General Ecology Laboratory Manual

BIOL 347

By

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First Edition 2006

Ecology Laboratory Safety Rules

"Leaves of three, let it be."

1. Cuts and scratches must be covered with Band-Aids. Disposable gloves will be provided on request.

2. All accidents, cuts, and any damaged glassware or equipment should be reported to the lab instructor immediately.

3. Long hair should be tied back while in lab.

4. Stereoscopes and other instruments are to be cared for as directed by the instructor.

5. It is the responsibility of the student to know the location and use of all safety equipment in the lab (eyewash, fire extinguisher, etc.)

6. Doors and windows are to be kept closed at all times.

7. For the best lab experience, read labs before coming to class. Make notes as necessary. Wait for a laboratory introduction by the instructor before starting work.

8. Be aware of poison ivy in the field and check for ticks upon your return.

9. Be prepared for inclement weather.

Limnology

Objectives

After completing this exercise you should be able to:

1. Use the following field equipment:

Seine net	Trawl net
Secchi disk	Oxygen meter
"Kemmerer" Bottle	Sounding line

2. Describe the sample sites using the highlighted terms.

Introduction

In this laboratory exercise we will be using various surveying techniques in order to understand some fundamental ecological phenomena associated with lakes. The study of lakes is referred to as Limnology, We will be sampling two lakes both in the Glacial Lake Souris basin: Buffalo Lodge Lake, just northeast of Granville and Lake Darling, northwest of Minot. Lake formation likely differs in each case (Bluemle, 1991), and may have consequent effects on limnological features, e.g., dissolved oxygen, productivity, and fauna.

Buffalo Lodge Lake (Appendix A) is probably an "ice-thrust feature." Giving the proper geology shortshrift, what this means is that during recent periods of glaciation, subglacier pressure conditions caused an enormous scooping phenomenon wherein a large amount of sediment is scooped up and deposited a short distance away. Many ice-thrust features thus are characterized by a lake set near to a relatively large hill (or small number of hills). Buffalo Lodge is the hill, and the pond that resulted from the ice-thrust is Buffalo Lodge Lake. Water enters Buffalo Lodge Lake in two ways: precipitation and stream flow from South Egg Creek.

Lake Darling (Appendix A) is a man-made lake within the Upper Souris National Wildlife Refuge.

What are we interested in? The hypothesis that we are testing is a classic one: that the different lake formation patterns and management practices have resulted in differences in present-day lake characteristics (Wetzel, 1985). (Of course, stocking of lakes with fish presents deviation from the natural condition; these lakes are stocked on occasion but are not actively managed in the same fashion as Lake Darling or Lake Sakakawea.) Along the way, we also gain exposure to some classical sampling techniques, e.g., trawl nets, seines, Secchi Disk use, dissolved oxygen titrations.

Thus, once again we will be performing a survey, and using *a posteriori* (i.e., after the fact) analyses to suggest hypotheses that could be tested experimentally. We will be collecting the following set of data from each lake for comparison:

Materials

Waders (24)
Oxygen meter
Secchi disk
Sounding Rope
Kemmer bottle
Seine Nets (say-ins)
Trawl Nets
Thermometers
Refractometer

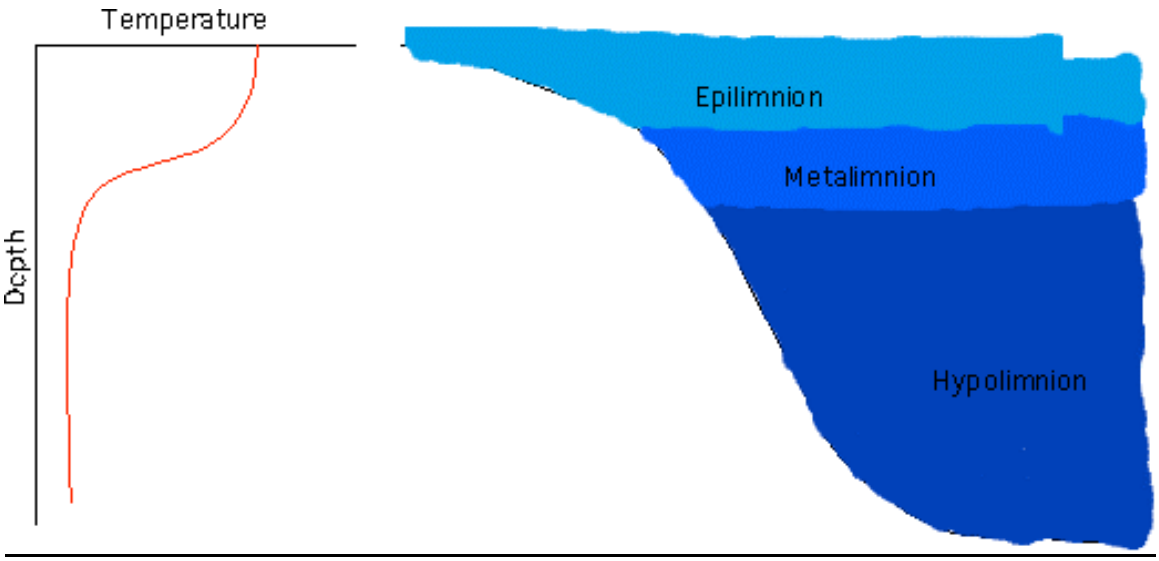
Collections jars
pH meter
disposable pipets
White tubs like autoclave tubs
Alcohol
GPS unit

Procedures

Measurements of Physical Parameters

Depth profiles: A fundamental feature associated with the geological origin of a lake is the shape of the basin. Natural lakes in North Dakota, such as Buffalo Lodge Lake, are essentially round relative to a man-made Lake, such as Lake Darling, a long lake. The way in which they can vary is in depth profile, i.e., is the lake deep or simply a very large flat puddle? Use a weight attached to a sounding line which has been marked off in meters to determine a depth profile across each lake.

Temperature profiles: Because cold water in more dense than cold water and therefore sinks to the bottom of lakes, thermal stratification can result. For example, in the midst of summer, there is often a distinct warm layer of water at the surface of a lake called the ***epilimnion*** that overlays the much colder deeper water called the ***hypolimnion***. The transitional zone between the two layers is sometimes referred to as the ***metalimnion*** and is marked by a drop in temperature. This drop in temperature can be quite rapid over the span of a couple of meters or more gradual. The transition in a physical parameter is referred to generically as a ***cline***; in this case a ***thermocline***. Water temperature is an important determinant of lake flora and fauna. Temperature also affects the solubility of dissolved gases, including oxygen. Not all lakes are stratified. Are the lakes that we are sampling stratified?



Dissolved Oxygen Profiles: Aside from water itself, oxygen is the most fundamental parameter in lakes. The oxygen dissolved in lakes has three primary sources. The first two are **allochthonous** sources – sources from outside of the lake. These are oxygen carried in by streams and rivers and atmospheric

oxygen introduced by wind driven mixing. The third source is autochthonous – from inside the lake – and is a result of photosynthesis by plants, algae and cyanobacteria. Oxygen may be an indicator of **primary productivity**. Not only does it indicate the productivity of the lake, but the amount of dissolved oxygen in water further affects the solubility of many other inorganic nutrients. As noted above the water temperature, in addition to mixing, effects oxygen solubility. It is not unusually for the water at the bottom of the hypolimnion to become ***hypoxic*** (little oxygen) or ***anoxic*** (no oxygen) as microbes consume the rich organic matter littering the bottom and deplete the surrounding oxygen in the process.

Using an oxygen meter establish an oxygen profile at the deepest sampling site.

Measurements of Productivity

Transparency: The Secchi disk measures transparency of the water. A limnologist is usually interested in using the Secchi depth to estimate the amount of phytoplankton, zooplankton and bacteria in the water. These organisms affect the penetration of light into the water and therefore, the Secchi depth. Light entering the water will be either absorbed or scattered by particles, dissolved colored matter, and the water itself. As the attenuation of light by dissolved colored matter or particles increases, the Secchi depth decreases. This inverse relationship produces the typical hyperbolic curve when Secchi depth is plotted against potential attenuating substances, such as algal chlorophyll, color, turbidity, or suspended solids. Secchi depths are typically used as surrogate measures of chlorophyll or planktonic biomass, and subsequently, as an indicator of the trophic state of the lake. The definition of trophic state may vary, but chlorophyll is often assumed to be a major indicator of trophic state. In theory, chlorophyll should be able to be estimated from Secchi depth. Secchi depth, therefore, should be able to be used as a surrogate estimator of algal abundance, either by producing empirical relationships between Secchi depth and chlorophyll or by deriving the chlorophyll concentration based on the theoretical relationship between transparency and chlorophyll. We will define the trophic status of our lakes based on the following Secchi depths:

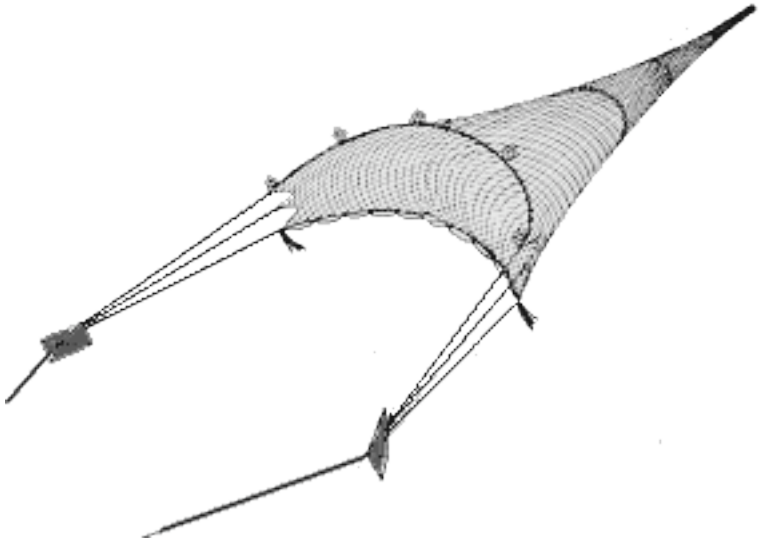
0-1.0 meters = Eutrophic (very murky and high in nutrients. Like most ponds)
1.1-7.9 meters = Mesotrophic
>8.0 meters = Oligotrophic (very clear, few nutrients. Crater Lake in Oregon has the record for highest reading of a Secchi Disk in the US at 39 meters)

To use a Secchi disk attach a weight to the white side and a sounding line to the side with the black and white quadrants. Lower the disk on the sunny side of the boat. Allow sufficient time (approximately 2 min) when looking at the disk near its extinction point for the eyes to adapt completely to the prevailing luminance level. Record the depth at which the disk disappears. Slowly raise the disk and record it depth of reappearance. The Secchi depth is the average of the depth of disappearance and reappearance (Davies-Colley, 1993).

Species Diversity and Abundance: Productivity can also be evaluated in a more qualitative way by assessing the relative abundance of plankton (both phyto- and zoo-), macroinvertebrates and fish. While the Secchi depth gives an indication of the abundance of organisms at the bottom of the food chain direct counts of plankton, macroinvertebrates and fish yield an estimate of the productivity at the top of the food chain.

