ECOLOGY LABORATORY

BIOL 366

Summer 2018

Compiled by the Ecology faculty of UNCW

Dr. Stuart R. Borrett (editor)

Table of Contents

SYLLABUS

Ecology Laboratory (BIOL366), Summer 2018

I. Introduction

Smith and Smith (2007) define ecology as "the scientific study of the relationship between organisms and their environment". Activities in this ecology laboratory will introduce you to some of the laboratory, field, and analytical tools and techniques that ecologists use. Our focus will be on learning to collect, analyze, interpret and communicate ecological data. Many of the field laboratory exercises will make use of sites on campus, including the remnants of the native Longleaf pine–wiregrass ecosystem that was once common in the southeastern United States.

A. Catalogue Description

BIOL 366. Ecology Laboratory (1) Prerequisite or co-requisite: BIO 366. Introduction to ecological sampling techniques and data analysis. Experience in field sampling, laboratory and computer modeling of sampling approaches, and scientific writing is also included. Three laboratory hours each week (6 during the summer).

B. Learning Outcomes

Through your experience in this course, you will be able to do the following.

- Understand and apply fundamental ecological principles.
- Select appropriate quantitative field sampling techniques (e.g. quadrat, transect sampling, mark-recapture techniques) for ecological investigations.
- Evaluate ecological hypotheses by designing and performing experiments, collecting and interpreting quantitative ecological data.
    - Apply mathematical models and statistical tests to describe data.
    - Critically evaluate field and test data and draw appropriate scientific conclusions.
    - Present your experimental data in graphical and table formats and use these to support you scientific conclusions.
- Apply software tools to organize, analyze, and present data and support your reasoning.
    - Learn to use a spreadsheet (e.g. Microsoft Excel) to organize and analyze data.
    - Learn basic statistics for describing a population and comparing traits between two populations.
- Demonstrate ability to conduct experimental and literature research to develop a formal scientific paper using correct format and style for scientific writing.
    - Identify and locate appropriate primary literature sources to provide context for introduction and discussion of your scientific paper.
    - Effectively present, evaluate, and interpret your data using appropriate graphical and table formats.
    - Evaluate and discuss your experimental findings and their significance in the context of appropriate literature sources.
    - Apply ethical standards of citation for supporting sources in all written laboratory reports and papers.
    - Demonstrate the ability to write critically, using concise scientific writing style in a standard scientific laboratory format.

II. Course Time and Location

Table 1. BIOL366 Times and Teaching Assistants

Section	Day	Time	TA
200	T, R	12:30 pm – 3:20 pm	None

III. Contact Information

Dr. Stuart Borrett
Office: Friday Hall 1057
Phone: 910.962.2411
Email: borretts@uncw.edu
Office Hours: by appointment

I will respond to email as soon as possible, but please allow 24 hours for a response. Also, please include **biol366** and an informative subject in the subject line of all email correspondence. Failure to do so may result in substantially longer response times.

IV. Materials and Readings

Readings and assignments for the course will be available through the course website at http://people.uncw.edu/borretts/teaching.html. You will need to click on the tab titled **Ecology Laboratory** for course materials. Please print out the laboratory instructions, data sheets, and questions and bring them to class with you. We expect you to have **read the laboratory assignment prior to class**. Showing up prepared is a key part of your course participation.

In addition to the lab manual and materials on the website, Pechenik's (2015) *A Short Guide to Writing about Biology* (9th ed., 2015, Pearson/Longman) is a required text. It is a useful guide to better writing that we will use this semester. Furthermore, the Biology Department has adopted this as a required text for all Biology and Marine Biology majors.

V. About this course

Field trips: Several on campus field trips are scheduled this semester. Because there are no adequate in-lab substitutions for these trips (aside from busy work!), we will go in all but the most severe weather. While most people do not like working in the rain, it is part of doing field science. You can be comfortable if you wear appropriate clothes. Please dress accordingly. Wear rain gear if it is forecast to rain (rain suits are best since umbrellas do not work well in the field), wear warm or cool clothes as appropriate, always wear shoes appropriate for walking over rough terrain or in the woods, and wear long pants when we are sampling in the forest to reduce the chance of scratches and insect bites. *Please bring a notebook for data collection and/or note-taking on field trip days*.

Lab attendance and assignments: Attendance is mandatory for all labs. You must attend the lab in which you are registered! Do not make of the mistake of thinking that you can make up a missed lab by simply showing up at another lab that week. Lab space is limited and TAs cannot keep track of students from labs other than their own. During semesters when multiple labs are taught, if you do miss a lab for a valid reason (e.g., sickness), you must obtain permission from your TA and a TA in another lab to make it up that week to complete the assignment (Note: you will only be allowed to do this once during the semester!). If you miss additional labs, you will lose the points for any assignment due for that lab, even if you try to attend another lab. Since the beginning of lab is an important time for introducing lessons and describing field procedures, you will be considered to have missed the lab if you are more than 10 minutes late. Students missing more than 3 laboratory periods will earn a zero in the course.

Laboratory Reports: Laboratory reports (short and long forms) must be submitted electronically (emailed) to the course instructor on the day due. Ask your TA as to whether they prefer Microsoft Word documents or PDF files. Note that Microsoft Word documents can be converted into PDF files in a number of ways depending on your operating and software (e.g., save as PDF, print to PDF, Adobe Acrobat, use online converters). Please save your files as "BIOL366_*name*_*assignment*.pdf" where *name* is replaced by your team name (group) or your last name (individual assignment) and *assignment* is replaced the laboratory number. For example, the blueberry team would name their short report for the sampling sedentary organisms laboratory as "BIOL366_blueberries_lab2.pdf". Reports with file names that do not follow this convention will not be accepted. When submitting group assignments, please copy the whole team on the email to insure they know it was submitted. This is an element of good teamwork.

iSTEM: The university will soon be starting a new minor for integrated science teaching. This minor requires students to take a number of courses designated as integrated science course (iSTEM) in which students have the opportunity to learn about how multiple sciences, mathematics and statistics, technology, or engineering work together. The BIOL366 will be designated as an iSTEM course because while learning about the science of biology (ecology), you will learn about and use simple statistical tools to analyze the data collected during the lab work and test hypotheses. In addition, you will learn to use technological tools, including how to use a spreadsheet (Microsoft Excel) to assist with organizing and analyzing their data.

VI. Schedule

The class schedule is specified on the course website at http://people.uncw.edu/borretts/teaching.html and then choose the Ecology Lab tab.

VII. Assessment

This course is built around four evaluation elements that are weighted as shown in Table 2. The first element is comprised of a team based question sets and graphs that will follow designated labs. The second element is a team based short laboratory report that follows 2 of the laboratories. This will give you practice writing in the scientific format and receiving feedback from your instructor. The third element is a more extensive individual laboratory report that you will write to describe your forest field-sampling laboratory. This report will be written in the form of a scientific manuscript to be submitted to the journal Ecology (see report format description and grading rubrics on the class website). This project is divided into two parts: (1) a report draft and (2) a final revised report (see schedule for due dates). The draft gives your instructor an opportunity to provide you with feedback on both your scientific analysis and writing. The final course element will be a comprehensive final examination.

There are a total of 100 points available for the class. The means that the short laboratory reports and question set are collectively worth 40% of your final grade, the Forest report is worth 35% of your grade (5% on the draft, 30% on final report), and the final exam is worth 35% of your final grade. Given the rapid pace of the summer session, late assignments will not be accepted.

Table 2. BIOL366 Assessment

Course Component	Percentage
Questions Set (2 x 5%)	10
Laboratory Reports (Short) (2 x 10%)	20
Forest Ecology Laboratory Report (Full)	
Draft	5
Final	30
Final Exam	35
Total points	100

VII. University Resources & Policies of Concern

A. Disabilities

If you are a person with a disability and anticipate needing accommodations of any type for this course, you must first notify (DePaolo Hall, http://uncw.edu/disability/index.html), provide the necessary documentation of the disability, and arrange for the appropriate authorized accommodations. Once these accommodations are approved, please identify yourself to your instructor in order that we can implement these accommodations.

B. Violence and Harassment

UNCW practices a zero-tolerance policy for violence and harassment of any kind. For emergencies, contact UNCW CARE at 910.962.2273, Campus Police at 910.962.3184, or the Wilmington Police at 911.

C. Academic Honor Code

The Department of Biology and Marine Biology and your instructors strongly support the Academic Honor Code as stated in the "Student Handbook and Code of Student Life," and we will not tolerate academic dishonesty of any type.

D. Writing Center

BIOL366 is a writing intensive course. Thus, you may find the resources available at the University Writing Center helpful (located in the University Learning Center). You can learn more about this great resource from their web page http://uncw.edu/ulc/writing/center.html.

1. DESCRIBING A POPULATION

I. Learning Objectives

At the completion of this laboratory, you should be able to:

- Identify Long-leaf pine trees in the field.
- Identify initial concerns regarding collecting a representative sample of a population.
- Describe a statistical population using estimates of the population's central tendency (mean, median, mode) and variation (variance, standard deviation, standard error, confidence interval). You should be able to calculate each of these metrics by hand.
- Explain why descriptive statistics are necessary in ecological studies.
- Create a histogram by hand.

This laboratory also serves as a first experience looking at the Longleaf Pine (*Pinus palustris*) forest that we will be studying in subsequent laboratories.

Figure 1. Pictures of the Longleaf Pine Forest (left) and Longleaf Pine needles (right) on the University of North Carolina Wilmington Campus

II. Introduction

We will be using the *Describing a Population* Laboratory from R. W. Kingsolver (2006). The text for this laboratory is available from the course website (http://people.uncw.edu/borretts/teaching.html). We will follow Method B, which looks at pine needle length.

III. Laboratory Assignment

Each *team* will complete the laboratory described in Method B: Needle Length in Conifers (Kingsland 2006). Please turn in one copy of your completed responses to questions and activities on pages 18, 19, and 20 of the laboratory assignment.

IV. References

Kingsolver, R.W. 2006. Ecology on Campus: Lab Manual. Pearson, New York.

2. SAMPLING SEDENTARY ORGANISMS

I. Learning Objectives

In this laboratory, the students will

1. learn basic approaches for sampling sedentary organisms;
2. investigate fundamental issues of experimental design (e.g., how many samples?);
3. distinguish between accuracy and precision;
4. experience the application of transect and quadrat sampling;
5. create and interpret appropriate tables and graphs; and
6. write a short laboratory report.

II. Introduction

Ecologists have the challenge of evaluating the nature and function of natural systems. However, they are often faced with the problem that they cannot accurately determine many important parameters strictly through qualitative observations. Problems with spatial scale often make it difficult to directly determine average densities in habitats such as large forested tracts or in aquatic habitats where one cannot directly see the animals or plants of interest. To measure functions in natural system, it is necessary to representatively sample both the biotic and abiotic components. Since it is usually impossible to sample an entire population within a study area, one must take smaller samples of the community or population of interest. The problem then becomes how to best estimate the parameters of some ecological system taking into account limited time and resources. The problem of sampling can be thought of as several issues: 1) what sampling method can be used to collect the needed data, 2) how do we select sampling locations to limit potential bias and obtain best estimates of target parameters, and 3) how can we minimize the number of samples we take while still providing an accurate estimate of parameters?

Ecologists have developed several sampling approaches to deal with sedentary (non-moving) organisms. The most common method is plot sampling. This method is relatively straightforward in that it simply involves counting the number of organisms of interest in a defined area (often, but not necessarily, a quadrat). Plot sampling is a highly versatile approach, providing information on densities, associations, dispersion patterns, and indirect evidence on a variety of community or population processes. However, three problems with this method that will be explored in this lab are the size of the sampling plot, how plots will be placed, and the number of plots to be sampled. The size of the plot must obviously vary with the size of the organisms to be sampled, but also may need to be varied depending on distributions of organisms or the size of habitat patches in the environment. It is often useful to have a plot smaller than the average patch size to get information of dispersion patterns, mean numbers within patches, and densities between patches. Also, larger plots tend to give more accurate estimates of overall densities, but are more cumbersome and time consuming to place and sample. The placement of plots needs to be done so that there is no bias in the data collected. This is usually achieved through random or haphazard placement (what is the difference?), but may also be limited by time and resources.

