

BIL 161 Laboratory: Biodiversity in Local Ecosystems and Microhabitats

The responsibility and rush of every day life sometimes distracts us from the natural world. The diversity of non-human species is immense and all around us, and yet most people rarely notice it. Today, we hope you will gain a naturalist's perspective.

The species you will encounter this semester--small and large, simple and complex--are the working cogs of that living machine we call the biosphere. Each species is important to the workings of its ecosystem. Even though we may not yet fully understand the ecological roles of every species, it is important to be able to identify them for further study. **Biodiversity** is defined as the degree of variation of living organisms within a particular ecosystem (or the entire planet). In today's lab, you will begin a small-scale exploration of biodiversity in the microhabitats of local ecosystems.

I. Biodiversity

You already know the biological definition of a **species** as a group of organisms able to interbreed in nature to produce fertile, viable offspring. A **population** is defined as all the members of a single species living in a defined area. All the populations in a given area comprise that area's **community**. And the communities interacting with the non-living components of the environment make up the **ecosystem**. Ecosystems are as varied as the surface of the earth, and within each ecosystem there are **microhabitats**—smaller areas within the ecosystem that are characterized by specific physical factors that determine what lives there. For example, a pond ecosystem might have microhabitats including (1) the interstitial space of pond plants, (2) the bottoms of stones, (3) the water column, (4) the sediment at various depths, and (5) the pond's surface, to name only a few.

All living organisms have evolved tolerance limits for various environmental factors, including temperature, light, humidity, nutrients, etc. Because different ecosystems and microhabitats have different levels and combinations of each of these factors, the species in each ecosystem 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.

A. Measures of Biodiversity

Ecologists use specific terms to describe the number, density, and variety of species living in a particular ecosystem. When you devise your study, be sure to use this vocabulary appropriately.

The simplest measure of biodiversity in an ecosystem is **species richness (S)**. This is no more than a count of the number of different species found in a collected sample, and it does not take into account species abundances. One index used to represent species richness is **Menhinick's Index (D)**: the number of species in the sample divided by the square root of the number of individuals in the sample.

$$D = \frac{s}{\sqrt{N}}$$

where **s** equals the number of different species in your sample and **N** equals the total number of individual organisms in the sample.

In ecology, the term **abundance** is used to describe the relative representation of species in an ecosystem. If you collect a sample from an ecosystem, the abundance of a particular species is simply the number of individuals per unit area or volume of your sample. The **relative abundance** of a particular species refers to how common or rare that species is in comparison to other species in the same locality or ecosystem. The relative abundance of any given species can be calculated as the number of individuals of that species divided by the total number of individuals of all species combined. For example, if you collected a one milliliter (mL) sample of pond water and found the following numbers of each of three species:

100 diatoms (*Neidium pseudodensestriatum*)
50 nematodes (*Psilenchus hilarulus*)
5 mosquito larvae (*Aedes aegypti*)

Then the relative density of each species would be:

<i>Neidium pseudodensestriatum</i> :	$100/155 = 0.64$
<i>Psilenchus hilarulus</i> :	$50/155 = 0.32$
<i>Aedes aegypti</i>	$5/155 = 0.03$

Many other measures of biodiversity are important in studies of community ecology, but we will restrict ourselves to using these basic measures for today. Review these indices and use them to decide on a problem to address. Your team may wish to determine whether there is a significant difference in species richness between two different types of ecosystems, or from two different microhabitats in the same ecosystem. Or perhaps you would like to see whether a certain taxonomic group is more abundant in one ecosystem (or microhabitat) than another. We will leave it up to you and your inquiring mind to decide on a specific project and how to design it.

B. Environmental Conditions and Biodiversity

Aquatic environments harbor many living organisms, and the biodiversity of an aquatic system depends, in part, on the salinity of the water. Fresh water is defined as having less than 0.5 grams dissolved salts per liter, or parts per thousand (ppt). It is **hypoosmotic** with respect to the cytosol of a typical living cell. Hence, a **freshwater** environment presents a challenge to living cells, which must expend energy pumping water out to maintain proper osmotic balance.

On the flip side, today's **seawater** is **hyperosmotic** with respect to a typical cell's cytosol. Marine saline water has between 30-50 grams of dissolved salts per liter (or ppt). Thus, many (though not all) marine organisms must expend energy to retain water in the cytosol.

Finally, a **brackish** environment is defined as having water with between 0.5 – 30 grams of dissolved salts per liter (or ppt). This is a wide range, and the term "brackish" is thus not very precise. But the biodiversity of brackish environments can depend on the salinity of the water.

