheet.
2. Using a calorie counter or nutritional label finder: record the calories, total fat, saturated fat, cholesterol, sodium, total carbs, fiber, and protein in Table 5 on the Answer Sheet.
 - a. This is a helpful website: <https://www.webmd.com/diet/healthtool-food-calorie-counter>
3. Add up the totals for each category and record them at the bottom of Table 5.
4. Using the [healthy diet calculator](#), enter as much of the information about yourself as possible. At the bottom hit next. Then record the customized dietary requirements provided into Table 5.
5. Calculate the percentage of daily value (total divided by daily recommended x 100) for each category that this meal used in the bottom row of Table 5.
6. Answer the questions in the Answer Sheet.

RESOURCES

There are lots of resources available to El Camino students for access to food. Click on the hyperlinks below for more information.

- [Warrior Pantry](#)
 - The Warrior Pantry offers healthy food items and toiletries. They offer both drive-thru and walk-up. Hours:
 - Walk-up Stadium Room #213 on the 2nd floor of the Bookstore Building: Tuesdays, 11:00 am - 4:00 pm; Wednesdays, 12:00 pm - 5:00 pm; Thursdays, 11:00 am - 4:00 pm
 - Drive-thru Parking Lot C-South: Wednesdays 10:00 pm - 2:00 pm
 - Make sure you complete the [Basic Needs Intake Form](#) before accessing the pantry
- [CalFresh](#)
 - CalFresh is a nutrition assistance program that helps low-income individuals and families buy the food they need, known also as the USDA-funded Supplemental Nutrition Assistance Program (SNAP). Eligible students will receive funds and use a EBT card to purchase food.

There are also lots of other [local Food Pantries off campus](#) that you can visit.

PROCEDURE 5: PRODUCE ITEMS

Next week, we will carry out an experiment comparing the amount of DNA we can extract from different produce items. The lab will provide strawberries, but each group should choose at least one more produce item for comparison. It should be something soft that can be easily squashed (like other berries or soft fruits).

1. List the produce item(s) the group will bring in next week on the Answer Sheet.

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CHAPTER OVERVIEW

8: Lab 8 Genetics and DNA Extraction

8.1: Lab 8 Background

8.2: Lab 8 Procedures

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8.1: Lab 8 Background

Learning Objectives

- Explain the steps of extracting DNA from cells.
- Compare and contrast the quantity of DNA extracted based on the organism's number of chromosomes.
- Define and recognize examples of the following terms: gene, character, trait, allele, genotype, phenotype, recessive allele, dominant allele, heterozygous, homozygous, P generation, F1 generation, and F2 generation.
- Solve genetics problems to determine offspring phenotypic and/or genotypic ratios, mode of inheritance, parental genotypes and/or phenotypes.
- Explain why common traits are not always the dominant allele.

Introduction: Observing DNA

The reason that all life looks the way it does is due to the molecule DNA. **DNA** stands for deoxyribose nucleic acid. DNA is passed down from parent to offspring and all living organisms contain this molecule. DNA provides living organisms with the instructions for doing everything from reproducing to growing to generally surviving. Every organism has evolved unique qualities and features even though the DNA molecule is structurally the same across all living organisms. There are small differences in the pattern of the DNA that results in the diversity of life we see across the planet.

Another requirement to be considered living is being made of one or more cells. Cells are made of lipids, carbohydrates, proteins and nucleic acids like DNA. In eukaryotic cells, like plants and animals, DNA is safely stored in the phospholipid membrane of the **nucleus**. During cell division, the DNA will become compacted into **chromosomes** making them easier to separate and for scientists to count. Generally speaking, most organisms have two copies of every chromosome, which is called **diploid**. For example, humans are diploid where we have 23 unique chromosomes with 2 copies of each resulting in a total of 46 chromosomes per cell. Now, some plant species can be more than diploid and are referred to as ____-ploid, where the blank is filled in with a different prefix relating to the number of copies. For example, strawberries are *octoploids* meaning they have 8 copies of each chromosome. Strawberry plants have 7 unique chromosomes with 8 copies of each resulting in a total of 56 chromosomes per cell ($7 \times 8 = 56$).

Today, we will be answering the question: how does the DNA extracted from a produce item compare to the DNA from a strawberry? To get the DNA out of the cells, we will use the directions below. By mashing the fruit, we begin to break down the tissues and carbohydrates surrounding the cells. Then we add soapy salty water (a.k.a. the DNA extraction buffer), which breaks down the fats/membranes and protects the DNA. We will pour the mixture through a strainer to remove any large chunks of produce and cell parts. Lastly, we add alcohol which causes the DNA to become solid but all other materials remain liquid. We have premeasured the strawberries to be used as our control group.

Introduction: Genetics

For this lab, there are a handful of vocabulary terms best learned by diving right in and using them.

- **Gene**- segment of DNA that is used during transcription to then be used during translation to make a protein, also called a protein coding region (ex. hair/fur color gene)
- **Allele**- different versions of the same gene (ex. brown pigment allele, blonde pigment allele, etc.)
- **Trait**- a feature of an organism (ex. hair/fur color)
- **Character**- various options for a specific feature of an organism (ex. brown fur, white fur, etc.)
- **Genotype**- the genetic make-up (or genes) of an individual
- **Phenotype**- the observable features of an organism, (ex. what the individual looks like)
- **Dominant**- the version of a gene always seen in the phenotype, represented as a capital letter (ex. A)
- **Recessive**- the version of a gene only seen in the absence of the dominant allele, represented as a lowercase letter (ex. a)
- **Homozygous**- having two of the same allele, represented as AA or aa depending on if they are dominant or recessive
- **Heterozygous**- having two different alleles, represented as Aa

When studying how genes are inherited and expressed in individuals, researchers found that sometimes things are not always as simple as dominant or recessive. Some traits are controlled by simple dominance where there are only two alleles and there aren't any other factors that affect the phenotype. But more often there are multiple alleles and outside factors, including in humans, that affect the phenotype. In the first part of this lab, we will collect data on some potentially simple dominance traits that are found within the classroom population.

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8.2: Lab 8 Procedures

PROCEDURE 1: DNA EXTRACTION

Equipment we'll need:

- Frozen or fresh strawberries
- Electronic Scales
- Ziplock bag
- Graduated cylinder
- Filtration apparatus (strainer, funnel, test tube)
- Cutting board
- Cutting utensil
- 15mL Extraction buffer
- Plastic pipettes
- Ice cold alcohol

1. Fill in questions in the Answer Sheet to build a controlled experiment.
2. Measure the mass of the provided strawberry in its plastic bag and record it in the Answer Sheet.
3. Obtain one plastic bag per produce item. Cut and weigh the produce item(s) brought in by the group to be nearly the same mass as the strawberry. Record the masses in the Answer Sheet.
 - a. Place produce items in separate plastic bags.
4. Remove the air and seal the bag shut. Mash the item for 2 minutes.
5. Measure 15mL of the DNA extraction buffer using a graduated cylinder.
6. Pour the DNA extraction buffer into the bag with the mashed produce. Mix for at least 1 minute.
7. Assemble filtration apparatus: place the strainer over the funnel, then place the funnel over the large test tube. Filter the produce fluids and DNA extraction buffer through the filtration apparatus. Allow to drip for 5 minutes.
8. Holding the test tube at an angle, slowly drip 2 pipettes full of ice cold alcohol down the side of the test tube. Do not mix. DNA molecules become solid in alcohol so we will begin to see the DNA clump and be pulled out of the fluids.
9. Compare the relative amounts of DNA extracted and record results in the Answer Sheet. Answer the follow up questions.

Clean up/Disposal

1. Pour liquids into the colander in the sink.
2. Replace pipettes to their original locations.
3. Dispose of ziplock bags in trash.
4. Rinse graduated cylinders, funnels, and strainers. Place them back in their original locations.
5. Rinse the test tubes. Remove any markings and tape labels. Place them on towels to dry.

PROCEDURE 2: SURVEY OF HUMAN TRAITS

Below is the list of human traits and their descriptions that we will be surveying.

Table 1. Summary of Human Traits

Trait (symbol letters)	Dominant Phenotype	Recessive Phenotype
Hairline across forehead (H, h)	Widow's peak	Straight line
Facial dimples (D, d)	Indents when loosely smiling	Indents absent
PTC taster (T, t)	Able	Unable
Bottom of earlobe shape (E, e)	Unattached	Attached
Shape of chin (C, c)	Indented (Cleft chin)	Smooth

Equipment we'll need:

- PTC Paper

1. Record your phenotype and possible genotypes in Table 1 in the Answer Sheet.
2. Write your results on the board then record the percentages in Table 2 in the Answer Sheet.
3. Discuss the results and answer the remaining questions.

PROCEDURE 3: APPLICATION OF GENETICS

Beyond figuring out how traits are inherited, genetic analysis can also be helpful to explain other aspects of biology. For example, scientists can record the number of characters they find in nature then use this to determine the inheritance rules. They could take this further to begin to piece together the evolutionary history of a trait and find explanations for how or why a species is changing over time. In this next procedure, we will trace the presence and absence of spines in threespine stickleback fish (*Gasterosteus aculeatus*) to understand its evolution and inheritance of spines. This activity has been adapted from the HHMI [The Making of the Fittest: Evolving Switches, Evolving Bodies](#) activity.

Threespine stickleback have a broad geographic range with populations in both marine and freshwater environments. The marine populations live in the ocean and typically have three spines, one on the belly and two on the back (Figure 1). The freshwater populations live in lakes and typically have two spines, lacking the one on the belly. The spine on the belly is called the pelvic spine and this is what we are interested in. Marine populations live most of their lives in the ocean but swim upstream to spawn in rivers and lakes. As the land changed in the past, some streams dried up, causing some populations to become stuck in the lakes. Researchers have hypothesized that the pelvic spine aids in protection from oceanic predators, resulting in higher survival for fish with the spine. In the lake environment, there are predatory insects that catch the fish by grabbing the pelvic spine (these fish are very small!). Thus, the fish without the spines have a higher survival rate in the lake environment. Today we will be looking at the results of two genetic crosses to help us determine if having the pelvic spine present is dominant or recessive. Here is a [video](#) that describes the Stickleback fish's evolution.



Figure 1. Image of a marine Stickleback fish. ([Image source](#))

Equipment we'll need:

- Envelope of Stickleback fish images

1. Fill in the Answer Sheet to predict the results of the two crosses.
2. Obtain an envelope with the fish samples. Sort the samples into F1 and F2 generations.
3. The fish have been stained with a red dye to help see the bony structures. The pelvic spines show as a W shaped zigzag on the fish's belly. See Figure 2 below.
4. Separate the F1 and F2 generation cards (if they are not already separated). Keep the F1 cards out of the bag; shuffle the F2 cards and return them to the bag.
5. Count the number of fish with or without the pelvic spine for the F1 generation. Record the results in the Answer Sheet.
6. Randomly draw one of the F2 cards from the bag. Record whether it has the pelvic spines in Table 3 in the Answer Sheet, then return the card to the bag. Shuffle the cards.
7. Repeat step 6 for a total of 30 draws.
8. Return the envelopes to the appropriate location. Answer the follow-up questions in the Answer Sheet.

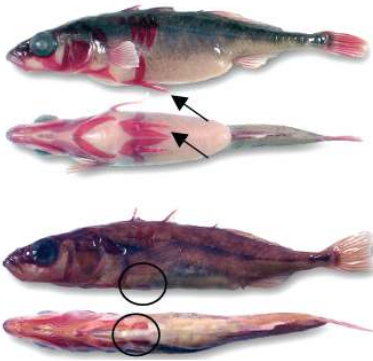


Figure 2. The top two images show a marine fish with pelvic spines. The bottom two images show a freshwater fish without pelvic spines.
([Image source](#))

PROCEDURE 4: BLOOD TYPING

The human ABO blood types represent an excellent example of codominant traits. There are three alleles, I^A , I^B , and i , that control the four blood type phenotypes A, B, AB, and O. The alleles I^A and I^B are codominant, meaning they both contribute to the phenotype when they are part of the genotype. Additionally, both I^A and I^B alleles are dominant to the i allele. Blood types refer to certain proteins that are found on the outside of a red blood cell, called antigens. Type A blood has A antigens, Type B blood has B antigens, Type AB blood has both A and B antigens, and Type O blood has neither A nor B antigens. Our bodies produce antibodies that attack foreign antigens. For example, if a person with Type A blood receives a blood transfusion that contains Type B blood, their immune system would produce antibodies that attack Type B blood. This forms a blood clot that can be lethal to the recipient of the foreign blood. The Rh factor is a similar antigen to the ABO antigens. If a person is Rh- (negative), they do not have the Rh factor on their red blood cells, whereas Rh+ (positive) blood does have the Rh factor on the red blood cells. For example, if a person has blood type A+ (pronounced A positive), their red blood cells have both the Type A antigens and the Rh factor. A person whose blood type is O- does not have any antigens on their red blood cells - neither Type A, Type B, nor the Rh factor.

In this lab, we will use simulated blood and antibodies to determine the blood types from four different samples: Smith, Jones, Brown, Green.

Equipment we'll need:

- Four simulated blood types
- Three simulated anti-serums
- Small trays for mixing blood with antibodies
- Toothpicks

1. Complete Table 3 on the Answer Sheet to become more familiar with the features and genetics of ABO blood types.
2. Add 4 drops of Brown's blood into each of the three wells in a tray. Mix 4 drops of the three different anti-serums into the corresponding wells. Use a different toothpick for each well so as to not mix chemicals. Deduce Mr. Brown's blood type from the patterns of agglutination.
 - Agglutination (coagulation, clumping, clotting) is a sign that the antibodies in the anti-serum attacked the antigens on the blood cells. If there is no agglutination, then there are no antigens for the antibodies to attack.
 - Sometimes it is difficult to see if the blood has agglutinated if the tray is resting on the desk. Try holding the tray above the floor or desk top or seeing if it's possible to read text through the blood sample.
3. Repeat step 2 for the other three samples.
4. Record the results in Table 4 on the Answer Sheet.
5. Answer the remaining questions about blood types on the Answer Sheet.

Clean up/Disposal

1. Return the dropper bottles to the proper locations.
2. Rinse the blood typing trays with the Alconox solution. Gently scrub off any remaining residue.
3. Place trays on the towel to dry.

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CHAPTER OVERVIEW

9: Lab 9 Central Dogma and Biotechnology

9.1: Lab 9 Background

9.2: Lab 9 Procedures

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9.1: Lab 9 Background

Learning Objectives

- Analyze their results to either support or refute their hypothesis.
- Explain the steps used during a bacterial transformation.
- Predict and explain the growth results for our bacterial transformation.

Introduction: Central Dogma

Last week we isolated DNA from within plant cells and then compared the amounts we obtained. We discussed how DNA is passed down from parent to offspring and that DNA is what makes all organisms unique. But we didn't go into how- that's what we're looking into today! DNA is commonly referred to as the blueprint for life. This phrase refers to the concept that DNA is used by cells to make proteins, which provide the cell with physical features and abilities that we can see. These features and abilities can be used to identify and differentiate organisms. For example, a strawberry is red because those cells contain a segment of DNA (a **gene**) that is used by the cell to make a pigment that is red in color. A banana does not contain this gene so those cells are not able to make the red pigment and be red. Let's take a deeper dive into how this happens.

