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EXPERIMENTS

How many species are there? Determining species richness.

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ABSTRACT
One of the simplest questions an ecologist can ask about a site is how many species live there. The answer is important for basic researchers and managers alike, but can be deceptively hard to obtain. This exercise introduces students to the issues surrounding the estimation of species richness, and can be completed in a single three-hour lab session. Students will learn to collect data in the field, obtain their own estimates of species richness, and evaluate the underlying assumptions and validity of these estimates. The exercise is written for estimating the species richness of trees in forests but could easily be adapted for other plant or animal communities.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** Species diversity, species richness, biodiversity, community ecology, plant ecology, forest ecology, scale, sample, estimate

- **Science Methodological Skills Keywords:** Collecting and presenting data, data analysis, field observation skills, field work, quantitative data analysis, quantitative sampling, use of dichotomous keys

- **Pedagogical Methods Keywords:** Assessment, informal group work, group work assessment, cooperative learning groups, authentic inquiry

CLASS TIME

One three-hour lab.

OUTSIDE OF CLASS TIME

On average, students will need two to three hours outside of the classroom to estimate species richness from the data and answer the associated questions.

STUDENT PRODUCTS

In the field, students will learn to identify common trees. Afterwards, they will produce a short report that shows their calculations and resulting estimates of species richness, and discusses the strengths and weaknesses of these estimates based on general principles and their own field observations.

SETTING

This lab was originally designed for estimating species richness of eastern deciduous forest trees, during the growing season. It can be adapted easily to other taxa or environments, with appropriate changes to sampling methods and materials.

COURSE CONTEXT

This experiment has been used successfully in an upper-level undergraduate and graduate ecology lab course. A simplified version of this lab could also be utilized in lower level biology, ecology, or conservation courses. We typically have four to six lab sections of 10-14 students each, with each section split into teams of three or four students. Data collected during the field portion of the exercise are pooled across all teams by the instructor, and shared with the entire class through email or the class website. Each student must analyze both the data collected by his or her own team and by the entire lab section, estimate species richness from a smaller and larger data set, and compare their values.

INSTITUTION

Clemson University is a land-grant (public) research university with a range of undergraduate and graduate programs.

TRANSFERABILITY

This study can be performed in any region where suitable habitat is accessible. The field observations are best performed during a season when the plants (or other taxa) are easily observed and identified. For example, we sample tree diversity between the months of March and October when leaves are present and tree species are most easily identified. Because students typically work in teams of 3-4 in the field, this exercise can be used easily by lab sections that range from a few students to 40 or more. In cases where classes include 20+

students, however, it may be desirable to have more than one instructor present to assist, or to assign students to larger teams.

ACKNOWLEDGEMENTS

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SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

How many species are present in a forest?

What Happens

Before lab, the students will read about species richness and the various methods and concerns that enter into estimating species richness in the field. The instructor will briefly review these points at the start of lab with a particular discussion of the terms “estimate” and “sample” and then summarize the actual procedures to follow in the field. Upon arrival at the field site, the students will briefly review field identification of the tree species present, and then work in teams of 3-4 to identify and count tree species in plots and along transects. These data will be combined by the instructor with data from other teams and then returned to the students for analysis. Finally, students will individually compute their estimates of local species richness, using species accumulation curves and total species estimators, based both on their own team’s data and that collected by the entire class. Each student will answer a series of questions that guide their discussion of the issues surrounding estimation of species richness generally, and the estimates produced through their work specifically.

Experiment Objectives

At the end of this exercise students will be able to:

1) Identify tree species present with the help of field guides.

2) Count numbers of each tree species encountered in plots and along transects.

3) Estimate species richness based on plot and transect data.

4) Understand and communicate the concerns and pitfalls inherent in estimating diversity in biological systems.

Equipment/ Logistics Required

- Instructor must identify appropriate field sites and guides to the selected taxa.
- 50 or 100 m transect tapes (1 per team)
- 10 m transect tape (1 per team)
- wire flags
- meter sticks (1 per student)
- clipboards (1 per team)

Summary of What is Due

Students will be assessed on their successful collection of data in the field by submitting their team's data to the lab instructor at the end of the exercise. Students will also be assessed individually based on their subsequent calculation, assessment and analysis of species richness, using both their team's data and that from the whole class (see sample data sheet). They will also be assessed individually on their responses to the questions for further thought and discussion that are listed at the end of the lab handout. The instructor may assign students additional work if desired, such as a literature review of methods or issues surrounding the estimation of species richness. This provides not only an opportunity to develop the students' scientific writing skills.

Rubric for student evaluation:

Activity	Pts
Pre-lab Quiz:	
Answer pre-lab questions, 2.5 pts per question	5
Field Study:	
Participation in gathering field data during lab	8
-Participation	2
-Working with group	2
-Submission of appropriate group data	4
Post-Lab:	
Analysis/Writeup	27
-Calculate Chao 1 and Chao 2 estimates of species richness for plotwise group data	2

-Calculate Chao 1 and Chao 2 estimates of species richness for plotless group data	2
-Calculate Chao 1 and Chao 2 estimates of species richness for plotwise class data	2
-Calculate Chao 1 and Chao 2 estimates of species richness for plotless class data	2
-Questions for further thought and discussion	15
Total:	40

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

The Importance of Species Richness

Determining the biological diversity of a site, ecosystem, or habitat is one of the most fundamental questions typically asked by ecologists. However, biological diversity can be measured at many different scales, from genetic diversity within an individual species, to species diversity within communities, and even to functional diversity within ecosystems. Perhaps one of the most straightforward and intuitive measures of biological diversity, however, is simply the question of how many species are present in a location. Determining this number, termed the **species richness**, is important both for fundamental and applied ecological research, and it is commonly used both by itself and as a component of more complex measures of biodiversity. For example, mapping patterns of species richness along latitudinal or elevational gradients may shed light on underlying ecological processes. Species richness would also be important in designing a system of protected areas that maximizes the species diversity for a region or country.

Extrapolation of Species Richness

Species richness is a simple measure of biodiversity but it can be surprisingly difficult to measure in the field. This can be true even for groups of organisms that are large, relatively stationary, and easily identified, such as trees, lizards, or grasses. In order to be certain of detecting every species present, it would be necessary to examine every individual in the population in a complete census. Obviously, this is almost never possible: imagine the effort it would take to examine every tree in even a 1 hectare forest! This is even more challenging when dealing with reclusive, tiny, or otherwise difficult-to-find species, as well as animals that move from place to place. No matter how hard we search, it is often impossible to be certain that we have found every single individual. Therefore, in order to measure species richness we generally sample only a small fraction of the individuals at a site, then extrapolate from our sample data to estimate the total number of species present.

