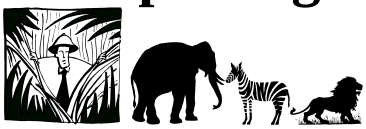
Chapter 1

Joe's Jungle: Exploring Biodiversity



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Contents

Introduction

This lab is written for an Introductory Environmental Science laboratory that meets one of two requirements for a general education laboratory science course. Students are primarily freshmen but typically include students in all classes. They enter with a wide range of abilities and interest. The course is structured to create an environment where students and teaching assistants can succeed at doing inquiry. The initial activities are guided inquiry; as the semester progresses there is less guidance and more onus placed on the students to develop their own research questions. This biodiversity activity occurs about midway through the semester. Students work in a model system, Joe's Jungle, where they learn how to quantify biodiversity. We have selected three of the simplest measurements so that students can be successful with the statistics. This activity can be scaled to any level. Adding more statistical analyses would be appropriate. You should refer to Wedlin (1999) for a better description of statistical measures of biodiversity.

Once students have learned the techniques for the data analysis, they apply what they have learned. They ask questions about biodiversity in the unseen jungle inhabited by microorganisms all around them. We use the microbial world for this simply because it is difficult to have access to natural populations in Northwest Ohio at the times we are doing this activity. Students are asked to think about where and why they might find differences in microscopic communities. This experiment leads to a series of activities where they then ask about the physical and chemical qualities of ecosystems and how they affect communities.

Materials

- Calculators
- Probes
- Nutrient Agar Plates (1 per group)
- Sterile Swabs (3 per group)
- Parafilm (sufficient quantity to seal petri plates)
- Scissors
- Grease pencils or sharpies (one per lab group is ideal.)
- Spray disinfectant and sponges
- Biohazard disposal bags and boxes (*Check the rules at your institution for disposal procedures*. We treat wooden swabs as sharps, putting them in boxes and bags.)
- Joe's Jungles (Figure 1) (one per lab group making sure there is an even number available. A few spares are wise.) These are 12 x 12 flats that are planted with a variety of succulents. There is a minimum of five species per flat. They are randomly planted so that there is some overlap between flats. Some flats have unique species. We have used seedlings in the past, but we have found that the cactus and succulents hold up through all 28 sections. Seedlings must be planted well in advance of the laboratory.

Figure 1. Joe's Jungles

Student Handout

Introduction to Biodiversity

After completing this activity, you will be able to:

1. Define the three levels of biodiversity.

One of the hottest topics in environmental science today is biodiversity. Biodiversity can be defined and therefore measured in three ways. The most common definition of biodiversity is **species diversity**. This is the number of different species in an ecosystem. (Remember that a species is a group of genetically similar organisms that are able to reproduce offspring that are, themselves, able to reproduce.) Species diversity -- how many different types of organisms (plants, animals, fungi, and bacteria) live in an area -- is what people most commonly think of when talking about biodiversity. In general, an ecosystem is said to be healthy if there is a greater diversity of species. This does not necessarily mean large numbers of organisms. In fact, an ecosystem that has large numbers of organisms all of the same species is often considered to be less diverse (think of a cornfield).

(Remember that humans tend to define the boundaries of the ecosystem using a human perspective. Natural populations may or may not fit precisely into human boundaries. Also, humans sometimes overlook populations that are less visible either because the organisms are small or they live underground.)

Ecological diversity examines not only the number of different species in an area, but also the complexity of a community. Someone studying ecological diversity will look at the food web and trophic levels, the kinds of niches within the community, and the ways in which energy flows and raw materials are recycled in the community. Understanding ecological diversity is an important factor in conservation of natural spaces.

The third type of biodiversity is **genetic diversity**. Someone studying genetic diversity looks at the variation within a single species. Very small populations with very little genetic diversity are at greater risk of extinction than populations with more genetic variation. Imagine a pond where all of the amoebas are genetically identical and perfectly adapted to a pH of 6.8. If acid rain causes the pH of the pond to be lowered to 6.3, all of the amoeba will die. If the fish in the pond are genetically varied where most of them are adapted to pH 6.8, but some can survive in higher pHs and some can survive in lower pHs, some will survive a change in the pH of the pond. The population will not become extinct. Considerations of genetic variability are particularly important in the conservation of extremely small populations of endangered species.

The region of Northwest Ohio you are living in is one of the most altered landscapes in the world. This area was originally a swamp. Even though humans do not necessarily appreciate a swamp as an ecosystem, they serve an important role in the environment, supporting life and filtering water. The Great Black Swamp was drained, the trees were cut, and the land was cleared. Now we are living in a very different ecosystem.