In eukaryotic organisms, DNA is stored in the nucleus of a cell in the form of chromosomes. Along the chromosomes are the many many genes that make up the organism. It is approximated that humans have 19,990 genes in our chromosomes! Those genes each contain the directions for a specific protein. Recall that a **protein** is a chain of amino acids that has a specific structure and function for the cell. Proteins are involved in many different processes in a cell such as movement, coloration, chemical reactions as an enzyme, etc. DNA is made of nucleotides, while proteins are made of amino acids. Thus, the cell must be able to translate between these two chemical languages. This process is actually called **translation**! In order to perform translation, the cell must first make RNA. **RNA** is related to DNA in that it is made of nucleotides but the difference is that those nucleotides contain the sugar ribose instead of deoxyribose. In addition, DNA contains the four nitrogenous bases of Cytosine (C), Guanine (G), Adenine (A), and Thymine (T). RNA contains Cytosine (C), Guanine (G), Adenine (A), and Uracil (U). See Figure 1 for a comparison.

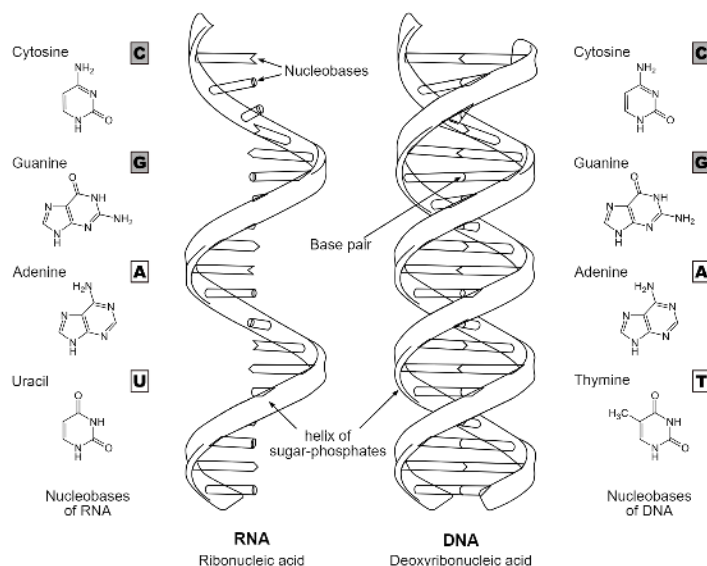


Figure 1. Diagram of RNA in comparison to DNA. ([Image source](#))

Inside the nucleus, various enzymes will open the DNA double helix then attach to one side and begin building a new piece of RNA. These enzymes will use the letters in the DNA as a **template** to determine the pattern of letters in the RNA piece being built. If there is a C in the DNA, a G will be added to the RNA and vice-ver-sa. If there's a T in the DNA, an A will be added to the RNA. Lastly, if there is an A in the DNA, a U will be added to the RNA. Table 1 summarizes these base pairing rules. This process is called **transcription**.

Table 1. DNA to RNA Base Pairing Rules

___ letter in DNA	Pairs to ___ letter in RNA
C	G
G	C
T	A
A	U

In the Answer Sheet, practice transcription with the sample piece of DNA.

There are three versions of RNA that are used to make proteins during translation. **Ribosomal RNA** (rRNA) forms a three dimensional shape combined with protein pieces (collectively called the **ribosome**) that has the ability to break and form chemical bonds. Thus, rRNA is responsible for building the actual protein. **Transfer RNA** (tRNA) has an amino acid attached to it and will bring that amino acid to the ribosome. The ribosome attaches surrounding a single stranded piece of RNA called **messenger RNA** (mRNA). If a tRNA can base pair to the mRNA, then the amino acid on that tRNA is removed and added to the growing protein chain. Scientists were able to decode the letters of DNA/RNA into each of the amino acids. They compiled this information into the **genetic code table** seen in Figure 2. The transfer of information from DNA to RNA then to proteins is referred to as the **central dogma**.

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } Ser UCC } UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } Leu CUC } CUA } CUG }	CCU } Pro CCC } CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } Arg CGC } CGA } CGG }	U C A G
	A	AUU } Ile AUC } AUA } AUG Met	ACU } Thr ACC } ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } Val GUC } GUA } GUG }	GCU } Ala GCC } GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } Gly GGC } GGA } GGG }	U C A G

Figure 2. Genetic Code Table. ([Image Source](#))

In the Answer Sheet, practice translation with the piece of RNA from above.

Introduction: Bacterial Transformation

Today we will be performing a **genetic transformation**. This is a process where a cell takes in a new piece of genetic material, i.e. DNA. The new piece of DNA can provide new beneficial features for the organism. This process happens naturally in the environment or can be manipulated in a lab. This is one method of creating **Genetically Modified Organisms (GMOs)**, organisms that have been altered in a lab to contain new genes.

Genetic transformation is a major procedure used in biotechnology. For example, researchers have been able to genetically alter plant species to be more resistant to pests, drought, or extreme heat. Then the plants can grow and survive in these unfavorable conditions, allowing farmers to produce more food for society. A second use of genetically modifying plants is to add genes that make the foods larger, sweeter, seedless, longer lasting, etc. Another application is to modify organisms to produce human proteins or hormones, which we can use as medication for patients. The most common example of this is the genetically modified bacteria species *E. coli* that is able to produce human insulin. Pharmacologists isolate this insulin and use it to treat patients with diabetes,

whose bodies don't produce insulin properly. Researchers are also working on creating bacteria species that can break down oil to help clean up oil spills. One other potential use of this technique is to cure human diseases, where a normal copy of a disease-causing gene can be inserted into the sick individual's cells, replacing the mutated version and curing their disease.

Something to note is how all of these examples involve adding DNA to the cell and then the cell will make a new protein. In this lab, we will be genetically modifying *E. coli* to glow green under UV light. We are using a pre-assembled experiment called the BioRad Biotechnology Explorer pGLO Bacterial Transformation Kit. We will be transforming the gene **Green Fluorescent Protein (GFP)** into the bacteria. The original source of this gene is from a bioluminescent jellyfish species. This gene in the jellyfish allows them to glow green in the dark ocean, helping them survive. If the *E. coli* gain the gene, they will make the mRNA related to the GFP gene. Then the ribosome will use that mRNA to build a protein that glows green.

In order to add this gene into the bacteria, we will be using a **plasmid**, which is a small circular piece of DNA. The plasmid is called **pGLO** for short. In addition to the GFP gene, the plasmid also includes a gene for antibiotic resistance. The subsequent addition of antibiotics to the food source allows us to kill the original *E. coli* cells that do not have the plasmid/GFP gene. Then the only cells that can grow are the ones that gained the plasmid/GFP gene, in other words the cells that were successfully transformed. Another part of this experiment is the ability to control when GFP protein is made. A common method of controlling the amount of a protein is to turn transcription on or off. On this plasmid, there is a special protein called a **repressor** that can block transcription based on if the sugar arabinose is present. If arabinose is *present*, then the repressor *cannot* block transcription so mRNA is made and then translation occurs making the GFP protein! If arabinose is *absent*, then the repressor *can* block transcription so mRNA is not made and translation *cannot* happen therefore no GFP protein is made.

For this lab, we will be using a few different control groups to determine if the transformation was successful. When performing the experiment, we will begin with two tubes, one labeled +pGLO and the other labeled -pGLO. The +pGLO tube will have the pGLO plasmid added to it; while, the -pGLO tube will not have the pGLO plasmid added to it. Both tubes will receive bacteria, transforming solution, and be treated exactly the same with the exception of adding the pGLO plasmid or not. At the end of the transformation procedure, we will be adding samples from the tubes onto four different plates. We will be using three different types of food also called **media**: (1) LB media which bacteria grow really well on, (2) LB +Amp media which has the antibiotic ampicillin added to it, and (3) LB +Amp +Ara media which has the antibiotic ampicillin and the sugar arabinose added to it. The sugar arabinose is included to control whether or not the *E. coli* bacteria make the GFP protein and glow green. When arabinose is present, the *E. coli* cells will make the green fluorescent protein and glow green. When arabinose is absent, the *E. coli* cells will not make this protein and will not glow green. A sample from +pGLO tube will go onto one LB +Amp plate and one LB +Amp +Ara plate. The sample from the -pGLO tube will go onto one LB plate and one LB +Amp plate.

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9.2: Lab 9 Procedures

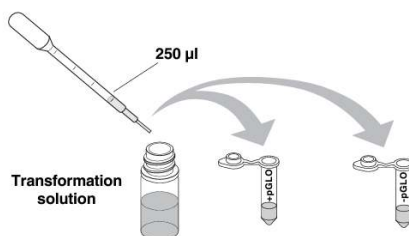
PROCEDURE 1: BACTERIAL TRANSFORMATION

The procedure involves a few important steps that allow the *E. coli* bacteria to take in the new DNA. We will be keeping the solutions and *E. coli* cells on ice to help everything remain cold and stable. We begin by adding the **transforming solution** made of **CaCl₂** (Calcium Chloride), which attaches to the DNA plasmid helping it enter the cells. We will perform a **heat shock** step where we rapidly move the tubes from ice to hot water then back onto ice. This causes the cell membrane to become unstable resulting in tiny holes that allow the plasmid to float into the cell. Next, the cells are given a food source, called **LB Broth/Media**, and some time at room temperature to recover and repair from the heat shock. Lastly, samples of the cells are added onto four different plates of growth media and allowed to grow. Images below from the manufacturer's protocol.

Equipment we'll need:

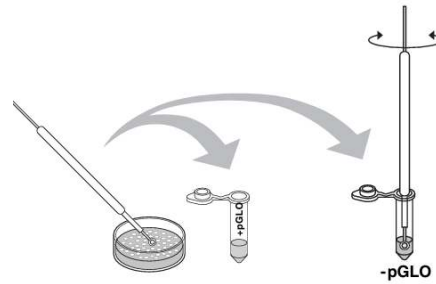
- 1 BioRad Transformation Kit
- 1 plate of *E. coli* colonies
- Ice buckets
- Incubator
- UV transilluminator
- Hot water bath
- 1 LB plate
- 2 LB + Amp plates
- 1 LB + Amp +Ara plate

1. Fill in the questions in the Answer Sheet to make predictions of our results.
2. Move all personal items to under the bench, keeping out the directions. Wipe down the bench with disinfectant.
3. Obtain a pGLO transformation kit with all of the materials needed, an ice bucket with LB and CaCl₂, and a beaker labeled biohazard.
4. Add 50mL of disinfectant into the biohazard beaker.
5. Put on the gloves from within the kit. Begin being mindful of touching personal items, face, water bottle, etc. These can all be sources of contamination!
6. Using the sharpie in the kit, label the tops of the two microcentrifuge tubes as either "+" or "-". Place them back into their rack.
 - a. The "+" tube will get plasmid added to it, whereas the "-" tube will not get plasmid added.
7. Obtain a sterile transfer pipette. Review the markings on the side. We will be using the second and third lines from the bottom to measure today.
8. Open the transfer pipette and pick up the CaCl₂ tube from the ice. Open the CaCl₂ tube. Using the pipette, suck up to the third line from the bottom, which is 250µL. Close the CaCl₂ tube. Transfer the liquid in the pipette to the "+" tube, making sure the pipette is fully empty. Close the "+" tube. Repeat this for the "-" tube using the same pipette.
 - a. Dispose of the pipette into the biohazard beaker.
 - b. Dispose of CaCl₂ tube in the red biohazard bin.

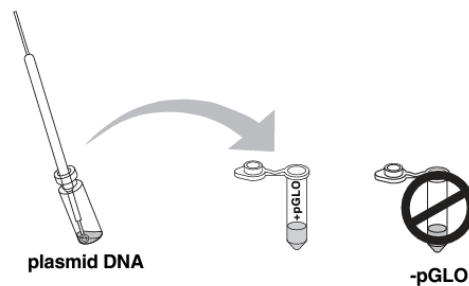


9. Place these tubes into the blue foam squares. The bottom of the tubes should be sticking out. Place the blue foam square into the ice bucket. Make sure the liquid in the tube is fully submerged.
10. Obtain one *E. coli* starter plate. Find the part labeled with the section number for your class.

11. Using a sharpie, draw a circle on the plastic plate surface around two separate colonies that are not touching any others. Use these for step 12.
12. Obtain the bag of yellow sterile loops. Carefully open the zip top and push one loop up from the bottom then select that one loop. Do not insert fingers into the bag as this could contaminate the bag.
13. Using the yellow plastic loop, open the *E. coli* plate and gently scoop up one of the circled colonies. Transfer this sample to the “+” tube by opening the tube then inserting the loop into the liquid and twirling the loop to get the cells off. Close the “+” tube. Place back into the ice.
 - a. Dispose of the loop into the biohazard beaker.
14. Repeat steps 12 and 13 with the other circled colony and the “-” tube. Place back into the ice.
 - a. Dispose of the loop into the biohazard beaker.



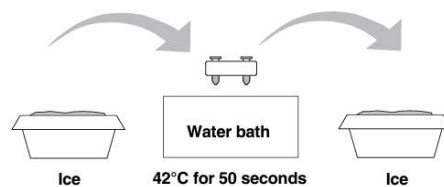
15. Go to the instructor bench with your ice bucket. They will add one loopful of plasmid to the “+” tube.



16. Keep the tubes on ice for 10 minutes. Make sure the liquid in the tube is fully submerged.
17. During this time, obtain 1 LB plate (green), 2 LB +Amp plates (blue), and 1 LB +Amp +Ara plate (red). Label by writing along the edges using the words/shorthand like shown below.

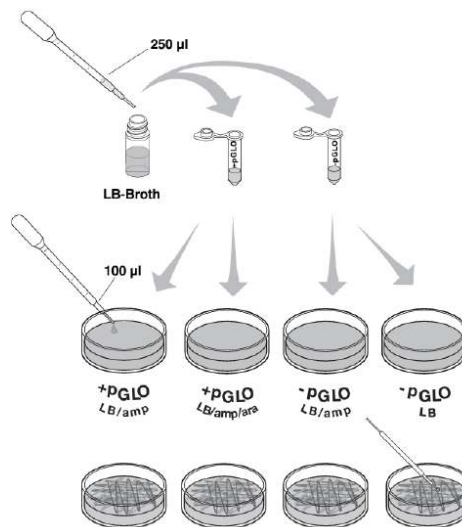


18. This step is quick and time-sensitive! Take the ice bucket and tubes over to the hot water bath at 42°C. Quickly, open the lid to the water bath, add the two tubes to the clear plastic rack in the water bath, and **close the lid**. Start a 50 second timer.



19. As soon as the time is up, immediately put the tubes back into the ice bucket. Let rest for 2 minutes.

20. Remove the tubes from the ice bucket and place onto a rack at room temperature.
21. Obtain the LB broth tube and two sterile transfer pipettes. Open the transfer pipette and pick up the LB tube. Open the LB tube. Using the pipette, suck up to the third line from the bottom, which is 250µL. Close the LB tube. Transfer the liquid in the pipette to the “+” tube, making sure the pipette is fully empty. Close the “+” tube.
 - a. Dispose of the pipette into the biohazard beaker.
22. Repeat this for the “-” tube using a new pipette.
 - a. Dispose of the pipette into the biohazard beaker.
23. Let tubes rest at room temperature for 10 minutes.
24. Set up the area so that the two -pGLO plates on the left and the +pGLO plates are on the right . Obtain two sterile transfer pipettes.
25. Open the transfer pipette and pick up the “+” tube. Open the “+” tube. Using the pipette, suck up to the second line from the bottom, which is 100µL. Close the “+” tube. Transfer the liquid in the pipette to the +pGLO LB +Amp plate, making sure the pipette is fully empty. Close the “+” tube.
26. Using the same pipette, repeat step 25 but add the sample to the +pGLO LB +Amp +Ara plate.
 - a. Dispose of the pipette into the biohazard beaker.
27. Obtain the bag of yellow sterile loops. Carefully open the zip top and push one loop up from the bottom then select that one loop.
28. Using the yellow plastic loop, spread out the liquid across the entire surface of the +pGLO LB +Amp plate evenly.
 - a. Dispose of the loop into the biohazard beaker.
29. Repeat step 28 with the other +pGLO plate.
 - a. Dispose of the pipette into the biohazard beaker.
30. Repeat steps 25 through 29 for the “-” tube and the -pGLO plates.