When organisms are known to vary across some defined gradient, a variation of the plot sampling method that is often used is a belt transect. This involves sampling replicate rectangular belts that straddle a known environmental gradient. Often these belts are broken into subsections. The problems of size (and shape), placement, and number of replicates also apply to belt transects.

An alternative to plot sampling methods are a variety of methods collectively known as plotless sampling approaches. These approaches are generally quicker to apply than plot sampling, but usually provide much more limited information. The most common plotless sampling method is point-quarter sampling. Point-quarter sampling can provide density measures if applied correctly and has the advantage over plot sampling in that it is less cumbersome and time-consuming than sampling of larger quadrats. However, it has the disadvantages that density estimates may not be accurate if organisms have severely clumped or uniform distributions and the technique does not provide as versatile information as obtained with quadrat sampling. The basic methodology for point-quarter sampling is: 1) randomly (or haphazardly) select a center point, 2) divide the area around the center point into 4 quadrants (pie sections), with the quadrants crossing at the center point, and 3) record the distance to the nearest tree (or one or more species of interest, e.g., conifer verus hardwood) in each quadrant.

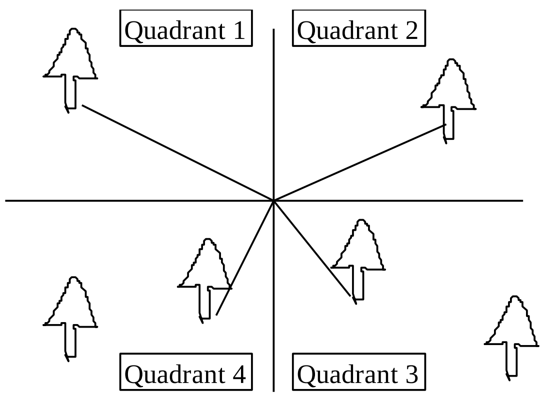


Figure 1: Illustration of Point-quarter sampling

This procedure is then repeated for additional replicates. Density of trees for Point-quarter sampling can be determined with the following formulas:

$$D=1/A \quad \text{and} \quad A=\left(\frac{\sum_{i=1}^{n} d_i}{P*Q}\right)^2$$

Where:
D = density in distance units squared
A = area occupied by trees
d_i = distance from the center point to the nearest tree in the i^{th} quadrant
n = number of trees
P = number of center points
Q = number of quadrants taken for each center point (usually 4)

Another method used to obtain qualitative information on the distribution and abundance of sedentary organisms is the line transect. This method simply involves placing a line of pre-determined length over an area and then recording the number of organisms intercepted by that line (or within a defined distance of the line). This method has the advantage of being quick and easy, but has the disadvantage of providing information on only relative abundances and not giving quantitative information on actual densities.

When using statistical sampling, we must consider the accuracy and precision of our estimates. The accuracy of our measurements is how close they are to the true value, which is often unknown. The precision of the measurements is how close multiple estimates are to each other. Precise estimates will have lower variability than imprecise measurements.

III. Methodology - Computer Sampling Simulation

In this laboratory we will conduct three experiments in Ecobeaker to determine how changing (i) the size of quadrats, (ii) the shape of the quadrats, and (iii) the number of samples collected influences the accuracy of our population estimates. In addition, we will evaluate how these results compare between populations that have an even, random or clumped dispersion pattern. Knowledge of how these sampling design issues influence the accuracy of your results is essential when you design your own experiments.

Before conducting the experiments described below, write a specific hypothesis for each experiment. What do you anticipate the results will be? Why?

A. The Ecobeaker Universe

The Ecobeaker universe presents a 50 X 50 m landscape with 3 different types of plants on it, each a different color. The green plants are growing in completely random places around the landscape. The blue plants grow much better when they are near other blue plants, so they are clumped together (a patchy distribution). The red plants compete strongly with each other, so they do not like to grow nearby other red plants. This makes the red plants space themselves out in a somewhat even pattern. There are exactly 80 individuals of each plant type in the whole area.

B. Getting into the program

1. Double click on the Ecobeaker icon
2. Open the file “Random Sampling” using the open command in the file menu.
3. When it is open, you should get a screen that shows the landscape to be sampled in the upper left of the screen. To the right of the landscape are the Species and Population size windows, which show the color and total number for each species. The Sampling parameters window lets you change the width, height and number of quadrats which you use to sample the populations. The Control panel window lets you actually take a sample (by clicking on "sample”).

C. Experiments

i. What is the effect of changing the quadrat size?

1. The Ecobeaker program has an initial, default quadrat size of 5 x 5m.
2. Click on the sample button in the Control panel to take a sample. A square will be drawn on the Landscape window, showing the area that is being sampled. Just below the Landscape window a dialog box will appear, telling you how many individuals of each species were found within this square. Write down both the number of individuals of each species and the width and height of the area sampled.
3. Repeat this procedure 14 more times (15 total), so that you can calculate the mean and standard deviation for your sample population. We want to know whether you can expect this sampling design to give you *accurate* and *precise* results.
4. Now increase the size of the sampling quadrat to a larger size such as 10 x 10m. To do this, go to the Sampling parameters window and type "10" for both the quadrat height and quadrat width items. Then click on the change button in the sampling parameters window to make the change in size you specified go into effect.
5. Repeat the sampling procedure for the larger quadrat size.
6. Repeat the entire procedure for at least two more quadrat sizes (e.g. 15 x 15m, 20 x 20m).
7. When you have finished your sampling, **estimate the population size** (number in the entire 50 x 50 m landscape) from each sample you took for each species. To do this, take the total area of the landscape (50 x 50 m = 2500 m^2) and divide by the area of your sample plot (e.g., 5 x 5 m or 25 m^2 in your first sample, 10 x 10 or 100 m^2 in your second sample, and so on). Next multiply this value by your sample count for each species (random, clumped, even) to estimate the population size for each of these in the entire landscape. Present the results in a table form.
8. Make a plot of population estimate (y-axis) versus quadrat area (x-axis) for each species type (random, clumped, even). This will show you visually what happened to your population estimate as you increased quadrat size. Use a bar graph to show the *mean* population estimate for each size, and add error bars to report the *standard error*. This graph will then indicate how both the central tendency of your estimate and the variation are affected by the quadrat size. You can visually compare these estimates to the known true value.
9. How does the accuracy and variability of your estimated population sizes versus quadrat area vary in the graphs for each species? Is there some point where increasing the size of the quadrat seems to make little difference in accuracy? Is this point the same for all the species?

ii. How does quadrat shape effect the sample estimate?

1. Change your quadrat size to 10 x 10m and do sampling as described above.
2. Repeat this procedure with 3 other plot shapes that have the same area (e.g., 5 X 20m, 4 X 25m, 2 x 50m). Notice that as the width gets smaller and the length increases, the quadrat is approaching a belt sample.

3. Estimate the true population sizes and construct graphs as described in #8 above (in this case, the y-axis would be estimated population size and the x-axis would be quadrat shape ordered from most narrow shape to exact square).
4. How did changing quadrat shape affect population estimates? Is the affect the same for all species?

iii. How many samples are required to describe the population?

1. Set the quadrat size back to 5m x 5m.
2. Now go to the Sampling parameters window and change the number of quadrats to 5. This time, when you take a sample, first one 5m x 5m quadrat will appear, as before, and a dialog box will tell you how many individuals of each species were found in that quadrat. Record these numbers. When you click on the continue button, a second 5m x 5m quadrat will be laid down, and again you'll receive a report of how many individuals were found in the second quadrat. Again, record these numbers. Continue clicking "continue" and recording the results until the dialog box appears that reports the **total number** of individuals found within the quadrats; you can ignore this total. From the data you recorded, calculate the mean number of individuals per quadrat and the standard error for this estimate.
3. Repeat the sampling procedure described in step 2 of this section for 10, 15, 20 and 25 quadrats, recording the means and standard errors for each.
4. Estimate the true population sizes and construct a graph that shows how your sample estimate change as the sample size increases. The x-axis in this case will be number of quadrats used and the y-axis will be the population estimates for each sampling set. Add error bars to your graph that report the +/- standard errors. You should plot the data for the three different dispersion patterns on the same graph to facilitate your comparison of how the dispersion influences your sample size effects.
5. How does increasing the sample size change the accuracy of your results? How does it influence the uncertainty (standard error) of your estimate? Do these results differ if the population has an even, random, or clumped dispersion?

IV. Laboratory Assignment

For this laboratory, each *team* will turn in a copy of their final 9 panel graph and the answers to the questions in sections Ci9, Cii4, and Ciii5.

3. DISPERSION AND ASSOCIATION LABORATORY

I. Objectives

In this laboratory you will:

1. Use quadrat sampling to obtain data on species density, distribution, and association patterns;
2. Perform a χ^2 based test to analyze dispersion for a species and association among two or more species; and
3. Learn to identify at least two plant species in the Longleaf Pine—Wiregrass ecosystem.

II. Introduction

Dispersion Ecologists who study populations and communities of organisms are often interested in the spatial distribution of individuals because the distribution patterns can provide information concerning the biology of a species and the factors limiting that organism. Individuals in a population may have one of three general types of spatial dispersions: clumped, random, or uniform (Figure 1). For example, a clumped distribution may indicate that a species is responding to fine gradations in the environment or that it has a form of reproduction that keeps juveniles near adults. Conversely, a uniform distribution may indicate territoriality or some other aggressive interaction among individuals. In this laboratory, we will use a combination of quadrat sampling and data analysis to characterize the dispersion and association of two populations of plants found in the longleaf pine forest on campus.

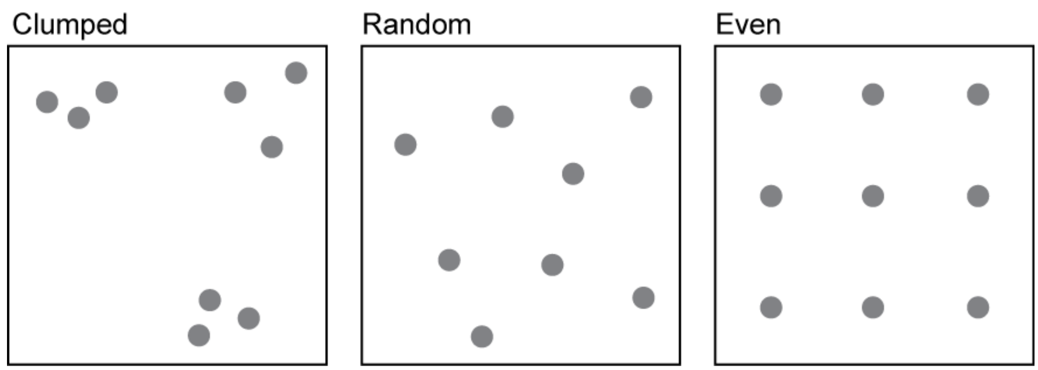


Figure 1: Three ways in which individuals of a population can be distributed in space.

Ecologists have developed several sampling approaches to count sedentary (non-moving) organisms. The most common method is plot sampling. This method is straightforward in that it involves counting the number of organisms of interest in a defined area (often, but not necessarily, a quadrat). Plot sampling is a highly versatile approach, providing information on densities, associations, dispersion patterns, and indirect evidence on a variety of community or population processes. We will use a quadrat sampling technique in this laboratory.

