

Biodiversity Laboratory: Does Biodiversity Vary Between Habitats?

The species you will encounter this semester--small and large, simple and complex--are the working cogs of the living machine that is our biosphere and our only home. Each is important to the workings of its ecosystem. Even though we humans have not yet fathomed the ecological roles of most non-human species, we must learn to recognize them as we search for their importance to us and to the biosphere.

As you recall from your first semester of biology, the most ancient living organisms, in the Domain Archaea and Domain Bacteria, are sometimes referred to as **prokaryotes**. Prokaryotic organisms lack membrane bounded organelles, and their DNA consists of a single, circular chromosome of double stranded DNA organized in what is known as the **nucleoid region** of the cell.

More recently derived, **eukaryotes** (Domain Eukarya) are believed to share a most recent common ancestor with the Archaea. Eukaryotes may have evolved from ancient prokaryotes that formed symbiotic relationships with one another (The Endosymbiont Model) and/or who underwent in-pocketing of their external plasma membranes to form an internal membrane network (The Autogenous Model). It is quite probable that both phenomena were involved in the evolution of eukaryotes.

Let's meet some of the results of these millennia of evolution.

I. Inquiry-Based Research: Species Diversity Variation in Two Local Ecosystems

A. Introduction

The responsibility and rush of every day life sometimes distracts us from the natural world around us. The diversity of non-human species is immense, and yet most people rarely stop to consider it. Today, we hope to change that for you.

Located to the west of the Cox Science Building is the Gifford Arboretum Native Biome. Nestled in its center is a small pond, complete with plants, animals, fungi, protists and bacteria of many species. A little bit farther off, towards the center of campus, lies Lake Osceola. What might we have in store for you? Hint: You never know just what you'll find in a bit of pond sludge.

All species of living organisms have different tolerance limits for various environmental factors, including temperature, light, humidity, nutrients, etc. Because different ecosystems have different levels of each of these factors, the species in each ecosystem will differ accordingly. The **abiotic** (non-living) components of any given habitat determine the composition and abundance of the **biotic** (living) components of that habitat, and the biotic components, in turn, affect each other's abundance and diversity.

Consider that a freshwater habitat presents more of an osmotic (water/salt balance) challenge to living cells than a saltwater or brackish environment. Also consider that urban habitats also are subjected to varying levels of human disturbance (e.g., pesticides, fertilizers, physical disturbance, sanitation/clearing efforts, etc.).

In today's lab, you will compare the species diversity in two different local habitats on the University of Miami campus:

1. The Gifford Arboretum Native Biome pond, a **eutrophic**, freshwater pond habitat isolated from other bodies of water
2. Lake Osceola, a eutrophic, **brackish** lake habitat that is contiguous with Coral Gables canals, and ultimately, connected to the Atlantic Ocean.

You will work in teams of four to locate, identify and catalog the different species collected from two different ecosystems or microhabitats of your team's choosing. For example, your team might wish to know whether biodiversity is greater in the water column or the muddy substrate of Gifford Pond. Or you might be curious to see whether there are more species in the mud of the freshwater pond or the brackish lake. We will leave it up to you to choose the most interesting problem.

B. The Pilot Study

A **pilot study** is a small scale, preliminary study conducted before the main research is begun. A pilot study can determine the feasibility of a larger-scale version of the research, or indicate a need to improve experimental design. In complex systems, such as ecosystems, pilot studies may be conducted in order to find out whether a suspected phenomenon is real, and worth further study.

If you and your team do not have a logically gained, pre-existing idea about what you might find in terms of differences in biodiversity in the microhabitats above, then today's lab will essentially be a pilot study: research undertaken to determine the property/ies of a system you wish to study. This preliminary research will determine whether there is variation between the systems. Once you know the answer, you will be better able to pose further hypotheses to test, the better to further explain your results.

Before your TA turns your team loose to collect samples, you need to have a plan. As a team, consider the differences between the two habitats to be sampled. Use any resources available to educate yourself about the two systems in advance. Will you collect samples from the water column, or the substrate? From shaded areas, or full sun? Are the larger organisms in the area (e.g., aquatic plants, overhanging plants, etc.) similar or different in your sampling areas? Be sure to consider all possible sources of variation in advance, and design your sampling technique to yield results that are as reliable and repeatable as possible.

Discuss the two systems you have chosen to compare, and devise mutually exclusive hypotheses about what you expect to see in terms of relative biodiversity of each system. List the possible outcomes below.