31. Stack the plates **agar side down**. Tape the four plates together and label the tape with the section number and bench letter. Place on the instructor bench

Clean up/Disposal

1. Return the plate of *E. coli* to its original location.
2. Return the plastic bag of other supplies and any remaining materials back to their original location.
3. Pour the disinfectant liquid, pipettes, and loops into the large bucket near the sink. Place the biohazard beakers back to their original location.
4. Dispose of the ice in the sink. Place the ice buckets back into the original location.
5. Dispose of all used microcentrifuge tubes and gloves in the red biohazardous waste container.

6. Wipe down the benchtop with disinfectant.

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CHAPTER OVERVIEW

10: Lab 10 Evolution

[10.1: Lab 10 Background](#)

[10.2: Lab 10 Procedures](#)

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10.1: Lab 10 Background

Learning Objectives

- Describe the process of evolution by different mechanisms.
- Predict which traits would be adaptive in different environments.
- Calculate the change in allele frequency in a population.

Introduction

In its simplest form, evolution means change. There are different mechanisms that can cause change. When we discuss the evolution of living things, we are referring to how they are changing over time. Biological **evolution** is the change in allele frequencies in a population over one or more generations. Two weeks ago, when we studied stickleback genetics, we learned that **alleles** are different versions of a given gene. The **frequency** is the percentage of that gene in a population. A **population** is a group of organisms of the same species that are interacting in an environment at a given time. Populations are also defined by their **gene pool**, the sum of all alleles of all genes from all individuals within a population. In this lab, we are studying whether evolution occurs in a population by measuring if the allele frequency is different in one generation compared to another. Thus, it is important to remember that evolution occurs in populations (not in individuals) across generations (not within an individual's lifetime).

There are four main **mechanisms** that cause evolution. **Mutations** cause new alleles to appear, which changes the frequency of the other alleles of that gene in the population. **Gene flow** occurs when an individual in one population disperses (or moves) to, and reproduces with, an individual in a different population. If the individual that dispersed has alleles that are not present in the new population, its offspring may inherit the new allele, changing the population's allele frequency. **Genetic drift** happens when the allele frequency of the population changes randomly. Genetic drift is more likely to occur in small populations and changes the frequency of alleles regardless of whether they are beneficial, harmful, or if there is no fitness difference between alleles. **Selection** occurs when an allele increases in frequency because it is advantageous in some way (or decreases in frequency because it is harmful). There are many different kinds of selection, but we will focus on **natural selection** in this lab. Natural selection is perhaps the strongest mechanism of evolution compared to the other three discussed here. However, it is important to note that natural selection does not make organisms "better" or "advanced", but rather it matches organisms to a particular environment with a particular set of conditions. Traits that increase an organism's survival or reproduction are considered **adaptations**. If environmental conditions change, then a trait that was once adaptive may no longer be adaptive in the new environment. Thus, natural selection depends very strongly on the environment.

In our lab this week, we will work through a few different experiments and simulations to explore how changing allele frequencies cause evolution by different mechanisms. First, we will explore how predator-prey interactions affect how traits evolve under natural selection. Then, we will use beans to simulate reproduction in a population to see how allele frequencies can change from mutations, randomly under genetic drift, or in different directions based on natural selection.

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10.2: Lab 10 Procedures

PROCEDURE 1: PREDATOR-PREY ADAPTATIONS

In this experiment, we will use various tablecloths to represent different environments, different types of beans to represent different “prey” phenotypes (such as field mouse fur color), different utensils to represent different “predator” phenotypes (such as hawk beak depth), and plastic bags to represent the predator’s stomach. This experiment works best in groups of 7 or 10, but the instructor may adjust these numbers based on class size.

Equipment we’ll need:

- Tablecloth
- 50 of each of four different kinds of beans
- Cell phone timer
- Plastic bag
- Forceps, chopsticks, spoon, fork, and knife

1. Answer the questions on the Answer Sheet to set up the experiment.
2. Layout the tablecloth flat on the desk.
3. Randomly scatter all the beans out on the tablecloth. Make sure they’re evenly distributed. There should be 50 beans of each type, for a total of 200 beans on the tablecloth.
4. Assign roles to group members. Groups of 7 should have 6 predators and 1 timekeeper/recorder. Groups of 10 should have 9 predators and 1 timekeeper/recorder.
5. As a group, decide on three utensils to use. Then, randomly assign each “predator” a utensil. There should be equal numbers of each kind of utensil (e.g., 2 forks, 2 spoons, 2 chopsticks; or 3 knives, 3 forceps, 3 spoons).
 - Be sure to record the predatory and prey phenotypes in Table 1 on the Answer Sheet.
6. Each predator needs one utensil and one plastic bag as the stomach.
7. The timekeeper/recorder sets a timer for 15 seconds. When the predators are ready, say “Go!”
8. Predators will collect as many “prey” (beans) as they can in 15 seconds.

General rules:

- No cheating! Each predator cannot use anything besides the assigned utensil.
 - No physical contact between predators. Do not tamper with another predator’s utensil or bag. This is a friendly competition!
 - No stealing prey from each other’s bags.
 - Do not scrape or shovel the prey directly into the bags. Each prey must be picked up and dropped into the bag. Though, prey do not need to be caught one at a time.
 - The collected prey must make it into the predator’s stomach to count! If a prey spills off the tablecloth or out of the bag, that prey is lost (killed) and cannot be counted towards a predator’s catch.
9. At the end of the 15 seconds, predators will get together with their same phenotype (based on utensil) and count the total number of prey caught. Record this in Table 2 Column B.
 10. Calculate the prey caught per individual for each phenotype by dividing the total prey caught (Table 2 Column B) by the number of predators (Table 2 Column A). Record this in Table 2 Column C.
 11. Rank the predator phenotypes in order from highest to lowest prey/individual. Record the ranks in Table 2.
 12. One of the predators with the lowest rank (least number of prey caught) will drop their utensil and pick up the utensil of the predators with the highest rank (most prey caught). Record the new number of each predator phenotype in Table 2 Column A for the next generation.
 - Example: There are 3 spoons, 3 forks, and 3 forceps at the beginning of Generation 1. Spoons catch 78 total prey ($78/3 = 26$ prey per individual), Forks catch 66 total prey ($66/3 = 22$ prey per individual), and Forceps catch 60 total prey ($60/3 = 20$ prey per individual). Because the forceps are ranked lowest and spoons are ranked highest, one of the forceps becomes a spoon. Generation 2 will start with 4 spoons, 3 forks, and 2 forceps.

- This simulates how natural selection would select for the trait that was most effective at catching prey (spoons) and select against the trait that was least effective (forceps). There will be more individuals born in the next generation with the advantageous allele, causing the allele frequency to increase.
13. Count the number of each type of prey caught across all predators. Record the number in Table 3 Column B.
 14. Calculate the number of surviving prey by subtracting those caught (Table 3 Column B) from those that started the generation (Table 3 Column A). Record this number in Table 3 Column C.
 15. All surviving prey will reproduce one offspring. Add the number of surviving offspring of each prey type (Table 3 Column C) to the tablecloth environment.
 - For example, if there are 20 pinto beans at the end of Generation 1, add another 20 pinto beans to the tablecloth. There are now 40 total pinto beans on the tablecloth.
 - Note: The **maximum** number of prey of a given phenotype is 150. Do not start a generation with more than 150 of any one kind of bean on the tablecloth environment.
 16. Calculate the total number of each prey phenotype that will start the next generation by multiplying the surviving prey by 2. Record these numbers in Table 3 Column D. These same numbers will go in Table 3 Column A for the next generation.
 17. Repeat steps 7-13 for a total of four generations. After four generations of hunting, record the number of prey and predators that would start Generation 5, but do not actually replenish the tablecloth environment with beans or do a fifth generation of hunting.
 18. Answer the questions on the Answer Sheet.

PROCEDURE 2: GENETIC DRIFT and MUTATION

In this experiment, we will have different kinds of beans that will represent different alleles. With a partner, we will simulate a “mating” event where we randomly select alleles (beans) for our offspring. Then, we will take on the genotype of our offspring in the next generation and do another round of “mating” for a total of 10 generations. This experiment works best if everyone is in the generation at the same time. During Generation 5, one person will be randomly selected to acquire a mutation (a third type of bean). We will see the effect, if any, of random events and mutations on a population’s allele frequency.

Equipment we’ll need:

- Beaker
- Pinto, great northern, and black beans

1. Before we start, please keep in mind that this simulation works best if every student is in the same round at the same time. Please wait for the instructor’s instructions before moving on to the next round.
2. Each person puts one black bean and one great northern bean into their beaker. The instructor may choose to use different beans depending on what’s available in the room.
 - The great northern bean represents the “a” allele and the black bean represents the “A” allele.
 - This means that each person starts with a heterozygous genotype Aa.
 - The initial allele frequency of both the “A” and “a” allele is 0.5 (or 50%).
3. Find a classmate to simulate a “mating” event with. Each person in the pair will randomly select one allele (bean) from their own beaker and show each other the allele (bean) at the same time. These beans represent the genotype their offspring will inherit. Remember this first genotype option.
 - Two pinto beans are “AA”, two great northern beans are “aa”, and one of each is “Aa”.
4. Put the beans back in the beaker, shake the beaker to shuffle the beans, and then repeat Step 2 with the same person, randomly selecting alleles (beans) from the beakers. Remember this second genotype option.
5. One person chooses the first genotype, the other person gets the second genotype.
6. Each person records their new genotype in the Answer Sheet in Table 4.
7. For the second generation, the beans in the beaker must match the genotype chosen from the “reproduction” in Generation 1 (from Step 4). Switch any beans in the beaker if necessary.

Example: To start Generation 1, Person 1 has a beaker with a black and great northern bean and Person 2 also has a beaker with a black and great northern bean. Person 1 randomly selects the black bean from their beaker, and Person 2 also randomly selects the

black bean from their beaker. They record the genotype “AA”. They each put the black bean back into their own beaker. For the second offspring, Person 1 randomly selects the great northern bean from their beaker, and Person 2 randomly selects the black bean again from their beaker. They record the genotype “Aa”. Person 1 decides to take on the first genotype “AA”, so Person 2 takes on the second genotype “Aa”. To start Generation 2, Person 1 replaces their great northern bean with another black bean so that their beaker now has two black beans (representing the genotype “AA”). Person 2 does not make any changes to their beaker since their genotype to start Generation 2 is “Aa”, just as it was to start Generation 1.

8. Find a *new* partner and repeat Steps 2-5 for three more generations, for a total of four generations. Remember to record the genotype starting each generation in Table 4 on the Answer Sheet.
9. To start Generation 5, the instructor will randomly select one student to acquire a mutation. This student will randomly select one of the beans in their beaker to be replaced with a pinto bean.
 - The mutant allele will be recorded as a’ (pronounced “a prime”; it is also recessive to the A allele).
 - Genotypes with the mutant allele include Aa’, aa’, and a’a’.
10. Then, repeat Steps 2-7 for Generations 5-10. Remember to record the genotype that starts each generation in Table 4 on the Answer Sheet.
11. Each person will add their genotypes for the 10 generations to the board.
12. Record the number of each genotype in each generation for the whole class in Table 5 on the Answer Sheet.
13. To calculate the total number of alleles in a generation, simply multiply the number of individuals (i.e., students in the class) by 2. This will be the same number in every generation. Record this in Table 5 of the Answer Sheet.
14. To calculate the allele frequency in each generation, follow the formulas on the Answer Sheet below Table 5. Record these frequencies in Table 5.
15. Answer the questions on the Answer Sheet.

Clean up/Disposal

1. Return the bags of beans to the appropriate locations.
2. Return the utensils to their locations. Return the plastic bags.
3. Put the piece of fabric back.
4. Return the beakers to their original location.

PROCEDURE 3: BACTERIAL GROWTH (Preparation for Next Week)

Next week, we will be learning about bacteria and protists. In order to look at the growth of bacteria, they need time to grow, so today we will start these experiments and then collect and interpret the results next week.

Equipment we’ll need:

- 2 agar plates
 - 2 sterile cotton swabs
 - Tape
1. Obtain two agar plates per group and one test tube of sterile cotton swabs.
 2. Swab the two different locations, being sure to twist the cotton swab as we sample and sample a large enough area.
 3. Add the sample to the agar plate by gently dragging the swab over the entire surface, zig-zagging back and forth while also twisting the swab. Tape the edges of the plate closed.
 4. Store the plates in our section’s area on the counter until next week.

Clean up/Disposal

1. Dispose of used cotton swabs in the trash.
2. Return the empty test tubes to their original rack.

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CHAPTER OVERVIEW

11: Lab 11 Survey of Small Things (Bacteria and Protists)

[11.1: Lab 11 Background](#)

[11.2: Lab 11 Procedures](#)

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11.1: Lab 11 Background

Learning Objectives

- Use observational skills to identify differences between microscopic species.
- Describe connections between microscopic organisms and everyday life.
- Distinguish between various types of microbial growth (i.e., colony morphology).

Introduction

Phylogenetic Tree of Life

★ = You are here

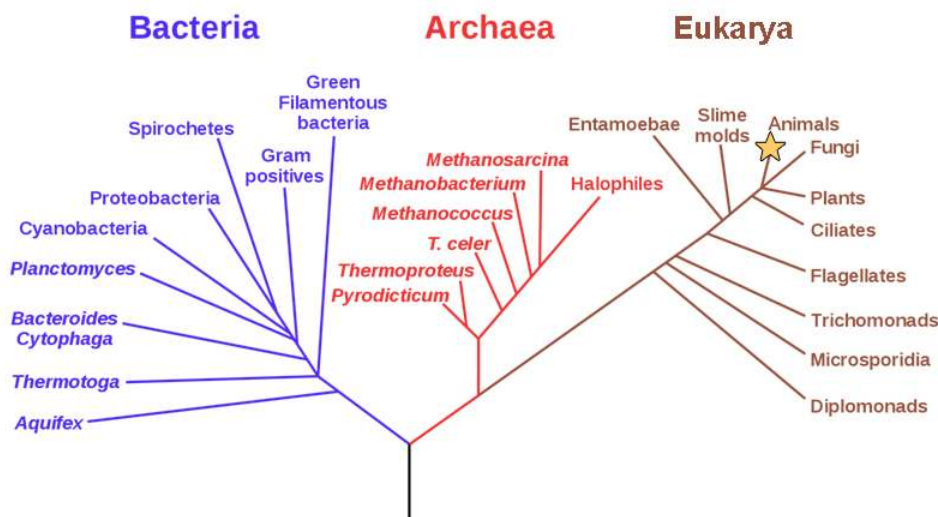


Figure 1. A phylogenetic tree of living things, based on RNA data, showing the separation of bacteria, archaea, and eukaryotes. (Public Domain; NASA via [Wikipedia](#))

The three **domains** of life are Bacteria, Archaea, and Eukarya. The domain Eukarya has four **kingdoms**: Protista, Fungi, Plantae, and Animalia. Today, we will be learning about Bacteria and Protista. Some similarities between these two groupings are that they are mostly all microscopic and unicellular (though some protists are large and multicellular), they are all living, they can all be found in environmental water sources, and they contain harmful species (known as pathogens, which cause disease). But mostly, these two groups are vastly different. Bacteria are usually 100 times smaller, they are found in every environment on the planet, and they are prokaryotic (meaning they don't have a nucleus or organelles). Protists are eukaryotic (so they have a nucleus and organelles), they can be consumers or producers (or even both!), and they are not found in as many environments as bacteria.