The primary approach to determining dispersion patterns is to compare **observed** patterns with what would be **expected** if the dispersion were random (using a statistical test). The *null hypothesis* for this investigation is that the individuals of the population are randomly dispersed (not clumped or evenly spaced). Why? If there is a difference between the observed pattern and the expected pattern, then you must further examine the data to determine whether individuals are found together (clumped) or are spaced apart (uniform).

There are several analytical methods to measure dispersion. The most common technique is to compare the data to the expectations of the Poisson distribution, but this approach is beyond our current skill level. Instead, we will use a Chi Square test (χ^2) to compare the sum of squares (a measure of variability) to the mean to determine dispersion patterns for the organisms we study. According to this test, if a species is uniformly distributed, variability should be low (similar numbers in all quadrats). If species are clumped, variability should be high (some quadrats with many individuals, others with few or none). Random distributions would have intermediate variability.

Figure 2. Longleaf pine forest with young turkey oaks on the UNCW Campus (photo credit Stuart R. Borrett, 2013)

Association A second interesting aspect of distribution patterns is whether two species (1) usually occur together (are positively associated), (2) seldom occur together (negatively associated), or (3) are randomly associated with respect to each other. If the species are positively associated, it may indicate some sort of obligate interaction, such as mutualisms or predation, or it may indicate similar habitat requirements. If they are negatively associated, it may reflect the results of competition or different habitat preferences.

III. Methodology

A. Data collection

We will count the numbers of two native plants within 1.0 m^2 quadrats along a transect through a natural forested area on the UNCW campus. Work with your instructor to select (1) two species to observe, (2) a starting point in the forest, and (3) compass bearing for your transect. Place a quadrat every 10 paces, using the compass bearing to help guide your travel. Each group (four students/group) will record the numbers of both plants within 60 quadrats. Record the data in the data sheet provided. After collecting the data, each team will analyze their data in the lab and we will summarize it on the whiteboard.


Figure 3. Students performing laboratory field work in the UNCW forest (photo credit Stuart R. Borrett, 2015)

D. Data Analysis

i. Analysis of Dispersion Patterns

To determine the dispersion patterns for the populations we are studing, you need to calculate the sample mean, the sum of squares, a χ^2 statistic, and the degrees of freedom (d.f.). We are using a χ^2 statistic because this is most appropriate for data like ours that are discrete count data. We will then characterize the dispersion pattern using the χ^2, the degrees of freedom (d.f.), and Figure 2. The calculations are as follows:

Mean $= \overline{X} = \sum_{i=1}^{n} x_i \Big/ n$, where x_i is the number of plants in the i^th qudrat and n is the total number of quadrats. We then use the mean to calculate the sum of squares is $SS = \sum_{i=1}^{n} \left(x_i - \overline{X}\right)^2$, which is a measure of size of deviation from the mean or how variable the sample set is. Using this information, we then calculate our test statistic $\chi^2 = \frac{SS}{\overline{X}} = \frac{\sum_{i=1}^{n} \left(x_i - \overline{X}\right)^2}{\overline{X}}$. For this study, the degrees of freedom is $d.f. = n - 1$. We can now determine the dispersion pattern for the candidate species using Figure 4. You will need to perform these calculations once for each native species.

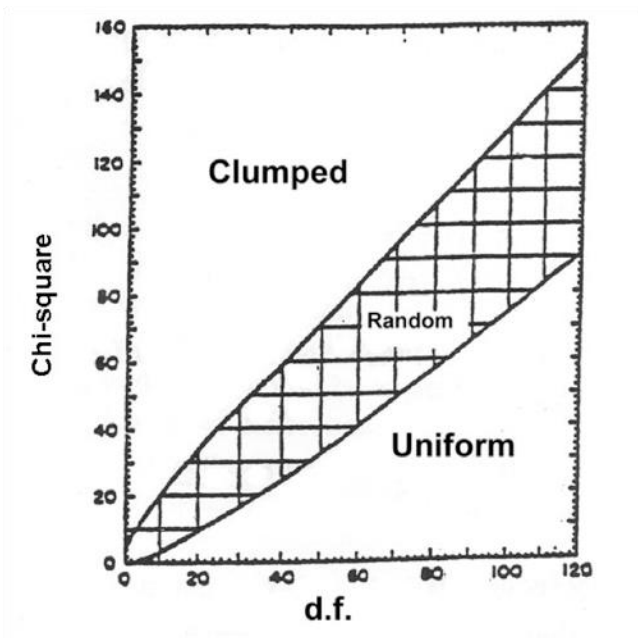


Figure 4: Relationship between degrees of freedom and chi-square statistic to determine the dispersion pattern of a population.

You are calculating this modified χ^2 statistic to test a specific null-hypothesis. Discuss with your team what the specific null hypothesis is and write it down.

ii. Analysis of Association

To determine how the populations of two species are associated, we first compare observed patterns to what would be expected if they are random. If they are not random, we can then calculate an index of association (V) to see whether the species are positively or negatively associated and how strong that association is. To do this, first construct the following table:

		Species 1 present	Species 1 absent	row sums
Species 2	present	a	b	m
	absent	c	d	n
	column sums	r	s	N (grand total)

a = no. quadrats where both species are present
b = no. of quadrats with only species 2
c = no. of quadrats with only species 1
d = no. of quadrats with neither species
m = sum of a+b
n = sum of c+d
r = sum of a+c
s = sum of b+d
N = sum of a+b+c+d

To determine if the association pattern is random, use the following modification of the chi-square (χ^2) test:

$$\chi^2 = \frac{N(ad - bc - N/2)^2}{mnrs} \text{ with 1 d.f.}$$

In this case, we are statistically testing the null hypothesis that "There is no difference between the association we observed and a random association" with 1 d.f. and we are assuming a decision criterion (α) of 0.05. Given the chi-square table in Appendix B,
if $\chi^2 > 3.84$, we have enough evidence to reject the null hypothesis and we can conclude that the association is not random and significant; if $\chi^2 < 3.84$, we don't have enough evidence to reject the null hypothesis and we conclude that our association is random. If the association is significantly different than random, we need to calculate the Index of Association (V) to determine the strength of the association and whether it is negative or positive.

$$V = \frac{ad - bc}{\sqrt{mnrs}}$$

V ranges from +1 to –1. A +1 means the species are completely positively associated (always found together). A –1 means they are completely negative associated (never found together). Values in between indicate weaker positive or negative associations, depending on the sign and how close the value of V is to 1 or –1.

IV. Laboratory Assignment

Each *team* will summarize their work using the Shortened Laboratory Report format (see Appendix A). Each team member will be an *author* of the report. Make sure to include the following:

1. Estimate the density of each population you are studying. Remember to report both the mean and standard deviation ($\bar{X} \pm SD$).
2. Determine the dispersion pattern for each species. Does species A have a clumped dispersion? Does species B have a uniform distribution? In your report, make sure to clearly report the evidence you are using to support your hypothesized dispersion patterns.
3. Determine the association between the two species. Again, make sure to include your *evidence* and calculations to support the association you claim.
4. In your discussion section, briefly consider likely *explanations* for the dispersion and association patterns observed for these species. What ecological mechanisms might generate the patterns you found?
5. In your discussion, describe some of the challenges you faced while collecting your data. Do you think your sample was representative of the forest? Why or why not?

Association and Dispersion Data Sheet

Quadrat	Number of Species A	Number of Species B	Quadrat	Number of Species A	Number of Species B
1			31		
2			32		
3			33		
4			34		
5			35		
6			36		
7			37		
8			38		
9			39		
10			40		
11			41		
12			42		
13			43		
14			44		
15			45		
16			46		
17			47		
18			48		
19			49		
20			50		
21			51		
22			52		
23			53		
24			54		
25			55		
26			56		
27			57		
28			58		
29			59		
30			60		

After collecting these numbers in the field, you will transfer them into an Excel spreadsheet for analysis.

4. FOREST ECOLOGY: EXPERIMENTAL DESIGN

I. Objectives

Through your experience in this laboratory, you will learn to

1. Design field population and community studies;
2. Apply quadrat, point-quarter, belt transect, and/or line transect sampling techniques for sedentary organisms;
3. Observe the importance of disturbances, such as fire, in structuring communities;
4. Use the scientific method to test ecological hypotheses;
5. Identify relevant literature and cite it appropriately in a laboratory report;
6. Write about an experiment and its results in the form of a scientific laboratory report.

Observe that this is the start of a multi-week laboratory that will be summarized in a full laboratory report. This report will undergo a full draft-revision cycle.

II. Introduction

A. Long-leaf Pine—Wiregrass Ecosystem is adapted to periodic fires

Fire disturbance is an important factor affecting the composition of forests and grasslands in many parts of the world, including coastal regions of the southeastern United States. Coastal forests from North Carolina to northern Florida are often dominated by one of several species of pines. However, these pine forests are often maintained as a persistent, disclimax forest type only through periodic fires. The pine trees dominating these forests, such as the long-leaf pine of southeastern North Carolina are often resistant to mortality from fires. Mature long-leaf pines have a thick bark that insulates them from quick, relatively cool ground fires that burn litter accumulated on the forest floor. The seedlings of long-leaf pines have a tuft-like stage that protects the growing tip during the first few years, and then exhibit a quick growth spurt that carries the growing tip several meters above the forest floor in only a few years (above the level of ground fire effects). The accumulation of needles in long-leaf pine forests, combined with characteristically dry summers and lightning storms, actually creates a situation that promotes periodic low-intensity fires.

In the absence of fires, hardwoods, including oaks, gums and maples, will begin growing under the mature pines. These hardwoods do not have the bark or growth characteristics of pines to make them resistant to fire mortality. However, many hardwood species are more tolerant of shading than pines. As they continue growing, the hardwoods will eventually form a canopy above the forest floor. This canopy greatly increases the amount of shading and eventually kills any pine seedlings that may germinate in the area. The mature pines eventually die and the former pine forest becomes dominated almost exclusively by hardwoods. Since hardwood seedlings can tolerate considerable shade, such a hardwood forest would be relatively persistent until disturbed. The overall succession from pine forest to hardwood forest can take several decades, with a variety of intermediate stages occurring in the interim.

Aside from fire, a variety of other factors also affect which trees dominate the coastal forests of the southeast. Important among these are local habitat characteristics such as drainage patterns, nutrient availability, soil moisture content and soil composition. A very different forest may form in a valley that is poorly drained with high soil moisture content compared to dry sandy ridges. Such habitat differences may produce observable differences in the composition of a forest community on a relatively small spatial scale.

B. Scientific Method

In this laboratory, we will be practicing applying the scientific method to ecological questions. Recall that the inductive scientific method can be idealized diagramed in Figure 1. The processes starts with initial observations that scientists turn into questions and then into hypotheses – a testable explanation for an observed phenomenon. Hypotheses generate predictions that can then be compared to the outcome of experiments. If the experimental data align with the prediction of the hypothesis, then we say that we have evidence to support the hypothesis. If the experimental data do not match the prediction, then we reject the hypothesis. Using the scientific method, **<u>we never prove anything</u>** – we seek evidence to support or reject suppositions about how the natural world works. Recall that a **null hypothesis** is a specific type of hypothesis in which scientists claim that the suspected explanation or processes has no real effect such that there is no significant difference between the two (or more) populations being compared.