Activity 1: Joe's Jungle

After completing these activities, you will be able to:

1. Determine the number of species in a defined area,
2. Statistically analyze biodiversity,
3. Statistically compare the biodiversity between two ecosystems.

Joe is the horticulturalist here on campus. He maintains the greenhouse plant collections and grows plants used in research. In order to learn how to compare plant biodiversity in the winter, Joe has created a series of jungles for us to examine. On your bench, you will see one of Joe's jungles. This will be the ecosystem you are sampling.

The first thing to do in sampling an ecosystem is to define the sampling technique and the area to be sampled. A line transect is used to sample large areas. The researcher runs a string for 10-100 meters in a random direction from a random starting point and then measures all of the plants that touch the string. Smaller samples can be studied by setting up grids. These can be done using 10x10 cm to 10x10 meter areas. The researcher then maps and counts all of the plants in that grid. These are also randomly placed in the ecosystem to make sure that the researcher doesn't bias her samples.

Since you are working in a model system, the area is already defined. You will count the number of plants of each species in your jungle. Choose one member of your group to serve as the botanist. This person will be responsible for describing and naming each unique species in your jungle. This person will have to communicate with the rest of the scientific community about the species. Careful records are critical. A second member of your group will be the recorder and the third member will serve as the sampling specialist. Record your data in the table provided on the data collection and analysis page. Make sure that everyone has the data. You will need it to successfully complete the statistical analysis. A set of sample data is given to you in Table 1.

Table 1. Sample data collection for Joe Jungle Q

Species	**Number of organisms**
1. Coconut tree	6
2. Fern	45
3. Lily	4
4. Palm	2
5. Bromeliads	18
Total number of species = S S= 5	Total number of organisms (all species included) = N N= 6+45+4+2+18 = 75

Measuring Species Richness (S)

The simplest measure of biodiversity is a count of the number of different species in a defined area. This number is called **Species Richness** and is represented by the letter *S*.

S= the number of species in the sample area

You can see from the set of sample data that the *S*, species richness, for Jungle Q is 5. Five different species were identified.

To compare *S*, species richness, between two different areas, a researcher must try to insure that the sample areas were close to the same size and that the sampling techniques were similar. In our sample data, we only looked at plants. There are more species in the area than just the ones you can see. If one sample includes bacteria and the other doesn't there will be a vast difference in *S*.

Measuring Dominance (d)

Looking at the number of species alone is a good first step in studying biodiversity, but it may not give the entire picture. A cornfield with six different weed plants would have an *S* value equal to 6. A meadow with approximately even numbers of six different wildflowers would also have an *S* value equal to 6. Simply looking at species richness tells us that these two ecosystems are exactly the same. Yet, we know that they are very different. In order to statistically show this difference, we need to include a measure of the relative numbers of each species in the ecosystem. Analysis that includes relative numbers of organisms and indicates the abundance of the most common species is referred to as **dominance**.

While there are many different methods to calculate dominance, one of the simplest is called the Berger-Parker Index. This index is represented by the letter *d.* This measures the proportion of the most abundant species relative to all of the organisms in the sample area. (The Berger-Parker Index is best interpreted by looking at a reciprocal. For the purposes of this activity, we will provide only the reciprocal equation and not the actual *d*.)

- To calculate *d* you simply divide the total number of organisms (N) by the number of organisms in the most abundant species ($N_{abundant}$).

$$d = \frac{\text{total number of organisms (N)}}{\text{Number of most abundant organisms } (N_{abundant})}$$

In our example, the total number of organisms is 75. The most abundant species is fern, which has 45 organisms. So $d = 75/45 = 1.7$. If there were only one species in the sample then *d* would equal one. Any number greater than one indicates greater diversity. The larger *d* is, the greater the diversity.

Comparing two ecosystems – calculating the Sorenson number

The analysis tools described above all describe a general comparison of two ecosystems. Many statistical tools can be used to do a beta analysis, which is an analysis that is able to compare two ecosystems. We will use the simplest test called the **Sorensen number.**

To do this comparison, first make a list of all of the species from each ecosystem you are comparing. Next, you need to know three things. (1) You need to know how many different species were found in the first ecosystem; this number is referred to as *a*. (2) You need the number of species in the second ecosystem; this number is *b.* (3) You also need to know how many species were common to both ecosystems. This number is *c*. Now we do a simple comparison of the number of common species to the total number of species in both *a* and *b.* If this number is equal to 1, that means that all of the species are found in both ecosystems and they are identical. If the number equals 0, there is no similarity. The closer this value is to the number one the more similar the ecosystems.