Introduction: Domain Bacteria

Let's start with talking about bacterial growth. Through our lives we have likely already stumbled upon bacterial growth before. Was there ever a time when you opened a bag of cheese or yogurt and noticed some small, shiny round dots on it? Those were colonies! Bacteria land on a surface with food as a single cell then divide and divide and divide, eventually forming a ball of cells called a **colony**. A colony is visible to the human eye and contains around 10⁹ cells. Each species of bacteria produces uniquely shaped colonies. This means that we can sometimes tell two different bacteria species apart based on what their colonies look like. Other organisms like molds (a class of fungus) also form colonies. The main way to tell bacterial and fungal colonies apart is based on their visual appearance, also called their **morphology**. Fungal colonies will look fuzzy or fluffy; bacterial colonies typically look shiny or wet. Today, we will practice identifying the colony morphologies we see on the plates we inoculated last class.

Below are some terms and categories used to describe the colony morphologies for bacteria.

- Shape - round, irregular, or punctiform (tiny pinpoints)
- Margin - smooth, undulate (wavy), lobate (lobes), or filamentous (unbranched strands)

- Surface - smooth, rough, or wrinkled
- Texture - moist, mucoid (sticky), shiny, dry, or dull
- Elevations - convex, umbonate (raised in center), or concave
- Pigment production (color)
- Opaque (can't see through) or translucent (can see through)

Introduction: Kingdom Protista

With increased access to genetic analysis in recent years, the taxonomic groupings of protists and other eukaryotes has become more complicated. The once separate kingdom of Protista is now intertwined and dispersed across the other three kingdoms, with some protists being more genetically similar to fungi and animals, and others more genetically similar to plants. For example, on the Eukarya branch in Figure 1, we see that there are groups labeled Plants, Fungi, and Animals, but there is no group labeled as Protista! So we can think of protists as all eukaryotes that are not animals, plants, or fungi. For our class, we will take a surface level look into this kingdom and its vast diversity.

The Protista grouping contains highly diverse species that live all over the world. Many protist species are microscopic and live in or near water. Some protist species are **autotrophs** where they use carbon dioxide, water, and light to produce their food by performing photosynthesis, making these species green, golden, or brown in color. Other species are **heterotrophs** that consume dead material or living cells, making these species either brown or clear/colorless. Yet other protists are called **mixotrophs**, which can both photosynthesize like autotrophs and consume other organic matter like heterotrophs. Protist species have evolved numerous ways of moving around, called **locomotion** or **motility**. One method is by having **cilia**, which are tiny, thin hair-like projections from the cell surface. These cilia will beat together forcing water to move around the cell and propel the cell forward. Another option some protist species have is a **flagellum**, which is a long, thin tail-like projection usually from one end of the cell (some species have more than one; plural = flagella). Flagella can either spin around functioning like the motor on a boat or undulate up and down similar to how a dolphin swims. The last option for movement is to have **pseudopodia** (meaning “false feet”), which are formed when the cell pushes the inner contents of the cytoplasm around, moving the cell forward like an inchworm. There are also some species of protist that cannot move and are considered **non-motile**.

Today we will begin by looking at some representative examples of Protist supergroups. Then we will build a flowchart, called a **dichotomous key**, for identification that we can use to try to classify unknown species in a pond water sample.

Table 1. Comparison of protist supergroups and their characteristics

Supergroup Examples	Morphology	Locomotion	Energy Harvesting
Amoebozoa (<i>Amoeba</i> spp.)	Microscopic single-celled; Cell shape varies as it moves; Colorless	Pseudopodia	Heterotroph
Archaeplastida (Green algae)	Single or multicellular; Shapes and sizes vary by species; Green color	Non-motile	Autotroph
Chromalveolata (<i>Paramecium</i> spp.)	Microscopic single-celled; Oval cell shape; Colorless	Cilia	Heterotroph
Chromalveolata (Diatoms)	Microscopic single-celled; Various cell shapes; Golden colors	Non-motile	Autotroph
Chromalveolata (Brown Algae aka seaweed/kelp)	Multicellular; Large plant-like organism; Green/brown color	Non-motile	Autotroph
Excavata (<i>Euglena</i> spp.)	Microscopic single-celled; Oval cell shape; Green color	Flagella	Mixotroph

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11.2: Lab 11 Procedures

PROCEDURE 1: BACTERIAL GROWTH

Equipment we'll need:

- Plates from last week
- 1. Obtain the group's plates. Do not open the plates.
- 2. Examine the plates carefully looking for even minor differences in the colonies growing on the plates.
- 3. Record the results in the Answer Sheet.

Clean up/Disposal

1. Dispose of agar plates into the red biohazard bin.

PROCEDURE 2: PROTISTA DIVERSITY

Equipment we'll need:

- Microscopes
- Live cultures
- Diatomaceous earth
- Cover slips
- Microscope slides

Part 1: Collecting Morphology Data

1. Obtain a compound microscope and 4 clean slides.
2. Make a wet mount of each specimen one by one. If the slide is left on the desk for too long, the samples will dry out. It is optional to spread out the labor where each student does one of the samples and then the group looks into each other's microscopes.
 - a. Mixed Protista: use the pipette to sample some of the green growth in the liquid near the bottom. Add a few drops to the slide then cover the liquid with a coverslip (use the plastic squares, do not use the tissue paper pieces).
 - i. Look for different species based on the characteristics in Table 1.
 - b. Mixed Green Algae: use the pipette to sample some of the green growth in the liquid. Add a few drops to the slide then cover the liquid with a coverslip.
 - c. Brown algae: using scissors and a scalpel, cut a very thin slice of the seaweed. Add a few drops to the slide then cover the liquid with a coverslip.
 - d. Diatoms: use the pipette to sample some of the brown clay-like in the liquid. Add a few drops to the slide then cover the liquid with a coverslip.
3. Once the slide is ready, place it on the stage and get the microscope in focus with the scanning objective (4x). Depending on the species, this might be enough magnification to draw what is seen. If more detail is needed, switch to the low power (10x) objective lens. Some species are best viewed at high power (40x) or highest power (60x). Remember the slide can be removed while in focus at the 4x objective so that the microscope stays in focus.
4. Draw as much detail as possible and include notes on locomotion seen.

Part 2: Building an Identification Flowchart

1. Using the data recorded from viewing the species under the microscope and the information in Table 1, build a flowchart that shows a process of elimination to be able to identify unknown protist species.
 - a. At the top of the flowchart, there will be a list of all six species.
 - b. Choose something that differs between the species that would fall into two categories such as size of organism or single/multicellular.
 - c. Write the two options on the opposite sides of the flowchart then fill in the species that fall into that category.
 - d. Repeat this process with other features such as locomotion or energy harvesting until all species are by themselves.

Part 3: Identifying Unknown Organisms

1. Make a wet mount of the pond water sample by using the pipette to transfer a drop of water to a clean slide. Cover it with a coverslip.
 - This may sound like a familiar procedure - we did it all the way back in the first microscopes lab! But now we have the background to allow us to identify these organisms and describe their biology in more detail.
2. Place the slide under the microscope and get into focus.
3. Find a cell or organism and record its features including how it is moving. Then use the flowchart to try to identify what possible grouping it could belong to.

Clean up/Disposal

Microscope

1. Remove slide from microscope.
2. Lower the microscope stage all the way down.
3. Put the 4x objective lens in place over the stage.
4. Turn off the microscope.
5. Unplug the microscope and wrap the cord neatly around the base.
6. Cover the microscope and return to its appropriate cabinet. Then lock the cabinet and return the key.

Slides

1. Remove the coverslip from the wet mounts. Dispose of the coverslip in the trash.
2. Rinse the slide clean.
3. Set slides out to air dry on the towels.

PROCEDURE 3: FUNGAL GROWTH (Preparation for Next Week)

As we saw from today's bacterial growth experiments, there are little microscopic species (**microbes** for short) everywhere around us and on us. These bacteria and fungi have evolved relationships with large organisms like humans. Some of the relationships can be harmful, causing us to become ill. However, the majority of interactions between humans and microbes are **mutualistic** where we both benefit from working together. What is amazing about nature is that humans are not the only species to have formed these positive relationships with microbes.

Next week, we will learn about another microscopic partnership between fungi and plants. In order to look at the growth of fungi, they need time to grow so today we will start these experiments then collect and interpret the results during the next lab.

Equipment we'll need:

- Rulers
 - 2 agar plates
 - Forceps
 - Tape
 - Cutting board
 - Cutting utensil
 - Beaker of isopropanol alcohol
1. Find a leaf that is at least 5 inches by 5 inches or at least the length of an index finger and width of 2 fingers. Work with other groups to choose leaves from many different plants.
 2. Return to the classroom and break the leaf in half.
 3. Dip the scissors in isopropanol alcohol and allow them to air dry. Wear gloves, wash hands with soap and water, or use hand sanitizer before continuing this experiment.
 4. Cut two 3x3cm or 4x4cm squares from the same leaf.
 5. Obtain two agar plates. Label one "dirty" with the group's bench letter A-I and the second plate label "clean" with the group's bench letter A-I.
 - a. Keep the plates closed as much as possible.

6. For the plate labeled “dirty”, place and lightly push one of the leaf sections into the agar. Tape the edges of the plate closed.
7. For the plate labeled “clean”, place the other leaf section into a beaker of isopropanol alcohol for 1 minute.
8. Using tweezers, remove the leaf section then place and lightly push the leaf section into the agar. Work quickly to avoid contamination from the air. Tape the edges of the plate closed.
9. Give plates to the instructor to store them until next class.

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CHAPTER OVERVIEW

12: Lab 12 Survey of Plants and Fungi

12.1: Lab 12 Background

12.2: Lab 12 Procedures

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12.1: Lab 12 Background

Learning Objectives

- Explain possible important roles of fungi and specifically endophytic fungi.
- Use observational skills to notice differences between fungal and plant species around us.
- Identify which major plant group a sample plant belongs to.

Introduction: Kingdom Fungi

In addition to Bacteria, Archaea, and Protists, Fungi are commonly microscopic. Similar to bacteria, Fungi are found in nearly every environment across the planet. Some fungal species are macroscopic like mushrooms; while, others are microscopic and form colonies like yeasts or molds. Fungi are part of the domain Eukarya, which means their cells contain a nucleus and organelles. Fungi can have lots of various shapes and structures. These organisms perform specialized versions of reproduction where multiple cells will form unique structures that can merge together during sexual reproduction. The production of microscopic spores is a unifying feature for all fungi. Spores will be released and spread across the environment through the wind or on animals and begin growing somewhere new. Fungi are heterotrophs, using other organisms as their food source. All fungi are important for the decomposition of dead material in the soil, helping to regenerate various essential nutrients for plants and other organisms to use.

During the fermentation lab, we actually experimented on a fungal species! **Yeasts** are round, single celled, microscopic fungi species. These species are unique in the type of cell division they perform called **budding** where the newly formed cell is smaller than the original one (left, Figure 1). Yeast are amazing at performing fermentation (a process of breaking down food sources without the use of oxygen) so much so that humans have harnessed this power to make all sorts of foods/beverages- bread, pizza, alcohol, kombucha, etc. When yeast are grown on an agar plate, they look very similar to bacterial colonies in size, shape, and color.



Figure 1. Representative examples of yeast species and their uses ([Left Image](#), Right Image from Polly Parks).

Have you ever wondered where the green or white fuzzy stuff on your bread/oranges/yogurt came from? That growth is a filamentous mold species that started as a single microscopic spore that typically becomes a large, round, fuzzy colony that is brown, black, white, or green in color (right, Figure 2). **Mold** growth is referred to as filamentous, where it grows as long thin tubes called **hyphae** (left, Figure 2). From the hyphae, many different and unique reproductive structures can form that will eventually begin to release spores.. Another major group of fungi are **mushrooms**. These species are macroscopic, meaning we do not need a microscope to see them. However, they still reproduce using microscopic spores. The spreading of mushroom spores can be noted in the random presence of mushrooms growing in the garden or along the sidewalk.

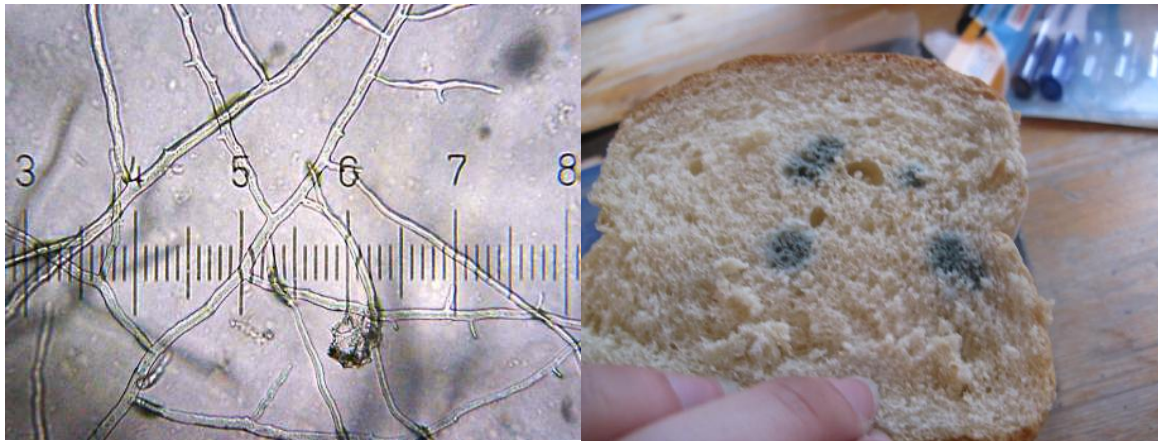


Figure 2. Representative examples of mold species ([Left Image](#), [Right Image](#))

For today's experiment, we are looking into the presence of specific types of fungi, called endophytes, that have evolved mutualistic relationships with plants. **Endophytes** live in between the plant leaf cells without causing disease or harm to the plant. Recent studies have shown that certain endophytes can provide plants with new abilities to survive various growing conditions. There are some endophytes that plants have in their seeds that, when removed, the plant seed won't sprout. Some endophytes remove or eliminate toxic molecules for the plants. Other endophytes can even help plants survive extreme heat or deadly pathogens, which is an important relationship to study for the maintenance of our agriculture and food supplies.

Last class, we took small leaf sections where the edges were cut so that any fungi cells inside the leaf tissue could flow out and grow on the agar plate. We did two samples: one where we did not do anything to the leaf labeled "dirty" and a second where we dipped the leaf in alcohol labeled "clean". The alcohol treatment would kill any fungi that were growing outside of the leaf tissue and thus is not considered an endophyte.

Introduction: Kingdom Plantae Diversity

All plants share the following characteristics: they are eukaryotic, multicellular, have chloroplasts, perform photosynthesis, are non-motile, contain a cell wall made of cellulose, and perform sexual reproduction (though many plants can also reproduce asexually). Ancient plants began as single-celled microscopic species living in the waters of the planet. Over time, these single-celled organisms began to work together forming tissues and eventually organs that then lead to the formation of plants.

Bryophytes, such as **mosses**, are most similar to those first plants that evolved. Mosses are incredibly simple plants, lacking underground root systems entirely. Mosses are generally very small and must live in wet environments (Figure 3). To reproduce, they make sperm cells similar to animal sperm that have flagella and actually swim through water to find an egg to fertilize. Mosses are unusual in the plant kingdom because male and female organs are found on separate plants (most species form both male and female parts on the same plant). Mosses typically have a short stem, called a caulid, with leaf-like structures called phyllids attached. A reproductive structure called the **sporophyte** will release spores that can grow into entirely new mosses (left, Figure 3).



Figure 3. Representative examples of moss species (images taken by Polly Parks).