Materials and Methods: Analytical Methods

Sampling effort

If we survey a site and find 25 species of trees, then we know there are at least 25 species there, but is that all of them? Might there be a few more, or a few dozen more? To estimate the total number of tree species, we need to know how many of each species were found, and how many total trees were observed. To understand this, think about how much effort is required to find each new species in the survey. In the beginning, it takes very little effort – by definition, the first tree encountered is a new species, and the second and third species are probably added quickly. However, as more individuals are examined, the rate at which new species are discovered typically declines. Once we have found 20 species, we may need to examine a large number of trees to find the 21^{st}, and an even larger number to find the 22^{nd} species, as most of the common species have likely been encountered. Fortunately, statisticians have developed a number of techniques that can use this decline in discovery rate to estimate the total species richness at the site.

Keeping Track of Effort

When estimating species richness, it is often important to monitor the amount of work required to discover each additional species. One way to do this is simply to keep track of each tree encountered, and record each time we find a new species, as in the example above. An alternative is to establish and survey plots, and record the cumulative number of species found as a function of the number of plots surveyed. When sampling other organisms, such as insects, we may substitute pitfall traps, light traps, or other passive or active sampling devices for plots, and record the cumulative number of species encountered as a function of the number of traps checked. In all these cases, we will end up with a graph of the cumulative number of species encountered on the Y-axis versus the sampling effort, as measured by the cumulative number of individuals, plots or traps sampled on the X-axis. This graph will increase as additional species are found, rise at a declining rate as it becomes more difficult to find new species, and remain horizontal when all species have been detected. The goal of extrapolation is to estimate where this curve becomes horizontal, thus becoming asymptotic to the true species richness.

Sample Forest

To illustrate some of the problems inherent in estimating richness, and some of the solutions, consider the sample of five plots from the forest in Figure 1. Each symbol represents an individual tree, and each distinct kind of symbol corresponds to a distinct tree species that inhabits the forest. The forest is divided into a grid, and those plots that were sampled are marked in bold. The species observed in each plot are also documented in Table 1. Note that not all species present were found in the sampled plots.

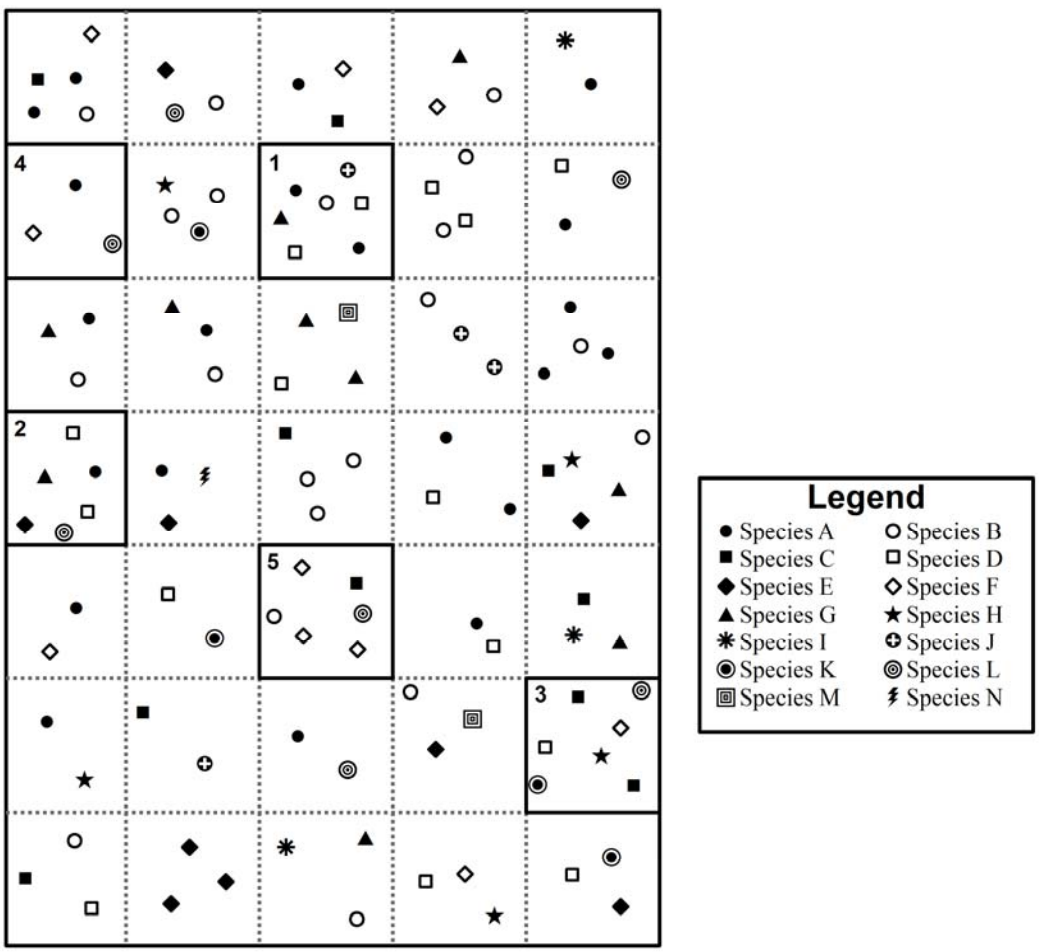

Figure 1. Sample forest data

Table 1. Observed Trees within Sample Forest

Plot #	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Species A	2	1	0	1	0
Species B	1	0	0	0	1
Species C	0	0	2	0	1
Species D	2	2	1	0	0
Species E	0	1	0	0	0
Species F	0	0	1	1	3
Species G	1	1	0	0	0
Species H	0	0	1	0	0
Species I	0	0	0	0	0
Species J	1	0	0	0	0
Species K	0	0	1	0	0
Species L	0	1	1	1	1
Species M	0	0	0	0	0
Species N	0	0	0	0	0
Number of species per plot	5	5	6	3	4
New species per plot	5	2	4	0	0
Total species found	5	7	11	11	11

Finding the Empirical Mean Species Accumulation Curve

Fig. 2 shows a graph of the species accumulation for the sample forest, using the data from Table 1. The species accumulation reflects the total number of species found as larger areas are sampled: plot 1, plots 1 and 2, plots 1, 2, and 3, and so on, in that order. Note that the rate at which new species are found does not continuously decrease as we might expect, but actually increases briefly before leveling off.

What does the curve tell us? It shows that there are at least 11 species of tree in the forest. The fact that the curve seems to level off after the first three plots are added up might indicate that most of the species have been found. Lots of species are found in plot one, two more with plot two, and four more when plot three is added, but none after that. Does this sudden stop mean that all the species have been found? Not necessarily. Fig. 3 shows what happens if we graph the data with the plots in the reverse order (5, 5+4, 5+4+3, etc.).