- This number is called the Sorensen number and is calculated as follows.

$$\text{The Sorensen number} = \frac{2c}{(a+b)}$$

Here is a simple example of calculating the Sorensen number:

Table 1. Sample data collection Joe Jungle Q

Species in ecosystem a	**Species in ecosystem b**
Coconut tree	Coconut tree
Fern	--
Lily	Lily
Palm	Palm
Bromeliads	--
--	Orchid
--	Focus
--	Rubber tree
5 total species in a	**6 total species in b**

By looking at this table you can see that $a = 5$ and $b = 6$.
(Remember *a* and *b* are simple species richness).

Of all the species in *a* and *b*, three of them are found in both ecosystems. This means that $c = 3$.

To solve for the Sorensen index you insert these numbers in the equation.

$$\text{Sorensen} = \frac{2 \times 3}{5 + 6} = \frac{6}{11} = 0.55$$

Data Collection and analysis page

Species	**Number of organisms**
Total number of species = S	Total number of organisms (all species included) = N

Calculate the Berger-Parker index *(d)* for your sample:

$$d = \frac{\text{total number of organisms (N)}}{\text{Number of most abundant organisms } (N_{abundant})}$$

Fill in the following table of class data.

Jungle name	**S**	**N**	**$N_{abundant}$**	**d**

Which jungle is the most diverse?

Now compare your jungle to the adjacent jungle using the Sorensen index.

Names of Species in your jungle	Names of Species in the adjacent jungle	Names of species common to both jungles
Total (a) =	Total (b) =	Total (c) =

Sorensen number = $\frac{2(c)}{a + b}$ =

How similar are the two jungles?

Activity 2: Biodiversity in the hidden jungle

After completing these activities, you will be able to:

1. Sample bacterial populations,
2. Use sterile technique,
3. Compare the biodiversity between two bacterial communities,
4. Make connections between areas that have greater biodiversity and the reasons for those differences.

By examining Joe's Jungles, you have learned to compare biodiversity in plant communities. The same techniques and analyses can be applied to any community. One of the most abundant forms of life on the planet is bacteria. Some bacteria are essential for life and some bacteria are harmful. In order to see bacteria, we need to collect them and culture them. Bacteria, like all living organisms require a suitable habitat, so think about where bacteria might be found. Bacteria have adapted to very harsh environments, and have been found where no other living things can exist. Yet, bacteria still require some moisture and many species may grow better at warmer temperatures. They need to be able to get nutrients from the environment and unless they are photosynthetic, they need to extract energy in the form of food from the environment.

- List some places that you are certain bacteria can be found.

- Pick two of these places. Why do you think that bacteria will be found here? What is it about each of these locations that causes you to think bacteria will present? *Use the Biodiversity concept map on the next page to organize your thinking.*

- Now think about which place would have greater biodiversity? Why?

(This is your hypothesis. Review your notes from above and see how you used your background knowledge, observations, and new information to make a prediction about which of the two locations has greater biodiversity.)

Now you can test your hypothesis. Your instructor will explain how to collect and culture bacteria (see the following pages). Design an experiment to test your hypothesis. Use the biodiversity tools from Joe's Jungle to collect and analyze your data. Keep careful notes about your experimental design. Make sure you write about the statistical tools that you will select to analyze your data.

- Two places that might have a difference in bacterial biodiversity are:

- Why I think there might be differences between these two places:

Use this Biodiversity concept map to organize your thinking:

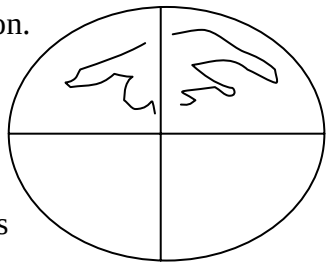
List the factors that might affect bacterial biodiversity:	Concept Map of how these factors relate to each other and to bacterial biodiversity:

Basic Procedure for Testing Bacteria

Bacteria are invisible to the human eye and therefore not readily identified or counted. Bacteria can be sampled from surfaces and from water, and grown on a nutrient medium in a petri dish. This allows bacteria to grow into sufficient numbers to observe and identify them. For the purposes of this laboratory, you will be asked to sample for bacteria and use limited tools for identification. Microbiologists have many more tools available to them to identify and count bacteria than we have time to explore in this lab.