From here ancient plants evolved **vascular tissues** to transport water and nutrients throughout the plant. This gave plants with vascular tissue a selective advantage because they could now move into drier environments. The next plant group to evolve was most likely the pteridophytes, seedless vascular plants including modern day ferns. **Ferns** have roots, true stems, and leaves that all contain vascular tissue (Figure 4). They are similar to the mosses in that they still have sperm with flagella. Thus, ferns survive best in moist environments so they can reproduce. Ferns have long frond shaped branches that come off of a central stem. Underneath their leaves are **sori** that contain structures to release spores, seen as the dots on the leaf surface in the middle image of Fig. 4. These spores will become separate male or female organs. Next, male and female gametes will fuse together and will grow into new fern plants.



Figure 4. Representative examples of fern species (images taken by Polly Parks).

Bryophytes and seedless vascular plants have swimming sperm and so cannot survive well in drier environments. However, a new group of plants evolved a new method of reproduction that did not rely on water, called pollen. **Pollen grains** are the microscopic male gametes that contain sperm cells for plants that can be transmitted by the wind, gravity, mammals, insects, etc. In addition to pollen, plants also evolved seeds as a method of reproduction. **Seeds** provide protection and a food source for plant embryos, allowing plants to occupy even drier habitats and wait until conditions are right before sprouting.

The first plant group to evolve pollen and seeds were the Gymnosperms. **Gymnosperm** seeds are considered “naked” because they do not have a protective fruit layer surrounding them. Gymnosperms contain three main plant groups: cycads, ginkgos, and conifers. **Cycads** live in milder climates and are usually mistaken for miniature palm trees. Cycads have frond shaped branches similar to ferns but the leaves are hard and spiky (Figure 5). In the center of the cycad contain the reproductive structures. These plants also have male and female organs on separate plants. Female cycads have a dome-like structure in the middle and male cycads have one to two large oval shaped cones.



Figure 5. Representative examples of cycad species ([Left Image](#), [Right Image](#)).

Ginkgos are incredibly fascinating because there is only one surviving species left within this genus. These trees have thin, fan-shaped leaves, seen on the right side of Fig. 6. They too have male and female organs on separate trees. They are very slow growing trees, where many planted in the United States are young and still have very thin trunks. Ginkgo leaves can also change color from green to yellow to orange to brown when the tree is mature enough (Figure 6).



Figure 6. Representative examples of ginkgo species ([Left Image](#), [Right Image](#)).

Conifers are cone-producing plants like pine trees, juniper trees, sequoia trees, and spruce trees (Figure 7). They have evolved to survive extremely dry conditions by making thin pointy leaves to reduce water loss (seen in Figure 8). This is the first group to contain both male and female reproductive organs within one plant. Male cones are smaller than female cones, are covered in a dusty orange material (pollen), and are located on the ends of branches (left, Figure 8). Female cones are the typical “pine” cones - they are large, and at the bottom of each scale is a microscopic egg (right, Figure 8). If the pollen happens to land on the egg, the fertilized egg will become a pine seed.



Figure 7. Representative examples of conifer species (images taken by Polly Parks).

The final group of plants to evolve were the Angiosperms. **Angiosperms** were the first plants to produce flowers and fruits, which were a major selective advantage. **Flowers** are modified leaf structures that contain a plant's reproductive organs. Once pollen and egg combine, the fertilized egg will turn into a seed that will be surrounded by the ovary, which develops into the **fruit**. Fruits can be dry or fleshy. Dry fruits are more similar to shells such as corn kernels, nuts, dandelions, and pea pods. Fleshy fruits are all the "fruits and vegetables" we like to eat such as apples, oranges, cherries, zucchini, bell peppers, tomatoes, etc.



Figure 8. Representative examples of male and female cones on conifer species ([Left Image](#), [Right Image](#)).

Angiosperms are divided into two groups called **monocots** and **dicots** based on their structures. The originating difference is within the seed each plant makes - there can be either one or two **cotyledons** that will form either one or two leaves when the seed begins

to grow. In addition, there are many other differences that have been identified between these two groups summarized in Table 1.

Table 1. Comparison of major Angiosperm plant groups.

Feature	Monocot	Dicot
Number of leaves that form when sprouts	One	Two
Root system	Web or thin, spread out	Single, large central root (called taproot) with root hairs
Wood formation	Rare	Common
Pattern of veins on leaf (left, Fig. 9 or 10)	Parallel	Network or weblike
Leaf connection (middle, Fig. 9 or 10)	Clasping around stem	Direct node to stem
Flower petal numbers (right, Fig. 9 or 10)	Threes or sixes	Fours, fives, or whorl (like a rose)



Figure 9. Representative examples of monocot species (images taken by Polly Parks).

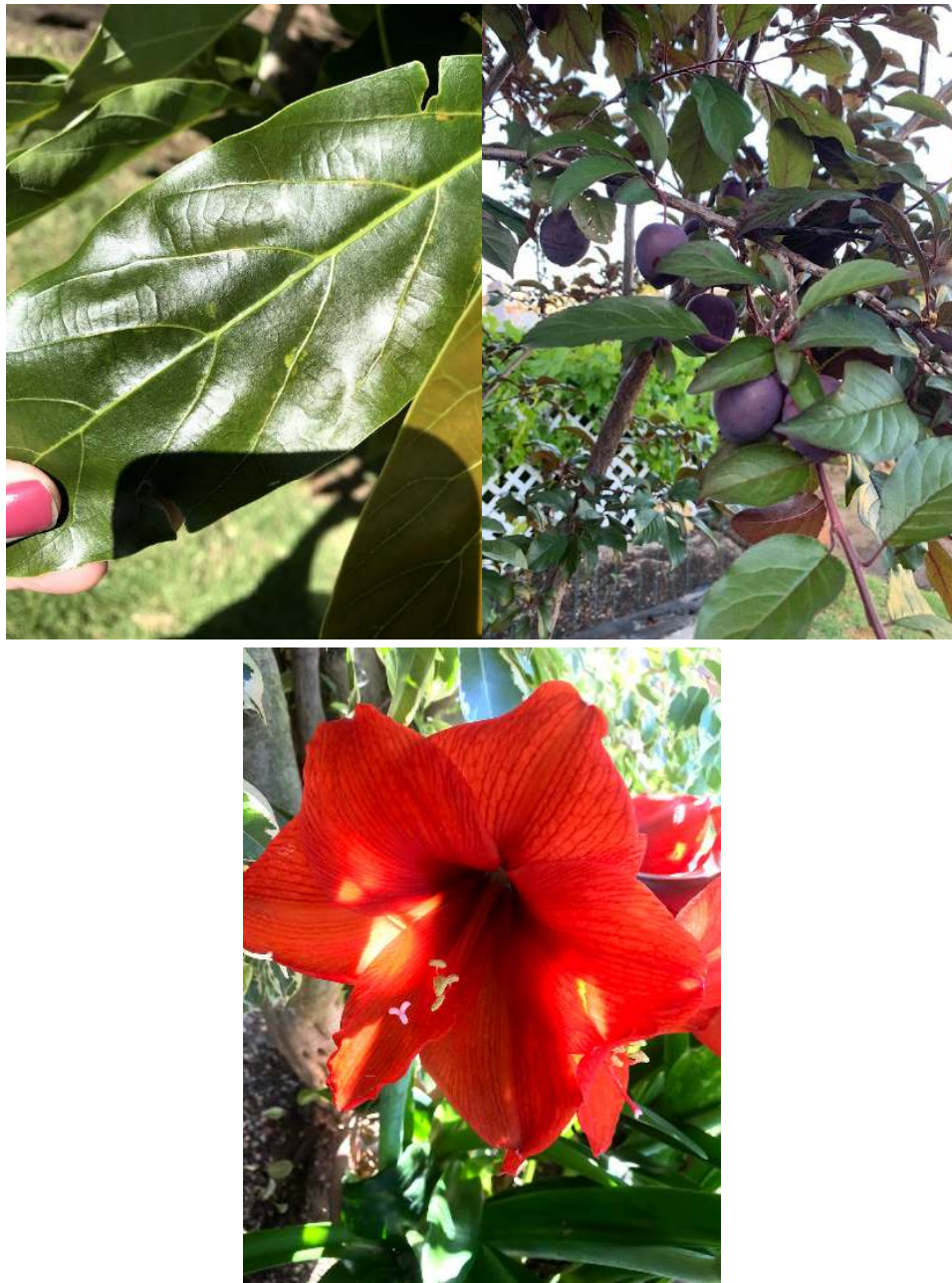


Figure 10. Representative examples of dicot species (images taken by Polly Parks).

Introduction: Plant, Leaf & Flower Anatomy

Plants can be divided into two general parts determined by their relationship to the ground. Parts above ground are called the **shoot system** (1, Figure 11). The parts below the ground are the **root system** (2, Figure 11). Some plants will produce a **flower** containing petals, stamen, and pistil as a method of reproduction (3-4, Figure 11). At the tip of the growing shoot system is an area of cells actively dividing called the **apical bud** (5, Figure 11). This is where vertical growth of the plant occurs. There can be additional apical buds lower in the plant for multiple stems to grow vertically (9, Figure 11). The plant stem is made up of various regions, where the locations that leaves grow outward from the central stem are called the **nodes** and the areas between branches or leaves are called the **internodes** (6-7, Figure 11). The location where the leaf is growing from the stem is called the **axillary bud** (8, Figure 11). This is one way that plants grow horizontally. Coming off of the stem is the **vegetative shoot**, containing the **leaf** and **petiole** (10-12, Figure 11). The leaf is where the majority of photosynthesis occurs for the plant, which is necessary to convert sunlight energy into usable chemical energy for growth and reproduction. Below the ground are the **roots** with thinner extensions called **root hairs** (13-14, Figure 11). These help the plant anchor in place and absorb water and nutrients from the soil.

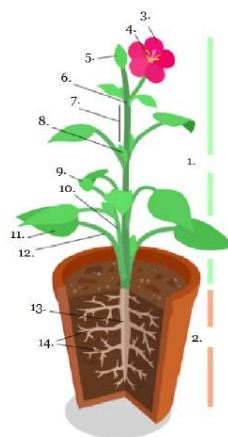


Figure 11. Diagram of a generic flowering plant. (CC BY-SA 4.0 International; [Sten via Wikipedia](#))

Now let's take a closer look at what is going on inside of a leaf and how these parts relate to photosynthesis. The top most portion of a leaf is called the **cuticle** and typically is made of waxy secretions that help to reduce water loss (1, Figure 12). Below that is a thin layer of cells called the **epidermis** that provides the leaf with protection (2, Figure 12). Next is a compacted, stacked region of cells that perform the majority of photosynthesis for the plant called the **palisade mesophyll** (3, Figure 12). The more pocketed and gapped region below this is the **spongy mesophyll** where some photosynthesis occurs and the spacing allows for more gas exchange (4, Figure 12). On the bottom of the leaf is another layer of epidermis (5, Figure 12). In addition, there are specialized cells called **guard cells** that form a microscopic gap in the leaf called a **stomata** (6-7, Figure 12). The guard cells expand and retract to open and close the stomata allowing CO₂ from the air to flow in and the O₂ product of photosynthesis to flow out. There is also the **vascular bundle** region of the leaf that is made of the **xylem** and **phloem** (8-10, Figure 12). The xylem is important in bringing water to the leaf cells to use as a reactant in photosynthesis. The phloem takes the sugar products of photosynthesis and disperses them throughout the plant.

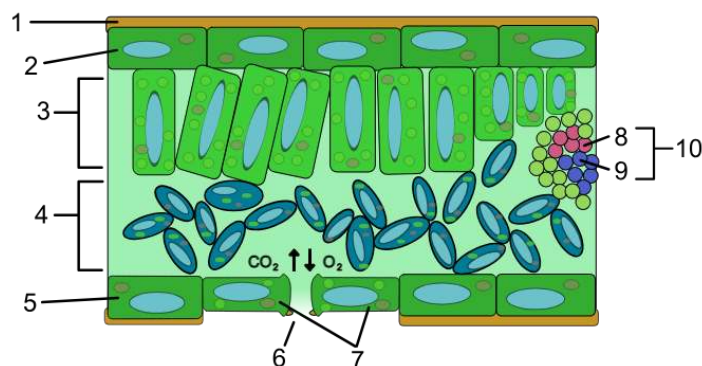


Figure 12. Diagram of the internal structures of a leaf. ([Image Source](#))

As mentioned above, flowers are modified leaf structures that contain both male and female organs (Figure 13). The base of the flower is called the **receptacle** which leads up to leaf-like supportive structures called **sepals**. The female organ is called the **pistil** and contains the stigma, style, ovary, and ovule. The **stigma** is where the pollen grain lands. It can also have substances like scents or sticky chemicals that attract pollinators. The **style** is a long tube that connects the stigma to the ovary. The **ovary** is the region of the flower that contains the flower **ovules** (i.e. eggs) and eventually swells into the fruit. The male organ is called the **stamen** and contains the anther and filament. Pollen is found in the **anther** and can stick to the stigma of the same flower, other nearby flowers, or onto a pollinator. The **filament** is a long tube that raises the anther higher in the flower structure. For today's lab, we will try to identify as many of these features as possible.

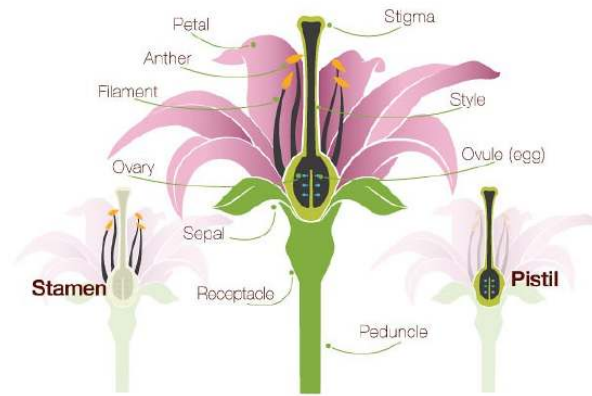


Figure 13. Labeled drawing of a flower and its parts ([Image Source](#)).

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12.2: Lab 12 Procedures

PROCEDURE 1: FUNGAL GROWTH

Equipment we'll need:

- Plates from last week
1. Review the answers to the design questions in the answer sheet from last class.
 2. Obtain the group's plates. Do not open the plates.
 3. Examine the plates carefully looking for even minor differences in the colonies growing on the plate.
 4. Record the results in the Answer Sheet.

Clean up/Disposal

1. Dispose of agar plates into the red biohazard bin.

PROCEDURE 2: PLANT SCAVENGER HUNT

1. Fill in Table 3 in the Answer Sheet to compare the different plant groups using the information from above.
2. With Table 3 complete, walk about our building and campus to find representative species of each major plant group. It is possible to find all seven groups from Table 3.
 - a. Option for inclement weather or evening classes: using your favorite search engine look for images of our campus and identify plants from those images. Get creative with ways to explore these plant groups and where they might grow.
3. Take a picture of each plant group you identify and bring them back to the classroom to share.

PROCEDURE 3: PLANT, LEAF, & FLOWER ANATOMY SAMPLES

Part 1: Leaf Structures - Stomata

Equipment we'll need:

- Clean microscope slides
 - Boxwood leaf
 - Cover slips
 - Black marker
1. Obtain a clean slide and coverslip.
 2. Remove a small Boxwood leaf. Color in the backside of the leaf with a black marker.
 - This helps us view the stomata.
 3. Place the specimen on the slide with the backside of the leaf facing up. Cover with a coverslip.
 4. Using the scanning (4x) objective lens, get the specimen into focus. Once in focus, move the objective to low power (10x). Draw what is seen in the Answer Sheet.

Part 2: Flower Structures

Equipment we'll need:

- One flower
 - Dissecting tray
 - Scissors
 - Scalpel/Razor blade
 - Needle
1. Obtain one dissecting tray, any necessary tools, and a flower specimen.
 2. Observe the flower and try to identify the parts using the image above.
 3. Remove the sepals and stem being careful not to harm the base of the flower.
 4. Remove all petals being careful not to harm the inner reproductive organs.