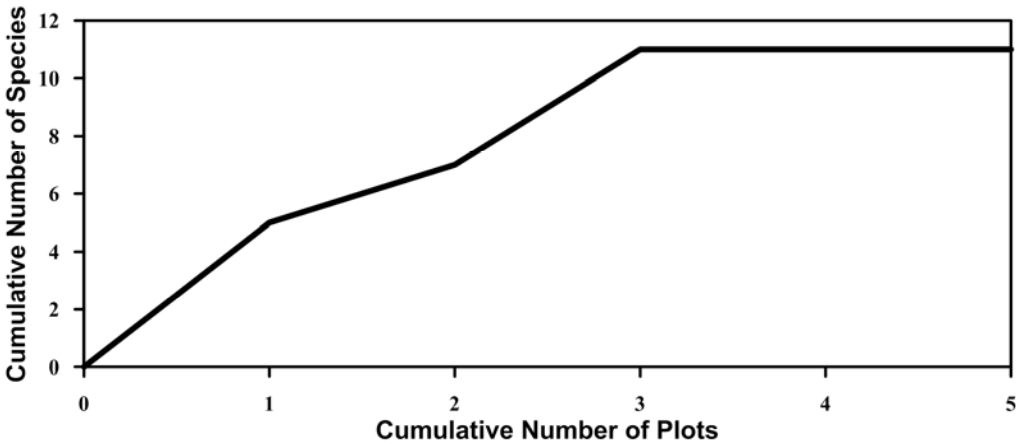

Figure 2. Plotwise species accumulation

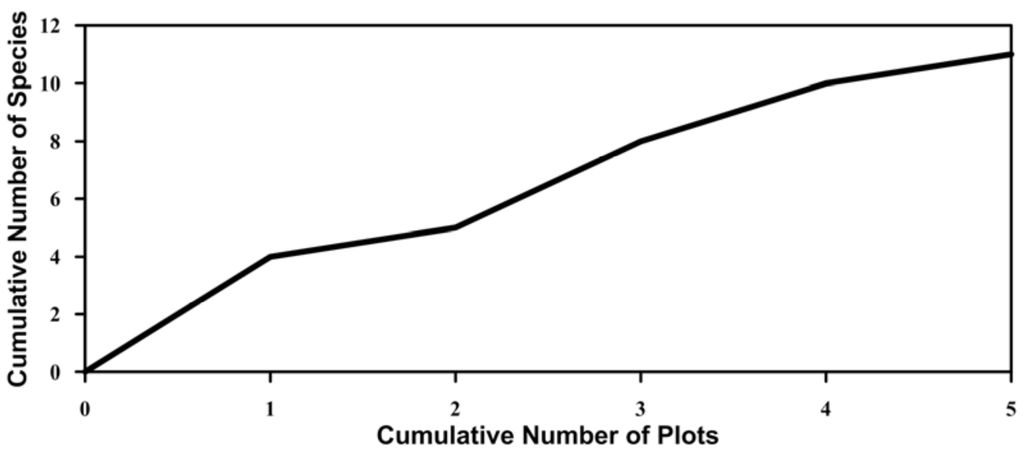

Figure 3. Plotwise species accumulation by different plot order

With the data tallied in this order, the graph looks very different from that in Figure 2. Although the rate at which new species are being found seems to be declining, new species are being found in every additional plot until all plots are sampled. Now it looks almost certain that there are more species to be found. Although both graphs end up with 11 species found by the fifth plot, the implications as to how many more species are out there and how easy they would be to find differ dramatically. But the order of the plots is essentially arbitrary, and has nothing to do with the species richness of the forest!

In order to remove biases caused by particular orderings of the plots, we can calculate the **Empirical Mean Species Accumulation**. Here, the species accumulation curve is calculated many times, each time scrambling the order in which the plots are entered. Then we average over all these results, and calculate the average number of species in the first plot, the average number after 2 plots, and so on, eliminating the bias from any particular ordering. This process is labor intensive, especially in large surveys with 50 or 100 plots, so the empirical mean species accumulation is almost always calculated using computers. Fig. 4 shows the result for our 5 plots above.

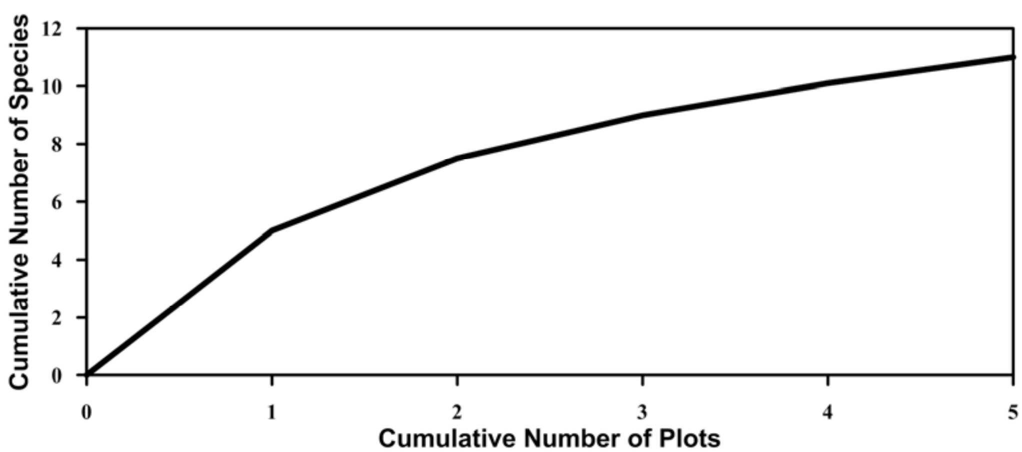

Figure 4. Empirical mean species accumulation

Notice that, unlike in the two previous graphs, the rate at which species are being discovered is constantly decreasing as more plots are added, and there are fewer sharp changes in slope. The empirical mean species accumulation data is usually the set of data used when estimating total species richness.

Heterogeneous Communities and the Expected versus the Empirical Means

It is common for communities of organisms to exhibit some spatial heterogeneity, with different species occurring in different parts of the site. Some trees will grow in clumps, or prefer the slightly moister sites, the more exposed areas, etc. If there are distinct subsets of organisms within an area, this may be reflected in tendencies for certain species to be found together in the same plots, or separately in different ones. These associations could bias the species accumulation curve, and simply randomizing the order in which plots are added to the analysis will not correct this. There are a variety of ways to remove the effects of these associations in plot data; one that is appropriate here is to randomize the data by individuals rather than plots. Since we know how many individuals of each species were found in each plot, we can independently and randomly disperse these individuals among “pseudo-plots”, eliminating any associations. If we do this randomization repeatedly, we can calculate a new **Expected Mean Species Accumulation** curve, in which the contents of the plots are randomized rather than the plots themselves. The new and empirical mean curves will be virtually identical if the community is homogeneous (Fig. 5). If not, then the new expected mean curve will rise less steeply, as species are no longer encountered in clusters (Fig. 6). The two curves will still meet at the end, but the fact that the site spans several distinct communities makes it tricky to estimate the full species richness.