A seine net is used to determine invertebrate and fish abundance near the shoreline. Operating a seine requires two people, one on each side of the net, with the weights oriented towards the bottom. When dragging the area keep the bottom of the net as close to the lake bottom as possible. empty the contents of the net into white bowls and count the number of different species of invertebrates and fish as possible. Determine the abundance of each species.

A trawl net is used to determine invertebrate and fish abundance in the lake interior. Operating the trawl net requires three people, two holding a rope attached to the doors or planes on each side of the net, with the weights oriented towards the bottom and the third driving the boat. Allow the net to reel out approximately 3 meters of rope. Trawl the lake for several minutes and quickly pull in the net. Empty the contents of the net into white bowls and count the number of different species of invertebrates and fish as possible. Determine the abundance of each species.



Water samples will be taken at various depths with a variant of the Kemmerer Bottle.

Results

At the end of this lab you will be asked to write a scientific paper based on your results from your surveys of these two lakes. You will be writing and submitting this paper as a group. The final version of this paper will be submitted to the U.S. Fish and Wildlife Service.

While collecting the data keep in mind that you will ultimately want to discuss the relationship between temperature and oxygen in each of the two lakes. You will also want to discuss the relationship between oxygen and PP in each lake, as well as contrasting the two lakes.

The following data sheets should help organize your data. These sheets may not include all the data necessary for your paper.

Lake name: _____

Station No.	GPS position	Depth (m)

Station No. _____

Secchi Disk:	reading #1	reading #2	reading #3
visible:			
not visible			

Profile: Depth	Temp. (°C)	Oxygen (ppm)

Species:	Species No,	No. Indiv.	Species No,	No. Indiv.

Station No. _____

Secchi Disk:	reading #1	reading #2	reading #3
visible:			
not visible			

Profile: Depth	Temp. (°C)	Oxygen (ppm)

Species: Species No,	No. Indiv.	Species No,	No. Indiv.

Lake name: _____

Station No.	GPS position	Depth (m)

Station No. _____

Secchi Disk:	reading #1	reading #2	reading #3
visible:			
not visible			

Profile: Depth	Temp. (°C)	Oxygen (ppm)

Species:	Species No,	No. Indiv.	Species No,	No. Indiv.

Station No. _____

Secchi Disk:	reading #1	reading #2	reading #3
visible:			
not visible			

Profile: Depth	Temp. (°C)	Oxygen (ppm)

Species: Species No,	No. Indiv.	Species No,	No. Indiv.

Literature cited

Bluemle, J. P. 1991. The Face of North Dakota, revised ed. Education series 21, North Dakota Geological Survey, Bismarck, ND.

Wetzel, R. 1983. Limnology, 2nd edition. Saunders College Publishing, Philadelphia, PA.

Davies-Colley, R.J, W.N. Vant, and D.G. Smith. 1993. Colour and Clarity of Natural Waters.

Writing a Scientific Paper

Introduction

In large part this class is about "doing" ecology. An integral part of any scientific endeavor is the dissemination of new knowledge. To that end you will be writing two peer-reviewed scientific papers, one on the limnology lab that you just completed and a second the terrestrial lab that you will conduct in the following weeks. This is the same process to which all active scientists submit. In this case the peer group reviewing your paper will be your classmates. The process will be broken three parts, submitting a paper and reviewing papers and revision of your paper.

Writing a Scientific Paper

A well-written scientific paper explains the scientist's motivation for doing an experiment, the experimental design and execution, and the meaning of the results. Scientific papers are written in a style that is exceedingly clear and concise. Their purpose is to inform an audience of other scientists about an important issue and to document the particular approach they used to investigate that issue. Clear, concise, proper English grammar is critical in science writing.

Most scientific papers have multiple co-authors. The first paper you will write will be a collaborative effort of all the individuals in your group. The group will submit a single paper with each group member listed as a co-author. Since the intent of this exercise is to mimic the real submission process we will assume that you have chosen to submit your article to the Journal of Ecology. Most modern submission processes are done electronically. Below is a link to the journal website where you will submit your article.

http://www.blackwellpublishing.com/journal.asp?ref=0022-0477&site=24

In the column to the right of the website is a link to "Author Guidelines". All scientific journals have a set of guidelines that detail which topics the journal accepts and the specific format that they expect the authors to follow. The cardinal rule in getting a manuscript published is to follow guidelines exactly! Follow the "Author Guidelines" link and read through the format requirements for a STANDARD PAPER. It may be helpful to print out this page from the website. Below I have provided an expanded explanation of each of the sections. You will also find an example of a **manuscript** (an unpublished article) in Appendix B. The manuscript in this appendix B is what your manuscript should look like before submission. Reading scientific papers (such as the articles you will use as your references for the Introduction and Discussion) and those we have discussed in class will give you good ideas and guidance as well. After all, these are peer-reviewed and published scientific papers, and they can serve as useful models for your own writing.

All scientific papers are written in the past tense. For example, you would not write: "fish mass **is** related to the trophic status of the lake". You would write: "fish mass **was** related to the trophic status of the lake."

The following sections should be included in your report:

- Title
- Abstract
- Introduction
- Materials and methods
- Results
- Discussion
- References

Title

The title should be succinct and convey exactly what you have studied. The title is often your first and only chance to grab a reader's attention.

Abstract

An abstract is a condensed version of the paper. A reader should be able to quickly ascertain the purpose and significance of your results, as well as, get a quick overview of your methods.

Often readers will only read the abstract, choosing to read at length those papers that are most interesting to them. For this reason, and because abstracts are frequently made available to scientists by various computer abstracting services, this section should be written carefully and succinctly to have the greatest impact in as few words as possible. The abstract is usually limited to a specific word count by the journal and is generally less than one double spaced page. Although it appears as the first section in a paper, most scientists write the abstract section last.

Introduction

This section identifies the issue that you are studying, what you hope to answer in the study, and the significance of your study.

This section also introduces your study in the context of previously published studies, to help explain why the current study is of scientific interest.

The Introduction generally moves from general information to specific information. This background must be summarized succinctly, but it should not be itemized. Emphasize your specific contribution to the topic. All references to previous studies should be properly documented in the format prescribed by the journal to which you plan to submit your manuscript. The introduction should end with a purpose statement (sometimes in the form of a hypothesis or null hypothesis): one sentence which specifically states the question your experiment was designed to answer.

This will also serve as a transition to the Materials and Methods section in which you will explain how you proceeded to meet your objectives and test your hypotheses.

The Introduction is usually around two double spaced pages in length.

Materials and Methods

This section provides all the methodological details necessary for another scientist to duplicate your work. Remember one of the essential features of science is reproducibility. This should be a narrative of the steps you took in your experiment or study and not a list of instructions such as you might find in a cookbook. You should assume that other scientists have the same basic skills that you have, but do not know the specific details of your experiment. For example, it is unnecessary to write:

"We lowered the Secchi disk on the sunny side of the boat and lowered it until it disappeared."

Rather, you should assume that the scientist is familiar with operating a Secchi disk and simply write:

"Water transparency was recorded using a Secchi disk."

An important part of writing a scientific paper is deciding what bits of information needs to be given in detail. The manufacturer of all equipment and the state in which the manufacturer resides should be enclosed in parentheses. Do not quote or cite your laboratory manual!

In the last paragraph, should provide a brief description of statistical tests you used (statistics are methods!). Be sure not to include extraneous information, though, as scientists know all about null hypotheses and when to reject them.

Although this section can be tedious to write due to the number of details, it is often intellectually the easiest section to write. **This is a good place to start writing the paper and often allows you to overcome the inertia involved when you sit down to write.**

Results

This section presents your observations and data but does not attempt to interpret their meaning. This is often hard for students because there is a tendency to include discussion within this section. You will not present the raw data that you collected, but rather you will summarize the data with text, tables and/or figures. Use the text of the paper to state the results of your study, then refer the reader to a table or figure where they can see the data for themselves. For example you may write:

"The average fish mass increased significantly ($p<0.05$) with depth regardless of the trophic status of the lake (Table 1)."

The sentence above is well written because: (i) the relationship between mass, depth and trophic status is stated concisely, (ii) the word significantly is accompanied by the statistical probability level ($p<0.05$), and (iii) the reader is referred to a table where the data to support the statement can be found.

Do not include the same data in both a table and a figure. Each table and figure has several lines of text in the legend (or caption) that explain the information that is being presented; this is, they are made to stand alone. A table's legend appears above it, while the legend for a figure appears below the figure. If your table includes the results of a statistical analysis, be sure to provide the information necessary for the reader to properly evaluate the analysis (probability levels, degrees of freedom, sample size, etc.). Tables and figures should be numbered separately (ie, Table 1, Table 2, Figure 1, etc). You must refer in the text to each figure or table you include in your paper.

The results section should be the longest part of your paper and generally covers anywhere from 2 to 10 pages of double spaced text excluding the figures and tables. Tables, figure legends, and figures are placed at the end of the manuscript, after the references.

Discussion

In the discussion section you explain what your results mean, why or how they differ from what other workers have found, and the significance of your findings.

Your results should be interpreted in light of other published results. Previously published results should be properly cited following the format required by the journal to which you are submitting. Be sure to avoid plagiarism, however. One useful way to avoid making errors in this regard is to read a section from your source, then restate in writing what you remember of the main points. You would then cite the source of that information in the text.

Relate your discussion back to the objectives and questions you raised in the Introduction section. However, do not simply re-state the objectives. Make statements that synthesize all the evidence (including previous work and the current work).

At this point it may be necessary to note problems with the methods and explain anomalies in the data. Do not simply list the problems but provide thoughtful discussion about the implications of the errors in terms of your conclusions.

Be conservative in your interpretations and avoiding drawing conclusions not clearly supported by your results. Some amount of speculation is acceptable but make it clear that it is just that – speculation.

Finally, you may want to suggest future directions for research, new methods, explanations for deviations from previously published results, etc.

References

This is the last section of the paper. Here you should provide a listing of all the published work you cited in the text of the paper. This does not mean every article you found in your research; only include the works you actually cited in the text of your paper. Be sure and follow the format prescribed by the journal to which you are submitting your manuscript. You should have a minimum of five references from the primary literature (meaning journal articles).

How do you find references? One source is google scholar (http://scholar.google.com/). Simply enter a keyword(s) related to your research topic and scan the titles and abstracts for those that seem most relevant. For example, in our case you may enter the keywords: "trophic status oxygen concentration".

You may find the help and advanced features, to the right of the search button, helpful. Pubmed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) provides a similar search engine. Many journals now have a moving window that allows free access to articles older than six months to a year. You may have to follow a link or two the journal's website in order to download the article.

If you have access to Endnote it will be a big help in organizing your references.