5. Identify and remove the stamen(s) from the flower. If possible, remove the anther from the filament. Another option is to rub the anther on the paper to see the dusty pollen grains.
6. Carefully using a scalpel or razor blade, cut the ovary directly in half to see the ovules.
7. Place or draw each flower part in the Answer Sheet.

Clean up/Disposal

Microscope

1. Remove slide from microscope.
2. Lower the microscope stage all the way down.
3. Put the 4x objective lens in place over the stage.
4. Turn off the microscope.
5. Unplug the microscope and wrap the cord neatly around the base.
6. Cover the microscope and return to its appropriate cabinet. Then lock the cabinet and return the key.

Slides

1. Remove the coverslip from the wet mounts. Dispose of the coverslip in the trash.
2. Dispose of the boxwood piece in the trash.
3. Rinse the slide clean. Set slides out to air dry on the towels.

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CHAPTER OVERVIEW

13: Lab 13 Survey of Invertebrates

[13.1: Lab 13 Background](#)

[13.2: Lab 13 Procedures](#)

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13.1: Lab 13 Background

Learning Objectives

- Identify invertebrates to the phylum level based on visual characteristics or a list of traits.
- Build a dichotomous key to describe differences in traits between animal phyla.

Introduction

All animals are multicellular, heterotrophic eukaryotes that lack a cell wall and are motile at some point during their life cycle. Take a moment to look over the phylogenetic tree in Figure 1 below. Identify which animals are considered invertebrates. These are the phyla we will learn about in this lab.

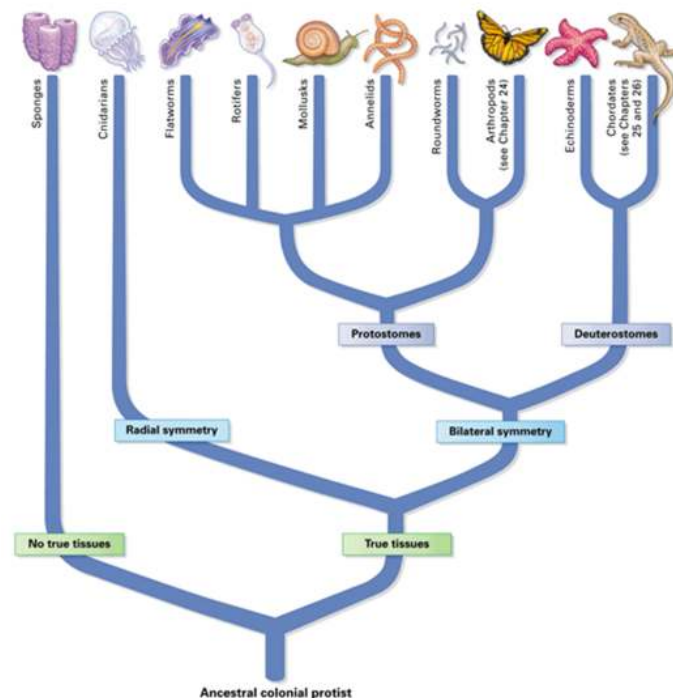


Figure 1. A phylogenetic tree of the kingdom Animalia showing only the ten most species-rich phyla. Together, these phyla cover the majority of living animals. From Discovery Biology, 6/e Figure 9.2.

Porifera

Animals in the phylum Porifera are commonly known as sponges. Porifera are among the first animals to have ever evolved! The cells of these organisms come together and collaborate, but are not true tissues. This means that Porifera are multicellular pore-bearing animals with no organs. It may be difficult to imagine, however, sponges only consist of individual cells that come together to make the whole work. An analogy is to think of a classroom with students who come together to be a whole class, yet remain as individuals. When sponges die, we can use their remnants for bathing, known as a natural sponge which is similar to a loofah (though, a traditional loofah comes from a fruit!). These animals may appear to have radial symmetry, however, animals with true radial symmetry also have true tissues. Therefore, sponges are considered to lack any symmetry. Sponges can be very large and can regenerate portions that get damaged or break off.

Cnidaria

Animals in the phylum Cnidaria have simple tissues and true radial symmetry, though they lack true organ systems. Examples include jellyfish (also called jellies because they're not fish), sea anemones, and coral. Cnidarians tend to have two body plans: polyps are tube-shaped with the tentacles facing upward (like coral and anemones), while medusae (singular: medusa) are bell-shaped with the tentacles facing downward (like adult jellyfish). Some cnidarians, like jellyfish, live in both the polyp and medusa

stage during different life stages. Tentacles have specialized stinging cells called cnidocytes that are used for catching prey and defense. With a simple nervous system, they can move their tentacles towards their mouth, and free-floating medusae contract simple muscular tissue for swimming. These animals can reproduce both sexually and asexually.

Mollusca

The mollusks have a relatively complex body plan with multiple types of tissues and organ systems. This is a very diverse phylum and contains the highest number of marine species of any animal phylum. Examples of mollusks include shellfish (like clams, mussels, and scallops), gastropods (like snails and slugs), and cephalopods (like octopi, squids, and cuttlefish). Mollusks have soft bodies with distinct regions, including a muscular foot and a mantle that sometimes secretes a shell. They have complex organ systems with most of their organs grouped together in the visceral mass. Cephalopods, especially octopi, are often considered the most intelligent invertebrates on Earth.

Platyhelminthes

These worms, commonly called flatworms, are the simplest of all worms because their bodies are made of simple tissue layers and do not have a distinct body cavity. As we see in Figure 1, flatworms, along with all other animals except poriferans and cnidarians, are bilaterally symmetrical. They were also among the first animals to evolve organ systems, including simple digestive and nervous systems. They lack a respiratory system, but their thin bodies allow gas exchange through diffusion, which makes them dependent on an aqueous environment. Flatworms may be parasites or free-living in fresh water, salt water, or saturated soils.

Annelida

The most complex worms are commonly called segmented worms, including earthworms and leeches. These worms have a true body cavity (coelom), advanced digestive & nervous systems, and distinct body segmentation. The cuticle that covers their soft cylindrical body allows them to absorb oxygen. Different species thrive in soil or in water. Some segmented worms have paired extensions that protrude from their bodies, called parapodia, for locomotion.

Nematoda

These worms are commonly called roundworms and may exist in aquatic or terrestrial environments. Their bodies are more complex than flatworms because of a distinct mouth, anus, and more complex digestive system surrounded by a semi-filled cavity called a pseudocoelom. Roundworms have a tough outer layer, soft cylindrical bodies, and no segmentation. Many species of Nematoda parasitize humans, including the hookworm and pinworm.

Arthropods

Arthropods are the most abundant and diverse group of eukaryotes, let alone invertebrates. Estimates vary, but of the 2.1 million eukaryotic species described, arthropods make up over 1.2 million of them. Of the arthropods, insects are the most diverse by far, consisting of over 1 million species! Insects were also among the earliest terrestrial animals. Other arthropods include crustaceans (like crabs, lobsters, and barnacles), arachnids (like spiders and scorpions), and myriapods (like centipedes and millipedes). Some species are aquatic and others terrestrial; some are even parasitic, like mites and marine isopods. The tissues and organ systems of these animals are well developed. Their sensory organs allow them to react quickly when hunting or escaping. All arthropods have a hard outer body layer called the exoskeleton, which is composed of chitin and provides support to pairs of jointed limbs. In fact, the word arthropod means “jointed foot”. The body is clearly divided into three sections: head, thorax, and abdomen. Insects’ exoskeleton and wings are two of the reasons they have been so successful for hundreds of millions of years.

Echinodermata

Echinoderms, including sea stars (also called starfish, but they’re not actually fish), sea urchins, and sand dollars, have a hard outer body made from calcium carbonate, as opposed to the chitin that makes up arthropod exoskeletons. In fact, the word echinoderm means “spiny skin”. However, there are some soft bodied echinoderms such as sea cucumbers. All echinoderms live in the ocean. Their embryonic development sets them apart from other invertebrates; as deuterostomes, the anus develops first before the mouth in the embryo. They have a true coelom, though their body shape may be round or star-shaped because of multiple appendages anchored at a central body. As larvae, echinoderms are bilaterally symmetrical, but they are radially symmetrical as adults. Many echinoderms move using a unique water vascular system and tube feet. Echinodermata is the sister phylum to chordates, which we’ll learn about next week.

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13.2: Lab 13 Procedures

PROCEDURE 1: BACKGROUND

1. Answer the questions on the Answer Sheet to become acquainted with invertebrates.

PROCEDURE 2: SPECIMEN SURVEY

Equipment we'll need:

- Various specimens of all eight phyla of invertebrates
-

1. Move around the room to examine the specimens in each phylum. Complete Table 1 in the Answer Sheet using what we learned above and our observations of the specimens.

PROCEDURE 3: DICHOTOMOUS KEY

A dichotomous key is a useful tool for identifying organisms based on traits they do and don't have. The key contains a series of questions that are typically yes or no, and the answer to one question leads us to another question. This video [Using Dichotomous Keys](#) by Science Sauce goes through a few examples to show how to use a dichotomous key. In the lab today, we are going to build a dichotomous key to identify the eight different invertebrate phyla.

For the Animal Kingdom, the first characteristic that divides groups is the presence of true tissues. Only animals in the Phylum Porifera (Sponges) are simple without true tissues. All other animals have true tissues with various levels of layer complexities. So, we can start our key there.

Next, animals are divided by their symmetry based on an imaginary bisection that goes through the middle of the body. Radial symmetry is found in animals that present two equal sides regardless of section direction (like a pizza). Bilateral symmetry is found in animals that can only be divided in equal left and right halves with a single line through the middle of the body. Figure 1 on the Answer Sheet has started the dichotomous key for us using information about the presence of tissue and symmetry.

Use the information we learned about the eight invertebrate phyla to complete the dichotomous key. Think about some of the major differences between the phyla. What major organ systems do they have, if any? Is the body cavity fully formed? Is their body segmented? Do they have limbs or are they tubular? How might they differ in embryonic development? Do they live in the water or on land or both?

1. Figure 1 and Table 3 on the Answer Sheet show the two different ways to build a dichotomous key using the same information. Pick one of these methods to complete the dichotomous key for identifying the other six invertebrate phyla.

PROCEDURE 4: MYSTERY ANIMALS

Equipment we'll need:

- Dichotomous key

1. Identify the mystery invertebrates using the dichotomous key from Procedure 2 to complete Table 3 on the Answer Sheet
-

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CHAPTER OVERVIEW

14: Lab 14 Survey of Vertebrates

[14.1: Lab 14 Background](#)

[14.2: Lab 14 Procedures](#)

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14.1: Lab 14 Background

Learning Objectives

- Identify vertebrates to the phylum level based on visual characteristics or a list of traits.
- Build a dichotomous key to describe differences in traits between animal phyla.

Introduction: Vertebrates

Thus far, we've learned about various phyla of invertebrate animals. The main trait that distinguishes invertebrates from vertebrates is the absence of a spinal column. So vertebrates are animals with a spinal cord protected by a spinal column. While we sometimes refer to the spinal column as a "backbone", it is not always made of bone! Some vertebrates don't have any bones and instead have skeletons made of cartilage. Many animals on land are vertebrates. However many aquatic animals like fish, whales, and sharks are vertebrates as well. These organisms are all grouped into the phylum Chordata.

Animals in the phylum Chordata all have a few traits in common, including a nerve cord, pharyngeal slits, a postanal tail, and a notochord that helps embryonic development can protect the nerve cord. Some chordates are actually invertebrates because their notochord is not a series of vertebrae - hard, hollow structures that surround and protect the nerve cord. But our focus in this lab will be specifically on vertebrates.

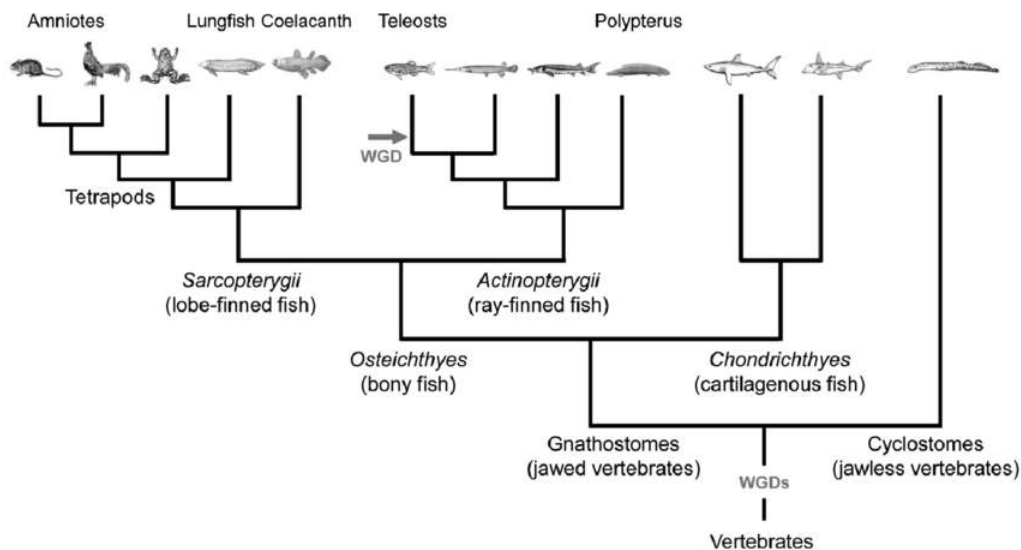


Figure 1. Phylogenetic tree of vertebrates, from Yamamoto et al. 2017.

Fish

As we can see from Figure 1, most vertebrates can be classified as fish. In fact, from a phylogenetic perspective, even the tetrapods (amphibians, reptiles, birds, and mammals) are fish! But we really only use the term "fish" to refer to the aquatic, non-tetrapod species. These animals are very versatile in size, color, shape, the depth they can survive in, and the type of aquatic biome they thrive in (salt vs fresh water). Despite all these differences, all fish share certain traits: they live in aquatic environments, have scales, gills, and a skeleton made up of bone, cartilage, or both.



Figure 2. Fish diversity ([image source](#)).

Most vertebrates, including fish, amphibians, and reptiles, are ectothermic (which we may also know as cold-blooded). Ectothermic animals generate little to no internal body heat and so rely on the environment for a source of heat. Their body temperature tends to match the environmental temperature. Review Figure 3 and think about how it depicts the difference between ectothermic and endothermic (warm-blooded) animals.

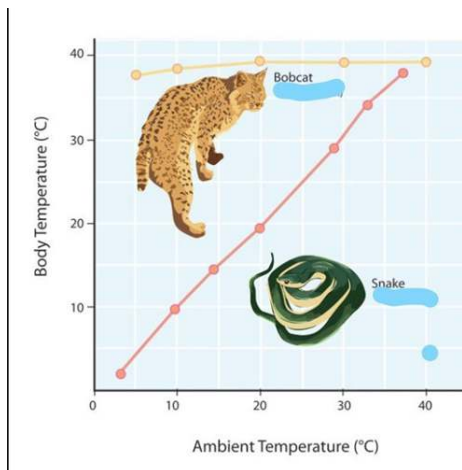


Figure 3. A line graph showing the relationship between body temperature (°C) and ambient temperature (°C) in endothermic organisms, such as bobcats, and ectothermic animals, such as snakes.

Agnatha (jawless fish)

As their name (both scientific and common) suggests, these fish do not have hinged jaws. However, they do have teeth! The most common examples include lamprey and hagfish. Lamprey are known for their circular rows of teeth that help them grasp onto the body of other animals where they consume the body fluids of their host. Some lamprey also eat algae and other microorganisms. Hagfish are better known for secreting a thick, fibrous, slimy mucus used as a defense mechanism.



Figure 4. Lamprey (left; [source](#)) showing its rows of teeth and a hagfish (right; [source](#)) secreting its mucus.