- Use the following procedure to design an experiment to test for the presence of bacteria on a variety of surfaces in your environment.

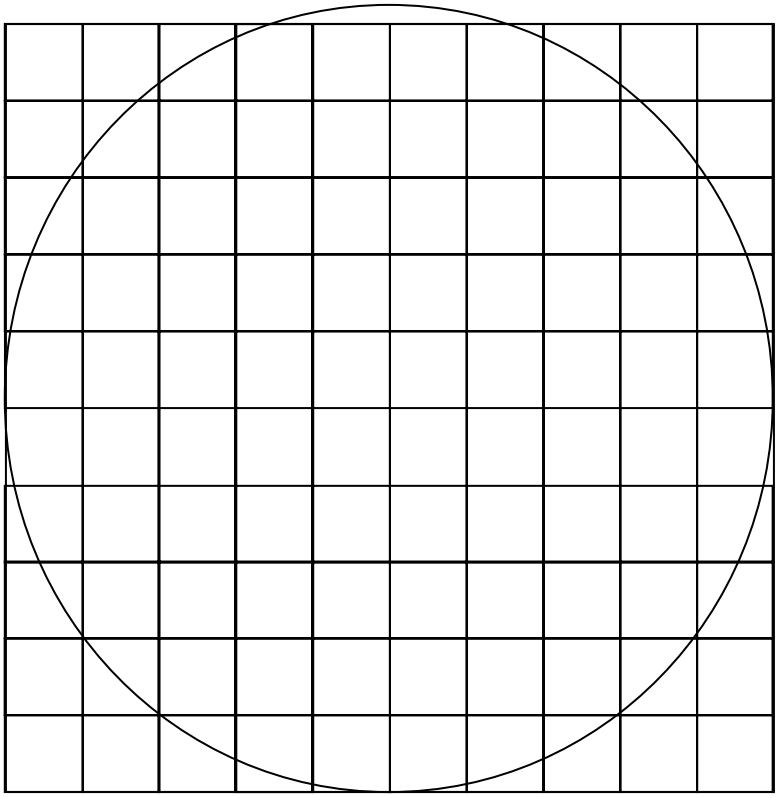
1. Obtain sterile swabs (they look like a long wooden Q-tip) and a petri plate that contains agar. Do not set the swab on any surface, or open the petri plate until you are ready to take a swipe.
2. Turn the plate over so that the half containing the agar is on top. Mark four sections in the bottom using a permanent marker. Label three of the sections with the surface or water you are sampling. Label the fourth section control. Write the name of your lab group and section number on the bottom as well.
3. Turn the petri dish over so that the agar is on the bottom.
4. Take one sterile swab and dip it into the water or wipe it across the surface you are sampling.
5. Lift the lid of the petri plate and wipe the swab on the labeled section. (See diagram to the right.) Do this quickly without breathing on the plate or touching it. Repeat for each of the other labeled sections. Do nothing to the section labeled control. Put the used swabs in the containers provided for biohazard disposal.



6. Seal the plate with parafilm and place upside down on the bench as directed by your instructor.
7. Spray your bench with the special cleaner and wash your hands with soap and warm water before leaving the laboratory.
8. Next time you return to lab, carefully describe the growth of the bacteria and record it in your journal. Be sure to list how many different types of bacteria are found in each sample. Use one of the grids below to help in counting your bacterial populations. Note that not all of the growth on your plate is bacteria. You are likely to encounter molds and fungi that have grown from microscopic spores. These species contribute to the biodiversity of the microscopic community you sampled.
9. When you finish your observations, put the plate into the specially marked biohazard container.

NOTE: Swabs and petri plates must be disposed of properly. There is a container in the laboratory labeled biohazard and containing a special liner. All swabs must be placed in the cardboard box. All petri plates must be placed in the container. **DO NOT PUT ANYTHING ELSE IN THE BIOHAZARD CONTAINER!**

Use this grid to help count your bacterial populations:



Notes for the Instructor

Safety Considerations

Be sure to check with your institution about all rules and procedures regarding the handling of potential biohazards and used petri plates. While the microbes collected by the students clearly are present in their environment, some of the bacteria are opportunistic and can cause infections under the right circumstances. We do not have students open the petri plates once swabbed. We use disinfectants to swab the benches before and after working with the plates.

Joe's Jungle

The purpose of this lab is to give students some experience with data analysis and statistical tools. A brief introduction can discuss the levels of biodiversity and the reasons we care about it. Have pots of succulents on each bench with a number of replacement pots available. Be sure to ask students to handle the plants carefully and to use the probes to count. Warn students not to touch the cactus spines, and to not pull the plants out of the soil.