1. _____

2. _____

3. _____

A complex problem, such as comparison of biodiversity between two different ecosystems, can pose difficulties for the investigator trying to make logical predictions. Since you will be collecting data to be analyzed statistically, an unbiased way to approach this problem is to pose appropriate null and alternative hypotheses about the possible outcomes. Write them in the spaces provided.

Your Null Hypothesis:

Your Alternative Hypothesis:

Type of Data you will be collecting (see Appendix 2):

Type of statistical test you will use to analyze the data (see Appendix 2):

Once all teams have hypotheses ready, your TA will lead a quick class discussion of each team's proposed research so that you, as colleagues, can help each other hone your experimental design. Then it's off to the wilderness!

C. Methods: Collecting Data

Now that you are well versed in experimental design, we will let you decide the precise methods to use in collecting your samples. We should no longer need to tell you that your methods must provide reliable data and meaningful results. Here are a few tips.

1. Always label your samples carefully and accurately, at every stage of your research.
2. Once you have collected your sample, return to the laboratory as quickly as possible to keep the organisms alive and comfortable.
3. A reasonable sample size is 5-10 ml of water or substrate.
 - a. How many samples from each habitat will you collect and analyze?
 - b. Will you count organisms in the entire sample, or just take a representative aliquot(s) from each sample? (An **aliquot** is a portion of a total amount of a solution.
4. Before you begin, read Appendix 5: Proper Use of the Microscope.
5. Use the syringes provided to collect samples, and carry them in properly labeled beakers. To observe and count organisms, do the following:
 - a. Place two drops of your sample on a concave microscope slide
 - b. Drop a coverslip onto the well
 - c. If necessary, place one drop of methyl cellulose at the edge of the coverslip, and allow to diffuse under the coverslip. (This will slow down any rapidly swimming microorganisms)
 - d. **STARTING ON LOW POWER**, observe your sample under the microscope.
 - e. Begin at one corner of the coverslip, and gradually work your way across and down, in a zig-zag fashion.
 - f. Whenever you find a motile organism (protist or animal), stop and identify it as completely as you can by using the Identification Guide following this section. If you're stumped, call your TA for help.

- g. Record your results on the appropriate tally sheet provided on the last pages.
- h. Do as many replications of this procedure as your team has decided are appropriate (and be sure to report all details in your presentation).

For additional help in identifying your organisms, you may check out a *Photographic Atlas to the Biology Laboratory* from your TA at the front desk. Just trade your Cane Card for the Atlas, and return it for your card back at the end of the lab period. There are enough Atlases to provide each team with one.

Additional very nice guides to pond life can be found here:

<http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/pond/index.html>

<http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/pond/index.html>

each with links to additional sites that will help you identify your wee beasties.

NOTE: Some lab sections may decide to do this project as a class, and pool all data. If this describes your lab, then be sure to give your data to your TA as soon as you have finished collecting it.

D. Organism Identification Guide

Since this is your first introduction to some of the vast diversity of the Kingdoms of Life, we don't expect you to be able to identify with any great resolution (i.e., to the species level) the many organisms we hope you will see. However, this guide should help you narrow down the identification of the living organisms in your sample, and help you fill your species diversity tally sheet. Refer to the Photo Atlases available in lab for more detail, once you have narrowed your organism down to one of the major groups listed below. Note that we have omitted the "Kingdom" and "Phylum" designations for the major taxonomic groups you will likely find in your habitats. This is because the rank of these taxa occasionally changes. What's more important than knowing that "Animalia" is a kingdom and "Arthropoda" is a phylum is knowing that the taxon Arthropoda is a subtaxon within Animalia.

If you find something you can't identify, call your instructor over for help! And if you find something particularly interesting, be sure to share with your classroom colleagues.

1. Protists (Several candidate Kingdoms)

These are the simplest of the eukaryotic organisms, and they are a very diverse assemblage now assigned to several different candidate kingdoms once subsumed under the defunct name "Kingdom Protista." The types you are likely to see today will be very small and usually very fast. To see them clearly, you'll probably have to use methyl cellulose to slow them down. Most common in daytime samples will be diatoms and small flagellates. But the occasional amoeba may also show up.

For a nice overview of diatom appearance and biology, visit:

http://arch.ced.berkeley.edu/kap2/php/Hidden_Ecologies/?page_id=197

2. Animalia, Porifera - The Sponges

The sponges are the simplest of animals, and they are found in both freshwater and marine habitats. They are characterized by an amorphous body shape with no distinguishable head or tail end. Lacking true tissues, these animals have an array of diversified cell types, each of which performs a specific function.