Chondrichthyes (cartilaginous fish)

As their name suggests, these fish have skeletons made of cartilage, not bone. For these organisms, there is not a single bone in their body! Examples include sharks, rays, and skates. Sharks are known for their rows of sharp teeth in a hinged jaw. However, sharks like the whale shark filter feed on plankton and microorganisms and therefore have reduced teeth with little to no function. Rays and skates both have more circular bodies with large pectoral fins that are fused to the head.

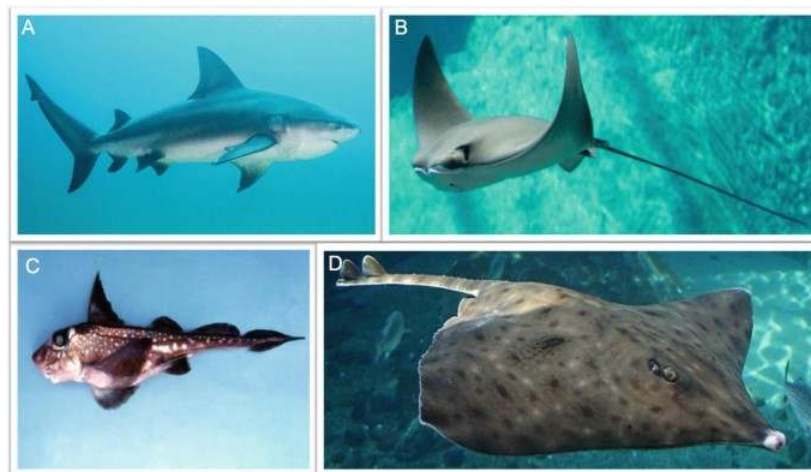


Figure 5. Examples of Chondrichthyes species, including (A) shark, (B) ray, (C) chimera, and (D) skate, from Wilson et al. 2021.

Osteichthyes (bony fish)

As their name suggests, these fish have skeletons made of bone. There are two main groups within the bony fish: ray-finned fish and lobe-finned fish. Almost all of the commercially available fish for consumption are ray-finned fish. In general, ray-finned fish make up a majority of all extant fish. These fish are characterized by relatively thin fins with thin bony spines connected by skin. Lobe-finned fish have much thicker, muscular fins with limb buds. Hundreds of millions of years ago, a lineage of lobe-finned fish began evolving fins that could also be used for moving on land, eventually leading to the tetrapods (animals with four limbs): amphibians, reptiles, birds, and mammals.



Figure 6. Many examples of the diversity in Osteichthyes ([source](#)).

Amphibia (amphibians)

Amphibians spend part of their life on land and part of it in freshwater. They are considered the first terrestrial tetrapods to have evolved from lobe-finned fish and represent an incomplete transition onto land because of their reliance on water for their life cycle. Most amphibians lay eggs in water. Larvae begin life with gills but metamorphose into adults with lungs and other traits necessary for life on land. Amphibians are the first to have true limbs and keratinized skin instead of scales. Gas exchange can also occur across the skin, so the amphibians' skin is typically thin and kept wet. Some species have glands that produce a mucus that help keep the skin moist, and some have a mucus that carries toxins. Examples of amphibians including frogs, toads, and salamanders. The image below provides examples of amphibians.

Types of Amphibians

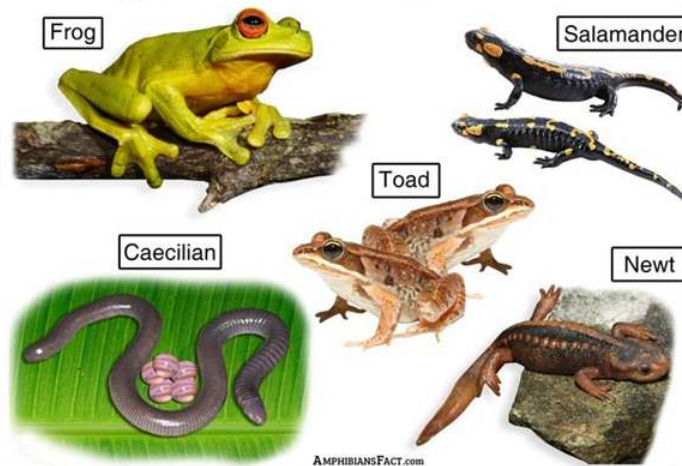


Figure 7. Different types of amphibians.

Reptilia (reptiles)

This class of animals is the first group to successfully survive on land independent of water. One of the reasons for their success on land is due to their scales that are highly keratinized, reducing dehydration. They are also the first taxa to evolve the amniotic egg. In fact, reptiles, birds, and mammals all inherited the amniotic egg from their common evolutionary ancestor. The amniotic egg has extra layers of membranes and fluids that help protect the developing embryo against the external environment. Reptiles lay this amniotic egg within a hard shell, enabling them to reproduce in drier habitats without a body of water. Examples of extant reptiles include turtles, crocodiles, snakes, and lizards.

Types of Reptiles



Figure 8. Examples of reptiles.

Aves (birds)

Data now suggest that birds belong within the reptile group (clade is the evolutionary term; see Figure 1). In fact, modern birds evolved from a specific group of dinosaurs called theropods, so in a way, dinosaurs still exist! However, birds display a number of unique adaptations that set them apart from reptiles. Unlike the reptiles, birds are endothermic, meaning they generate their own body heat through metabolic processes (like the bobcat from Figure 3). One of the most distinctive characteristics of birds is their feathers, which are modified reptilian scales. Birds have several different types of feathers that are specialized for specific functions, like contour feathers that streamline the bird's exterior and loosely structured down feathers that provide insulation. Feathers also often act as a signal for males to attract females for mating. Of course, birds also have wings, although not all wings enable flight. Flightless birds, like ostriches and emus, often have reduced wings with little or no function. But others, like penguins, may have their wings modified for other functions like swimming.



Figure 9. Bird diversity, from Tobias 2022.

Mammalia (mammals)

Mammals are vertebrates that have hair and mammary glands used to provide nutrition for their young. Fun fact: mammary glands are actually just modified sweat glands! Certain features of the jaw, skeleton, skin, and internal anatomy are also unique to mammals. The presence of hair is one of the key characteristics of a mammal. Although it is not very extensive in some groups, such as whales, hair has many important functions for mammals. Mammals are endothermic and hair provides insulation by trapping a layer of air close to the body to retain metabolic heat. Hair also serves as a sensory mechanism through specialized hairs called vibrissae, better known as whiskers. These attach to nerves that transmit touch stimuli, which is particularly useful to

nocturnal or burrowing mammals. Hair can also provide structural protection, as in porcupines and hedgehogs, or even in its color, as in skunks.

There are three groups of mammals, defined by their modes of reproduction. Monotremes, including the platypus and echidna, are the only oviparous mammals, meaning they lay eggs instead of giving live birth. Marsupials, including kangaroos and opossums, give birth to very underdeveloped offspring who then climb up into the mother's pouch to continue developing. Eutherians, also known as placental mammals, nourish their embryos internally with an organ called the placenta. About 94% of extant mammals are Eutherians.

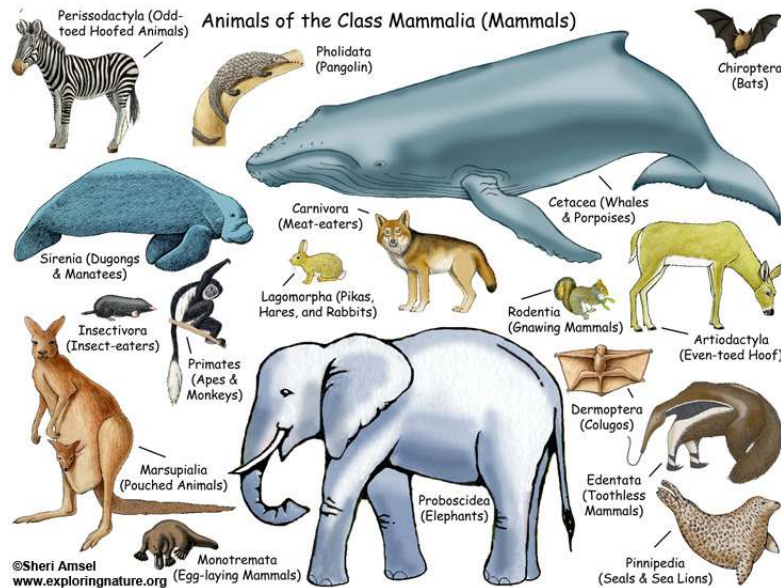


Figure 10. Mammal diversity.

Introduction: Food Webs

In our labs, we've grown microscopic bacteria and fungi species from all kinds of surfaces. We've looked at microscopic protist species in pond water. We even grew microscopic fungi species from within plant leaves! We also surveyed the plant life around us on campus and learned about the enormous diversity within the animal kingdom. We can conclude that life is all around us and all around each other. Thus, we know that organisms must have evolved to live in the presence of one another, interacting in many different ways.

Ecology is the study of how life interacts with other living organisms and the surrounding environment. Long term evolutionary and ecological interactions between species (interspecific) are called **symbioses**. For example, **exploitation** describes an interaction where one organism consumes another organism, such as **predation** or **herbivory**. Similarly, some species feed off others but not so much that it kills the other organism, which is called **parasitism**. **Mutualisms** are a very different kind of interaction where organisms have evolved to live in the presence of one another *cooperatively*. In this case, both species benefit from their interaction. Lastly, **commensalism** occurs when only one species benefits while the other is unaffected.

There is an amazing and diverse community of life that exists alongside humans in our own neighborhoods and on our own campus. Humans often don't see it, notice it, or recognize it while we go about our day-to-day activities and busy lives. This part of our lab challenges us to take notice of, and document, the community of organisms living here around us. We will also observe how various species are interacting, label their relationships, and define their roles in the ecosystem. Organisms that perform photosynthesis will be labeled **producers**. Any organisms seen in a predatory relationship will be labeled some level of consumer. If the organism is eating living plant material, they are called **primary consumers**. If the organism is eating something that would eat plant materials, they are called **secondary consumers**. Then building from there, an organism that eats a secondary consumer is called a **tertiary consumer**. Some species, such as bacteria and fungi often found in soils, are **decomposers** that use dead plant/animal materials as a food source.

A visual representation of how species within an ecosystem interact with one another is called a **food web**. Below is an example of a food web for the savanna ecosystem (Figure 11). Notice how there are multiple arrows between some of the organisms - this is important because it shows the interconnectedness of species within an environment and highlights the reality that organisms don't eat only one food source. Today we will be observing the ecosystem around us and attempting to draw a representative food web.

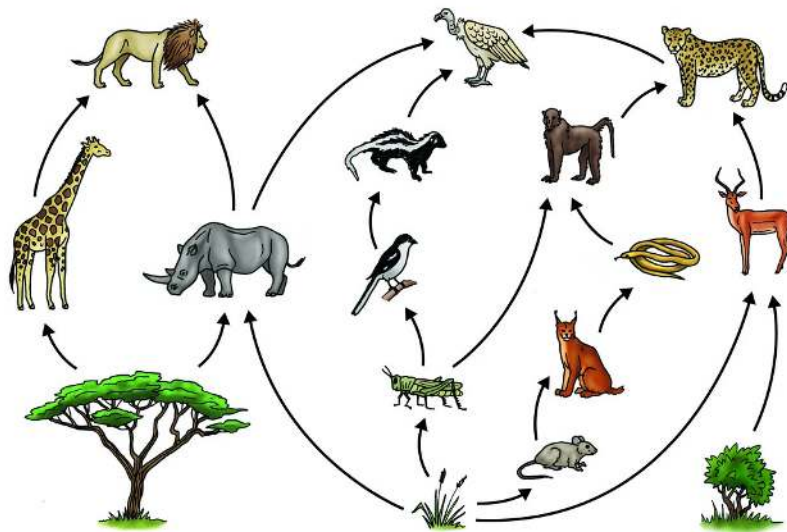


Figure 11. Diagram of a simplified food web for the savanna ([image source](#)).

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14.2: Lab 14 Procedures

PROCEDURE 1: SPECIMEN SURVEY

Equipment we'll need:

- Various specimens of vertebrates

1. Answer the questions on the Answer Sheet to become acquainted with the vertebrates.
2. Move around the room to examine the specimens in each group of vertebrates. Complete Table 1 in the Answer Sheet using what we learned above and our observations of the specimens.

PROCEDURE 2: DICHOTOMOUS KEY

In the previous lab, we learned how to use and make a dichotomous key to identify different organisms based on their unique traits. We are going to make another dichotomous key in this lab to help us identify vertebrates. Review the video [Using Dichotomous Keys](#) by Science Sauce for a reminder. Use the information we learned about the various vertebrate groups to complete the dichotomous key. Think about some of the major differences between the groups.

1. Create a dichotomous key for identifying the different groups of vertebrates either using the flowchart or list format we learned in the previous lab.

PROCEDURE 3: MYSTERY ANIMALS

Equipment we'll need:

- Dichotomous key

1. Identify the mystery vertebrates using the dichotomous key from Procedure 2 to complete Table 3 on the Answer Sheet

PROCEDURE 4: FOOD WEB

1. Walk outside and find a location you would like to observe. Describe the location chosen in the Answer Sheet.
2. First, spend 2 minutes making observations to become familiar with the community.
3. Move around the area and record 12-15 different living organisms in Table 4 of the Answer Sheet.
 - Both macroscopic and microscopic organisms that we know are in the soils are eligible.
 - Try to find a variety of organisms from the groups we learned about the last few weeks.
 - This might involve getting low to the ground or plants, looking closely for what else might be present.
 - If the specific name of the organism is unknown, use a numbering system ex. Bird 1- small brown, Bird 2- medium black.
4. Spend 15 minutes observing how the organisms interact. Record notes in Table 4.
5. Take photographs of the different organisms to use for the food web.
6. Either staying outside or returning to the classroom, begin building a food web to describe the ecosystem by following these steps:
 - Categorize each organism as a producer, primary consumer, secondary consumer, tertiary consumer, decomposer, or some combination of trophic levels. Add this to Table 4.
 - Either draw or insert images of the species that are producers at the bottom of the page in the Answer Sheet. Label each organism by its name, type of organism, and as a producer.
 - Insert the organisms that are primary consumers as a next level. Then repeat with secondary and tertiary consumers. Label each organism by its name, type of organism, and as its appropriate consumer level.
 - Draw or insert arrows to show the interactions between the species. Arrows travel from the prey and point to the consumer. There should be multiple arrows flowing between the species to represent the many interactions seen.
 - i. Food webs are not simple linear progressions (those are food chains, which are overly simplistic).
7. Once the food web is complete, try to identify examples of the following ecological concepts:

◦ Predator-Prey

- ✓ ~~Reciprocity~~
- Mutualism
- Commensalism
- Competition
- Non-Native species impact on the community
- Human Impact on the community

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CHAPTER OVERVIEW

15: Lab 15 Ecology

[15.1: Lab 15 Background](#)

[15.2: Lab 15 Procedures](#)

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15.1: Lab 15 Background

Learning Objectives

- Explain the connection between life and plastics in the environment.
- Isolate and identify the composition of unknown plastics pieces from sand.
- Predict the effects of overharvesting on fish populations.
- Explain the ecological and economic benefits of sustainable fishing.

Introduction: Plastics

Sand is made of tiny rock grains created by erosion from the land. While, **plastic** is a man-made material that is melted, shaped, and cooled. Most plastics are made from **petroleum** (also called fossil fuels and is similar to the gasoline that goes into our cars). Petroleum fuels are a limited resource on the planet and their extraction creates other environmental issues such as increasing carbon emissions. To make plastic products, the fuel must first be extracted from deep within the earth's crust. Then it is processed into a polymer, which is a long chain molecule of many repeating sections. When these polymers are connected, they will begin to form a solid plastic. Most commonly, the companies produce **resin pellets**, which are tiny beads of plastic that can be melted down and molded into any shaped object. The pellets are then purchased by molding companies who work with various brands to make their bottles, bags, or other products.