Different habitats not only have variable abiotic factors, and in our suburban area they also are subjected to human disturbance such as pesticide and fertilizer runoff, physical disruption, sanitation/clearing efforts, etc. How might these factors affect species diversity and species composition of an ecosystem.

C. Local Environments and Their Biodiversity

Close to the center of campus lies brackish **Lake Osceola**, which contains more species than you might imagine if you merely glance while you walk by. It is connected to the ocean by a long network of canals, so species from both fresh water and marine environments have access to the lake.

Until recently, there was a small, isolated freshwater pond, the **Gifford Arboretum Pond** just behind the Cox Science Building. Before the demolition of the area commenced to make way for the new Cox Annex, we were able to sample the pond and save some of the microscopic biota for your examination. It can be found in the small aquarium on the window ledge of each lab. Note that the species living in the water column itself, those in the algae, and those living in the sludge at the bottom might not be the same.

Coral Gables and surrounding communities are dotted with many man-made ponds and canals. Some are connected to each other, and some are closed systems. Some are freshwater, and some are brackish. In the lab today, you will find two small aquaria containing samples from such a local body of water (**Dr. Krempels's pond**). One aquarium contains samples from the sediment at the bottom, and the other contains a sample from the water column.

II. Inquiry: Species Diversity in Local Ecosystems

Some of you might already have some background knowledge about relative diversity in the microhabitats and ecosystems we will be sampling. If not, then take the opportunity before lab to do some background reading about this topic so that you will come to lab armed with ideas for exploration. If you know of an observation made in a published study that you would like to explore further, then you may commence by posing competing hypotheses that might explain the observation. If, however, you have no knowledge at all about the systems we will be sampling, you might need to begin with a pilot study.

You will work in teams of four to consider what you know about our local aquatic habitats and use this to pose a question about the relative biodiversity of two different types of samples. Your team will choose the two systems to compare, but note that you must do background reading in order to ask a meaningful question.

A. No Observation? Design a Pilot Study.

If you and your team have no idea about what to expect from biodiversity observations in your ecosystems, then your experiment should take the form of a **pilot study**. Your pilot study will be a preliminary experiment designed to yield a statistically supported **observation** that can then be subjected to further exploration.

For example, if you do not know whether there is a difference in species richness between the water column of a freshwater pond and the sediment of the same pond, then you would perform a pilot study to determine whether there is a statistically significant difference, and to characterize—to the best that your data allow—the nature of that difference. State valid null and alternative hypotheses based upon the question you are asking (e.g., “Is there a significant difference in biodiversity between the water column and the sediment of XXX freshwater pond?”), then take an appropriate number of samples from each ecosystem, collect and record species data. From these data, you will be able to calculate an appropriate measure of biodiversity (species richness,

abundance, etc.) and then subject the data to an appropriate statistical test. The hypothesis not falsified will serve as your observation for further experimentation.

For example, if you found that the biodiversity in the water column was significantly higher than that in the sediment, your next step would be to hypothesize why that difference exists by posing as many competing hypotheses (i.e., tentative explanations) as are reasonable. Each of these should be testable with proper, scientific methods, and if you are clever you will be able to word them in such a way that you can eliminate all but the correct hypothesis with carefully designed experiments.

If your team chooses to do a pilot study, be prepared to pose competing hypotheses to explain your experimental observations, and to propose experiments that would allow you to test them. These should be included in your presentation.

If your work today is intended to be a pilot study, then state this clearly, and provide a logical rationale for why you are performing this pilot study. What do you predict you will observe?

If you are doing a pilot study and using a statistical hypothesis, What is your **null** hypothesis regarding the system you are examining?

What is your **alternative** hypothesis?

What **parameter** will you measure? _____

What statistical test will you use to analyze your data? _____

Describe your experimental methods.

B. Designing an Experiment Based on an Existing Observation

Consider the differences between the two habitats to be sampled. What do you already know about their relative biodiversities? If you do have an observation that you wish to explore experimentally, then write it here:

Make a list of as many possible (competing) hypotheses to explain this observation as you can.

It is not likely that you will have time to address each of these hypotheses in today's lab. So choose one that your team feels able to test, realizing that all the other hypotheses would also have to be tested in order for your study to be thorough and unbiased. Which hypothesis will your team test?

If you are posing a statistical question, what is your **null hypothesis**?

Your **alternative hypothesis**?

What **parameter** will you measure? _____

What statistical test will you use to analyze your data? _____

Describe your experimental methods.