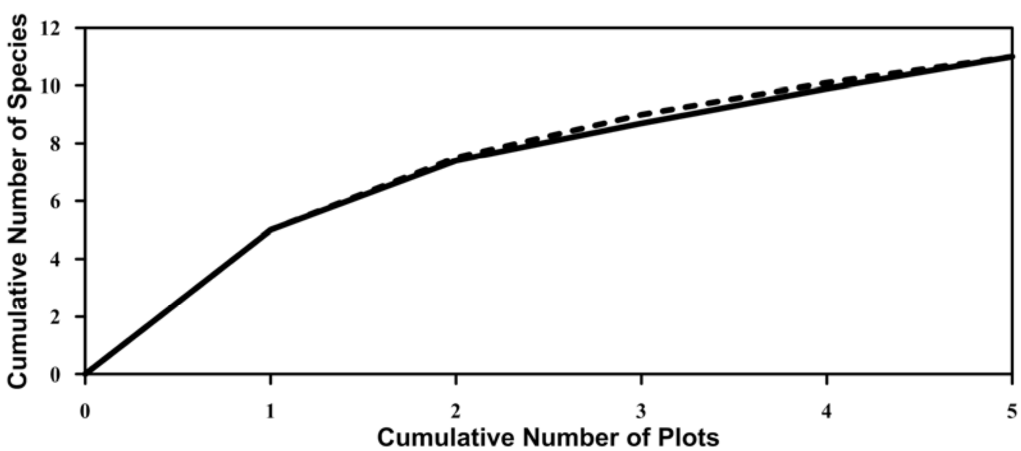

Figure 5. Empirical (solid line) vs. expected (dashed line) mean species accumulation for homogeneously distributed species.

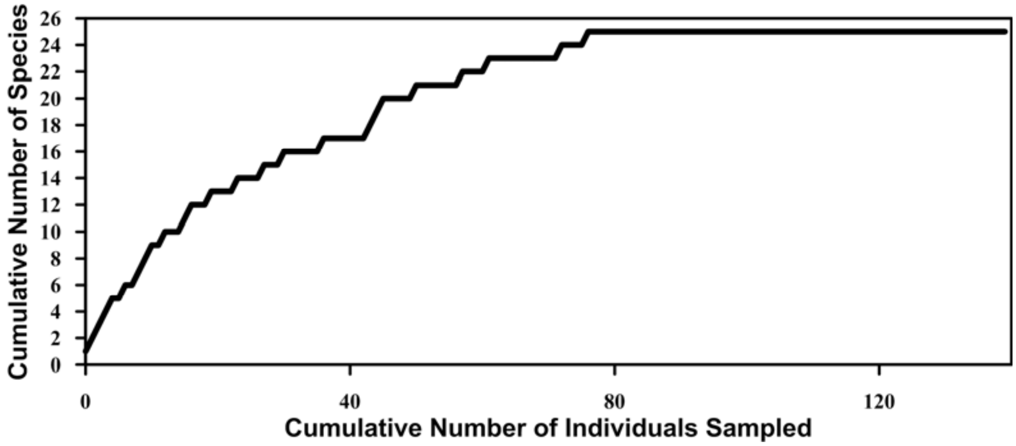

Figure 6. Empirical (solid line) vs. expected (dashed line) mean species accumulation for heterogeneously distributed species.

Individual Data and the Expected Curve

In general, species richness is surveyed using plots, as described above, or equivalent groupings (trap-nights, etc.). However, data may be collected by plotless methods (Fig. 7), such as recording a list of all individuals encountered along a transect. Here we face the same problem as before: the graph of the number of species found versus the number of individuals encountered may rise more or less rapidly, and level off or not, depending on the order in which the transect is walked!

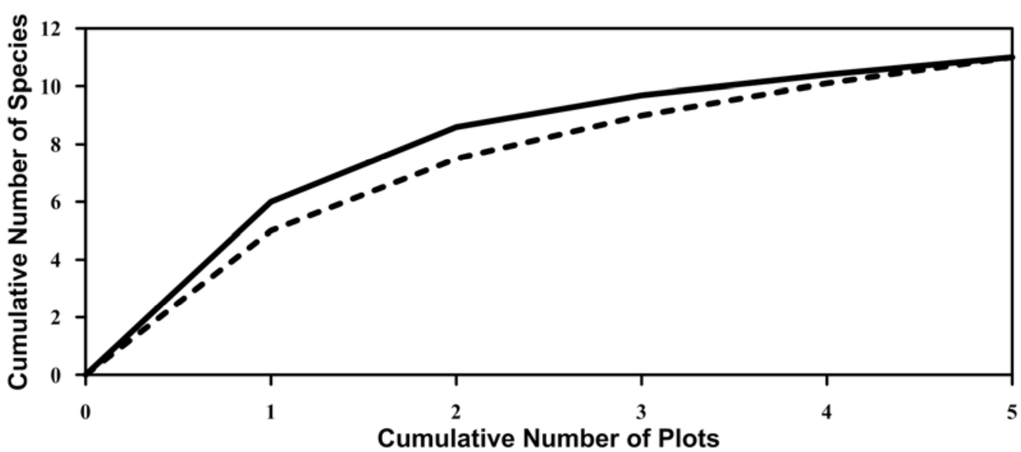

Figure 7. Plotless Species accumulation

The solution to this problem is also similar. We use a computer to randomize the order in which species in our sample are encountered, take an average over many such simulations, and this yields the **Expected Species Accumulation** (not shown). When the community is relatively homogeneous, the expected curve from a plotless survey is roughly equivalent to the empirical mean accumulation from a plot survey, described earlier. If the community is not homogeneous, then groups of species will be encountered together in plots and the empirical mean species accumulation obtained from plots will rise faster initially than it would from a plotless survey.

Calculating species richness

There are many different methods to estimate species richness. Some simply fit algebraic equations to the data, where the equations have no theoretical basis other than that they describe curves that rise then level off, like the ones generated above. We can then ask, “What is the asymptote of the best-fitting equation to the mean species accumulation curve?” Other methods are based in statistical ecology, where the relative number of common and rare species is known or believed to follow some theoretical pattern, allowing us to estimate the additional number of species too rare to be detected. The choice of best estimator (and how intensively to sample plots or individuals) will depend on the relative distributions of species, the homogeneity of the area, and the size of the sample. As a result, researchers often use several different estimators together.

There is always uncertainty in science when extrapolating beyond what is known, but the alternative, to only count species that are seen, is certain to be wrong. Some theory-based estimators require computers, but we will use two that are widely used and can be calculated by hand. These are the Chao estimators, named after their originator, the statistician Anne Chao.

The Chao 1 Estimator

The first Chao estimator (Chao 1984), referred to as Chao 1, predicts species richness based on the total number of observed species in the samples, S(n), and the number of those species that were represented by just one or two individuals. This estimator can be used with plot-based or plotless data, and is given by

$$S_{\max} = S(n) + \frac{a_n^2}{2b_n}.$$

Here a_n is the number of species for which only one individual was found, and b_n is the number of species for which only two individuals were found. Clearly, the more species that are found only once, the more additional rare species remain to be discovered. This model is commonly used for diverse communities, and is especially accurate when there is a high proportion of rare species.