The activity is straightforward. Students will count the number of species and the numbers of organisms of each species. Assign roles: Botanist/skeptic, recorder, counter/doer. Everyone needs to have a written record of each species. They can name them anything they want as long as they are consistent. You may choose to have a quick botanical convention to decide the names of the species. You may also refer to keys of local interest you can find.

The biodiversity indices we use are:

- S= number of different species
- Berger-Parker index, *d*, is a measure of relative abundance of species along with different species.
- The Sorensen number allows you to statistically compare two different ecosystems.

These statistics were chosen for their simplicity rather than their appropriateness. You are probably aware of better analyses. Many students may be "mathphobes" who will be intimidated by the word statistics. You may get around this by asking questions: How do we know these ecosystems are different? How different are they? Remind students that they can use statistics to help answer these questions. This is the process of analyzing raw data so that patterns become visible. You may wish to talk about how statistics can be manipulated to make a point. You should remind them that an understanding of the utility of statistics helps them assess data when people are presenting it to them. Once students have collected their data, walk them through the calculations. Make sure that they understand them. Make sure they understand the purpose.

Hidden Jungle

So far, you have addressed how to assess biodiversity; now students are going to look for it. They need to think clearly about the causes of differences in biodiversity. Use the concept map (in the student handout) to start this conversation. Students will sample the hidden jungle -- bacteria and fungi. They must first identify two different areas that they expect to have differences in bacterial biodiversity. They will use the concept map to focus their thinking about what factors affect biodiversity. Have them start by listing all of the factors that are needed by living organisms in order to survive. You should expect your list to include water, appropriate temperatures, and

sources of energy, raw materials, and micronutrients. Use the concept map to connect environmental factors with the two places in which they expect to find differences in microbial biodiversity. They will hypothesize that two areas have differences in biodiversity based on these environmental factors. For example, many will believe that the floor in the restroom is more likely to have a greater biodiversity than the hall because it has more access to bacteria or because it is wetter. Some other characteristics of this experimental design:

- The Independent variable will be the location/physical characteristics
- The Dependent variable is the measure of biodiversity
- Controls include swabs, time, coverage, *etc.*
- Some assumptions are that all bacteria sampled in the environment will grow on standard nutrient agar, and all will grow at room temperature.

Analysis of bacterial growth in "hidden jungle"

Have students wipe the bench. The "doer" of the group should get the plate from you. Place the plates on the grid. As with Joe's Jungles, they should agree upon what visible characteristics make up unique microorganisms. They can use color, colony size, or colony shape as cues. Have photos available in the lab to identify specific types of bacteria.

Data collection should identify "species" type and number for each area sampled. Analysis should include use of all of the biodiversity indicators. Remind students that they are comparing the two sites that they selected to sample. They do not need to compare with another group, although choosing to do so and justifying it scientifically should be encouraged. Summary must include a direct reference to the hypothesis.

Making a Concept Map for Factors Affecting Bacteria Growth

In this lab, students have the tools to make a map about factors that they think will affect the growth of bacteria. In the student handout, there is a page with two rectangles, a small rectangle to the left of the page and a large rectangle to the right of the page. On this page there are a couple of questions, start this part by doing the concept map, as it will help them answer the questions at the top of the page. Many students have never used a concept map, so here are some simple instructions on how to get students to use and understand their concept map.

1. Ask your students to take about 5 minutes and write down (on the left-hand side of the concept map page) as many factors they can think of that would affect the growth of bacteria.
2. Ask your students to share some of the factors they thought of and make a list on the board. Hopefully, some of the factors they have thought of are temperature, light, and moisture.
3. Students will create their concept map in the rectangle to the right of where they made their list of environmental factors. To construct a concept map you place the main topic (which here is the growth of bacteria) either in the middle of the rectangle or off to one of sides. I personally prefer to place it in the middle, but I am always sure to instruct my students of both options and have them do what is best for them.
4. Then depending on where you have placed your main topic, you will begin placing the list of factors that affect growth either around the main topic if you placed it in the middle, or off to the other side if your main topic is to one side.