Submitting a Scientific Paper

A few steps are required before you submit our manuscript. First, each page and line of the manuscript should be numbered so that reviewers can easily convey to which paragraph, sentence and word they are referring. To add line numbers to your Word document do the following:

1. Switch to page layout view.
2. Click Select All on the Edit menu.
3. On the Format menu, click Document, and then click the Layout tab.
4. If you're adding line numbers to part of a document, click Selected text in the Apply to box.
5. Click Line Numbers.
6. Select the Add line numbering check box, and then select the options you want.

To add page numbers, simply choose Page Numbers from the Insert menu.

Typically, the references section is followed by any tables (with headings), a figure legends page, and finally each figure on a separate page. The journal to which we have chosen to submit our manuscript does permit the figures and corresponding legends to be embedded in the document rather than at the end. You may adopt either format.

Figures must be submitted as either tiffs, jpegs or gifs.

Finally, email your Word document to me at: paul.lepp@minotstateu.edu. I will convert your document to a pdf and distribute it to the reviewers.

Reviewing a Scientific Paper

Most requests to review a manuscript are accompanied by a one page sheet that asks you to briefly summarize your impression of the paper. This allows the editor to quickly decided which of the dozens to hundreds of papers they receive each week to publish. Generally, reviewers are given three options with regards to the recommendation of the manuscript:

1) accept as is, without revisions.
2) accept with revisions.
3) reject.

Appendix C contains the form which you must complete on your assigned manuscript and return to the instructor.

This short summary form is not very helpful to the submitting authors, however. The summary is usually accompanied by a more detailed review of the manuscript. The goal of the peer review process is to improve the quality of the manuscript in terms of the science and the "readability". This goal may be accomplished by returning the edited (using a red pen) manuscript to the authors or a list of proposed improvements such as the example in appendix D. The anonymous reviews will be returned to the authors so that they may revise their manuscript.

When reviewing a manuscript please adopt a positive, impartial, but critical attitude toward the manuscript under review, with the aim of promoting effective, accurate, and relevant scientific communication.

You are not required to correct deficiencies of style, syntax, or grammar, but any help you can give in clarifying meaning will be appreciated. In particular, point out the use of scientific jargon, misspellings of chemical names, use of outmoded terminology or incorrect genetic nomenclature, and use of misspelled, incorrect, or outdated scientific names of organisms.

Your criticisms, arguments, and suggestions concerning the paper will be most useful if they are carefully documented. Do not make dismissive statements. Substantiate your statements. Reviewer's recommendations are gratefully received by the editor; however, since editorial decisions are usually based on evaluations derived from several sources, reviewers should not expect the editor to honor every recommendation. You will be asked to suggest acceptability as noted on the specific review form (e.g., accept; accept with revision; reject;).

Please remember that very few papers qualify for an immediate, unconditional acceptance.

There are many reasons to reject a paper. In general, if there are serious flaws in experimental design, incorrect interpretation of data, extensive additional experiments required, or any organizational or English usage flaws that prevent critical review of the manuscript, then recommend that the manuscript be rejected.

Terrestrial Community Ecology - Quadrat Sampling

Objectives

After completing this exercise you should be able to:

1. Estimate species abundance and co-occurance

Introduction

The principal activity of **community ecology** is to estimate species abundance and diversity, determine how many species coexist and elucidate the processes that govern this coexistence of species. The first step in examining the interactions of species is to test the hypothesis that they interact at all, i.e., testing the null hypothesis that two species are distributed independently. We will test this null hypothesis for four species of plants.

This lab exposes you to **quadrat sampling**. This sampling technique essentially consists of laying a quadrat (an grid of any shape/size) on a predetermined location or a randomly or arbitrarily (our case) chosen location and assessing various parameters, e.g., number of species, species diversity, and biomass. We will examine the distributions of six plant species in a prairie meadow that consists of several subcommunities. The six that we will survey are:

Medicago sp. (purple alfalfa)
Atemisia spp. (sagebrush)
Symphoricarpos occidentalis (buckbrush)
Solidag spp.(goldenrod)

Excellent descriptions of these plants may be found at *http://www.ext.nodak.edu/extpubs/ansci/range/eb69-2.htm*. We are going to use a 0.8 m diameter quadrat to assess the likelihood that two species are found together, apart, or have no relationship at all.

Materials

Hula hoops
GPS unit
Clip boards

Procedures

The class will be divided into pairs. Each pair will be given a 0.5 m diameter hula hoop. You will conduct 40 quadrat surveys per pair. "Randomly" toss your quadrat several (6-20) feet. Examine the sample contained within the margins of the quadrat. Count the number of each of the four species within the quadrat. After this, simply pick up the quadrat and toss it several feet again. Repeat the other steps. Repeat the entire procedure 40 times. This activity should take approximately 30-45 minutes.

Results

The data collected in part 1 will be combined with the data from part 2 and submitted as a lab report. You will use the same format that you used in presenting the limnology data. As with the limnology data you will be submitting a paper as a group. The final paper will again be filed with the U.S. Fish and Wildlife Service.

Use the table below to record your sampling.

Quad.	*M. alba*	*Grindelia*	*Aster*	*Medicago*		Quad.	*M. alba*	*Grindelia*	*Aster*	*Medicago*
1						21				
2						22				
3						23				
4						24				
5						25				
6						26				
7						27				
8						28				
9						29				
10						30				
11						31				
12						32				
13						33				
14						34				
15						35				
16						36				
17						37				
18						38				
19						39				
20						40				

From these data you will want to determine the mean abundance of each species per square meter of the sample site. You will also want to determine the standard deviation. What is the importance of the standard deviations in this context? Do they give you any clue to patchiness or evenness?

Next you will determine the whether two coexist for reasons other than random chance. To do this you will create a **matrix** based on the presence or absence of one species relative to another. Record the number of quadrats in which both species were either present, one or other of the species was absent or both species were absent in the upper left-hand triangle of each box. These are your **observed (O)** results. Place the sum of each column and row in their respective total cells. The lower right-hand corner should contain the sum of the column above it or the row to the left (they should sum to the same value). If you have had a genetics class the chi-square (χ^2) test may sound familiar. The chi-square test is the most commonly used non-parametric (meaning you don't need to know all the parameters effect the distribution of the variable) statistical test. To calculate the chi-square statistic (χ^2) you need to your **expected (E)** distribution. Calculate the expected distribution for each cell by multiplying each row total by each column total and dividing by the overall total from the lower right-hand corner. Place this value in the lower right-hand triangle of each cell (see example).

Grindelia

Melilotus alba	present	absent	total
present			
absent			
total			

Medicago

Melilotus alba	present	absent	total
present			
absent			
total			

Grindelia

Aster	present	absent	total
present			
absent			
total			

Aster

Melilotus alba	present	absent	total
present			
absent			
total			

Grindelia

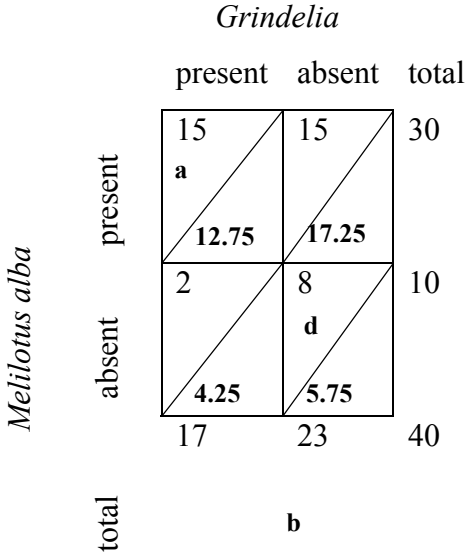
Medicago	present	absent	total
present			
absent			
total			

Medicago

Aster	present	absent	total
present			
absent			
total			

Example,

(30*17)/40=12.75
(30*23)/40=17.25
(17*10)/40=4.25
(23*10)/40=5.75



To ascertain whether two species positively or negatively interact we will calculate a value we call the "index of association":

$$S = \frac{ad - bc}{\sqrt{(a+b)(c+d)(b+d)(a+c)}}$$ **c**

This value **S** will be negative or positive (or zero). A **S** of '0' indicates that the distribtution of species 1 is independent of the distribution of species 2 (and vice versa). If **S** does not equal zero, then we must conduct a statistical test to determine if **S** is statistically different from zero. For example, while we all would agree that a **S** of 0.00001 is not terribly important, we don't know what to do with a **S** of 0.33. As an alternative to arm-wrestling or a fistfight, the statistical test gives us an objective way to address this question.

Determine the χ^2 statistic for each **pair** of species using the following formula:

$$\chi^2 = \sum \frac{(0-E)^2}{E}$$

For example, $\chi^2 = \frac{(15-12.75)^2}{12.75} + \frac{(15-17.25)^2}{17.25} + \frac{(2-4.25)^2}{4.25} + \frac{(8-5.75)^2}{5.75}$

A χ^2 value greater than 3.84 is significant with a corresponding p-value less than 0.05 (our analysis has one degree of freedom).

The chi-square analysis will only tell you if your results are significantly different than what you might expect to find in a comparison of randomly distributed populations. It will not tell you if two species more likely to be found together or separately.

Can you hypothesize what factor may result in two species co-occuring or not?

Terrestrial Community Ecology - Transect Sampling

Objectives

After completing this exercise you should be able to:

1. Describe the sample sites using the highlighted terms.
2. Employ several sampling techniques to estimate species diversity..

Introduction

In this laboratory exercise we will be using a classical surveying technique called **transect sampling** to document different patterns in species diversity. We will be using this technique to explore various hypotheses concerning the generation and maintenance of species diversity. We are choosing to sample the arthropods (i.e., spiders and insects) and plants of a riparian meadow of the Glacial Lake Souris Basin. We could sample any of the flora (plants, fungi, bacteria, etc.) and fauna (animals) that exist here, but choose arthropods and plants for their tractability (i.e., we can count 'em and/or catch 'em).

PRAIRIES, FIRES AND SUCCESSION

The prairies of central North America contain ecosystems that are -- under natural circumstances -- dominated by fire. Grasses and other relatively small plants dominate these ecosystems because they are fire-adapted, that is, they place most of their energy in underground root systems and they can recover quickly after the above-ground portions of the plants are burned to the ground (mowing has a similar effect in many ways). In the absence of fire, grasses are eventually shaded out by shrubs and trees, and unless fires continue to occur, this type of grassland ecosystem disappears. On North Dakota farms, natural fires (initiated usually by lightning) are suppressed, with the result that shrubs often dominate certain areas. These areas are ideal places to study **secondary succession: the replacement (one group "succeeding" another) of one community of plants and animals by another**.

The questions that you should keep in mind are: (1) Is there a difference in species diversity between the center of a early and late succession riparian meadow (the area most affected by the succession) and the farming-dominated edge (where succession is in earlier stages); (2) Are there differences in the species found in each place; and (3) What are some of the possible causes of differences in diversity and community-composition? See Appendix E.

To assist you in addressing question #3, I have outlined several prominent hypotheses concerning species diversity. Keep in mind that these are all hypotheses about **processes**, and that one or more of them may be sufficient to explain the **patterns** that we see in our survey. These hypotheses are outlined in greater detail in your text.