The Chao 2 Estimator

The Chao 2 model (Colwell and Coddington 1994) is a variation on the one above, but adds to the number of observed species S(n) an additional amount based on the number of species that were encountered in just one plot, or two plots. It is written

$$S_{\max} = S(n) + \frac{L^2}{2M},$$

where L is the number of species found in only one plot or trap, and M is the number of species found in exactly two. This is more accurate for heterogeneous areas or communities where rare species tend to occur in small clumps. In cases where several individuals of a species are found together in one spot but nowhere else, it is more accurate to treat them as one individual rather than as multiple individuals. This estimator is only valid for plot-based data, for which the two models are often used in conjunction.

Calculating Species Richness for the Sample Forest with the Chao Estimators

Chao 1 Looking back at the sample forest data, we see that we observed 11 species in all. Of these, four species (E, H, J, and K) were represented by a single individual, and two species (B and G) were represented by two individuals. Therefore, the Chao 1 estimate of the total number of species present is

$$\mathbf{S_{max}= 11+ (4^2/(2x2)),} \text{ or } \mathbf{S_{max}=15}.$$

Chao 2 Alternatively, 4 species (E, H, J, and K) were found in only one plot each, while three more (B, C, and G) were found in two plots each. Therefore, the Chao 2 estimate of the total species richness is

$$\mathbf{S_{max}=11+(42+(4^2/(2x3)),} \text{ or } \mathbf{S_{max}=13.7.}$$

These two estimators agree that there are a small number of additional species in the site that were not found, and in this case, they provide similar solutions that are close to the actual number, 14. There are formulas to estimate the variance, standard error, and confidence limits of these estimators, but they are tedious to calculate by hand (Colwell 2000), and are beyond our goals for this lab. It is enough to know they exist, if you are conducting ecological research in the future.

Other Issues in Estimating Species Richness

Species identification: For many taxonomic groups it can be surprisingly hard to assign all individuals to known species. This is not just a problem for Amazonian explorers, but is true even for scientists working in the relatively well-studied areas of the United States. In Southeastern forests, for example, there are many oak species, some of which hybridize, making identification challenging. Even experts may have difficulty with certain groups of sedges, grasses, herbaceous plants, or insects. This problem may be worse in other regions and especially with other taxa for which local field guides may not exist or are poorly developed.

Unknown species: In field studies of diverse or poorly documented groups such as fungi, plankton, some insects, etc., it is not uncommon to find organisms that cannot be assigned readily to any known species. Sometimes researchers will count them as morphospecies, that is, organisms that differ in appearance and are assigned at least temporarily to species A, B, C, etc. (Hammond, 1994). This probably underestimates the true species richness, since some species are hard to distinguish visually, especially among the very groups that require this approach - the small, highly diverse, poorly known taxa such as ground beetles or flies. It is important to classify morphospecies consistently, to allow comparison of richness at different times or sites, or as sampled by different researchers.

Trapping bias: Many organisms are not sampled directly in counts, but rather indirectly with nets, light traps, plankton tows, and so on. However, organisms vary in how readily they are sampled by such means. Some species may be too small or fast to be caught, may avoid or escape from nets or traps, or may simply fly higher or swim lower than the nets or traps reach. Even some common species may rarely be captured. Furthermore, without a complete species survey in the first place, it can be difficult to determine if a trap is effectively sampling all the species present. As a result, ecologists will generally combine several different sampling methods to document the entire fauna of a site. An ant survey might include pitfall traps combined with timed searches and baited traps to find all ground-dwelling species, and arboreal traps to get those additional species that live in trees. Such combined data sets will identify a larger fraction of the total species present, but their different forms make extrapolation to additional, undetected species even more difficult. In addition, since different traps or sampling methods catch different organisms, at different rates, it will be hard to reconstruct other community-level parameters such as the relative abundance of the species (Longino *et al.* 2002). *Sampling effort bias*: Plot- and individual-based estimates of species richness can differ greatly, though they are often treated as equivalent. Plot-based methods are susceptible to bias if densities vary among plots (Magurran 2004). If we sample the same number of plots in young and old-growth forests, it may appear that we have sampled with equal effort and that young forests have far more species than the older ones. However, the young forest may have 20 times as many trees per hectare, so the sampling effort is not equal. Plotless methods would not be biased, since they ask how many species are detected among the first 100, 200, 300 trees, and proper analysis of plot data would also correct for this sampling bias. We now know that some old-growth forests are quite diverse, though less dense than younger forests.

Materials and Methods: Experimental Procedures

Study Site(s):

The southern Appalachian region from northeast Georgia and along the North Carolina-Tennessee border has the highest diversity of tree species of any region in the continental United States. This area was never glaciated, which can reduce diversity. Instead, past ice ages increased local diversity by pushing many northern species south, where they persist in localized cool, moist sites. More generally, the varied topography, elevations, soils, and climates in the region allow the mixing of species from across the eastern US, including the coastal plain, piedmont, gulf coast, and mid-Atlantic regions. We restrict our surveys to one local site, but it is immediately clear that there are many tree species present, and that it would be hard to find them all in survey. To estimate the total species richness in southeastern forests, we shall require the statistical techniques described above.

Overview of Data Collection and Analysis Methods:

1. Divide into teams of 3. Ensure that each team has at least one 50 or 100 m transect tape, a 10 m transect tape, two meter sticks, wire flags, and a clipboard.

2. Each team will measure and mark with flags the first 10 x 10 m plot, with one corner set at the 10 m mark on a 100 m transect (or two 50 m transects on end).

3. The team will then sample all trees in the first plot that are greater than 2 cm diameter at breast height (dbh), identifying them to species, and recording their number on the data collection sheet under “Plot #1”. For example, plot 1 might have 6 flowering dogwood > 2 cm dbh, as well as 2 white oaks, 1 American holly, and 2 redbuds (see data collection sheet below). Some trees are clonal so that nearby stems are all from the same seed, but for the purposes of this lab we shall consider them as separate individuals unless they clearly arise from the same root.

4. Move 20 m along the transect; mark and survey the second plot. Identify and record the number of all trees greater than 2 cm dbh on the data collection sheet under “Plot #2”. Repeat three more times, or as instructed, keeping the data for each plot separate.

5. In the same area, walk along a 100 m transect and record every single tree that is encountered within 1 meter of the transect line, and at least 2 cm dbh. Use a meter stick for this. This will sample an area of only 200 m^2, compared with 500 m^2 by the plot method, so additional transects may be desired. You will not use the actual order in the analysis here, just the total number of species encountered, and the number that were encountered exactly once, and exactly twice. Record your observations in the data collection sheet under the “Plotless Data” column.

6. After the formal data collection is done, explore the immediate area outside the plots and try to find any additional species that were not detected in your samples.

7. Submit a copy of your group data to your lab instructor for grading and compilation with data collected by the other groups in your class.

8. After receiving the compiled data from the instructor either through email or the class website, plot the cumulative number of species encountered versus the cumulative number of plots or individual plants surveyed.