3. Animalia, Cnidaria - The Radially Symmetrical Animals

Found in both freshwater and marine habitats, these animals are radially symmetrical (i.e., the body is divisible into identical "pie shaped" wedges) and have two true tissue layers (endoderm and ectoderm).

4. Animalia, Platyhelminthes - The Flatworms

If the body is dorsoventrally flattened (i.e., flattened from "top" to "bottom") and there is a distinct head end that guides the animal's movements, there's a good chance you're looking at a flatworm. (If you're not sure, call the instructor for a positive I.D.) These animals have three true tissue layers (endoderm, ectoderm and mesodermal mesenchyme) and simple organ systems.

5. Animalia, Rotifera - The Wheel Animalcules

These tiny animals are no bigger than a large protist, yet they have three true tissue layers and complex organ systems. They feed by means of a cephalic (head end) corona of cilia which beats food particles from the water into the mouth. They also use the corona for swimming; it pulls the animal through the water like a little propeller when it decides to weigh anchor (pull up its sticky pedal disk) and move.

6. Animalia, Nematoda - The Roundworms

These worms are very thin, symmetrical, and tapered at both ends. There is no evidence of body segmentation, and they move with a characteristic sinusoidal wave motion unique to this phylum. This is because the body wall has only longitudinal muscles, another characteristic unique to this phylum.

7. Animalia, Annelida - The Segmented Worms

The familiar earthworm is a member of this large, diverse phylum. You can identify a segmented worm by the ringlike markings on its body, which delineate the body segments. Internally and externally segmented, the body design and function is based on this characteristic metamerism, which is found in many other more derived (i.e., not primitive) animal taxa. In freshwater, you are most likely to find annelids that resemble tiny earthworms. In brackish or salt water, you are most likely to find polychaetes. A free-swimming polychaete can be distinguished by its long, segmented body bearing paired, paddle-like or bristle-like appendages. There is usually a distinct head with tentacles and eyes. Burrowing species have reduced appendages and less obvious heads.

8. Animalia, Mollusca - The Mollusks

Closely related to the Annelids, the mollusks have secondarily lost their body segmentation, though it is present in larval forms that you might see in your sample today. Mollusks can usually be identified by the presence of a distinct head and a

muscular foot, though if you happen to find a bivalve (e.g., a clam or mussel), these features will be hidden inside the two shells.

9. Animalia, Arthropoda - The Arthropods

This is the most diverse of all animal phyla, with hundreds of thousands of species (The beetles alone comprise more than 350,000 described species!). Arthropoda includes the familiar insects, crustaceans, and spiders, as well as other less familiar forms. Like the annelids to which they are closely related, the arthropods show distinct body segmentation. And if it has distinctly jointed appendages, it's an arthropod.

10. Animalia, Echinodermata - The Spiny-Skinned Animals

Our closest invertebrate relatives that you're likely to see today are the starfish and their relatives, though you'll probably see only ciliated larval forms. Adults are pentaradially symmetrical. These animals are strictly marine, and may not be present in either of your samples.

11. Animalia, Chordata - The Chordates

This familiar group includes the sea squirts (subphylum Urochordata), the lancelets (subphylum Cephalochordata) and the vertebrates (subphylum Vertebrata). All are united by the presence of a cartilaginous skeletal support rod (the notochord) present at some time during development, a muscular, post-anal tail, segmentally arranged muscle bundles (at least in development) and pharyngeal gill slits. **AVOID COLLECTING ANY VERTEBRATES IN YOUR SAMPLE. If you do accidentally collect a small fish or tadpole, take down the data in your notebook (bring it with you into the field), and release the animal without harming it.**

Using the general identification guide above along with any other sources you can find (Google image search is always a treasure trove!), try to locate and differentiate as many different species within the listed taxonomic groups as you can. Simply use hashmarks to keep count of the number in each category, and enter these on the tally sheets provided for each of the habitat types you sample.

When you are finished with your samples, RETURN THE LEFTOVER WATER AND/OR MUD TO ITS ORIGINAL LOCATION in the pond or lake. There should be no samples left in the room when the lab is over!

RINSE ALL GLASSWARE AND SYRINGES THOROUGHLY, AND LEAVE THEM NEATLY WHERE YOU GOT THEM FOR THE NEXT LAB'S USE.

E. Analyzing your Data

When all teams have finished their survey and reported them to the TA, subject your data to statistical analysis in the way you deem most appropriate. (HINT: The contents of each 1cc sample can be considered one data set, and so a mean number of species can be calculated for each of the two habitats. Are these paired or independent samples? Beware of false replicates! You be the judge.) Use the space provided for notes and preliminary calculations.