Time hypothesis: communities diversify as they age. Thus older communities have greater species diversity.

Intermediate disturbance hypothesis: areas that have experienced intermediate levels of disturbance should be more diverse.

Competition/climatic stability hypothesis: In more constant (e.g., consistently wetter) areas, competition is suggested to occur and may increase diversity as competing species occupy narrower niches.

Predation hypothesis: Predators act as a biological control on the most abundant herbivorous prey. Because the most abundant prey may be the best competitors, competition is reduced. This allows inferior competitors to persist, thus increasing diversity. (Testing this hypothesis requires that we be able to determine which arthropods are predators and which are herbivores)

Productivity hypothesis: As productivity (the amount of plant biomass produced per m^2) increases, this increase the available resources for other species in the food web. Thus high productivity should yield high diversity.

Bear in mind that these hypotheses are not mutually exclusive. This means one, all, or even none of them may serve to explain the patterns that we document.

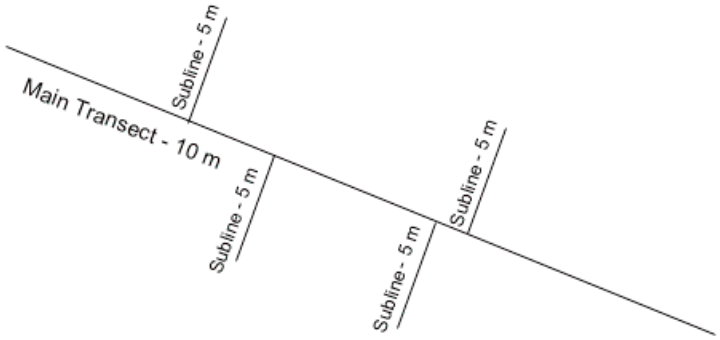
Materials

Transect line (twine), 35 meters
Wooden stakes
Utility Knives
Meter sticks
Bag o' chits
GPS unit
Clip boards
Collection jars
Alcohol
Sweep nets
Sorting dishes
Plant Keys
Arthropod Keys

Procedures

Because one cannot record every organism in a given region, one takes samples and assumes that these samples mirror the true diversity of that region. One can take quadrat samples (we did that in our last lab) or transect samples. A transect sample consist of a line or lines along which one records organisms. The placing of the transect line in an ecosystem is usually a combination of random sampling and specific placement (e.g., one of our transects needs to be through a shrub dominated area).

Each group will set up a transect line such that each group is sampling a different community. Each group will receive 35 meters of twine. Use 10 meters and stakes to setup the main transect line. Cut four 5 meter sublines. Draw a distance chit and a direction chit and tie-off the subline to the mainline at the distance and direction indicated. Repeat the process for the other three sublines. You should now have a transect line that looks similar to the figure below.



Each person in the class will be given the task of identifying one plant species along all of the transects. Each person will pick one plant, which we will later identify in the lab. We will not collect any

plants other than this single specimen. We will only count each occurrence of the specimen. To do this cut a one meter length of twine for each member of the group. To perform the plant sampling, one walks each subline and records every plant within 1 meter of the subline. Use the 1 meter length of twine held perpendicular to the subline to maintain the proper distance. Each person will walk both sides of each subline while recording the number of specimens of their species that fall within one meter of the line. (We could also record biomass, but that does not play a role in estimating species diversity.)

We will also collect arthropods (using sweepnetting) for later identification in the lab. Two members from each group will sweep the sublines. Place the arthropods in the collection container containing 70% ethanol, which acts as a preservative. We will return to the lab with our specimens and record the different species (and numbers of each) that we collected. This is a time-consuming exercise that may require non-lab time. We will use guides and keys to help us identify them. It will not be critical to name them correctly, but it will be essential that we all give the same organisms the same names. (The species diversity indices don't care what you find. They only care about how many of each.)

It will be enlightening if we are able to determine which species are predators and which are simply herbivores. Using the videotape "Backyard Bugs" -- admittedly a bit below your superior intellectual capacities -- will help in considering the importance of different groups of arthropods in our transects.

To assist you in making sure that each critter gets a different names, use the following lists and links:

http://www.kendall-bioresearch.co.uk/key.htm
http://caplter.asu.edu/explorers/protocol/arthropods/key.htm#Anchor-42251

Phylum Arthropoda (meaning "jointed foot")
- **Subphylum Chelicerata**
  - **Class Arachnida** -- body has 1-2 segments, four pairs of legs, no antennae
    - **Order Araneae** -- the spiders
    - **Order Opiliones** -- daddy-long-legs
    - **Order Acari** -- mites and ticks
- **Subphylum Crustacea** -- potato bugs, pill bugs (and also crayfish, lobsters, etc.)
- **Subphylum Uniramia**
  - **Class Chilopoda** -- centipedes, one pairs of legs per body segment
  - **Class Diplopoda** -- millipedes, two pairs of legs per body segment
  - **Class Insecta** -- insects, three body segments, three pairs of legs
    - **Order Orthoptera** -- grasshoppers and crickets
    - **Order Odonata** -- dragonflies and damselflies
    - **Order Isoptera** -- termites
    - **Order Hymenoptera** -- bees, wasps
    - **Order Diptera** -- flies
    - **Order Hemiptera** -- true bugs, have mouth parts modified for sucking
    - **Order Homoptera** -- cicadas, leafhoppers, aphids
    - **Order Neuroptera** -- lacewings
    - **Order Coleoptera** -- beetles
    - **Order Lepidoptera** -- butterflies and moths
    - **Order Siphonaptera** -- fleas

There are other many classes of insects, but they will be less common.

Results

As in quadrat sampling you will want to calculate the mean density of each species per square meter and SD assuming you have five independent samples (one for each subline). How many square meters did you cover? It will take some simple geometry to determine this. Create a type written list of taxa and the corresponding number of individuals in each taxon from your sample site. Turn this list in to me. I will make copies of each list and each group will get a copy of the data. This will allow each group to compare the species diversity in each of the different habitats.

Calculating Species Diversity

We will use two classical two classical methods of quantifying how many species (and how many individuals of each species) are found in a given area. Each one gives slightly different information. We will compare the effect of succession on diversity using the Shannon-Wiener diversity index (H') and the Species Evenness index (J'), which is just an extension of H'.

Method #1 -- the Shannon-Weiner index (H')
The formula used to calculate this index is

$$H' = -\Sigma[p_i \times \log_n(p_i)]$$

This formula is the most commonly used index of species diversity. Its value can range from 0 to infinity, the higher values meaning one sample is more diverse than another. If H' = 0.0, then nothing was found in the sample. For example,

Taxon	abundance	Sample calculation proportion of total sample (p_i)
grasshopper	35	35 / 97 = .36
garden spiders	12	12 / 97 = .12
fruitflies	8	8 / 97 = .09
wombats	1	1 / 97 = .01
jaberwockies	41	41 / 97 = .42
Total	97	totaled=1.00

$$H' = -[(.36 \times \log_n.36) + (.12 \times \log_n.12) + (.09 \times \log_n.09) + (.01 \times \log_n.01) + (.42 \times \log_n.42)]$$

$$H' = -[(-.37) + (-.25) + (-.22) + (-.05) + (-.36)]$$

$$H' = -[-1.25]$$
$$H' = 1.25$$

Method #2 -- the species evenness index (J')
Once H' is calculated, the calculation of J' is trivial:

$$J' = H'/H'_{max}$$

H'_{max} is calculated by resetting the abundance of each species so that they are all equal.

Taxon	abundance	Sample calculation using the above data proportion of total sample (p_i)
grasshopper	35	35 / 175 = .2
garden spiders	35	35 / 175 = .2

fruitflies	35	35 / 175 = .2
wombats	35	35 / 175 = .2
jaberwockies	35	35 / 175 = .2
Total	175	totaled=1.00

Now calculate H'_{max} just as you would for H':

$$H' = -[(.2 \times \log_n .2) + (.2 \times \log_n .2) + (.2 \times \log_n .2) + (.2 \times \log_n .2) + (.2 \times \log_n .2)]$$

$$H' = -[(-.32) + (-.32) + (-.32) + (-.32) + (-.32)]$$

$$H' = -[-1.6]$$
$$H' = 1.6$$

Now, $J' = H'/H'_{max}$

$$J' = 1.25/1.6$$
$$J' = 0.78$$

The values for J' can range from 0 to 1. A value of 1 means that all species are equally abundant in your sample and that the sample has high evenness. A low value (near 0) means that your sample is dominated by a single species.

Two important notes to remember:

1. **$\log_n$** (meaning "the base of the natural logarithms") may be represented on your handheld calculator as **ln**.

2. A sample that has higher species diversity (H') than another sample does not necessarily have a higher species evenness (J').

The data you have gathered from part 2 should be combined with the data in part 1 and submitted as a single group report. Discuss your findings with respect to the hypotheses presented in the introduction. Provide tables and/or figures (i.e., graphs) that summarize the data on species diversity. Simply providing a list of species may not be the most efficient strategy for relating our diversity estimates to the hypotheses listed above. Our goal is to understand what processes may be responsible for generating the variation in diversity indices that we see in our transect sampling.

The following questions may help guide the development of your paper:

- Do any of the hypotheses outlined above (and expanded upon in the text) seem to match the patterns that we documented?
- Based on our data, is the diversity of arthropods in any way associated with the diversity of plants? Are there any arthropods that seemed to be associated with other groups of arthropods?
- Many prairies and pastures in North Dakota are intentionally burned every several years. Is this a "good" or "bad" idea in the context of prairie biodiversity? In the context of farming? In the context of natural resources (e.g., hunting and fishing)?

Again, keep in mind that other people will be reading the final draft of these paper so put in a little extra effort.

Multifactorial Experimental Design

Objectives

After completing this exercise you should be able to:

1. Design multifactorial experiments.
2. Carry out statistical analysis on multifactorial experiments.
3. Describe the effects of salinity and temperature on *Artemia* sp.

Introduction

In this laboratory exercise we will be using a more modern approach to document the effects of various processes that occur in communities. The approach uses a true experiment, that is, initial conditions are known and controlled and experimental and control conditions are established.

In addition, we will go beyond the typical experiment wherein one control is established and compare to the results of one experimental condition. This traditional approach is known as a single-factor experiment, i.e., only a single factor varies between the experimental and control conditions. We will manipulate several factors, and this approach is known as a multifactorial experiment. The advantage of such an approach is that it allows one to simultaneously evaluate several factors and also allows one to investigate how those manipulated factors can interact. Interaction is a complex phenomenon that can be difficult to understand. In essence, interaction between two factors means that knowledge of each factor alone will not give you the information that you need to understand the factors if they are present together.