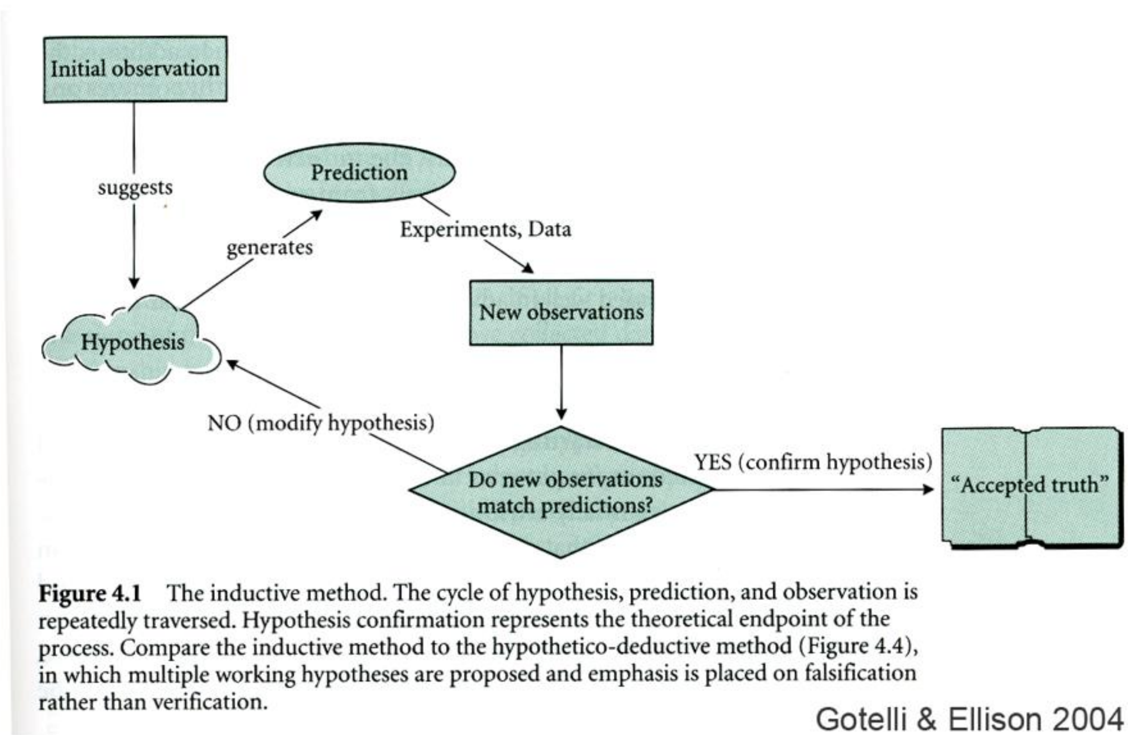


Figure 1: Diagram of the idealized inductive scientific method (From Gotelli & Ellison 2004).

In previous laboratories you have spent time in the Long-leaf pine forest on campus and tested hypotheses with experiments planned by your instructors. In this laboratory, you will make some additional observations of the Long-leaf pine forests on campus, and be responsible for developing your own questions, hypotheses and null hypotheses, and then designing experiments to test the null hypotheses.

II. Methodology

A. Defining the question:

Using one or a combination of techniques, your laboratory team will design a field sampling project to answer the following questions:

- How does fire affect southeastern North Carolina forest communities?
- Can you distinguish a forest that has been burned more recently than another?
- What criteria might you expect to observe to best show these differences?

These questions are actually much broader than they seem and your lab will not be able to address all variables associated with them in just the two sampling periods allotted. Below are some examples of ways in which this question can be narrowed to a more manageable topic:

1. Do pine trees dominate in forests that have been recently burned compared to forests that have not been burned for an extended period? To answer this question one may want to look at mature trees and seedlings separately because seedlings may be affected long before the composition of the adult trees changes significantly.
2. Are pine trees more abundant in forests that have been recently burned compared to forests that have not been burned for an extended period? Once again, the pattern may be different for seedlings versus mature trees, depending on time since burning.
3. Does fire affect total tree density (density of seedlings and density of mature trees)?
4. Do more frequent fires affect the type and amount of herbaceous plant cover and litter cover in the forest floor community?

These are a few examples of how you can narrow the broad question into a more manageable, smaller topic. You will do so by dividing into teams of up to four people within your lab. You will then visit the forests on campus, **devise at least four null hypotheses** that address the above or other questions, and develop a sampling design for testing those hypotheses. Ultimately, and with advice from your instructor, your group will narrow your research design towards testing two null hypotheses. Then, you will collect and analyze the data to complete these tests.

Each person in the group will complete an individual laboratory report on this work using a standard scientific manuscript format and terminology. This report will follow the "Long Laboratory Report" format shown in Appendix A.

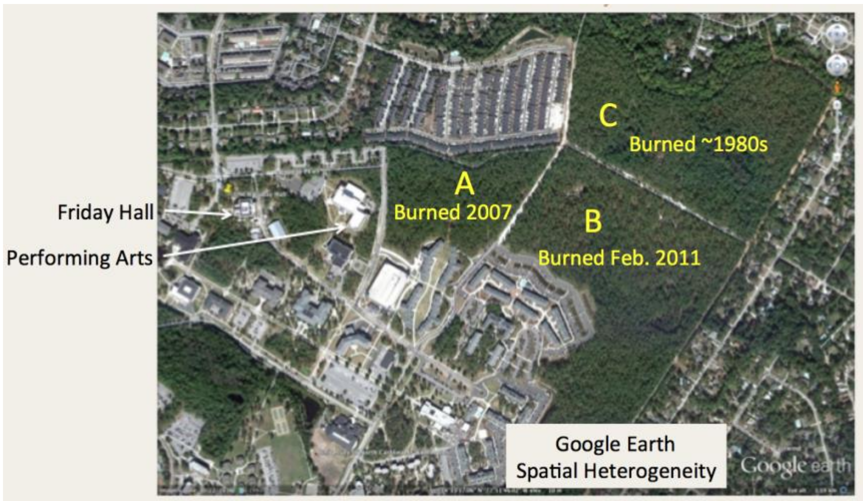


Figure 2 Google Earth Satellite image of the forest in the North East corner of the UNCW campus with three sections designated as A, B, and C. These forest patches were most recently burned in different years.

B. Designing the Sampling Program

Your sampling will be conducted in 2 forests patches in the North-East Corner of the UNCW campus (Figure 2). In an effort to preserve the remnant Longleaf Pine forest, the patches have been periodically burned. However, patches have different burn histories; the most recent year a patch was burned is shown on Figure 2. As can be seen when you visit these areas, the communities are in different stages of transition from pine forest to hardwood community.

In a previous lab you have been introduced to 4 of the basic sampling techniques used to determine abundances of sedentary organisms: quadrat, belt transect, point-quarter, and line transect. After you have defined your specific questions and hypotheses, you need to use one or a combination of these techniques to collect the appropriate data.

In designing the sampling program, you will make use of information from your previous labs, class discussion, and suggestions from the laboratory instructor. However, following are some general points to keep in mind:

- Some sampling techniques, such as line transects, can be used to measure relative numbers, but they do not provide any information on actual abundances (density).
- Some sampling techniques are best used to answer specific questions such as changes across environmental gradients (certain kinds of transect sampling).
- You need sufficient replication to adequately describe the sample populations and perform statistical analyses. The more replication, the more statistical power you will have to pick up significant patterns. A bare minimum to detect moderately strong patterns in a community as variable as a forest community would be 15 replicates per forest.
- If you decide to use quadrats, you need to decide on the correct size. Larger quadrats may provide better density estimates, but they take longer to do and thus reduce the number of replicates that can be completed in a limited lab period.
- Sampling points (location of quadrats, start of transects, point-quarter center points) need to be selected in an unbiased manner. Throwing a frisbee and walking 5 spaces in a direction indicated by a marker on the frisbee is one method we have used before. Laying out a measuring tape and sampling at set intervals (e.g. placing quadrats at every 10m) is another method. Overlaying a grid on a photograph of the area and selecting random sampling areas is another.
- Do you need to collect any physical data (temperature, light penetration, canopy cover, etc.)? For some questions, this is not necessary. For others, additional physical data may be very useful.

Your completed sampling design must include:

1. Where the work will be done? (we have already decided this for you).
2. What type of sampling method(s) will you use (e.g. quadrat, point-quarter, belt transect, and/or line transect)? If you are doing quadrats, how big will they be? Will you use different-sized quadrats for seedlings versus mature trees? If you do transects, how long will they be?
3. What is your replication (how many quadrats, center points and/or transects will be taken per site). Provide a rational for this decision. How will their location be selected?

4. What type of data will you collect? For example, will you identify and count all tree species or only numbers of pines and hardwoods? Will pine or hardwood seedlings be sampled separately from adults? Do you want relative numbers or actual densities? Do you want to take counts or percent cover, which might be particularly useful if you want to look at ground covers? How will you distinguish seedlings, saplings, and mature trees? (In the past we have said everything that was at least 2 m high and you could NOT close your thumb and forefinger around its trunk was a mature tree.)
5. How will you statistically analyze your data (e.g. what tests do your plan to use to compare data within or between sites)? We will talk about this more in a later lab.
6. Details of any physical measurements to be taken including the units and the instruments needed to make the measurements.

III. Assessment

This is the start of a multi-week laboratory project. At the end of lab today you will submit a written summary of your proposed research questions, ***four*** specific null hypotheses to test, a sampling design that you could use to test each hypothesis, and a list of at least ***three*** primary literature references you expect to be relevant for your report that are formatted as appropriate for the journal Ecology. During the next lab period you will implement two of the experiments at the discretion of your instructor. In week three of this laboratory, you will analyze the data in class.

The final part of the Forest Ecology Laboratory is for you to write a full report of the results of your research efforts as a full Laboratory Report (see Appendix A). Please carefully follow the departmental guidelines for writing a full laboratory report, which are posted at http://people.uncw.edu/borretts/courses/biol366/BMB_LabReport_Instructions_revised.pdf. Note that your report must include citations to at least (minimum) five papers from the primary peer reviewed science literature. The report will be evaluated with a rubric (http://people.uncw.edu/borretts/courses/biol366/BMB_LabReport_Rubric_366Long.xlsx), which you are encouraged to use to help you as you write your report. To help you improve your science writing skills, students will turn in a complete draft of this report that will be reviewed by their instructor. The students will then use the instructor feedback to revise their report. The revised report represents a substantial portion of the course grade.

To recapitulate, we are using three steps to building your report for this laboratory.

Report Steps	Due Date
Step 1. Draft Hypotheses and Sampling Plan	In Class Today
Step 2. Draft report including all required components.	See Syllabus Schedule
Step 3. Revised extended lab report	See Syllabus Schedule

IV. References

Gotelli, N. J. and A. M. Ellison. 2004. A primer of ecological statistics. Sinauer Associates, Sunderland, Mass.

5. FOREST ECOLOGY: SAMPLING

In this laboratory you will implement two of the studies you designed in the last lab. The entire lab period is devoted to your data collection

Once the data is collected in the field, I encourage you to return to the laboratory and enter it into an Excel spreadsheet. Before you leave, you should email a copy of the data to all members of the team.

a)

b)

Figure 1 BIOL366 Students sampling for their experiment in the forest on the University of North Carolina Wilmington campus (photo credits Stuart R. Borrett)

6. Forest Ecology: Statistical Analysis of Ecological Data

I. Objectives

1. Explain why scientists employ statistics to understand ecological problems.
2. Calculate the following descriptive statistics: mean, variance, standard deviation.
3. Describe the concept of normal distribution.
4. Select the appropriate statistics to analyze laboratory data
5. Understand and apply the t-test to differences in populations.
6. Understand and apply the chi-square test to frequency or count data.

II. Introduction

Ecologists are often concerned with numbers of organisms (density) and their patterns of distribution in nature. This makes ecology a quantitative science. However, ecologists cannot count and determine the location of every organism in a given area. Rather, ecologists must collect and analyze data from samples taken within the population. The quantitative data collected by ecologists can be (actually, must be) analyzed using statistics.

A. *Terminology*

Before we begin our work, some definitions are needed:

x_i = single observation or data point
n = sample size (number of data points)
$\sum_{i=1}^{n} x_i$ = sum of $i = 1, 2, \ldots, n$ observations
s^2 = variance
s = standard deviation
SS = sum of squares
df = degrees of freedom, often = n-1

B. *Descriptive Statistics*

Descriptive statistics summarize some aspect of the population. The most commonly used metrics to describe the *central tendency* of the data are the mean, median, and mode, while the most common metrics to describe the *variation* in the data are variance, standard deviation, and standard error. For ecological studies, the mean, variance, standard deviation, and standard error are most often used.