9. Calculate the richness of this habitat using the Chao 1 and Chao 2 methods, for both the plot and transect data collected by your group and by the entire class. (See attached sample data sheet for an example.) Be sure to include your calculations in addition to species richness estimates.

Data Collection Sheet

Use this table to record species observations. Commonly encountered species are listed. Additional space is listed below for students to record any additional species they encounter that are not listed here.

Table 2. Record of species observations.

Tree species	Plot #1	Plot #2	Plot #3	Plot #4	Plot #5	Plotless
American Beech						
American Elm						
Basswood						
Black Cherry						
Black Tupelo						
Black Walnut						
Chestnut Oak						
Dogwood						
Mockernut Hickory						
Pignut Hickory						
Post Oak						
Shagbark Hickory						
Shortleaf pine						
Sourwood						
Southern Catalpa						
Southern Red Oak						
Sugar berry						
Swamp Chestnut Oak						
Sweetgum						
Tulip Poplar						
Virginia pine						
White Oak						

Questions for Further Thought and Discussion:

1. How does your estimate of species richness change when you estimate it based on your entire class's data versus only the data your team collected? Did other teams find species that were not present in your team's plots? Would a guess of total richness just from your plots have anticipated these additional species? Did you have enough data in your team's plots, or across all plots surveyed by the class to be confident of your estimate? Explain your answers.

2. How many samples or plots do you think would be sufficient to reliably estimate local species richness? Explain your answer. (Assume that it is not practical to survey every tree.)

3. Explain the difference between a sample and a census: what are the advantages and disadvantages of each?

4. You have been commissioned to assess the species richness of trees throughout a highly diverse forest. The site includes a combination of old growth forest and young forest that was logged only 30 years ago, as well as a mixture of dry upland forest and forested bogs in the lowlands. How would you approach assessing the species richness of this site? What methods would you recommend and why? What concerns or potential problems do you foresee?

5. Find a published peer-reviewed study that estimates the species richness of another taxonomic group or habitat such as forest birds, meadow wildflowers, or stream invertebrates? What challenges did that study face in identification, sampling, logistics, and so on, and how did the authors address those challenges? How did they estimate species richness and why? What are the key differences between their methodologies or concerns, and the ones you encountered in this assignment? What are the reasons for these differences? How do the habitat, taxa, and scale of the study affect the choices they made in the methodology of their assessment? Be sure to include proper citations for the paper you cite.

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Tools for Assessment of Student Learning Outcomes:

In the course of this lab, students learn to identify local tree species and utilize plot and transect methods for ecological sampling in the field. We assess students' knowledge of the local trees, both informally by questioning in the field and more formally with quizzes.

Students also learn to create and interpret species accumulation graphs, to calculate Chao estimators of species richness, and to evaluate the differences between Chao estimators. We assess students' ability to calculate and understand these statistical estimates by checking student work and solutions, and student discussions of the issues and concerns surrounding creation of these estimates. We also assess students' ability to interpret these estimators of species richness estimators, the importance of determining an appropriate sample size, and student comprehension of the various other conceptual issues surrounding them through written discussions of the challenges that this question poses, and the strengths and weaknesses of the field and statistical solutions we use. In addition, we assess students' ability to integrate information gleaned from these metrics by asking students to comment on their results based on what they actually saw in the field, such as particular species occurring in clumps, perhaps in microsites, being difficult to identify, or being missed entirely by our sampling.

Finally, we assess students' conceptual understanding of the work by asking them to suggest alterations that would make it more accurate or efficient, and to compare and contrast their work and a study on another taxon or habitat from the scientific literature.

NOTES TO FACULTY

Challenges to Anticipate and Solve

1. **Selecting field sites.** Most college campuses will have a forest or woodlot nearby that is relatively unmanaged (thinned, mowed, etc.) where this study can be conducted. There may be particular reasons to choose particular sites because of accessibility, species diversity, absence of brambles or poison ivy, and so on.

2. **Incorporation into other studies.** We have sometimes incorporated this lab into a larger one, for example, asking whether species richness is greater in burnt areas, or on south versus north-facing slopes, etc. There are many directions an instructor can go with this, which would also influence site selection.

3. **Selecting field guides.** It is important that each group have a field guide to the taxa of the region, which strikes a balance between ease of use for novices and comprehensiveness. For trees, there will often be many choices including ones that err in both directions. We provide a simpler guide to the common species to all groups, and the instructor keeps a comprehensive field guide handy for individual trees that prove difficult or for individual students who want to learn more.

We expect that most instructors in field ecology will already know of a range of field guides to their region, that vary from pocket guides for children to comprehensive guides used by professionals such as the 1987 edition of *Gray's Manual of Botany: A Handbook of the Flowering Plants and Ferns of the Central and Northeastern U. S. and Adjacent Canada*– 1987 by M. L. Fernald and A. Gray, available at www.amazon.com. Still, for new instructors we offer a few places to search for guides.

Some guides only identify trees, such as the ones listed at the Arbor Day Foundation website, http://www.arborday.org/trees/whattree/?TrackingID=908, which lists ones that can be purchased in book form, accessed online, or downloaded to a smartphone or tablet. The National Audubon Society Field Guides include editions on Eastern and Western Trees, at http://marketplace.audubon.org/products/national-audubon-society-field-guides. A little research should turn up more local guides in most regions of the US, such as in our region, *Native Trees of the Southeast: An Identification Guide Paperback* – by C.L. Brown, L. K. Kirkman and D. J. Leopold, available at http://www.timberpress.com/books/native_trees_southeast/kirkman/9780881928280.

If the students will be studying other organisms as well during the semester, the instructor might prefer a more general guide to natural history which identifies common trees but also wildflowers, birds, reptiles, etc., such as the National Audubon Regional Field Guides at http://marketplace.audubon.org/products/audubon-regional-field-guides.

4. **Identifying species.** In cases where field guides are not available or inadequate, it may be necessary to create field guides for the taxa and site(s) where the labs take place. The instructor may prepare this in advance, based on his or her own collections at the site and after referring to monographs and other sources to work out distinguishing field marks. Alternatively, this task could be assigned to honors or other top students one year, in preparation for use in the lab the following year. In order to encourage students to familiarize themselves with identification of the more common taxa prior to the onset of the lab, we also often hand out field guides the week before this lab is conducted, and allow students to identify a sample or image of one of the local tree species as an extra-credit portion of the quiz at the beginning of the lab.

5. **Differences among students.** There will likely be wide differences in students' abilities to identify tree species. We sometimes have forestry

students who know all the species, working alongside students who have never done field labs before. Where possible, we try to assign one knowledgeable student to each group. We also move between groups helping students identify species that are challenging, and confirming ones they have worked through.

6. **Ensuring equal participation.** It is often challenging to ensure that all students in a group participate equally, and here it can be tempting for motivated ones to try to do this themselves, in order to get it done, and for unmotivated students to let them. All it takes is for the instructor to continue to make rounds among the student groups and ask occasional questions, and this can be avoided.