Keeping in mind that Ecology is the study of the distribution and abundance of species, we are going to explore to cause-effect relationships of two abiotic factors (**salinity** and **temperature**) and one biotic factor (**density**) on survivorship of brine shrimp (*Artemia* sp.). Brine shrimp are oceanic, planktonic crustaceans that can be found in both shallow and deep waters. They can also be found in salty pools formed on land by ocean spray and flood. In addition, they can be found in large inland salt lakes, e.g., our animals come from the Great Salt Lake in Utah. They often reach extremely high densities, can experience a large variation of salinity regimes (e.g., the salty pools become saltier as evaporation occurs), and are often exposed to extreme thermal variation (e.g, as thermal pools warm and cool throughout a daily cycle). These very real and natural conditions can be mimicked in the lab. Brine shrimp are also very easy to hatch and culture in the lab. (Many of you know brine shrimp as "sea monkeys." You may also find it interesting that *Artemia* sperm cells are actually longer than the adult male that produces them!)

Procedure

We are conducting a three-way factorial experiment and analysis. Our statistical analysis will be a three-way analysis of variance (or ANOVA). The data that you will each collect and analyze will be % survival, measured simply by counting the number of live and dead brine shrimp in a sample drawn from each mesocosm (i.e., artificial population in a cup, bin, cage, etc.) and determining

(# live shrimp) / (total # counted in sample) = % survival.

We will use three salinity conditions, two densities and two temperatures. Because there are three factors, one with three levels and the other two each with two levels, there are 12 experimental conditions (3 X 2 X 2 = 12; see Fig. 1).

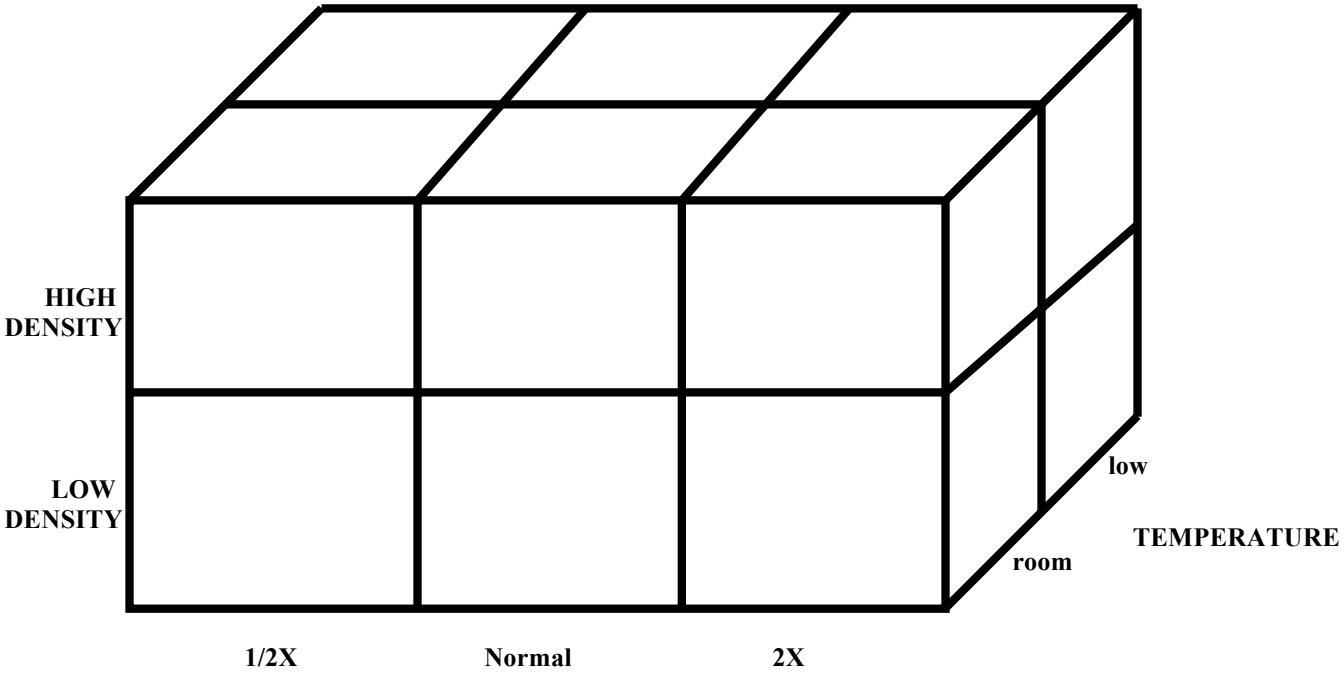


Fig. X. -- Diagrammatic representation of experimental design. There are three levels of salinity and two levels of temperature and density, thus 12 possible conditions (3 X 2 X 2 = 12). Because all possible combinations are used, the experiment is called a full-factorial design.

In other words, we will have twelve different conditions:

1) 1/2X ocean salinity, **low density**, low temperature
2) ocean salinity, **low density**, low temperature
3) ***2X ocean salinity***, **low density**, low temperature
4) 1/2X ocean salinity, **low density**, *high temperature*
5) ocean salinity, **low density**, *high temperature*
6) ***2X ocean salinity***, **low density**, *high temperature*
7) 1/2X ocean salinity, high density, low temperature
8) ocean salinity, high density, low temperature
9) ***2X ocean salinity***, high density, low temperature
10) 1/2X ocean salinity, high density, *high temperature*
11) ocean salinity, high density, *high temperature*
12) ***2X ocean salinity***, high density, *high temperature*

Each group will set up each of the 12 experimental conditions. Thus, each experimental condition will be replicated four times, thus a total of 48 containers with salt water and brine shrimp in them.

I will provide then assist your team with the spreadsheet on it for ease of data entry. Your team will all collect data and enter it onto a spreadsheet. I will then assist your team in the statistical analysis.

Data collection is accomplished simply by counting the number of living and dead animals in a 1 ml sample taken from the mesocosm. Use a graduated tranfer pipet to transfer 1ml of sample from the mesocosm to a petri dish. Count the number of living and dead shrimp. Repeat this process two more times. You should count three different samples from each mesocosm, resulting in 36 data points.

Results

As before, provide tables and/or figures that efficiently summarize the data. Make sure that you choose a method that best conveys the meaning contained in the data (i.e., the hypothesis tests).

As usual, support your responses with references to your section on data presentation. Answer the following questions.

1. Did any of the main effects result in significant effect on survivorship? Briefly (i.e., one-two sentences) what this means.

2. Were there any interaction terms that resulted in significant effects on survivorship? Briefly (i.e., one-two sentences) what this means.

3. Can you use these results to make any inference about what happens in nature? For example, what conditions are likely to provide ideal circumstance for brine shrimp survival? What conditions are most stringent and likely provide a selective filter for shrimp survival (i.e., intense natural selection on survival capabilities like physiological control of water balance)?

Demography

Of primary interest to ecologists and evolutionary biologists is demography. The study of demography includes documentation of average age at first reproduction, average age at death, mortality schedules (i.e., when individuals are likely to die), generation time, expectation of years of life remaining, and reproductive value. These values are critical fo understanding how natural selection will change a population under study (i.e., it addresses how a population will evolve, or how it has evolved). Notice that the key features concern birth and death rates, the same features that determine population growth.

This "field" exercise will expose you the demographic concepts found in Chapter 6 of Stiling (2002). In this exercise, we will construct a **life table** of the population of humans associated with the Minot area. To construct a life table, it is required that one obtains mortality estimates of many individuals, i.e., how old is an organism when it is likely to die? This is, at best, a daunting task in natural populations, and at worst an impossible one even in two 3-hour lab sessions. However, humans neatly keep mortality records in several forms, the primary one being the tombstones found in a cemetary. Every tombstone has the birthdate and deathdate of an individual, thus the lifespan is simply the difference between the two. Knowing only these lifespans (and given some information about fecundity), we can construct a very nice life table. An interpretation of this life table can provide some very meaningful information, e.g., when is death most likely during any individual's lifetime? (This bit of knowledge is obviously important for insurance companies when they calculate your life insurance premiums.)

(The whole point is that the principal aspect of organisms that is affected by natural selection is age at first reproduction [also called "age at maturation"]; natural selection also influences reproductive value.)

Before proceeding, you must be aware of the numerous terms that get used in this process:

STRAIGHTFORWARD DATA

x = age (we will use 5 yr increments)
l_x = proportion of population surviving to age x
m_x = age-specific fecundity (# of offspring at age x)

DERIVED STUFF

$l_x m_x$ = realized fecundity (how many offspring are actually born considering that many individuals die)
$x l_x m_x$= age weighted fecundity
E_x = expectation of future life
v_x = expected reproductive value from x forward.

$$v_x = \frac{\sum_{y=x}^{\infty} l_y m_y}{l_x R}$$

STUFF DERIVED FROM ENTIRE LIFE TABLE

GRR = gross reproductive rate (the summation [Σ]of all fecundity data)

R_o = net reproductive rate (the summation [Σ] of realized fecundity; equals 1 in a stable population, less than 1 in a shrinking population, and is greater than 1 in a growing population)

T = generation time (the summation $\Sigma x l_x m_x / R_0$); the time between generations.

(Please keep in mind that these symbols are only important in that they represent something; the actual symbol used doesn't matter. Stiling uses several different ones; I use these. Which are better? Doesn't matter. What does matter is what they represent, and both Stiling and I are representing the same thing.)

Procedure

1. Collect data on age for 100 individuals and record these data in the data sheet provided. Attempt for as little overlap among data collectors; we are attempting to record each individual only once.

2. Combine data from the entire lab. Record these data on your data sheet.

3. Fill in the remainder of the **life table**. I have already provided fecundity data (collected from another source of mortality and fecundity: obituary sections). Note that no indivuals reproduce prior to age 11, and none reproduce after age 65.

DATA SHEET

Age	Frequency
0-5	
6-10	
11-15	
16-20	
21-25	
26-30	
31-35	
36-40	
41-45	
46-50	
51-55	
56-60	
61-65	
66-70	
71-75	
76-80	
81-85	
86-90	
91-95	
96-100	
101-105	
106-110	

TABLE 1 – Life table for human population associated with Minot, North Dakota during the period 1850-2000.

x	l_x	q_x	m_x	$l_x m_x$	$x l_x m_x$	$x l_x m_x / R_0$	E_x	v_x
0-5								
6-10								
11-15			0.02					
16-20			0.15					
21-25			0.62					
26-30			0.51					
31-35			0.31					
36-40			0.19					
41-45			0.15					
46-50			0.11					
51-55			0.09					
56-60			0.08					
61-65			0.06					
66-70			0.01					
71-75								
76-80								
81-85								
86-90								
91-95								
96-100								
101-105								
>105								

GRR =
R_0
T

Species Competition

Note: This lab was taken from the article "Exploring the Lotka-Volterra Competition Model using Two Species of Parasitoid Wasps" by C.W. Beck, J.A. Guinan, L.S. Blumer and R.W. Matthews in the August 2004 issue of Teaching Issues and Experiments in Ecology.

Objectives

At the conclusion of this lab, students will be able to...

1. Describe the life cycle of *Nasonia vitripennis* and *Melittobia digitata*,
2. Explain the possible interactions between two parasite species competing for the same host resource,
3. Design an experiment to determine the nature of the interaction between these two species when competing for a common host,
4. Conduct a consensus experiment to determine the effects of intraspecific and interspecific competition on reproductive output in *Nasonia vitripennis* and *Melittobia digitata*,
5. Use the resulting data to estimate the parameters of the Lotka-Volterra competition model,
6. Relate class research outcomes to the principle of competition exclusion.