Mean

The mean is a measure of the central tendency (average) for a population.

$$\text{Mean} = \bar{X} = \sum_{i=1}^{n} x_i$$

Example 1: In population #1, the following numbers of trees are counted in 5 quadrats: 1, 6, 11, 16, 21

Mean = 55/5 = 11

Example 2: In population #2, the following numbers of trees are counted in 5 quadrats: 10, 11, 11, 11, 12

Mean = 55/5 = 11

Variance

As shown above, two populations with the same mean may have quite different variation in numbers. In the example given above, population #2 has a narrow range of abundances per quadrat (10-12) while population #1 has a relatively wide range of numbers per quadrat (1-21). The variance is a measure of this range of possible results.

$s^2 =$ SS/df

SS can be calculated as $SS = \sum_{i=1}^{n} \left(x_i - \overline{X}\right)^2$. However, this is a cumbersome equation to use when there are large numbers of data. A simpler way of calculating SS on most calculators is:

$$SS = \sum_{i=1}^{n} x_i^2 - \left[\left(\sum_{i=1}^{n} x_i \right)^2 \Big/ n \right]$$

Example 1: For population #1 described earlier:

SS=855-[3025/5] = 855-605 = 250

s^2=250/(5-1)=62.5

Example 2: For population #2 described earlier:

SS= 607-605=2

s^2=2/(5-1)=0.5

Standard Deviation

The standard deviation (SD) is determined as the square root of the variance. For population #1, the standard deviation would be 7.9. For population #2, the standard deviation would be 0.7. The standard deviation is important because it provides an easily visualized measure of the variation from the mean for normally distributed data.

Standard Error & 95% Confidence Intervals

Standard error (SE) is a measure of the data variability that is adjusted by the number of samples. Thus, SE is standard deviation divided by the square root of the number of observations $SE = SD/\sqrt{n}$. For population #1, the

What is normally distributed? A normal distribution is a typical bell curve, with the peak of the curve corresponding to the mean. However, a bell curve can be narrow and tall or broad and short, depending on whether the data has a low or high variance. The standard deviation provides an easily understood estimate of this variability. For normally distributed data, 95% of all possible observations (such as counts in quadrats) will lie within 2 standard deviations of the mean.

Scientists sometimes report the 95% confidence limits for their estimated mean values. This is calculated at $\bar{X} \pm (1.96 \times SE)$. For population #2, the SE is 0.3162, so the 95% confidence interval would be (10.38, 11.62). This means that if we took further quadrat samples from this population, on average 95% of these additional quadrats would have densities between 10.38 and 11.62 trees per quadrat.

C. Comparative Statistics

What are comparative statistics?

Statistics can also be used to determine whether populations (or measurements of population characteristics) are similar or different. For example:

Is the density of pine trees in two areas similar or different?
Is the number of crabs in the Cape Fear estuary more now than a decade ago?
Is my sample population distinguishable form one that is normally distributed?

This use of statistics is called significance testing. Using the scientific method, even using statistics, the scientist cannot prove anything. Statistics can only demonstrate that an event is very unlikely, but nothing is ever proved in the process. Typically, the investigator establishes a hypothesis and then tries to determine if that hypothesis is likely by showing the alternatives (called null hypotheses) are not likely. For example, one may have a hypothesis that densities of pine trees are different between 2 forests. However, because statistics do not prove differences, the investigator actually seeks evidence that the null hypothesis of no difference between the forests is unlikely to be true (confusing isn't it!).

One of our tasks as scientists is to *select* the best statistical tests to analyze our data. This requires that we know a bit about statistical tests and the assumptions that they make. In this laboratory, we discuss five comparative statistic methods: the Student's t-test, Welch's t-test, the Mann-Whitney U test, the Shapiro-Wilk goodness of fit test, and the χ^2 goodness of fit test. Again, these tests are appropriate for different purposes in part because they make different assumptions about the nature of the data under consideration. For this laboratory, we will follow the data analysis flow chart shown in Figure 1.

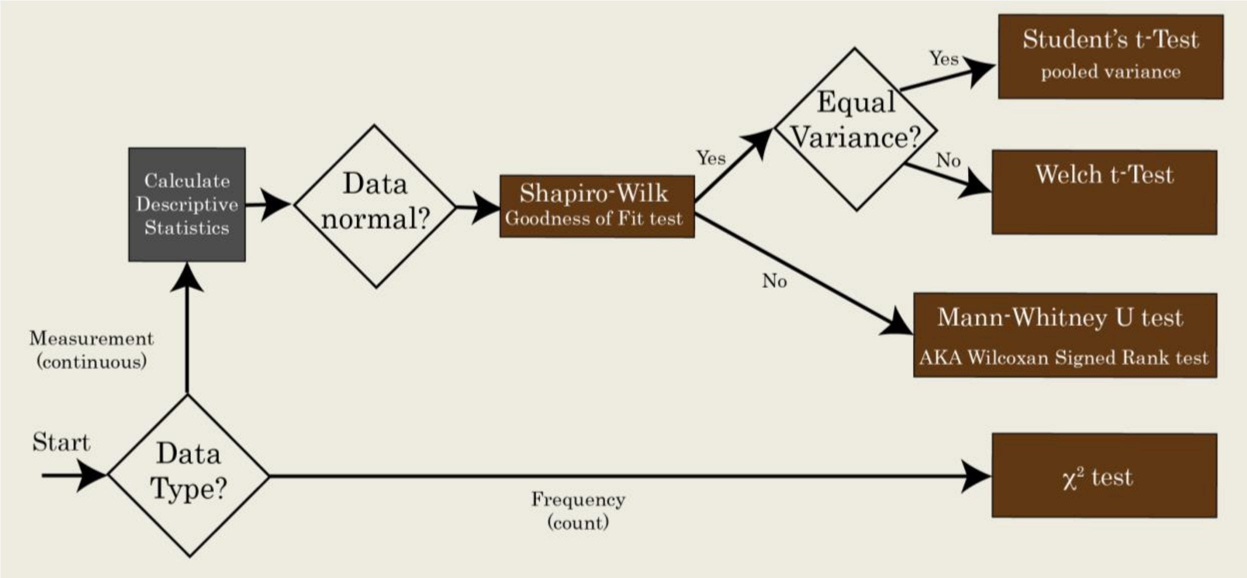


Figure 1 Data analysis decision tree for the BIOL366 Forest Ecology laboratory.

Scenario
We will use the following scenario to illustrate our data analysis problem.

Step 1: State Hypothesis
A researcher develops the following hypothesis:

H_a = There is a difference in the mean circumference of pine trees between a recently burned forest and a forest that has not been burned for 25 years. We might expect there to be more surviving younger trees in the unburned forest to be older and thus have a larger circumference.

Step 2: Form the null hypothesis
H_o (the null hypothesis) = There is no difference in average circumference between the two forest types (Burned and Unburned).

Step 3: Data collection
Next, the researcher collects data. In this case, we measured the circumference of the long-leaf pine trees encountered along a 25 m transect through each forest. The measurements recorded in cm were:

Unburned: 48, 41, 26, 30.5, 35, 25
Burned: 50.5, 60, 46.5, 47.25, 30.5, 49, 48, 43

Step 4: Data Analysis
The scientist now needs to select the most appropriate statistics to test H_o.

Our first question to consider focuses on the type of data we have (Figure 2). Is the response variable measured on a continuous scale (e.g., circumference) or is it a count/frequency variable?

To compare two populations of a continuous variable, we could use the Student t-test, the Welch t-test, or the Mann-Whitney U test, depending on several characteristics of our data. The Student t-test and Welch t-test are parametric statistics, and thus assume that the data come from a known distribution – in this case a normal distribution. The Mann-Whitney U test is a non-parametric test and therefore does not make assumptions about the underlying data distribution. The disadvantage of the non-parametric statistics is that they tend to have less power to discriminate real differences, so parametric statistics tend to be preferred when possible.

This means that before we can select the most appropriate statistical test to compare the means of the two populations, we need to know if our data are approximately normally distributed. To answer this question, we will use the Shapiro-Wilk test.

Shapiro-Wilk Goodness of Fit Test

The Shapiro-Wilk Goodness of Fit test compares your continuous measurement data (e.g., tree circumferences, litter depths, etc.) distributions to a normal distribution and tests the null hypothesis that *there is no difference between the distribution of your data and a normal distribution.*

We will use the JMP IN statistical software to perform this test on each of the sample populations (burned and unburned).

To complete this test in JMP, do the following:

1. Open a web browser and navigate to https://tealware.uncw.edu/.
2. Double click on the JMP icon. NOTE: If you are using the university computers you can go to the START menu in the bottom left hand corner of the desktop and search for JMP Pro 11 and open it from there.
3. Select “New Data Table” in the JMP Starter window that opens.
4. A table will appear. If there is only 1 column (labeled column 1), you will need to create a second by double clicking in the right side space (to the right of “column 1”).
5. Click on the column 1 square and then click on the name. Type in “forest type”. Do the same for column 2, typing in “circumference”. For forest type, change the default data type to “Character”. The Modeling Type should be “nominal”. For the “circumference” data, please ensure that the data type is “numeric” and the modeling type is “continuous”.
6. Enter the data in the following format (u=unburned forest, b=burned forest):

	Forest type	Circumference
1	U	48
2	U	41
3	U	26
4	U	30.5
5	U	35.25
6	B	50.5
7	B	60
8	B	46.5
9	B	47.25
10	B	30.5
11	B	49
12	B	48
13	B	43

7. Next, select **Analyze → Distribution** from the menu. You will be given a dialogue box with the columns listed on the left. Select the circumference data to be the y variable and fit the distributions **by** the forest type. Choose okay and you will be given a graph and some descriptive statistics and a plot for each population. Record the sample size (N), mean, standard deviation, and range (minimum and maximum values) for your data. These are your descriptive statistics.
8. To fit the normal distribution to your data, click on the red triangle next to “Circumference” header, and select **Continuous Fit -- Normal**. A small data box will appear at the bottom of the page that gives you the test parameters. Click on the red arrow in the Fitted Normal box and select **Goodness of Fit**. Another small box will appear with results of the Shapiro-Wilk W test. Record the W value and W<p value. The latter value is your p value and tells you if your data are normally distributed ($p > 0.05$) or not normally distributed ($p < 0.05$).
9. Repeat the steps in 8 for the other forest type (burned or unburned). Record the data for this forest as above.

For our example, you should find that according to the Shapiro-Wilk test we do not have enough evidence to reject the null hypothesis that our data are from normal distributions in both the burned (W=0.902960, p = 0.3071) and unburned forests (W = 0.982116, p = 0.9456). Thus, we can assume are data are normally distributed and use either the Student or Welsh t-test to test our starting null hypothesis that there is no difference between the mean tree circumference between the two forests.

If the data from either of our forests was not normal, then the data violates the assumption of the t-test. In this later case we would probably want to use the Mann-Whitney U test as it is an appropriate non-parametric statistic that does not assume the data are normally distributed.

The Student t-test

The t-test is a parametric statistic used to compare the means of two populations. This test is useful for comparing variables whose measurement use a continuous scale of measurement such as length, height, or weight.

To calculate the t-test we start by calculating the t-test statistic using the following formulas:

t-test statistic: $t = \frac{|\bar{X}_1 - \bar{X}_2|}{S_{x1-x2}}$, where

$$S_{x1-x2} = \sqrt{\left(s_p^2/n_1\right) - \left(s_p^2/n_2\right)} \quad \text{and} \quad s_p^2 = \left(SS_1 + SS_2\right)/\left(df_1 + df_2\right)$$

Notice that the numerator of the t statistic directly compares the means of two sample populations labeled #1 and #2. The denominator of the statistic considers the variation in the sample sizes as well as the number of samples. In the case of the t-test, the degrees of freedom are n_i-1, where n_i is the number of measurements made in each of the cases compared.