7. **Choosing the right season.** We try to match field exercises with topics being covered in the accompanying ecology lecture course, which places community labs such as this later in the fall. This presents a scheduling challenge for two reasons: the sky can become dark before our 3:30-6:30 pm labs are done, and in some years drought or other factors will cause the trees to lose their leaves early, making species identification challenging. Therefore, we always monitor the field site through the fall, and switch this field exercise to an earlier week when necessary.

Comments on Introducing the Experiment to Your Students:

We require the students to read the lab handouts prior to coming to lab each week, and hold occasional pop quizzes at the start of lab to ensure that they have done so. We then give a 5-10 minute power-point talk that explains the research question and why it is important, then describes what the students will actually do in the field. Then we gather our gear and travel to the field site to carry out the exercise.

In our experience, students will often have difficulty calculating Chao 1 and Chao 2 unless care is taken to ensure that they understand how to assess the number of doubletons and singletons prior to the field portion of this lab. For this reason, we will typically go through the full process of determining S(n), a, b, L, and K and then calculating Chao 1 and Chao 2 using a pre-existing or ‘dummy’ set of data before proceeding to the field. If data from a previous semester are used for this purpose, then the instructor can engage the students by comparing their estimates of species richness with those produced previously, and solicit thoughts as to why the precise estimates vary.

We have found that the process of identifying species can be aided by reviewing the dominant or distinctive species when arriving at the field site, and by giving advice on how best to identify the other species, including how to use the field guide. This is especially helpful if dichotomous keys are being used. As mentioned elsewhere, an extra credit question involving plant identification at the start of the class is often helpful in encouraging the students to develop a baseline familiarity with the identification techniques to be used in the lab prior to class.

Comments on the Data Collection and Analysis Methods:

Faculty may choose to create a data entry table for students to use in the field. These would ask for the usual information on date, location, plot or transect number, and student members of the group, and have the most commonly encountered species already listed. There should be additional rows where the students can write in the names of other species that they find. As it is often impractical to return to a computer lab following the field component of this exercise, we typically have the students turn in their raw data sheets at the end of the exercise. However, instructors may prefer to have students submit the data electronically as Excel files, particularly in cases where indoor lab facilities are close at hand.

We introduce students to Excel early in the semester, and show them how to enter and manipulate data and graph results. It might be possible to create a worksheet that will calculate the Chao 1 and Chao 2 estimates, but we believe that the calculations are not difficult and that there is educational value in working through them individually. We do provide a worksheet that allows students to make preliminary *ad-hoc* estimates (see data set), though not the Chao estimates. We do ask students to show their calculations, to prove that the work was theirs and to pinpoint errors when they occur.

Comments on Questions for Further Thought:

General Comments:

Proper responses to questions 1 through 3 will vary among teams and lab sections, depending on species richness estimates deriving from each team's data. Thus, these questions are typically assessed from the perspective of ascertaining the students' grasp of the theoretical and practical consideration of the issues surrounding the collection, processing, and interpretation of the data they have available. While the detail of responses may vary depending on the level at which the class is taught, we typically found that thorough responses to each of these questions would require no more than a page of text each. However, we have found that a series of specific questions are often more successful than a single, broad question in stimulating the students to discuss the various issues surrounding the estimation of species richness. A sample set of student responses is also presented as a separate file.

Itemized Comments:

1. *How does your estimate of species richness change when you estimate it based on your entire class's data versus only the data your team collected? Did other teams find species that were not present in your team's plots? Would a guess of total richness just from your plots have anticipated these additional species? Did you have enough data in your team's plots, or across all plots surveyed by the class to be confident of your estimate? Explain your answer.*

This question is typically considered to have been addressed adequately if students give a thoughtful response as to why or why not the available sample was adequate. Discussions of the apparent differences in species estimates produced by smaller group data versus the larger classwide dataset are also appropriate here.

2. *How many samples or plots do you think would be sufficient to reliably estimate local species richness? How might the placement of the plots that were sampled affect our species richness estimates? Explain your answer. (Assume that it is not practical to survey every tree.)*

As with the previous question, the critical question is why the students feel the study area does/does not require additional sampling for estimates to be accurate. If the students make a reasoned argument for either case, this question may be considered to be adequately answered.

3. *Explain the difference between a sample and a census: what are the advantages and disadvantages of each?*

Make sure that students understand that a sample in this context includes only a (typically small) portion of the individuals in a population, while a census is exhaustive, and includes every single individual. A census does provide a definitive answer to the question of what is species richness, though it typically requires an impractical amount of effort, and it is often impossible of sampling every individual. Samples, on the other hand, require a more manageable amount of effort, but can only be used to estimate species richness. In addition, these estimates are susceptible to many forms of bias depending on factors such as the distribution of the organisms themselves and the sampling methods used.

4. *You have been commissioned to assess the species richness of trees throughout a highly diverse forest. The site includes a combination of old growth forest and young forest that was logged only 30 years ago, as well as a mixture of dry upland forest and forested bogs in the lowlands. How would you approach assessing the species richness of this site? What methods would you recommend and why? What concerns or potential problems do you foresee?*

Students should think about all the various aspects of species richness estimation that they encountered throughout this lab, both in the context of potential biases in their estimates, and logistical concerns. Potential issues would include: does this area really constitute a single community, or multiple discrete communities? If one forest is old growth and the other young, there may also be concerns stemming from differences in canopy size and density, as well as different ratios of rare/common species.

5. *Find a published peer-reviewed study that estimates the species richness of another taxonomic group or habitat such as forest birds, meadow wildflowers, or stream invertebrates? What challenges did that study face in identification, sampling, logistics, and so on, and how did the authors address those challenges? How did they estimate species richness and why? What are the differences between their methodologies or concerns, and the ones you encountered in this assignment? What are the reasons for these differences? How do the habitat, taxa, and scale of the study affect the choices they made in the methodology of their assessment? Be sure to include proper citations for the paper you cite.*

Clearly, the students' responses to this question depend greatly on the paper they choose. This question presents an opportunity to evaluate and comprehend the practical concerns involved in estimating species richness in another context. This also gives the students an excellent opportunity to ensure that they have a good grasp of the reasons behind the choices that go into designing an ecological survey. If students are not familiar with finding peer-reviewed papers, a brief discussion of how best to find and cite such publications may be warranted. This also presents an opportunity to discuss the campus library search functions, Google Scholar, Web of Science, etc. if necessary**.**

Comments on the Assessment of Student Learning Outcomes:

Students are given credit for participating in the field portion of this lab in order to ensure all students are active in collecting the data. Significant weight is placed on the collection and proper formatting of each group's data, as the remaining portions of the lab are not possible without it. While some portions of the questions for further consideration do focus primarily on basic understanding of the concepts presented during this lab, many of the later questions focus more on the students' ability to connect concepts and concerns in various situations that differ from the study conducted in this lab. For this reason, it is often helpful for the instructor to encourage students to think in this manner by asking questions that require this mode of thinking throughout both the initial presentation and during the field portion of the lab. We also typically provide students with a grading rubric to increase transparency and reduce student questions regarding why they received the scores they did.