Introduction

In this investigation, we will examine the effects of competition for resources on reproductive output within and between two species of parasitoid wasps.

Ecological communities are composed of populations of all species in a habitat. The structure of a community will be determined in part by the dynamics of the interactions between the species in the community. Interactions between two species can be direct or indirect (i.e., mediated through other species).

In even a simple natural community, hundreds of different species of plants and animals interact with one another. In spite of this diversity, however, we can identify categories of interactions that have different effects on population growth (Table 1). The categories are defined by the direction of the effects on the interacting species.

In addition to interactions among species, interactions among individuals within a species can also be important in structuring a community. Within-species interactions can affect the population dynamics of the species, which in turn will influence interactions among species. *Intraspecific* competition occurs when different individuals of the same species or population compete for a resource. These interactions can be fierce because the individuals require the same limited resources to survive and reproduce. When different species are vying for the same food, habitat, or some other environmental resource it is called *interspecific* competition. These interactions are typically somewhat less intense. This is because while the requirements of two species might be similar, they can never be as close as they are for individuals of the same species.

Table 1. Categories Of Direct Interactions Between Two Species In The Same Community

Name of interaction	Type of contact	Direct effect on species #1	Direct effect on species #2	Other aspects of the relationship
Neutral relationship	Two species are linked only indirectly through interactions with other species.	0	0	Each species has a neutral relationship with most species in its habitat
Commensalism	A relationship that directly helps one species but does not affect the other much, if at all.	+	0	Commensalism, mutualism, and parasitism are all cases of symbiosis.
Mutualism	Benefits flow both ways between the interacting species.	+	+	Better viewed as two-way exploitation than as cozy cooperation.
Predation True predators Grazers	Predator attacks and feeds upon a series of prey but does not take up residence in or on them.	+	–	Prey generally dies. With grazers, plant might or might not die.
Parasitism Parasites Parasitoids	Parasite feeds on tissues of one or more hosts, residing in or on them for at least part of their life cycle.	+	–	A host might or might not die as a result of the interaction.
Interspecific competition	Disadvantages may flow both ways between species, or the superior competitor may be largely unaffected	–	–	Generally less intense than competition among members of the same species.

0 means no direct effect on population growth.
+ means positive effect; – means negative effect.

Consider, however, the theoretical case of two species that occupy the identical niche. Gause (1934) studied two protist species that both fed on the same bacterial cells. When he combined them in a single culture, one always drove the other to extinction. Many other experiments have since supported "Gause's Law," now called the *Principle of Competitive Exclusion*. It states that any two species that utilize identical resources cannot coexist indefinitely or "complete competitors cannot coexist" (Hardin 1960).

Many experiments have demonstrated that the more two species in a habitat differ in their resource use, the more likely it is that they can, in fact, coexist (Krebs 1994). Even two species with a great deal of overlap may live together for some time, although competitive interactions often suppress the growth rate of one or both of them. Over time, an interesting phenomenon called *resource partitioning* may occur. Members of each species may come to specialize in a subdivision of some category of similar resources. For example, if both feed upon apples, one may feed upon small green fruits and the other upon larger, riper ones.

The Lotka-Volterra model was developed to allow ecologists to predict the potential outcome when two species are in competition for the same resources. Basically, the model attempts to account for the effect that the presence of one species will have on the population growth of the other species, relative to the competitive effect that two members of the same species would have on each other.

The equation for the population growth of species 1 is:

$$\frac{dN_1}{dt} = r_1 N_1 \left[\frac{K_1 - N_1 - \alpha_{12} N_2}{K_1} \right]$$

And for species 2, it is:

$$\frac{dN_2}{dt} = r_2 N_2 \left[\frac{K_2 - N_2 - \alpha_{21} N_1}{K_2} \right]$$

where:

N_1 and N_2 are the population sizes of species 1 and 2,
r_1 and r_2 are the intrinsic rates of increase for these species,
K_1 and K_2 are the carrying capacities of the habitat for each species,
$alpha_{12}$ and $alpha_{21}$ are the effects of one species on the population growth of the other. Specifically, $alpha_{12}$ is the effect of species 2 on the growth of species 1, and $alpha_{21}$ is the effect of species 1 on the growth of species 2.

If the values for each equation are known (or can be estimated empirically from the results of an experiment), then the equation can be used to predict the potential outcome of a competition (i.e., whether they can co-exist or if one will eventually exclude the other). The values for K_1, K_2, $alpha_{12}$, and $alpha_{21}$ are used to plot the isoclines of zero growth (i.e., where dN_1/dt or dN_2/dt equal zero) for both species on the same graph, and the resulting sums of population growth vectors (trajectories) are used to determine the outcome of the competition (Figure 1).

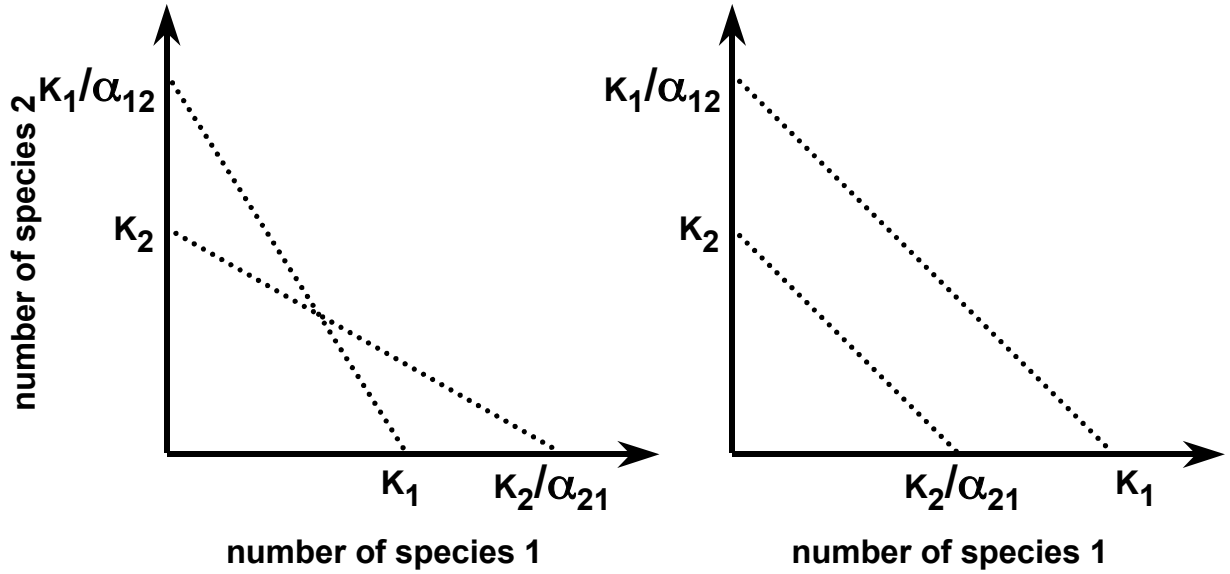


Figure 1. Example graphs of isoclines of zero growth for which species 1 and species 2 coexist (at left), and species 1 competitively excludes species 2 (at right).

The Lotka-Volterra competition model describes the outcome of competition between two species over ecological time. Because one species can competitively exclude another species (Figure 1) in ecological time, the competitively-inferior species may increase the range of food types that it eats in order to survive. However, the response of species to interspecific competition in evolutionary time is often the opposite of what occurs in ecological time. Competitors generally will specialize on particular resource types. This resource partitioning that occurs over evolutionary time actually results in decreased or the absence of competition between the two species.

Although they are not particularly closely related to one another, the life histories of two parasitoid wasp species, *Melittobia digitata* and *Nasonia vitripennis*, are quite similar. Both species are capable of using the same host, although in nature they used different hosts. *Melittobia* are about half as large as *Nasonia*, but both are quite small and completely harmless to humans.

Their complete life cycles are relatively short (2-4 weeks at 25^{o} C), and also quite similar (Figure 2). Females lay numerous eggs through the host covering. The eggs hatch to become larvae that consume the host, then change to pupae, and finally metamorphose to an adult stage. In *Melitobbia digitata*, the adult females may have either normal or stunted wings. The normal winged adults disperse from the host to search for new food resources. The flightless females will lay their eggs on the same host from which they emerged, or disperse to a new host within the same nest (Freeman and Ittyeipe 1976, Côsoli and Vinson 2002).

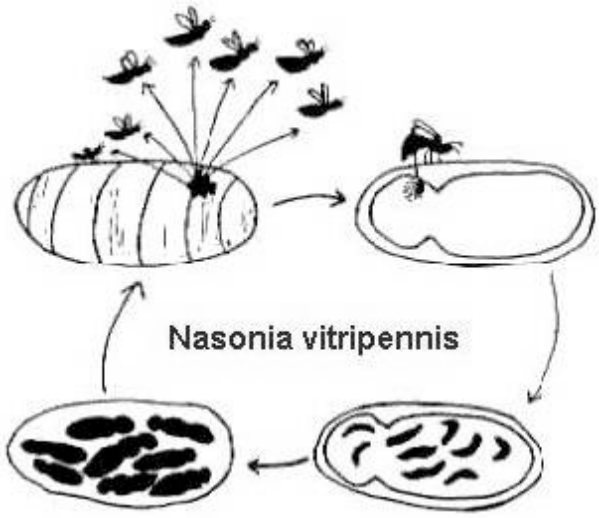


Figure 2. The life cycle of *Nasonia vitripennis* on a *Neobellieria bullata* host pupa (drawing by Bethia King). The life cycle of *Melittobia digitata* is the same, although individuals at all stages are smaller.

There are about 70,000 known species of parasitoids worldwide (9% of all insects), but estimates of their number run as high as 800,000 (Strand 2002). Of the described species, about 80% are members of the order Hymenoptera, as are the two species you are using in this lab. In nature, hosts can be parasitized by more than one species of parasitoid, all competing for the same resource (Stand 2002). Since the host species may itself be a parasite on another species, the ecological effects of that competition on community structure can be very complex. For example, Swaine jack pine sawflies (*Neodiprion swainei* Midd.), which can attack, defoliate, and kill large stands of jack pines in North America, play host to 11 different species of parasitoid wasps. It appears that most of these species coexist because they partition the host resource among them by parasitizing different stages of the host's life cycle, or different segments of the host population (Price 1972). One member of the parasitoid guild that attacks sawfly cocoons is an introduced species (*Pleolophus basizonus*). It is a superior competitor and its presence determines the abundance of some of its competitor species (Price 1970). Although competition can affect parasitoid community structure, spatial and temporal variation in host resources may be even more important in determining parasitoid species richness, even in the presence of interspecific competition among parasitoids (Hawkins 2000).