There are many types of plastics. *Type 1 PETE polyethylene terephthalate* is found in water bottles. *Type 2 HDPE high-density polyethylene* is found in milk jugs and grocery store bags. *Type 3 PVC polyvinyl chloride* is found in clear food packaging and shampoo bottles. *Type 4 LDPE low-density polyethylene* is found in squeeze bottles, bread bags, and some food bags. *Type 5 polypropylene* is found in bottle caps, straws, and medicine bottles. *Type 6 polystyrene* is found in Styrofoam, disposable utensils, and cups. Certain molecules can be added to change the properties of the plastic such as increasing hardness, making it more bendable, or improving flame resistance. The addition of these other substances commonly makes the plastic non-recyclable. Also, each different type of plastic is recycled in a unique way.

Generally speaking, plastic products are not currently recycled and are definitely not recycled at the rate they are produced. Most plastics even when put into the recycling bins are never reused. One example is plastic wrap used to store foods cannot be recycled because they are too thin and get caught in the separating machines. Another example is plastic chip bags cannot be recycled because they also contain aluminum foil on the inside that cannot be taken apart from the plastic exterior. Small plastic items like toothbrushes, tampon exteriors, eating utensils, twist ties for bread, straws, and many others are all too small and get caught in the sorting machines. Deodorant containers and yogurt cups are made of multiple types of plastic that cannot be separated so neither is recyclable. Water, soda, and other beverage bottles are the most commonly recycled items. They are large enough and generally made of only one type of plastic. Some bottles have a different type of lid that is recommended to be removed prior to recycling. Other bottles like shampoo, peanut butter, and condiments are also recyclable but need to be cleaned prior to throwing them away.

When items don't get recycled, there is the possibility of them ending up in our environment. Historically, other countries bought plastic trash from the United States. Some of those countries would try to reuse items that they could and others burned the materials as an energy source. Burning plastic is not a great option because it produces many toxic chemicals. Ultimately, loads of plastic are ending up in the oceans. One reason is that many plastics are simply not meant to be recycled. These plastics then end up in landfills that possibly pollute the nearby waterways. Then the plastic floats along eventually reaching the ocean. When plastic is exposed to the sun for long periods of time, the sunlight causes the plastics to break down into smaller pieces, a process called **photodegradation**. Scientists are finding that the large plastic objects are breaking down into **microplastics** that are smaller than 5mm and hard to see with the naked eye, making them challenging to track. Even more challenging to track is when the plastics break down into **nanoplastics**, which are smaller than 1mm and need to be chemically tested for because they cannot be seen.

Plastics are an incredibly useful tool in modern society. Plastics are lightweight, colorless, and durable. They are essential in the medical field where diseases could spread if other materials are used. Plastics keep our foods fresher for longer. The goal of today's lab is to learn about plastics, identify the types of plastics found in our local beach sand, and discuss this issue. This lab is not meant to shame anyone for their use of plastic. It is also not trying to convince anyone to switch to using non-plastic alternatives. While working through these exercises, keep an open mind and considerate tone during discussions.

Introduction: Population Ecology

The field of **ecology** can be studied at many different scales. Population ecology studies the changes to a population's size and demographics. Community ecology studies interactions between species. Ecosystem ecology measures environmental effects, such as energy and nutrient cycling, on populations and communities. Applied ecology attempts to apply the different kinds of ecology to real world problems. For example, conservation ecology works to protect vulnerable, threatened, or endangered species from extinction. As we may have noticed, many of these fields of ecology overlap and are also **interdisciplinary** with other fields of study such as evolution, physiology, chemistry, physics, and more.

In this lab, we will apply the principles of population ecology to determine how we might be able to manage fish populations more sustainably. A **population** is a group of individuals of the same species interacting in a common area. The **population size** is the number of individuals in the population, and **population growth** measures how much the population size changes over time. It is important to note that, in this case, the term growth measures both the increase in population size (positive growth) and decrease in population size (negative growth). Population ecologists attempt to determine the main factors that affect population growth.

Commercial fishing operations harvest many thousands of tons of fish each year. Seafood is an important source of nutrition for many human populations, including here in southern California. When done responsibly, commercial fishing can feed humans and provide people with income; while leaving enough fish in the sea to both sustain other predators and allow the harvested species to repopulate. This is the goal of **sustainable fishing**. However, many species of fish have been, and continue to be, **overharvested**. Some commercial fishing vessels use large nets, called drift nets. Drift nets were widely used throughout the 20th century and could be as long as 50km (31mi)! Vessels could harvest thousands of tons of fish at a time leading to the decline, and in some cases complete collapse, of various fish species. Drift nets also cause significant amounts of bycatch, which is when non-target species are caught in the large nets. Bycatch has significantly contributed to the decline in global populations of sensitive species like sea turtles and dolphins. As a result, in 1987, the United States limited drift nets to 2.8km (1.7mi) when used in American waters. The United Nations agreed to similar limitations in international waters. Other commercial fishing practices cause similar ecological damage. For example, bottom trawling tows large nets along the ocean floor, allowing for similar levels of harvesting and bycatch, but also damages sensitive ocean floor ecosystems that may take long periods of time to recover. Recent focus on sustainable fishing practices is helping to maintain healthy fish populations, but much work still needs to be done to ensure the longevity of fish populations. This lab, which is adapted from Biol 213 at CSULB, will simulate both the economic and biological aspects of commercial fishing.

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15.2: Lab 15 Procedures

PROCEDURE 1: REFLECTION

1. Starting from when you wake up, create a list of all of the plastic items you use throughout the day. Then next to the item, put a star if the item is a single use container that would be thrown away after you're done using it (such as a chip bag, yogurt container, etc.). Record your thoughts in the Answer Sheet.

PROCEDURE 2: SYNTHETIC SAND

In this activity, we will be looking at beach sand and what is commonly found in it. There are a lot of things that are in beach sand, some things are from animals and plants that live in the ocean or on the shore. Shells are hard material that some animals use to protect themselves. Plant pieces like seeds, sticks, and leaves are from land or water plants. Also, there are commonly protist materials such as seaweed. We can also find anthropogenic items (man-made) such as plastics, metals, glass, papers, and fabrics. Today we will be comparing two sand samples from local beaches: Torrance Beach and Seal Beach.

Equipment we'll need

- Tweezers (4)- labeled oil, isopropanol, glycerol and water
- 3 small beakers filled with either oil, isopropanol, or glycerol
- Four sample collecting trays
- 650mL beaker
- 1 dropper bottle of acetone
- Sand sifters/sieves
- Sand samples from local beaches

Part 1: Initial Observations

1. Fill in the Answer Sheet to build an observational experiment.
2. Walk over to the sand samples. Fill the sieve with 3 beakers full of sand then gently filter the sand back into the bucket. Avoid creating large amounts of dust.
3. Transfer all items collected by the sieve into one of reusable sampling collecting trays.
4. Repeat steps 2 and 3 for the other sand sample.
5. Record the initial identities and number of each item found in Table 1.
 - a. If the group is unsure of an item's composition, ask for the professor's opinion.

Part 2: Plastic Materials Identification

6. Fill the 650mL beaker with water.
7. Choose a piece of separated plastic material and place it into the beaker of water. Be sure to fully submerge the item in the water and gently let go of it from the tweezers. Make sure not to introduce any bubbles that would cause the item to float. Note if the material sinks or floats.
8. Based on if the item sank or floated, transfer the piece of material to the appropriate beaker based on the next step in Figure 1 flowchart below.
 - a. For example, if the material sank the next step is to transfer the piece to glycerol testing if it sinks or floats in this solution.
 - b. Use the specific tweezers to transfer into for each specific solution.
9. Follow the flowchart until identification is reached. Record the results in Table 2 on the Answer Sheet.
10. When finished with a piece of material, remove it from that beaker and place it on a separate reusable collection tray.
11. Repeat steps 7 through 9 with all of the plastic separated materials.

Clean up/Disposal

1. Add the separated plastics to the large beaker on the instructor's desk.
2. Return the used beakers of different solutions to their original locations.. Return the dropper bottle of acetone.
3. Pour water down the drain.
4. Rinse tweezers, beakers, and collection trays. Place on the towel to dry.

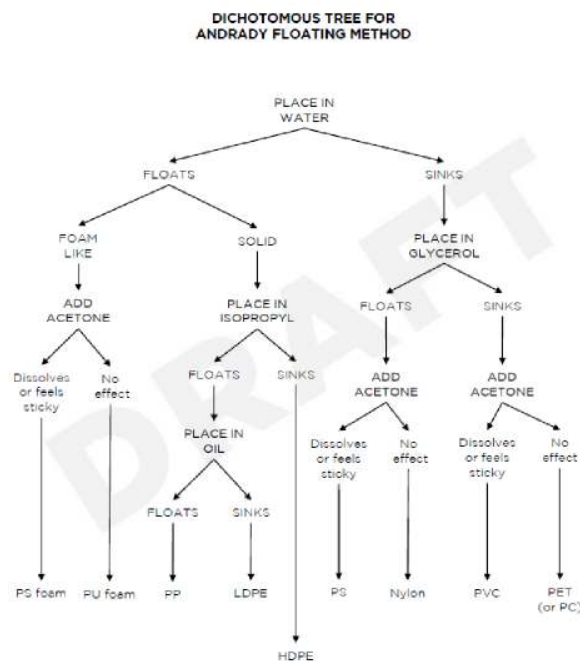


Figure 1. Flowchart of how to identify plastics materials from [Algalita](#).

Minimizing Plastics Usage Resources

- Get active! Look into the events [Algalita](#) in Long Beach or [The Golden Triangle](#) in Gardena are hosting
- Educate others!
- Avoid and reduce usage:
 - Shop used clothes and shoes
 - When buying new items, choose non-plastic options (ex. bamboo toothbrushes, metal water bottles, cloth grocery bags)
 - Go reusable and re-fillable! Stores like Sprouts and [The Golden Triangle](#) sell products and foods in bulk- bring small containers or cloth bags to fill them
 - Shop local and at farmers markets

PROCEDURE 3: SUSTAINABLE FISHING

Equipment we'll need:

- Three types of beans, each a different size, representing three different types of fish in the ocean
- One large tray, one small bowl, and one large bowl
- Two pairs of forceps
- Two spoons

The Rules of the Game

1. Each group should rotate through the following tasks: note taker and timer, bean counter, and fishing persons (two per season).
2. The different sized beans represent different species of fish with different values.
 - Large beans represent a species of fish like tuna that is of high quality (H) and worth \$15 each.
 - Medium sized beans are a commercially valuable fish like pollock that are of medium quality (M), but garner less money than the large fish so they are worth \$5 each.
 - The smallest beans represent species that are of low quality like stingrays, sea stars, and bonito, which are considered *bycatch* (B). These cannot be sold and so cost the fishers \$2 each.
 - Bycatch includes a variety of species of fish and sometimes other organisms like invertebrates, dolphins, and sea turtles. Bycatch species are caught along with other fish but have no commercial value and are often thrown back dead. The shrimp fishery has the highest bycatch of all fisheries, with an 80% bycatch rate!

3. Our *first* fishing season will cost us nothing because our uncle has left us a boat, some gear, and some gas in the tank. However, each subsequent fishing season will cost us **\$20** to begin (“**Operation cost**”).
 - In our **first and second seasons** we are limited to only using fishing poles of low quality because our uncle did not leave us any expensive gear. To represent this, all fishers of the group must use **forceps** and their **NON-dominant hand** (right-handed folks must use their left hands and vice-ver-sa).
 - After the second season, we may purchase **upgraded** technology. This consists of better fishing poles and gear, bigger boats, and moving to other oceans. All upgrades are one-time expenses, and paying the fee gets the upgrade for both fishers.
 - To start the third season, we can pay the **\$50** “Upgrade cost” to use our **dominant hand** when fishing with the **forceps**.
 - To start the fourth season, we can pay the **\$100** “Upgrade cost” to use a net (**spoon**) with our **non-dominant hand**.
 - pay the **\$300** “Upgrade cost” to use to a bigger boat (**large bowl**)
 - To start the fifth season, we can pay the **\$200** “Upgrade cost” to use our **dominant hand** with the net (**spoon**).
 - To start the sixth season, we can make two final upgrades:
 - pay the **\$2,000** “Upgrade cost” to move completely to a new ocean (think about how expensive that would be in real life!)
 - Pay a **\$15 searching fee** for *each* ocean you explore before moving.
 - The instructor may decide to offer occasional discounts!
4. If an organism is **dropped** when transferring it from the ocean to the boat, it dies. Remove this bean (fish or bycatch) from the ocean; if it is bycatch, deduct the cost from your profit, but if it is a medium or large fish, do not count it toward your profit.
5. If you have a few bad years, you may take out a **loan**. Loans can allow you to purchase upgrades if you do not have enough profit to buy one. Loans incur a \$20 loan fee. Remember to include the loan and the fee in the spreadsheet against your profit.

Beginning the First Fishing Season:

1. Place 20 bycatch (small beans), 40 medium-quality fish (medium sized beans), and 20 high-quality fish (large beans) in the ocean (large tray).
2. Each fishing season will last 30 seconds. When the timer calls out “Fish!”, the two fishers will fish at the same time with the same tool/upgrade.
3. After 30 seconds, the timer calls out “Stop!” and both fishers must stop immediately. Only fish that have made it into the boat (small or large bowl, depending on upgrades) count.
4. At the end of each fishing season, count the total number of each type of organism caught by both fishers together and record it in Table 3 (column B) in the Answer Sheet.
5. Calculate the team’s profit in Table 4 in the Answer Sheet. Decide if the group wants to upgrade to better fishing technology.

Fish Reproduction:

1. After each season, in column C in Table 3 in the Answer Sheet, calculate the number of individuals of each fish species remaining in the ocean by subtracting column B from column A.
 - Add this number of each type of organism (bean) to the ocean (tray).
2. Multiply the population size after harvest (column C) and record this number in column D. This is the number of each type of organism (bean) that will start the next season, so record this number in column A for the next season.
 - Note: these numbers become large quickly, so we may estimate by counting out 10 beans then estimate how many groups of 10 we might have based on the size of the pile.
3. Each population has a **carrying capacity**. Larger organisms tend to have lower carrying capacities. Once each population reaches its carrying capacity, its population stops growing.
 - **Large: 250 Medium: 350 Bycatch: 500**
4. Remember to subtract \$2 for every bycatch individual we catch!
5. Continue this cycle of fishing and fish reproduction through the 7th season.
6. Clean up: Make sure we separate and return the beans back to their original bowls.

FISHING GAME RULES QUICK REFERENCE GUIDE

COSTS

- Season 1: \$0
- Season 2-7: \$20 per season operation cost
 - Plus any loans you take out
 - If you have less than \$20 to start a season, you **MUST** take out a loan!
- Seasons 3-7: any upgrade costs

UPGRADES (can be purchased at the START of the season)

- Upgrades costs are per team, not per person
- Seasons 1-2: NO UPGRADES! Only forceps with non-dominant hand
- Season 3: \$50 to use dominant hand with forceps
- Season 4: \$100 to use a spoon with non-dominant hand; \$300 to get a bigger boat
- Season 5: \$200 to use dominant hand with spoon
- Season 6: \$2000 to move to a different ocean (with \$15 search cost)

HARVEST

- H = High value = Large beans = \$15 each
- M = Medium value = medium size beans = \$5 each
- B = Bycatch = small beans = -\$2 each
- Carrying capacities:
 - H: 250 M: 350 B: 500

Clean up/Disposal

1. Return all items to their original locations.

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