We use the t-statistic and the degrees of freedom to look up the probability (p-value) of recovering the value given that the hypothesis is true. If the p-value is less than an α-value = 0.05, then the value is unlikely and we must reject the hypothesis. If the p-value is larger than the α criterion, then we determine that there is not enough evidence to reject the hypothesis.

Example Application of the T-test

Continuing our earlier example, the investigator uses the Student t-test to compare the means of the two populations.

The following numbers are calculated to determine the t-statistic for the two populations (b=burned, u=unburned)

Burned	**Unburned**
$mean_u$: 36.15	$mean_u$: 46.843
$n_{u:}$ 5	$n_{u:}$ 8
SD_u: 8.655	SD_u: 8.234

Thus, $t = \frac{|36.15 - 46.84|}{4.782} = 2.235$

The degrees of freedom (df) for this test are $(n_u\text{-}1) + (n_b\text{-}1) = (5\text{-}1) + (8\text{-}1) = 11$
This t-value can be looked up in a t-table. If the calculated values is greater than the value under the df. row for 0.05 probability level, then you reject the null hypothesis and conclude there is a significant difference between the burned and unburned forests. In this case, the t-value has a probability of 0.047; therefore, we can conclude that pine tree circumferences are statistically different between the two forests, and given the means we know that they are smaller in the burned forest.

Calculation using JMP IN (version 7.0)

To repeat the analysis above in SAS JMP IN, complete the following steps:

10. From the menu bar, choose **Analyze – Fit Y by X**
11. Choose forest type as X and circumference as Y
12. Do the group means/one-way ANOVA comparison (which will be the default comparison for your data).
13. You will get a graph of the data. Click on the small red arrow by the name of the graph and choose the **means/ANOVA/Pooled t**. This will actually run the statistical tests.
14. The results of the t-test will be displayed along with the results of several other tests. Please note that the calculated t-value is the roughly the same as we calculated by hand. The "Prob > |t|" of 0.0470 is the p-value for our null hypothesis of no difference.

Welch t-Test

The Student's t-test assumes that the variances in the two populations being compared are equal. This is rarely the case for ecological data. Welch's t-test uses the same t-test logic as the Student's t-test shown above, but it does not assume equal variances. Thus, if you want to compare the mean values of a continuous type variable in two populations with unequal variance, then the Welch t-test is more appropriate.

To calculate the Welch t-test in JMP, select Analyze → Fit Y by X → (y = circumference, by = type). Then, select the t-Test from the menu that appears when you click on the red tab next to the "Oneway Analysis of Circumference By Forest Type" header that appears. The results of the Welsh t-test for our data show that there is no statistically significant difference between the mean tree circumferences (t=-2.20782, df = 8.28, p-value = 0.0571). We interpret the analysis in this way because the p-value (Prob >|t|) is greater than our critical value of 0.05. Notice that this is a different conclusion than we reached using the Student t-test; the test assumption makes a difference in the outcome here.

Mann-Whitney U Test (alternatively Wilcoxan sign-rank test)

If our continuous measurement data do not come from normal distributions, we cannot use either the Student or Welsh t-test. Instead we need a non-parametric test. For the question our scientist posed, the Mann-Whitney U test is most appropriate. This is alternatively called the Wilcoxan sign-rank test. How the test works is interesting, but as this is not a statistics course we will not show how it works here. However, we encourage you to look it up.

To calculate this test using JMP, select the **nonparametric → Wilcoxan test** from the dropdown menu on the red tab next to "Oneway Analysis of Circumference By Forest Type" on the box that previously appeared when you selected **Analyze – Fit Y by X** in step 10 above. The information you need will be under the "2-Sample Test, Normal Approximation" header.

Like the Welch t-test, the Mann-Whitney U test suggests that there is not enough evidence to reject our null hypothesis that there is no difference between the circumference means in the two forests (S=22, Z=-1.83, p-value=0.065). Again, our test p-value exceeds our critical value of 0.05.

Please note that for our example scenario, we calculated the Student, Welch, and Mann-Whitney U statistical tests for our data. This is important for pedagogical reasons; however, in practice *you should only report the statistical results from the most appropriate statistical test*.

Chi Square (χ^2) Goodness of Fit Test

Figure 2 shows that if our data is count or frequency data, it should be analyzed using the Chi Square (χ^2) Goodness of Fit Test. Using the χ^2 test, scientists can determine if observed values are the same as values expected for a given situation. For example, you survey the number of crabs under "large" rocks and "small" rocks in a swift current to determine if there is a difference in the number of crabs under each rock type.

The total number of crabs under 20 rocks was:

	Large rocks	Small rocks
Observed	200	10
Expected	105	105

The expected number is established by determining the number of crabs expected if the null hypothesis were true. In this case there is a hypothesis (H_a) of a difference between the rocks and a null hypothesis (H_o) of no difference. So, if there are a total of 210 crabs collected, with no difference in the number found under each rock type, there must be an expected number of 105 for both large and small rocks (105+105 = 210).

The χ^2 statistic is then calculated by:

$$\chi^2 = \sum_{i=1}^{k} \frac{(observed_i - expected_i)^2}{expected_i}$$

In this example, $\chi^2 = (200-105)^2/105 + (10-105)^2/105 = 171.9$

For this case, the degrees of freedom (df) for the test is determined by the number of groups minus 1 (2-1=1). For 1 degree of freedom at a 0.05 significance level, the critical table value is 3.84 (see Appendix B). Since your calculated value is greater that the table value, the null hypothesis is rejected and you conclude there is a difference in the number of crabs under large rocks versus small rocks.

Some statisticians argue that if the df = 1 in a Chi Square test then a Yate's correction needs to be used to avoid a Type I error (rejecting the null when it's true). The argument is that the correction makes the test more rigorous with small samples. However, recent evidence suggests that the Yate's correction is too conservative. Thus, we will not add this complication to our calculations.

III. Questions to Consider

For this lab, you will use the flow chart in Figure 2 to guide your analysis of your Forest Ecology Laboratory data. For the **Results** section of your Forest Ecology laboratory report, you should do the following.

1) Briefly describe the data your group collected and determine which statistical tests are most appropriate to analyze your data.

2) Measurement data
   - Calculate descriptive statistics (N, mean, s.d., range) for each forest
   - Construct a bar-graph of the means with standard error error-bars to visually be able to compare the populations
   - Test for normality (W value, p) with interpretation for each forest
   - Report the results of the Student, Welch, or Mann-Whitney tests (test statistic value, degrees of freedom, p) with interpretation

3) Count data.
   - χ^2 results (χ^2 value, degrees of freedom, p) with interpretation

In addition, please address the following questions in the **Discussion** section of your report:

(1) For your forest data, what are your null and alternative hypotheses, and how will you decide to reject/accept them? What decision-making went into your experimental design to allow you to be confident in the way you chose to test these hypotheses?

(2) How do your results compare to similar research previously reported in the primary literature? Have other scientists conducted similar studies? If so, do your results agree with the previous work? What might explain any differences you found? Please put your work into the context of the existing ecological literature and questions related to your topic area.

(3) Write a brief summary you could use to explain to someone with no connection to this lab why and how we were exploring scientific and statistical differences in populations in these forests labs and what your main conclusions are based on your results.

(4) If your job were to report to a land management advising council about how best to manage this forest, what would you recommend to them based on your results? Why?

7. WETLAND COMMUNITIES AND DELINEATION

I. Objectives

1. Identify the three distinguishing attributes used to delineate a wetland habitat from other habitat types.
2. Collect and use hydrology, soil, and vegetation data to delineate the boundary between the upland forest and the Greenhouse Carolina Bay
3. Practice identifying native plant species in the Longleaf pine forest.

II. Introduction

In one sense, all communities on Earth are wetlands because they depend on water to some degree. Environments vary, however, in the amount of water that they contain. At one extreme are very wet (aquatic) habitats while at the other are very dry upland habitats (deserts). Between these two extremes is a transition zone. At some point in this transition, the impact of water becomes great enough that the soil becomes **anoxic**. **Wetlands** are habitats that have anoxic soils for at least some portion of the growing season. Historically, wetlands have been vilified as the source of diseases and as obstacles to human progress. Throughout human history wetlands have been modified through filling or drained and converted to farmland or sites for houses and factories. A large proportion of wetlands around the world have been destroyed as a result. Ecologists that studied transition zones between upland and aquatic habitats traditionally did not try to determine the exact boundary between them. They recognized the unique nature of flooding and the plant and animals. The term wetland was not used, instead swamps, marshes, moors, bogs, etc., were used to describe the habitat under study.

When the **Clean Water Act** was passed in 1972, **Section 404** of that legislation prohibited the filling of "Waters of the United States," which included wetlands (a copy of the relevant laws are posted at http://www.epa.gov/owow/wetlands/laws/). This meant that a clear definition was necessary so that those attempting to obey the law would know where wetlands existed and those in charge of enforcing this new law could enforce it. Unfortunately, the zone between an aquatic habitat and the upland terrestrial habitat is seldom marked by an abrupt boundary. Instead there is a gradual change from the aquatic habitat that is almost always dominated by the effects of water, to the upland side where water is a limited resource. Where wetlands end and upland habitats begin has occupied a great deal of scientists' and attorneys' time since the act was passed.

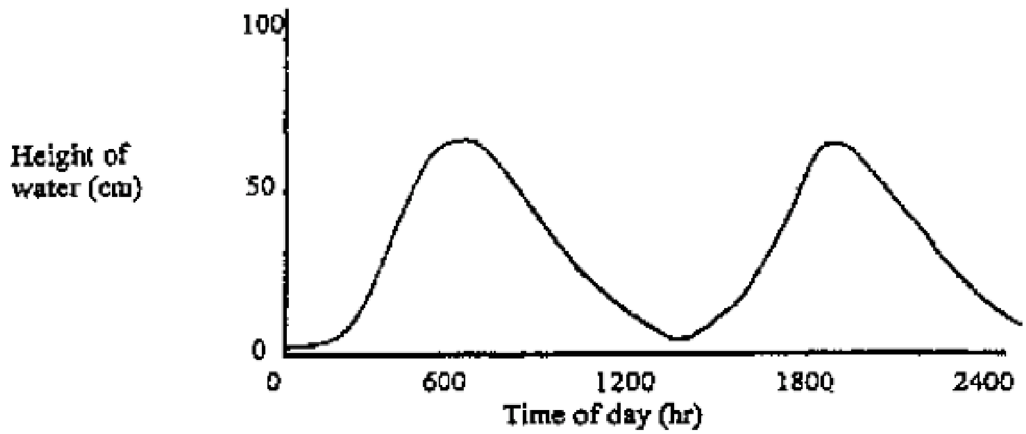
The current definition of a wetland is based on the presence of three parameters: 1) **hydrology**, 2) **hydric soils**, and 3) **wetland plants**. A wetland is an area that is flooded for a sufficient length of time so that it is dominated by **hydrophilic** (water loving) or at least water tolerant plants, and where the soil has responded such that it is anaerobic within the top 12 inches (30.5 cm) for a least 5% of the growing season, or about 14 days in southeastern North Carolina. Although the definition used by federal, state, and local agencies may vary, this concept forms the basis for most definitions. While this may seem like a straightforward definition, it is often difficult to determine exactly where a boundary begins or even if a site is a wetland. Rainfall is not uniform over the year or even over many years, so wetlands can often be extremely dry and uplands can be flooded for long periods of time. It often takes wetland scientists to make boundary determinations and even then, scientists may argue over exactly where the boundary should be. Some state agencies, frustrated over the difficulty of determining where wetlands exist, use another approach. If certain plants dominate, then the habitat is considered a wetland, no matter if it floods or not. A recent review of several rapid methods for wetland conditions is posted on the EPA website at (http://www.epa.gov/owow/wetlands/monitor/RapidMethodReview.pdf)

Most people are familiar with large classical wetlands such as the Everglades or the Okeefenokee Swamp. Few, however, realize that wetlands are everywhere, even in their backyards, on mountainsides, and in the desert. Wetlands have important ecological functions, including some of the same ones as nearby upland and aquatic habitats. Within the classification of wetlands are giant swamps covering large areas of the Earth and very small areas of damp earth. Each type has certain functions that contribute to the function of the entire landscape. While most of us recognize the importance of preserving large systems such as the Everglades, which harbors Endangered Species and nurtures the coastal fisheries of south Florida, we often neglect the small temporarily flooded pools in our backyards, which may be the only nesting habitat for frogs and toads in the area. Without these small wetlands, important predators may be missing from the nearby landscape. Thus, each wetland has value, no matter how small or temporary. Federal, state, and local regulations protect most large wetlands from destruction, but small ones are afforded little protection and are disappearing rapidly from the landscape, taking with then many species that depend on them. UNCW's campus has a number of wetlands that are in various states of health. In this laboratory, we will further consider the three factors that legally define a wetland.