Parasitoids whose hosts are important pests on crops or forest trees are sometimes intentionally released as biological controls on pest populations. Where more than one species of parasitoid attacks the same host, it is important to understand the nature of that competition before making releases. If the different parasitoid species are capable of co-existing by means of resource partitioning, control of the pest host may be best achieved by introducing some or all of the parasitoid species. On the other hand, if the competitors limit each other's populations because of their competitive interactions, then maximum control of the pest species might be achieved by releasing the most efficient of the parasitoid species by itself (Amarasekare 2000).

Materials and Methods

Overview of Data Collection and Analysis Methods:

Lab 1

The categories of interactions discussed in Table 1 can seem quite straightforward when one is simply reading about them. But if you were to observe two unfamiliar animals interacting, how would you decide what "label" to apply? Could you predict the outcome of the interaction? How could you test your prediction?

The two parasitoid wasps presented in this laboratory investigation seem to occupy similar niches. We are interested in the interactions between the two species. In addition, we want to be able to quantify the effect of one species on the other.

Each group should:

- discuss and list all the possible experimental combinations that could be set up involving two parasitic wasps, *Melittobia digitata* and *Nasonia vitripennis*, and a single host, *Neobellierria*,
- predict what you think might be the outcome for each possible interaction,
- identify and list variables that you would manipulate in your experiment,
- identify and list variables you would keep constant in your experiment,
- identify and list dependent variables you would want to measure to quantify the effect of each species on the other.

Each group will share their experimental design with the rest of the class. Together as a class, we will develop a consensus experimental design. Based on the consensus experimental design, each person should set up one replicate culture for each treatment. See "Handling Parasitoids" below. We will pool the data from the entire class for analysis.

Weekly checks:

Each week, each person should inspect their cultures to see if any adult wasps have emerged. You should record the date that you first see emerged adults for each culture. *Nasonia* cultures should be frozen 21 days after they were established. *Melittobia* and mixed species cultures should be frozen after 42 days.

Lab 2 (6 weeks after Lab 1):

Each person should count the number of offspring produced in each replicate culture. Enter your data into a spreadsheet so that the data for the class can be pooled. Use the pooled data for estimating the parameters for the Lotka-Volterra competition model and for statistical analysis of the effects of competition.

In your groups, discuss how the data can be used to quantify the parameters of the Lotka-Volterra competition model (see "Quantifying the Lokta-Volterra competition model" below). Also, discuss what particular treatment comparisons can tell us about the relative importance of intraspecific and interspecific competition in these two species of parasitoids (see "Guidelines for Data Analysis" below).

Handling Parasitoids:

Adults of both parasitoids, *Melittobia digitata* and *Nasonia vitripennis*, are very "user friendly." Although females possess normal wings and can fly, they do not do so readily. However, they are negatively geotaxic (i.e., they move up, away from gravity). When a few females from a culture are shaken out onto a horizontal surface, then covered with an inverted glass vial, they will readily climb into the vial and up the sides. Once you have wasps in a vial, you can easily add a host pupa, then plug the vial tightly with cotton. Large numbers of individuals can be efficiently handled in this way. The adult wasps can also be manipulated with short pipe cleaners, to which the wasps will temporarily adhere.

Quantifying the Lotka-Volterra Competition Model:

The Lotka-Volterra competition model was described and defined with equations in the Introduction. As noted there, if the values for each equation can be estimated empirically from the results of an experiment, then the equation can be used to predict the potential outcome of a competition (i.e., whether the two species will co-exist or if one will eventually exclude the other). The values for K_1, K_2, $alpha_{12}$, and $alpha_{21}$ are used to plot the isoclines of zero growth (i.e., where dN_1/dt or dN_2/dt equal zero) for both species on the same graph, and the resulting sums of population growth vectors (trajectories) are used to determine the outcome of the competition.

Based on our experimental design, we need to determine the values of these parameters. Recall that the carrying capacity for a population is the maximum number of individuals that can survive in a habitat. For simplicity in this experiment, we have defined the habitat of the parasitoids as a single host. In reality, of course, a habitat would likely contain more than one mud-dauber nest or blowfly puparium, and so there would be many potentially exploitable hosts. To determine the carrying capacities of the two species, we need to know the maximum number of offspring of a given species that can be produced on a single host when only that species is present. With this in mind, data from which treatment would be used to estimate the carrying capacities of *Melittobia* and *Nasonia*? (Remember that at carrying capacity all host resources will be used.)

Estimating the competition coefficients ($alpha_{12}$, and $alpha_{21}$) is a little more complicated. Recall that the equation for the population growth of species 1 is:

$$\frac{dN_1}{dt} = r_1 N_1 \left[\frac{K_1 - N_1 - \alpha_{12} N_2}{K_1} \right]$$

When all of the host resource is used by the parasitoids, then a population can no longer grow. In other words, $dN_1/dt = 0$. This condition will occur when $K_1 - N_1 - alpha_{12}*N_2 = 0$. To find $alpha_{12}$, we need to solve for it (i.e., do a little algebra) and then substitute values for K_1, N_1, and N_2. Above, we described how to find the carrying capacities. Assuming that *Melittobia* is "species 1," use its carrying capacity for K_1. The number of *Melittobia* and *Nasonia* offspring produced in interspecific competition are N_1 and N_2, respectively. With this in mind, data from which treatment would be used to estimate N_1 and N_2?

The same approach that you used to calculate $alpha_{12}$, can be used to calculate $alpha_{21}$.

Now that you have calculated all of the parameter values, you can use these values to plot the zero growth isoclines and predict the outcome of competition between *Melittobia* and *Nasonia*.

Guidelines for data analysis:

We can use comparisons between different treatments to explore the relative importance of intraspecific and interspecific competition. First, identify what type of competition, intraspecific or interspecific, if any, is occurring in each treatment. After you have done this, think about all of the comparisons between pairs of treatments. What does each of the comparisons tell us? It might be helpful to produce a chart that lists the comparisons and what they mean. Since all of the comparisons involve two treatments, they can be analyzed statistically using t-tests.

Questions for Further Thought and Discussion:

1. Based on the parameter values that you calculated for the Lotka-Volterra competition model, what is the predicted outcome of competition between the two species? Was the predicted outcome achieved in every replicate of interspecific competition? If not, why not?

2. “Gause’s Law” states that competitors that share exactly the same resources in the same way cannot coexist. This means that the species that most efficiently uses the contested resource will eventually eliminate the other at that location. Does Gause’s Law seem to apply to the interaction between *Melittobia* and *Nasonia*? Why or why not?

3. If these two species were to use the same host in nature, how might resource partitioning allow them to coexist?

4. Based on the results of your experiment, why don’t the two species use the same host in nature?

5. Given the estimated values for carrying capacities and competition coefficients, predict the outcome of competition between *Melittobia* and *Nasonia* using the Lotka-Volterra competition model in *Populus* (see References and Links). Is the predicted outcome of competition affected by initial population sizes or population growth rates? If so, how? How is the time to reach equilibrium affected by these values?

6. The carrying capacities and competition coefficients are just estimates. What factors might affect the carrying capacities and competition coefficients for these two species?

7. If interspecific competition occurs in these species, how might we determine what mechanism of competition (interference or exploitative) is occurring?

*** Note: Answers to many of these questions and numerous other comments by the contributing author can be found in the "NOTES TO FACULTY: Comments On Questions for Further Thought" page.

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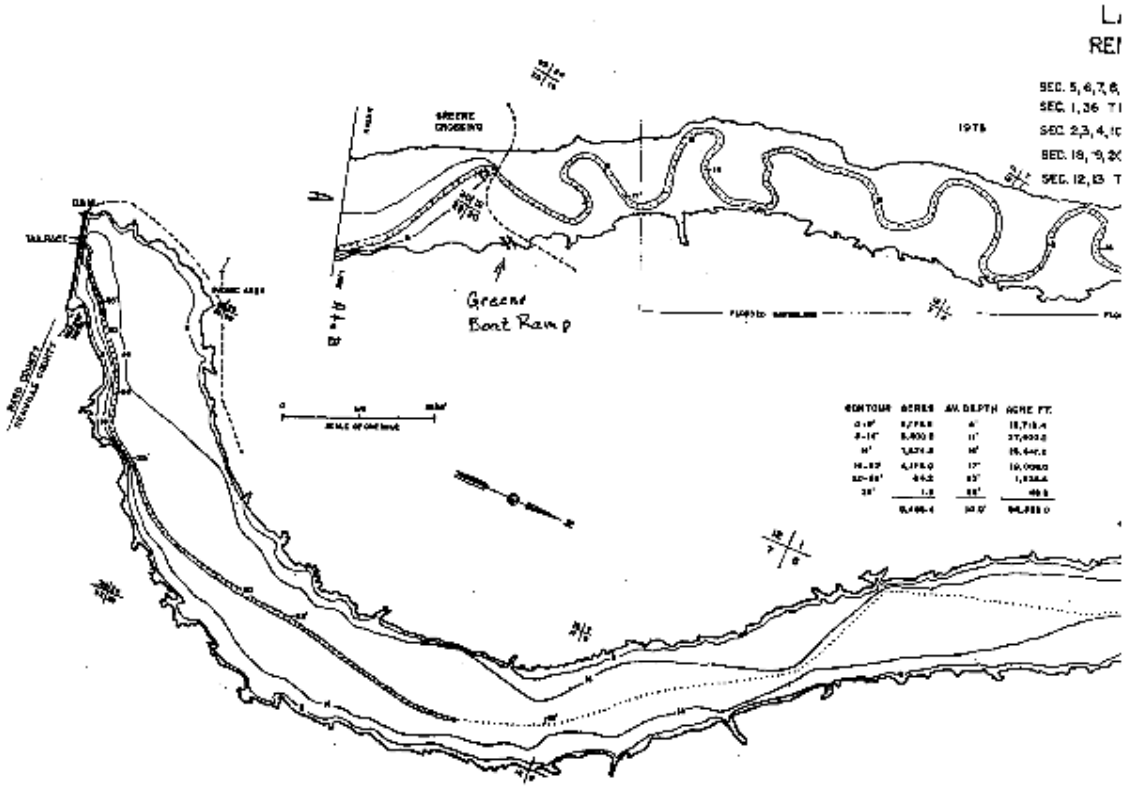
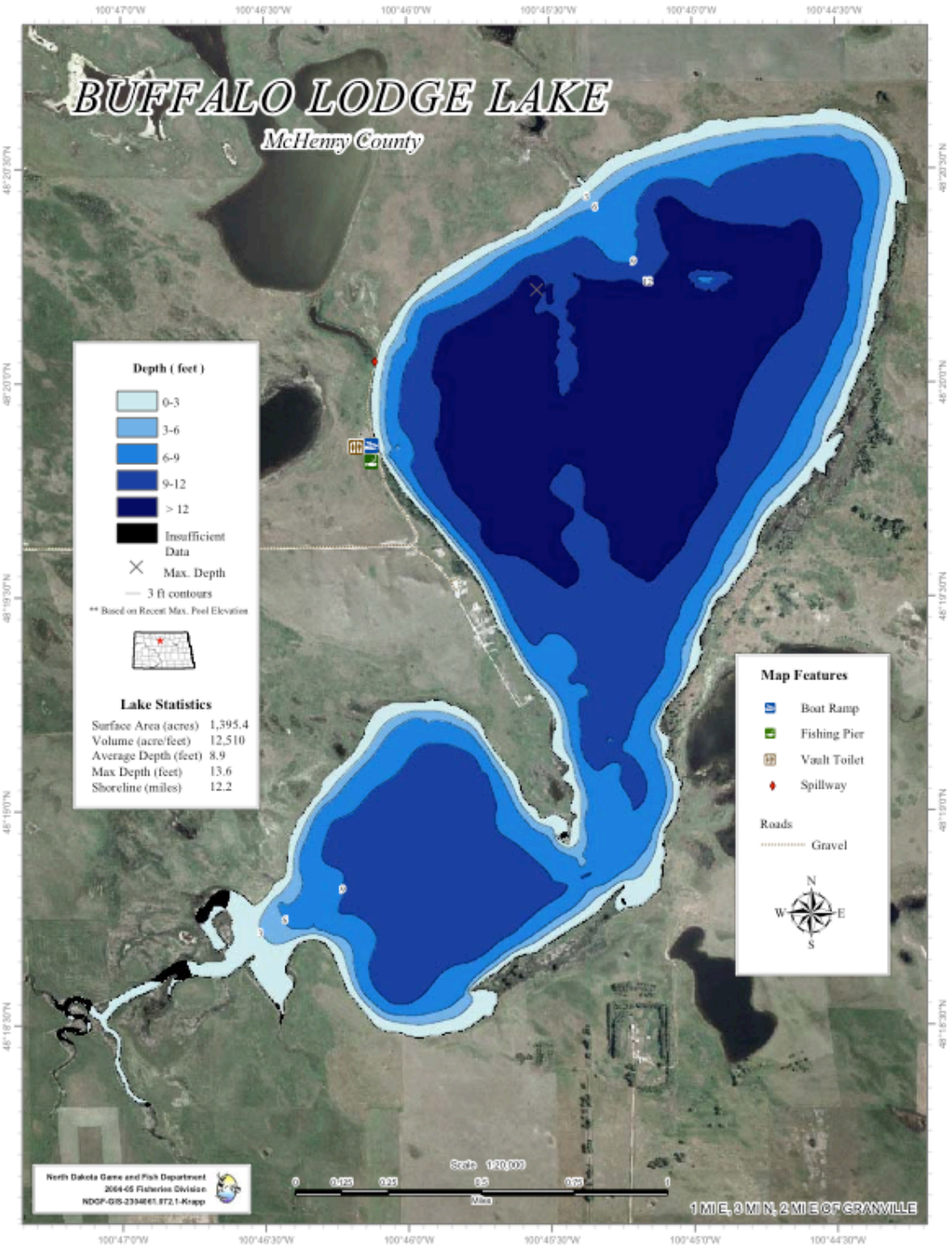
Appendix A – Maps