5. Once all the factors are placed on the concept map, have the students draw a line from each factor to the main topic (since they have already determined all these factors affect bacterial growth).
6. The second part of a concept map is determining how each of these factors also affects one another. So let us use temperature, light, and moisture and determine their relationship in affecting one another. For example, the amount of light is going to affect the temperature, so draw an arrow from light to temperature. The temperature is going to affect the amount of moisture, as well as the amount of moisture affecting the temperature, so draw an arrow pointing in both directions from the temperature to the moisture since they affect one another directly. Continue to draw arrows connecting as many of the factors as you can, this will help determine the relationship and importance of each individual factor. Instruct students that not all of the factors will be connected to another factor, as some factors are independent in nature. The point of the arrow goes in the direction of the effect, and some may affect one another equally.
7. Now students can add in the locations they believe have different levels of biodiversity. Connecting these areas to the factors affecting growth will give them a clearer picture of why they might expect such differences.
8. Once students have connected all the factors, this should help them in determining the factors that are truly important for the growth of bacteria. Knowing these factors and their impact on one another, the students should now be able to think of two locations that will have difference levels of bacteria growth.

Counting Bacteria in the Hidden Jungle

Students will hopefully have some growth on their plates so they can compare bacterial diversity between sites. Counting the number of species and the number of organisms present is easy and straightforward with each colony being counted and considered one organism (Figure 2).
However, at times, a type of bacterium will become overgrown so the section contains many colonies (Figure 3) that cannot be counted in the same manner as the section with all individual colonies. When there is a situation in which individual colonies cannot be counted then a grid can be used to estimate the population size.

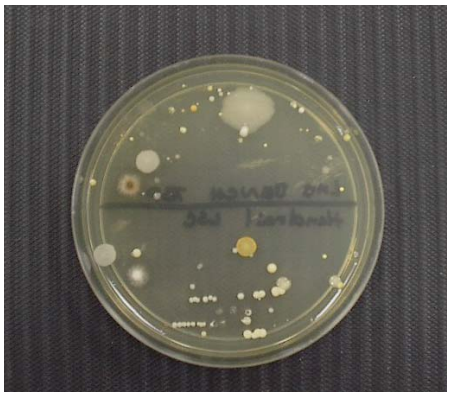


Figure 2. Unseen jungle plate with unique bacterial colonies.

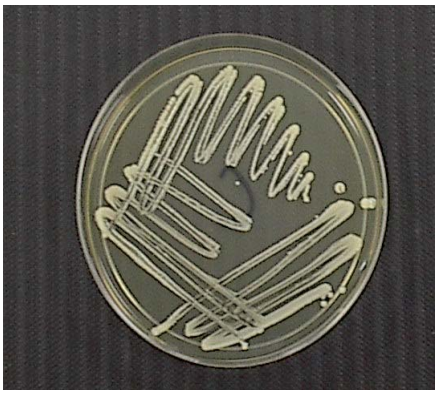


Figure 3. Unseen jungle with indistinct colonies.

The grid provided to the students has large squares but you could modify this to have smaller squares if you find it necessary. Provide students with transparency copies of the grids and washable markers, then have students trace the outline of the overgrown bacteria. Students will need to count the total number, rounded to the nearest 0.25, of grid squares covered in bacteria. Once they have estimated the amount per square, using simple addition the student can now calculate the total number of grid squares that contain each bacterium, thus estimating the population size of the overgrown bacteria. If students can find one individual isolated colony, they can use it to estimate the actual size of an individual colony of this species. Students now estimate how many of those individual colonies would fit in one of the grid squares. The number of individual colonies for one grid square multiplied by the total number of grid squares with bacteria growth equals an estimation of the total number of colonies. For example, if a student estimates there would be 25 individual colonies per one grid square and they calculated a total of 10 grid squares have bacteria growth, then 25 X 10 would equal ~250 colonies (organisms for our purposes) of this species of bacteria present. Students need to be sure to indicate that the total number of organisms is an estimate and not an actual count.

With this method, whether students have individual, isolated colonies or overgrown bacteria they can calculate the *S* and *d* values they learned in Joe's Jungle, as well as comparing the bacterial growth in each ecosystem (location swabbed) using the Sorensen Number.

Disposal of Biohazard Materials.

The lab has a container for contaminated plastic and paper products (biobag), a container for contaminated broken glass (either a sturdy cardboard box inside a biobag, or a sturdy plastic bottle with a lid that has the biohazard symbol on it), and a sturdy box for NON-contaminated broken glass and glass for disposal.

Literature Cited

Wedlin, David and William Draper. 1999. Using Animal Crackers to Understand Indices of Diversity. Page 505 *in* Tested Studies for Laboratory Teaching, Volume 21 (S.J. Karcher, Editor). Proceedings of the 21st Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 509 pages.