A. Water

Obviously, water is the critical component of these wetlands. Both the depth of flooding and the duration of flooding are important. To represent this aspect of a wetland's hydrology, we use a **hydrograph**. A hydrograph typically shows the flooding frequency and duration over a specific period of time, for example a tidal marsh in the Cape Fear River over one 24-hour cycle (Fig. 1a) or a bog in New England over the course of one year (Fig. 1b). Note that the marsh and bog are not flooded all of the time.

(a)



(b)

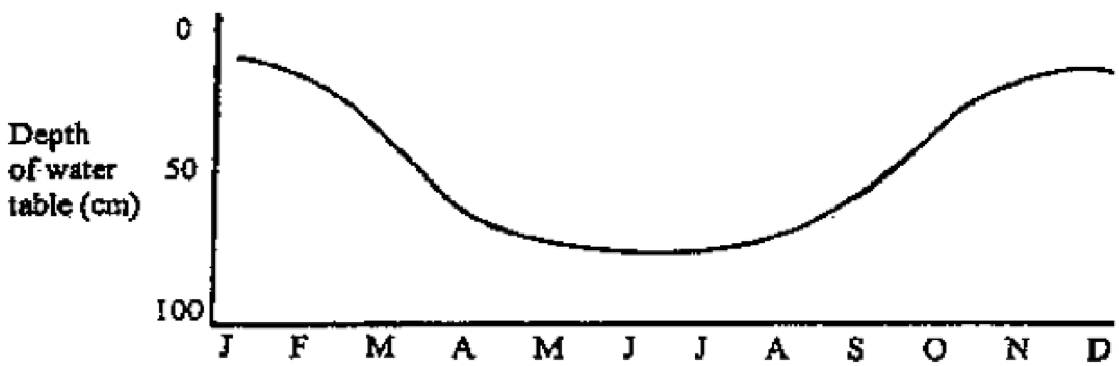


Figure 1. Example hydrographs. (a) hydrograph of a tidal marsh showing the surface flooding during each high tide. (b) hydrograph of a bog showing the depth of the water table during each month of the year.

B. Ecological Functions

Each wetland has ecological functions that differ depending on its position in the landscape. Key functions of wetlands can include providing habitat for different species thereby increasing an areas biodiversity, protection from floods, functioning as biogeochemical hotspots where pollutants are chemically and biologically transformed. Wetlands are often important for animals that are not wetland species, but need wetlands for breeding, protection, or for food. For example, a tidal wetland serves as a feeding area for juvenile fish and shrimp, and so contributes biomass to the adjacent ocean. Swamps along rivers remove sediment and nutrients from the adjacent river, purifying water. Isolated wetlands serve as reservoirs, holding water so that parts of the landscape below them do not flood as often or severely. Some hold floodwater and allow it to percolate into the ground, thus recharging our ground water. Not all wetlands have all functions, but every one has an important role in the landscape. For more on the values and functions of wetlands see http://www.epa.gov/owow/wetlands/pdf/fun_val.pdf.

C. Two Wetlands on the UNCW Campus

There are two wetlands on campus near Friday Hall that we will inspect in this laboratory. Figure 2 shows the location of both McCrary's Bog and the Greenhouse Bay wetlands.

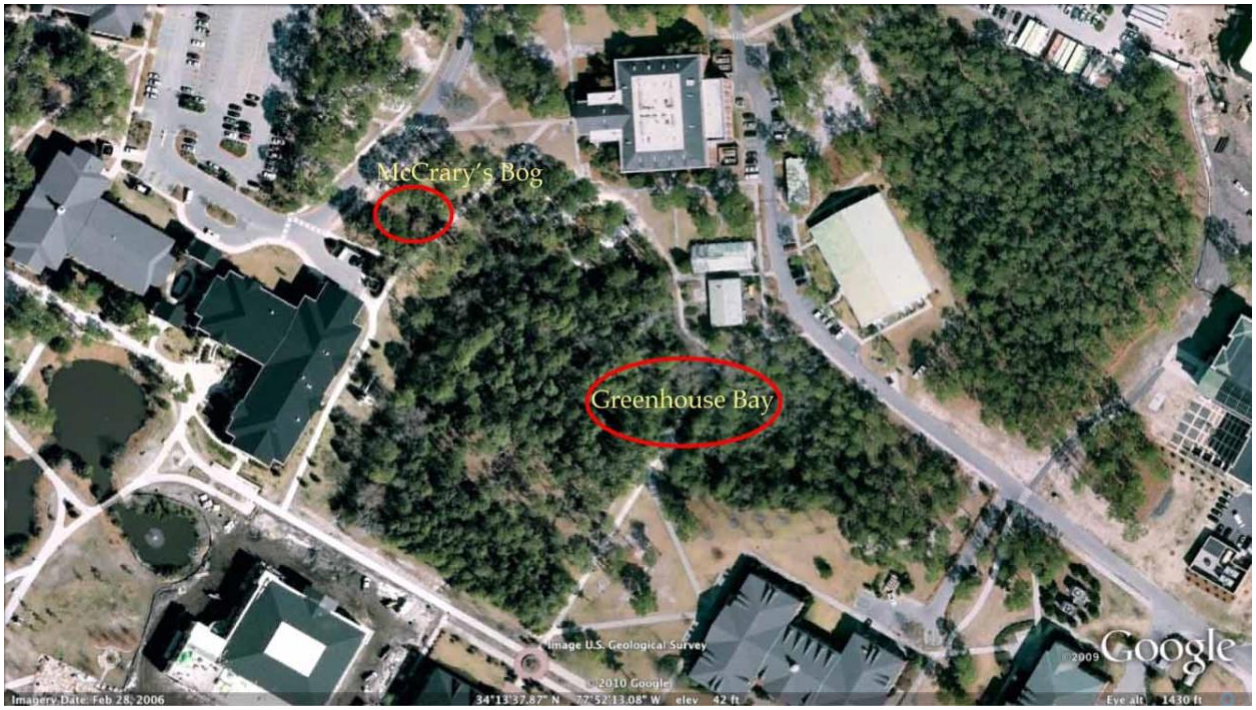


Figure 2. Locations of McCrary's Bog and the Greenhouse Bay wetland on the UNCW campus.

McCrary's Bog

McCrary's Bog is a wetland that is periodically wet near Friday Hall (Fig 2). This area was once connected to the wetland behind the greenhouse, but was separated from it by the sidewalk and fill placed along this corridor. At one time, Venus flytraps and sundews were abundant here. Although small, it contains a clam shrimp and a fairy shrimp that have been found in only a few areas near Wilmington. The only other known sites for these species were located where the present Walmart is now and in what in now the Landfall golf course. When this area is flooded by rainfall, these small crustaceans hatch, grow, mate, lay eggs, and die, all in the course of a few weeks. During this period of time, various frog and toad species will also use the area for breeding. A few buttressed black gum trees are the only overt sign that this area is a wetland. There may be no flooding here for years at a time followed by months of flooding. Runoff from the adjacent road, fertilizer, and inadvertent filling continue to degrade this site. A hydrograph of McCrary's Bog would indicate ponding after heavy rains, especially during the winter months when **evapotranspiration** is minimal. Figure 3 illustrates what the wetland looks like when full.


Figure 3. McCrary's Bog filled with water during the winter of 2009 (Photo by S.R. Borrett)

Greenhouse Bay

Figure 2. Dr. Willard standing in the Greenhouse Wetland while teaching an Osher Life Long Learning class about the wetland ecology -- including two tree frogs. (photo by Stuart R. Borrett, 2016)

Behind the greenhouse is a relatively large area of dense shrubbery called a Carolina Bay. In this part of North Carolina, such bays may occupy many square miles and are important areas for wildlife. For example, Lake Waccama is the largest Carolina Bay in the Southeastern US. These evergreen shrub bogs exist because water is trapped temporarily, causing the accumulation of organic matter, which in turn produces very acidic peat that limits the types of plants and animals that can live there. These wetlands are usually flooded during the winter. When the vegetation begins to grow in mid-April, evapotranspiration removes the water, lowering the water table. During these times, the ground water level may be four feet or more down in the soil. These wetlands usually evapotranspirate more that the 50+ inches of rainfall that falls each year. When plants cease growth in mid- to late September, these wetlands rehydrate and remain wet until the cycle begins again.

Ditches connected to the University's storm-drain system have drained this wetland. Several years ago some of these ditches were filled, restoring some of the wetland's water retention capability. In addition, some of the water that flowed into the gutters from the roof of Friday Hall was diverted into this wetland. Recent projects alongside this wetland and the introduction of exotic species, such as water hyacinth, and garbage into this wetland threaten its natural state. In addition, fire has been eliminated. Fire is a natural part of this community, and favors the growth of herbaceous plants such as the carnivorous Venus flytrap, pitcher plants, and sundews over woody species.

III. Methodology

1. Visit the south side of the Greenhouse Bay (cross the bridge and immediately turn right into the upland forest). Face north towards the bay. You are now standing in an upland forest and facing a wetland habitat. Note the gentle slope downward towards the bay. At what point does the upland forest stop and the wetland begin?

   We will answer this question by examining the hydrology, soil, and vegetation at several sites along a transect that extends from the upland forest and into the bay. At each site, there is a hydrology well that has been inserted into the ground. By sticking a pole into the well, we can determine where the water table is in relation to the surface of the soil. Record the depth of the water table (in cm) at the first well on the data sheet. Use a soil auger to measure the depth of the litter layer and the depth where the A-horizon meets the B-horizon near the first well. Identify the four most dominant plant species at the first well.

   Repeat this procedure at each station.

2. Draw a graph depicting the depth to the top of the water table in relation to the surface of the soil at each station. Why isn't this a hydrograph? What would you need to do to make it a hydrograph?

3. Draw a graph depicting the thickness of the O (litter) and A-horizon at each station. At what point does the soil become anoxic along the transect?

4. Compare your dominant vegetation with the list provided in lab. At what point do wetland plants begin to dominate the vegetation? Why did you select this boundary?

IV. Assignment

For this laboratory, each *team* will report their results using the Shortened Laboratory Report format (see Appendix A). Therefore, each team member will be an *author* of the report. Make sure to report your data using appropriate figures, tables, and summary statistics and address the questions asked in the discussion section. Use this evidence to determine the boundary between the upland and wetland area.

Data Sheet

Station number	Water table	Depth (in cm) of Litter	A-horizon	Dominant vegetation
1				a. b. c. d.
2				a. b. c. d.
3				a. b. c. d.
4				a. b. c. d.
5				a. b. c. d.
6				a. b. c. d.
7				a. b. c. d.
8				a. b. c. d.