BUFFALO LODGE LAKE
McHenry County
Depth (feet)
0-3
3-6
6-9
9-12
> 12
Insufficient Data
Max. Depth
3 ft contours
** Based on Recent Max. Pool Elevation
Lake Statistics
Surface Area (acres) 1,395.4
Volume (acre/feet) 12,510
Average Depth (feet) 8.9
Max Depth (feet) 13.6
Shoreline (miles) 12.2
Map Features
Boat Ramp
Fishing Pier
Vault Toilet
Spillway
Roads
Gravel
N
W
E
S
North Dakota Game and Fish Department
2004-05 Fisheries Division
NDGF-GIS-2304#01.ST2.1-Knapp
Scale 1:10,000
Miles
1 MI E, 3 MI N, 2 MI E OF GRANVILLE

Appendix B- Unpublished Manuscript

Appendix C – Reviewer's Form

Please Return with Your Review

To:
Paul Lepp, editor
Journal of Ecology
Dept. of Biology
221 Cyril Moore Hall
Minot State University
Minot, ND 58701

From:

email address:

Journal of Ecology

1. Please provide on a separate sheet of paper your review of the manuscript. Your comments are critical decision. Suggested items for commentary are:

- Is this subject suitable for publication in the Journal of Ecology?
- Is this a new and original contribution?
- Significance to the target scientific community
- Are the presentation, organization, and length satisfactory?
- Are the references adequate and are they all necessary?
- Are the interpretations and conclusions sound and justified by the data?
- Appropriateness of the approach or experimental design
- Appropriateness of the statistical analyses
- Adherence to correct scientific nomenclatures
- Adequacy of experimental techniques
- Relevance of discussion
- Adherence to the Instructions to Authors
- Adequacy of title and abstract
- Appropriateness of figures and tables

2. Please check one

☐ Accept without Revision
☐ Accept with Revision
☐ Reject

Appendix D – Sample Manuscript Review

The manuscript "Assessing shifts in microbial 16S rRNA amplified from uncomtaminated and TNT-contaminated soil with functional ANOVA of hybridizations to oligonucleotide microarrays" by Eyers, *et a*l is an incremental and necessary step in addressing the problem of cross-hybridization in a complex microbial community. The data and methods appear solid and interesting but I have some concerns regarding the clarity, intent and interpretation of the manuscript.

Most significantly, the value of the functional ANOVA in evaluating differences between two environmental samples is unclear to me. What is the significance in a biological sense of the MAX_{DCSD} between two environmental samples? The authors never really address this question. My expectation would be that normalized dissociation curves of a highly discriminatory probe should be nearly identical between samples harboring the same taxa regardless of the environmental source as is illustrated in figure 1C presented by the authors. The difference in the MAX_{DCSD} between samples (last column of table 1) would appear to speak more to the complexity of cross-hybridization than to differences in the relative abundance of various taxa. For example, both environmental samples may contain equal numbers of the same *Plantomycetes* species and yet be statistically different at MAX_{DCSD} for probe EUB33-II because the difference, in composition and abundance of species other than *Platntomycetes,* between the two samples contributes unequally to the shape of the dissociation curve. Perhaps it was the authors intent to highlight this possibility. If so then it was not clearly presented in the discussion on pp 14 -15. If the authors wish to infer something regarding the relative abundance of particular taxa, as implied by paragraph 2 on p. 15, then it seems to me that the most appropriate sample comparison would be the fluorescent intensities at the most discriminatory PM/MM MAX_{DCSD}. This would probably require an internal control, however, to normalize between chips (like a human alu sequence perhaps).

The remaining comments are points that need clarification:

p.4, line 13. A transitional sentence would help this paragraph enormously.

p. 4, line 23. comprized → comprised.

p. 6, lines 19-21. What kit was used? I assume some sort of T7 expression kit.

p. 10, lines 3-13. The intent of this paragraph is unclear and the wording awkward. I would suggest inverting the paragraph. Moving the last sentence to the beginning of the paragraph would provide a topic sentence.

p. 10, line 8 add "…MM probes (Table S-2) among either contaminated, uncontaminated or both samples." or some other clarifying statement. Only 23 of the probes are significantly different in the uncontaminated sample.

p.11, lines 4-7. Would you expect a difference inTds between the samples? See discussion above.

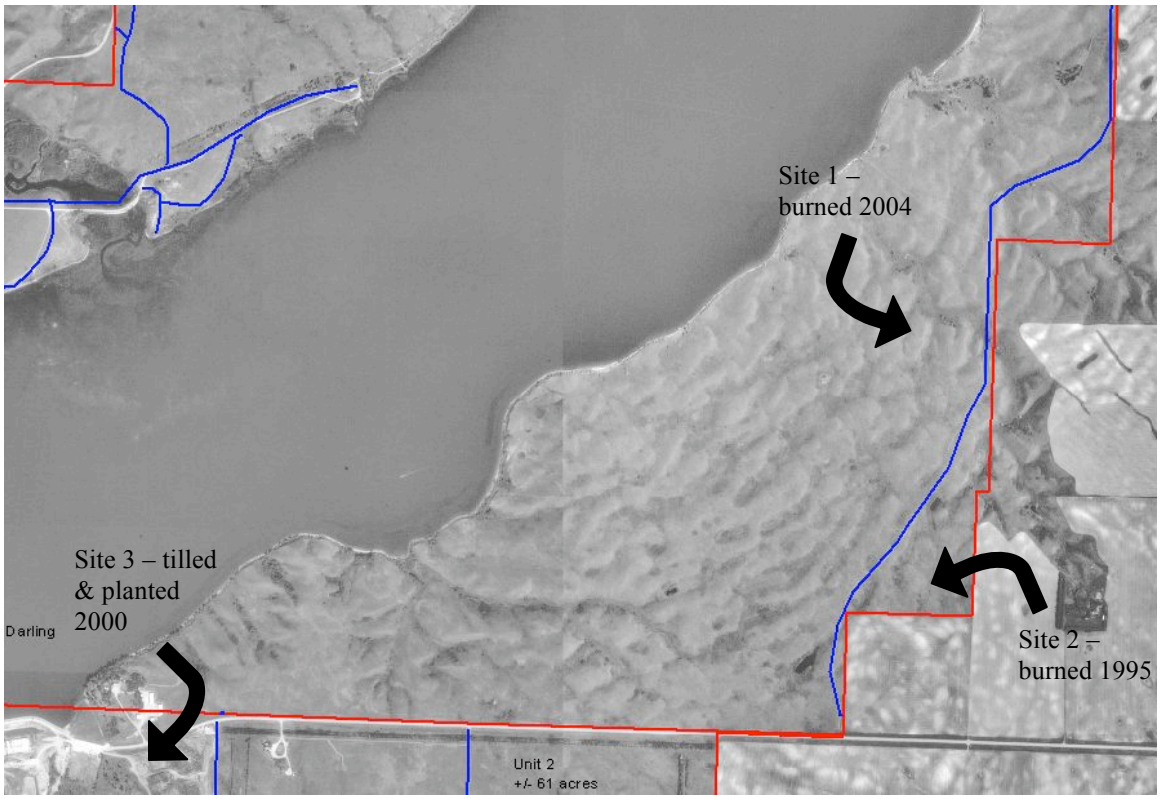
p.14, line 6. I think "…ten PM…" should be either 15 if considering all probes that were significantly lower in uncontaminated versus contaminated or 13 if considering only SSU probes.

p. 14, line 19. "…ANOVA (Figure 2; **Table 1**)."

p.14, line 20. "…for 3 of them **(Table S-2)**, …."

p. 22, line 6. End sentence after "…CodeLinks microarrays (A)." and start a new sentence. Same in line 12.

Appendix E – Lake Darling



Teacher's Notes

1. The following species are a possibility in the terrestrial quadrat lab:

   Melilotus alba (white sweet clover)

   Grindelia (Yellowtop Gumweed)

   Aster sp. (white aster),

   Medicago sp. (alfalfa with purple flowers)

   Great descriptions and pictures are given at
   http://www.ext.nodak.edu/extpubs/ansci/range/eb69-2.htm