Once all teams are finished deciding what type of study to do and have drawn up a plan, your TA will lead a brief discussion of each team's ideas. Each team will describe your observation or pilot study proposal, your prediction(s), hypotheses, etc. to the class, and briefly describe the experimental methods, including the statistical test to be employed for data analysis.

Be ready to modify your study, as necessary, based on the critiques of your colleagues and lab instructor, and to provide thoughtful critique for other teams.

C. Collecting Data

Each team should collect a known volume of water (or sediment, as the case may be) from each of your chosen ecosystems/microhabitats. Place your sample in a labeled container provided (and return to the laboratory as quickly as possible if you are collecting in an outdoor locality on campus).

Once your team has returned to the laboratory, sampling can begin. Each team member should draw up one cc (= one milliliter (mL)) of sample. Be sure everything is properly labeled so that your samples won't be misidentified. To count organisms in your sample:

1. Place two drops of your sample on a convex microscope slide
2. Drop a coverslip onto the drops
3. Place one drop of methyl cellulose *at the edge of the coverslip*, and allow to diffuse under the coverslip. (This will slow down the rapidly swimming microorganisms)
4. STARTING ON LOW POWER, observe your sample under the microscope.
5. Begin at one corner of the coverslip, and gradually work your way across and down, in a zig-zag fashion.
6. 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 as well as your Photo Atlas. If you're stumped, call your lab instructor for help.
7. Record your results on the appropriate tally sheets provided.
8. Do as many replications of this procedure as your team deems appropriate for valid statistical analysis.
9. Use your raw data to calculate the parameter (e.g., species richness) your team has chosen. Calculate the index for each sample so that you can calculate a mean for your statistical analysis.

D. Organism Identification Guide

Since this is your first introduction to some of the vast diversity of Life, we don't expect you to be able to identify with any great precision 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 your Laboratory Photo Atlas for more detail, once you have narrowed your organism down to one of the major groups listed below.

Feel free to use online resources for identification. Once you have identified an organism as a diatom, for example, a Google image search might well yield a possible identity for your organism. (Be careful, though. When it comes to protists, it sometimes takes a real expert to tell them apart. We'll be happy if you learn to tell a flagellate from a ciliate at this stage.)

A broad overview of the types of organisms you might encounter can be found at these handy sites:

<http://tinyurl.com/tmhz>

<http://www.microscopyu.com/moviegallery/pondscum/>

If you find something you can't identify, ask your instructor for help.

1. Protists

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 now-defunct taxon "Protista." The types you are likely to see today will be very small and usually highly motile. To see them well, 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 will show up.

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 - Radially Symmetrical Diploblasts

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.

8. Animalia, Mollusca - The Mollusks

Closely related to the Annelids, the mollusks have secondarily lost their body segmentation, though it is present in the larval forms which 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, 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 might 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. Their lack of an excretory system makes osmoregulation in freshwater or brackish water impossible for them, so you will find them only in oceanic ecosystems.

11. Animalia, Chordata - The Chordates

This familiar group includes the sea squirts (Urochordata), the lancelets (Cephalochordata) and the vertebrates (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. The only kinds you're likely to see today are fish or amphibians, if any. We just thought you'd like to know they're there.

Using the general identification guide above along with your Photo Atlas, 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 two habitats sampled.

E. Analyzing your Data

The contents of each 1cc sample can be considered one data set. Depending on what your team has decided, calculate species richness, abundance, or diversity for your two sample types. Whichever index you have chosen as your parameter can then be subjected to statistical analysis, as your team deems appropriate.

(Consider whether your samples are paired or independent, in order to use the correct statistical test.)

Mean parameter for ecosystem #1: _____

Mean parameter for ecosystem type #2: _____

Your statistic value: _____

P-value: _____ **> P >** _____

Accept or reject your null hypothesis? _____

With your team, discuss the significance of your results. List as many competing hypotheses as possible to explain your observation. How might you test each one? Include this in your presentation.

Before you leave lab today, check with your lab instructor to be sure you have all the instructions and materials you need to start working on your poster presentation. Instructions for preparing an effective poster can be found linked to the BIL 161 online syllabus under Laboratory 5. All team members must participate in the presentation's creation and presentation, so be sure you have contact information for all your team members, and that everyone knows in advance when you plan to meet.

Species Diversity Tally Sheet for Locality: _____

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							

Species Diversity Tally Sheet for Locality: _____

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Rotifera (wheel animalcules)							
Nematoda (roundworm)							
Annelida (segmented worms)							
Mollusca (mollusks)							
Arthropoda (arthropods)							
Chordata							
Other							