Wetland Communities Plant List

Upland Plants

Long-leaf Pine (*Pinus palustris*)

Turkey Oak (*Quercus laevis*)

Post Oak (*Quercus stellata*)

Live Oak (*Quercus virginiana*)

Sassafras *(Sassafras albidum*)

Wire Grass (*Aristida stricta*)

Bracken Fern (*Pteridium aquilinum*)

Pixie-moss (Pyxidanthera barbulata)

Wetland Plants

Pond Pine (*Pinus serotnia*)

Jessamine (*Gelsemium sempervirens*)

Red Bay (*Persea borbonia*)

Loblolly Bay (*Gordonia lasianthus*)

Sweet Bay (*Magnolia virginiana*)

Bull Bay (*Magnolia grandiflora*)

Red Maple (*Acer rubrum*)

Greenbrier *(Smilax laurifolia*)

Inkberry (*Ilex glabra*)

Gallberry (*Ilex coriacea*)

Water Oak (*Quercus nigra*)

8. Indirect Measures of Population Size

I. Objectives

1. Learn techniques used to estimate population size through indirect measures
2. Describe the assumptions of these sampling techniques
3. Describe the conditions under which these techniques may be most appropriate for population estimates.
4. Compare a different forest to the Longleaf pine forest we studied previously.
5. Practice using field guides to identify species.

II. Introduction

Sometimes it is not possible to estimate the size of a population using mark-recapture or capture-removal sampling techniques. This is especially true for species of wildlife that are difficult to capture, cannot be easily marked, or which you do not want to remove from an area. In those cases, ecologists, wildlife biologists, and fisheries biologists may use indirect measures to estimate population density, such as counting the number of squirrel nests in a hectare of forest or recording numbers of bird vocalization at several points. Indirect measure typically cannot be used to get precise estimates of population density, but rather provide information on relative abundance that may be used in determining which area has greater numbers of an organism or in determining if there are changes in population size. Relative abundances of species can be sufficient to monitor seasonal or annual changes in population levels and to characterize faunal communities.

Indirect measures of population size include both direct observations of organisms that provide information on relative abundances as well as observation of indirect signs of the organisms' presence. Direct observation techniques include strip censuses, point surveys and roadside counts. Strip censuses involve walking (or in aquatic systems, swimming) a measured distance in a fixed time period (e.g. 1 km in 1 hr), with abundances expressed as the number of animals seen per kilometer or hectare. This is a variation on the line transect approach and provides an efficient method to collect a large amount of data quickly and with relatively little effort. The technique has been used for bird, mammal, insect and fish populations (where a diver swims a set distance). Roadside counts are a modification of the strip census technique. Observers travel a specific route, usually at the same time of day and at the same speed, and count the number of individuals seen. Care should be taken to census during the animal's peak activity times and to avoid poor weather conditions when the animals may not be active. Another modification of the strip census technique are point surveys. In point surveys, the observer spends a specific period of time (e.g. 5 min or 1 hr) at sites that afford suitable viewing. An index of abundance can be expressed as the number of animals seen per time unit of observation. This technique has been used widely for birds, mammals, reef fish and insects.

The above techniques involve ways of directly observing animals to obtain information on relative abundances. However, such direct observations of an organism may not always be practical, and one may have to resort to observations of indirect evidence of an animal's passing. Examples include counts of tracks, pellet or scat counts, nest counts, tree markings for species that mark their territories, bird calls (often censuses from individual points), and feeding stations (as evidenced by shell remains or seed remains). These various indirect measures can be sampled through standard transect or quadrat approaches or by using one of the methods described above, such as a strip census or a point survey.

III. Methodology

In this lab we will visit the Bluethenthal Wildflower Preserve on the UNCW campus (Figure 1). We will practice observing the forest through the lens of ecology, using guide books to identify species, compare the forest to Longleaf pine forest we studied at the back of campus, and develop observational skill for indirect measures of populations. The exact methodology may be varied depending on weather conditions and season of sampling.

You can learn more about the Bluethenthal Wildflower Preserve online at http://www.uncw.edu/ba/physicalplant/arboretum/bluethenthal.htm.

During your visit, please keep a list of the species you identify in the forest. Make sure to look high and low, consider different kingdoms, and focus on different ecologically relevant scales.

No laboratory report is required for this activity.

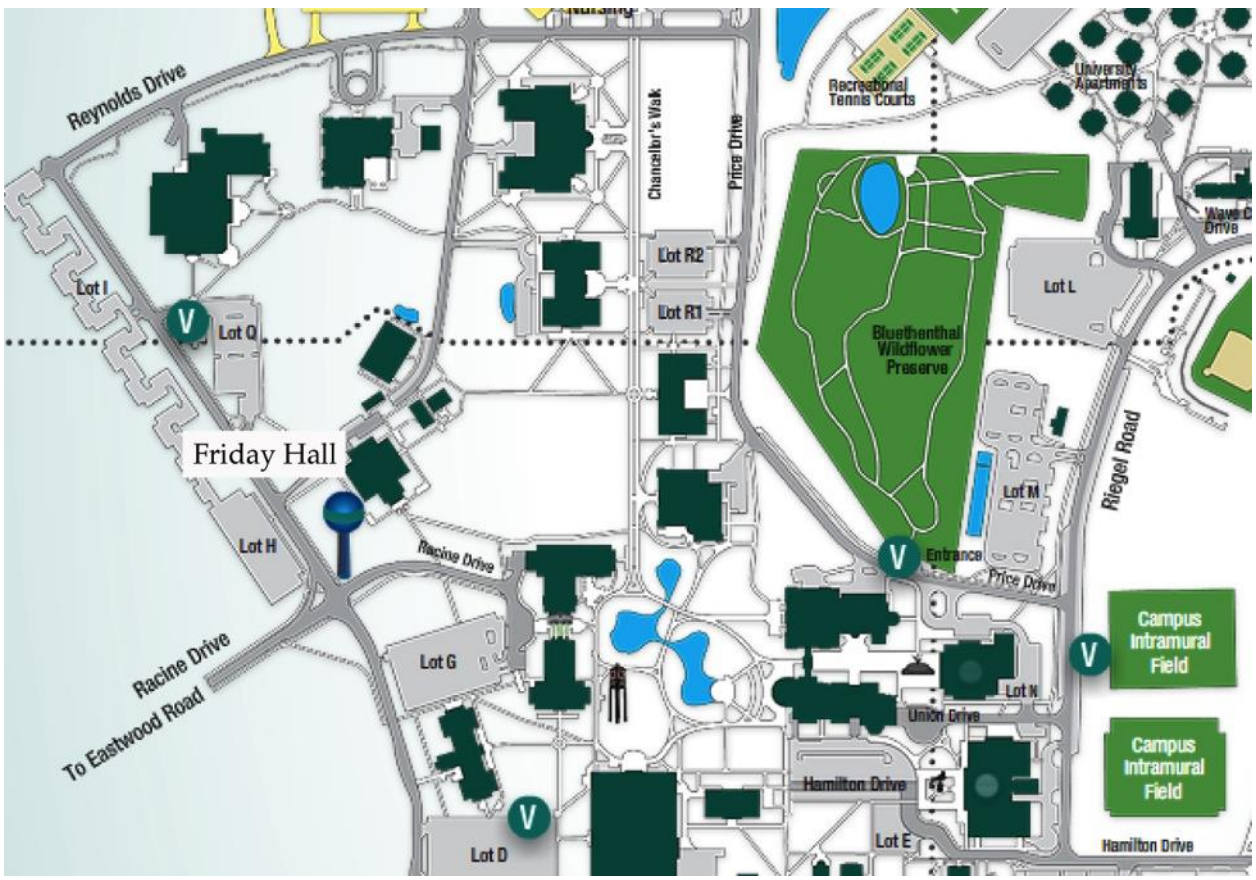


Figure 1: Map to Bluethenthal Wildflower Preserve

APPENDIX A: LABORATORY REPORT INSTRUCTIONS

Laboratory reports for this class will follow the UNCW Department of Biology and Marine Biology laboratory format as described in this document (http://people.uncw.edu/borretts/courses/biol366/BMB_LabReport_Instructions_revised.pdf). The reports will be assessed with the associated rubrics posted on the class website. The Guidelines, rubrics, and the assigned Pechenik's *A Short Guide to Writing about Biology* are resources to help you do well on the scientific writing exercises. We will practice two forms of this report: shortened and full.

Shortened Laboratory Report

For some of the laboratories in BIOL366, we will be using a shortened version of the laboratory report. This will let you focus on developing your writing and reporting skills on the Results and Discussion sections, and on developing appropriate tables and figures for the reports. For the Shortened Laboratory Reports, follow these additional guidelines:

- The **Introduction** will be limited to only a single, short paragraph that introduces the laboratory objectives and the hypotheses being tested.
- The **Methods** section will primarily be a simple citation to the laboratory manual as follows: "We used the methods specified in the Ecology Laboratory Manual (Borrett, 2016)". If you need to make deviations from the specified manual, then you can also specify these. There is little point in you re-writing the methods already specified in the lab manual.

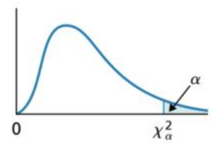
Full Laboratory Report

For the multi-week Forest Ecology Laboratory, you will write a full laboratory report following the department Laboratory Report Format guidelines. You will be responsible for developing all sections of the report. For this report, you are also required to appropriately cite at least **five** sources from the *primary literature* (peer-reviewed journal articles). In keeping with Writing Intensive courses at UNCW, you will initially turn in a draft report. This will be reviewed by your instructor and returned to you with feedback. You will then have an opportunity to make necessary changes before turning in a revised report.

APPENDIX B: CHI-SQUARE TABLE

What follows is a slightly modified portion of a chi-square table published online by Pearson (2012, http://media.pearsoncmg.com/aw/aw_weiss_introstats_9/cw/tables/Values_of_chi-square_alpha_II.pdf). The critical χ^2 value is indicated when the degrees of freedom (df) are 1 and the decision criterion (α) is 0.05.

Detailed chi-square table
Values of χ^2_α II



$\alpha = 0.05$

df	$\chi^2_{0.25}$	$\chi^2_{0.20}$	$\chi^2_{0.15}$	$\chi^2_{0.10}$	$\chi^2_{0.05}$	$\chi^2_{0.025}$	$\chi^2_{0.02}$	$\chi^2_{0.01}$	$\chi^2_{0.005}$	$\chi^2_{0.0025}$	$\chi^2_{0.001}$	$\chi^2_{0.0005}$
1	1.323	1.642	2.072	2.706	3.841	5.024	5.412	6.635	7.879	9.141	10.828	12.116
2	2.773	3.219	3.794	4.605	5.991	7.378	7.824	9.210	10.597	11.983	13.816	15.202
3	4.108	4.642	5.317	6.251	7.815	9.348	9.837	11.345	12.838	14.320	16.266	17.730
4	5.385	5.989	6.745	7.779	9.488	11.143	11.668	13.277	14.860	16.424	18.467	19.997
5	6.626	7.289	8.115	9.236	11.070	12.833	13.388	15.086	16.750	18.386	20.515	22.105
6	7.841	8.558	9.446	10.645	12.592	14.449	15.033	16.812	18.548	20.249	22.458	24.103
7	9.037	9.803	10.748	12.017	14.067	16.013	16.622	18.475	20.278	22.040	24.322	26.018
8	10.219	11.030	12.027	13.362	15.507	17.535	18.168	20.090	21.955	23.774	26.124	27.868
9	11.389	12.242	13.288	14.684	16.919	19.023	19.679	21.666	23.589	25.462	27.877	29.666

Pearson Education, Inc. 2012
http://media.pearsoncmg.com/aw/aw_weiss_introstats_9/cw/tables/ Values_of_chi-square_alpha_II.pdf