More work space:

Your statistic value: _____

P-value: _____ > P > _____

Do you accept or reject your null hypothesis? _____

III. Further Research: Solving Problems

Now that you have preliminary results from your pilot study, the work has just begun. Which of your initial hypotheses was correct? Each possibility has interesting implications about the nature of the two systems, and how hospitable they are to a diversity of species.

A. Identifying the Problem and Formulating Hypotheses

Was the species diversity different between your samples? If so, why? How, precisely, did the two habitats differ? Give a detailed summary.

Next consider: Why do you see certain types of organisms in each habitat? What are the physiological requirements of some of the organisms you found in your samples? Were there certain types of organisms you found in one habitat, but not the other? Were there some types of organisms found in both habitats?

In the space below, give a brief introduction to the problem you are addressing. Give as much background as possible, considering what you know about the physiological requirements and natural history of the species you found in your samples. This will, of course, require a bit of background reading from your text and

other scholarly sources.

Try to explain your results by posing additional hypotheses about WHY your results turned out the way they did. Discuss this as a team, and write down as many possible explanations for your results as you can.

Hypothesis 1: _____

Hypothesis 2: _____

Hypothesis 3: _____

Hypothesis 4: _____

Hypothesis 5: _____

Hypothesis 6: _____

Are any of these hypotheses mutually exclusive? Consider this, and include a discussion of possible multiple factors in your presentation. Explain what experiment(s) you could perform to determine, by process of elimination (falsification) which of your hypotheses is correct. Consider all possibilities, and--as a team--decide what you would manipulate to test your hypotheses. List all possible outcomes for each of your experiments. Make predictions about what you expect to observe if a given hypothesis is true, or not true. A pilot study is only the beginning. In your presentation, you must map out a specific course for further research.

B. Creating a Poster Presentation

Scientific meetings of every type include both lectures and poster session. In the latter, researchers are provided with a backdrop on which to post their research paper in a large format that can easily be read by attendees of the poster symposium. The results of the Biodiversity Safari will be presented in the form of a poster symposium during your lab session (see the syllabus for the exact dates).

In the time between your lab and the poster symposium, your team will work together to create a poster presentation of your work. A poster, like any other presentation of scientific data, should be presented in the basic format for a scientific paper described in Appendix 3. Be sure to include all appropriate sections.

Unlike a journal article, however, a poster is meant to be concise, easy to read, and relies heavily on graphics to get the point across. You and your team must all refer to the instructions and guidelines for preparing an effective poster presentation found here:

<http://www.bio.miami.edu/ktosney/file/PosterHome.html>

This site, created by Dr. Kathryn Tosney, is an excellent guide for anyone needing to learn both the basics of poster presentations, and how to make your poster effective and engaging to the right audience.

Your poster should be printed on regular paper, all the parts of which should be designed to fit on a space 4' x 3'.

At the symposium, each team will be provided with a poster board with the dimensions 4' x 3'. **YOU ARE NOT TO PERMANENTLY ATTACH ANY ITEMS OR PAPERS TO THESE BOARDS! THEY ARE RE-USABLE, AND MUST NOT BE DAMAGED.** You will be provided with pins with which to attach your poster pages for the duration of the poster symposium. At the end of the symposium, you will take down and submit your poster to your TA for grading. (This means that you should NOT bring in a poster with pages already glued on. Use the poster boards provided in lab, treat them with respect, and all will be well.)

Species Diversity Tally Sheet:

Taxonomic Group	species 1	species 2	species 3	species 4	species 5	species 6	Total #
Protist (diatom)							
Protist (flagellate)							
Protist (amoeboid)							
Protist (ciliate)							
Porifera (sponge)							
Cnidaria (cnidarians)							
Platyhelminthes (flatworm)							
Rotifera (wheel animalcules)							
Nematoda (roundworm)							
Annelida (segmented worms)							
Mollusca (mollusks)							
Arthropoda (arthropods)							
Chordata							
Other							

Total number of different species: _____

Time of day sample was collected: _____

Notes: _____

Species Diversity Tally Sheet:

Taxonomic Group	species 1	species 2	species 3	species 4	species 5	species 6	Total #
Protist (diatom)							
Protist (flagellate)							
Protist (amoeboid)							
Protist (ciliate)							
Porifera (sponge)							
Cnidaria (cnidarians)							
Platyhelminthes (flatworm)							
Rotifera (wheel animalcule)							
Nematoda (roundworm)							
Annelida (segmented worm)							
Mollusca (mollusks)							
Arthropoda (arthropods)							
Chordata							
Other							

Total number of different species: _____

Time of day sample was collected: _____

Notes: _____