While we have historically limited assessment to the elements presented here, instructors could expand this assignment to include a variety of additional products if desired. The later questions for further thought could be expanded to a research paper consisting of a basic literature review of species richness analysis, or a more in depth examination of another published study on species richness. Alternatively the students could present their work in a research paper, or a short lecture, such as an 8-10 minute research talk that a graduate student might give at a conference, and allowing several minutes for questions.

Comments on Formative Evaluation of this Experiment:

Classroom Component:

Use of a brief quiz at the beginning of the lab allows a quick assessment of the students' understanding of the various issues surrounding this lab, ensures that the students have read through the lab prior to the start of class, and also often stimulates questions from the students surrounding any material they failed to understand. This allows for an instructor to better tailor the focus of the lecture to the understanding of each class. We use questions such as:

1. What is species richness?
2. Why is it typically not possible to measure species richness conclusively?

3. Identify to species the leaves presented by the lab instructor. (1pt extra credit)

Prior to the field portion of the lab, we also typically walk the students through evaluation of species accumulation curves and the calculation of Chao species richness indicators. In order to ensure that students are comfortable with the mathematical portions of this lab, we may also ask students to calculate these metrics using the sample forest data in the handout, but using a different set of plots than are presented.

Field Component:

Problems arise continually in the field, which provides an opportunity for continual formative evaluation as the lab progresses. By interacting with the students and addressing various problems as they arise, the instructor receives continual feedback on the progress of each student team throughout the field portion of the assignment, and to the content that students may be consistently having difficulty with. Instructors will also typically evaluate the data collected by each group during the field portion of the lab, as well as at the end when group data are submitted for compilation. This allows the instructor to identify and correct any flaws in student-led data collection prior to analysis. Groups whose data shows serious problems can gain a better understanding of their mistakes by viewing compiled data among other groups. The instructor can prompt the students to make (and justify) many of the decisions on the sampling both before and during the field portion of the exercise as a way to obtain immediate feedback on the students' grasp of the various practical issues of assessing species richness. Examples of such decisions would include: What is species richness going to tell them about the site? How do they think their estimates of species richness could be more accurate? What species are difficult to distinguish, and how should they be distinguished? What strategies are they using for identifying different species both accurately and quickly? Should the team start sampling near the trail, where thinning, trampling or other disturbances may be issues, and in what direction should the transect go? Should we count trees that are rooted outside the plot but extend over it? What about one with multiple or broken stems, and what other practical concerns might arise? Students often assume that science is cut and dried, and one of the learning outcomes from trying to answer this simple question is the realization that it is almost impossible to anticipate all the issues that will arise in research. Here they will see that scientists must be adaptable and sometimes make judgment calls on issues with no clear answer.

Written Component:

Student comprehension of the content in this lab may also be assessed by evaluating patterns in the questions students are most likely to answer inadequately. Cases where students fail to comprehend certain aspects of the lab content or where students fail to adequately assess the role of local conditions, taxa, or scale in dictating methodological and analytical concerns can also help assist the instructor in better directing students' attention to those issues during the course of future labs.

Comments on Translating the Activity to Other Institutional Scales or Locations:

This activity is highly transferable. It can be carried out by college freshmen in introductory biology, or by advanced undergraduates in biology, ecology, forestry, wildlife biology, etc. It could be done at a small college with a small group of students, or at a large institution with many lab sections, provided a field site is available that will allow the students to spread out and not overlap or trample the landscape. This study can be performed in any region in almost any kind of vegetation that is accessible. Of course, the plots might need to be larger in desert ecosystems and smaller in alpine ones.

The field collection must be performed during a season when the plants (or other taxa) are easily observed and identified. Some schools teach winter ecology and plant identification, but sampling trees that have lost their leaves increases the difficulty and may leave students questioning their own results. This work is best conducted in mild weather, since the students will be working outdoors for several hours. It is probably best conducted in smaller classes, because the students may be spread out in a forest and need supervision and assistance both in the setup of transects and plots and in species identification. With larger classes, students may be placed into larger groups and asked to survey more plots, or multiple instructors may be used. If used in a freshman-level course, it may also be desirable to remove evaluation of species richness through specific statistical estimators, to limit the scope to evaluation of species accumulation curves, and possibly to simplify some of the questions for further comment, as well as possibly providing the students with a specific paper for comparison in question 5 rather than expecting them to find their own papers to review.

The questions and general principles of this lab could be applied to animals. However, methods for surveying animals vary widely depending on the organism and habitat, and this lab may only be applied if the animals are sedentary or can be observed without disturbance. For example, soil-dwelling animals might be surveyed using Berlese traps, which would replace plots in the analyses above. When studying animals, the data sheet may also be modified to include additional parameters such as weather conditions and time of day, if those factors may affect presence, activity level, life stage, or visibility of the relevant taxa.

In principle, we can imagine taxa and sites that could be surveyed by students with physical or other disabilities. An alternative would be to use pre-identified data such as the sample forest data included in the lab handout, though this would have to be carefully designed and monitored.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

See the Excel file for an actual data set obtained by Clemson University students for a site on the Clemson Experimental Forest, Clemson, SC. Fields highlighted in yellow represent data that would typically be left blank for students to fill out or calculate themselves.

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GENERIC DISCLAIMER

Adult supervision is recommended when performing this lab activity. We also recommend that common sense and proper safety precautions be followed by all participants. No responsibility is implied or taken by the contributing author, the editors of this Volume, nor anyone associated with maintaining the TIEE web site, nor by their academic employers, nor by the Ecological Society of America for anyone who sustains injuries as a result of using the materials or ideas, or performing the procedures put forth at the TIEE web site, or in any printed materials that derive therefrom.

Species richness is the number of different species represented in an ecological community, landscape or region. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distributions. Species richness is sometime considered synonymous with species diversity, but the formal metric Species diversity takes into account both species richness and species evenness. global species estimates insects species richness. This is a preview of subscription content, log in to check access. Preview. Unable to display preview. Download preview PDF. References. Adis, J. (1990) Thirty million arthropod species-too many or too few? J. Trop. Ecol.Â Erwin, T.L. (1982) Tropical forests: their richness in Coleoptera and other arthropod species. Col. Bull. No one knows how many species of bacteria there are, but it is thought to be between 10 million and 1 billion species. Bacteria...Â Another approach to determining the quantity of bacteria in a sample is to use a technique called DNA reassociation. In DNA reassociation, scientists use chemicals to "unzip" the two strands of the double-helix in bacterial DNA, then mix them up. Compatible DNA strands will re-link up with one another. The longer the reassociation process takes, the more species are present in the sample. These measurements of time can be used to estimate bacterial biodiversity in the ocean or soil. When this technique was applied to a soil sample in the late 1990s, a 16,000 species were found.