Evolution and Biodiversity Laboratory
Activity: Data Collection

You have some expertise in using the microscope to identify microorganisms. Your team has a research protocol ready to follow. You are ready to collect data.

For the next two weeks, your team will collect samples shortly before lab and bring them to lab for biodiversity census and analysis. This chapter will guide you through data collection.

I. Analyzing Your Samples

Each team member should take one concavity slide.
Begin with one of your three samples from Habitat #1.
Each team member should extract a set volume (2-3 drops is usually sufficient) *from the same sample* with a disposable plastic droppers and place it on his/her concavity slide.

Most organisms will settle near the bottom of the cup, though some species might be swimming free in the water column. You will need to decide what to do with this information in order to keep your census as consistent as possible.

Use the same procedures you used to observe cultures in the last session:
Carefully drop a cover slip over the filled concavity.
If desired, place a drop of methyl cellulose at the edge of the coverslip.
Gently move the coverslip to promote methyl cellulose diffusion into your sample.
This can take a few minutes.
You are then ready to count organisms.

If you have trouble seeing organisms, you may wish to stain the sample with methylene blue. Place a small drop at the edge of the cover slip and allowing it to diffuse into the sample for a few minutes.

This will kill the organisms, possibly making them more difficult to identify if they are not moving. However, you may be able to see cellular structures more clearly.

We recommend you try identifying with methyl cellulose first. Use methylene blue stain only if you are having difficulty without it.

Use the same observation method you learned last week, starting at one corner of the slide, counting and identifying all organisms in a single field of view, then moving the slide over one field of view and counting again. Repeat until you have surveyed the entire slide.

- Use the **tally sheet template** linked to the online syllabus to record your results.
- Use **one tally sheet for each replicate (three per habitat)**.
- **Pool counts** of a single replicate (from all team members) on the same tally sheet.
- **All slide counts from a single sample cup comprise a *single replicate*.** (Figure 1)
- **Each team member should count two slides for each sample.**

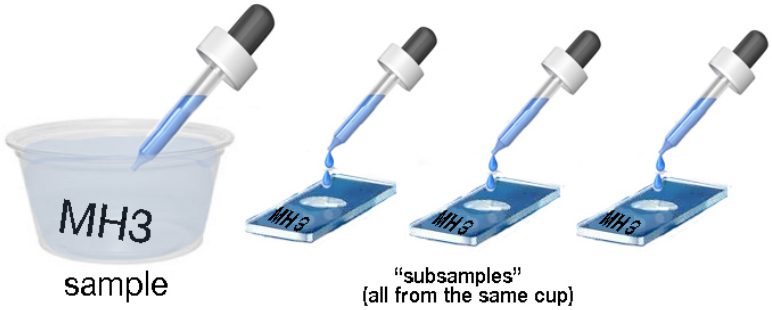

Figure 1. One sample/replicate is the material contained in one collection cup. A "subsample/subreplicate" is one slide (2-3 drops of sample) surveyed by each team member. Multiple subreplicates are done to increase replicate sample size.

When you have finished counting your first sub-replicate

- rinse the concavity slide with deionized (DI) water
- dry with a Kimwipe
- re-use the same concavity slide for the next sample
- **census each of your six samples in the same way**

IMPORTANT: DO NOT WASTE OR DISCARD YOUR SAMPLES!

Once you have completed your counts, you may wish to subject the water in your samples to various tests of water quality. (See Section II)

By the end of today's census, you should have

Habitat X	**Habitat Y:**
Eight pooled subreplicates of sample X-1	Eight pooled subreplicates of sample Y-1
Eight pooled subreplicates of sample X-2	Eight pooled subreplicates of sample Y-2
Eight pooled subreplicates of sample X-3	Eight pooled subreplicates of sample Y-3

REMEMBER: *DO NOT THROW AWAY CONCAVITY SLIDES!* THEY ARE REUSABLE AND EXPENSIVE! When you have finished all your survey counts, wash your concavity slide gently with clean tapwater, rinse with deionized (DI) water, and dry well with a Kimwipe. Replace the slide in the proper box marked "concavity slides".

DO NOT CONTAMINATE YOUR SAMPLES! Do not use the same pipets or other tools to pull from different sample cups, and especially not from different habitats. Rinse your pipet with deionized water between samplings.

By the end of the entire data collection phase of your project you will have:

- eight counts (two by each team member) for each replicate
- six replicates from each habitat, each with the same number (8) of pooled counts

II. Collecting Water Quality Data

There are various tools available in the lab for you to sample aspects of water quality in your sample. Your instructor will show you where to find them.

Do the water tests after you have finished your counts, to be sure the tests do not interfere with the organisms in your samples.

Although you cannot definitively assign differences in biodiversity between your two habitats to differences in particular water quality parameters, those parameters may still give you a starting point for suggesting further research questions that might answer those questions.

III. Beware the Perils of Pseudoreplication

Truly independent samples are necessary for valid comparison. A common error made by the novice (and sometimes the NOT-so-novice) is **pseudoreplication**.

If you collect six samples from each of the two habitats you are comparing, and then each team member counts two slides' worth organisms from each of the six samples, then you should have eight counts comprising a single sample, not eight independent samples. Counts from a single cup came from the same transect, and are considered part of a single data set. They must be pooled.

IV. Clean Up is Part of Good Lab Technique

When your team is completely finished, all members must clean up the lab station.

- Discard all coverslips and any flat slide in the sharps container at the front desk.
- Wash, dry, and replace concavity slides in their proper box on your lab table.
- Replace all lab equipment you used in its proper place.
- Collect and discard all trash and debris from your station.
- Properly stow your microscope, as per your lesson last week.
- Wipe up all spills and be sure your lab countertop is tidy.

Teams leaving a dirty workstation will be docked five points per member.