BIOLOGICAL INVESTIGATIONS

A HANDBOOK for BIO 110 and beyond

Lewis & Clark College

Name:_____

Section:_____

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Foreword

The goal of this handbook is to provide information that will help you succeed in Biology courses, and, if you so choose, as a Biology major. It includes sections about studying successfully for biology, expectations for college-level work in biology, field and laboratory procedures, experimental design, methods for data presentation, and basic statistics -- all common skills that are critical for good scientific practice. This handbook will be an essential resource for Biology 110, but will also be relevant for other courses in the major. Instructors will refer to its content in both introductory and advanced courses. If you decide to continue taking courses in Biology, keep this handbook throughout your time at L&C and use it as a reference. We encourage you to take notes in it and to give us feedback on sections you found unclear and also on sections you found most useful.
-- the Faculty and Staff of the Department of Biology

 Other sections are adapted from the lab manuals of earlier introductory biology courses (BIO 141, BIO 151, BIO 200).

We are grateful to a group from a fall 2021 section of Bio 110 for the use of their lab notebook as an example.

THE BIOLOGY CURRICULUM AT LEWIS & CLARK COLLEGE

The Philosophy behind the Biology Curriculum

The biology curriculum at Lewis & Clark has been shaped by guidelines in the American Association for the Advancement of Science's 2010 report, "Vision and Change in Undergraduate Biology Education: A View for the 21st Century"(https://live-visionandchange.pantheonsite.io/wp-content/uploads/2011/03/VC-Brochure-V6-3.pdf). This report recognized that biological discoveries have led to an explosion of knowledge, and have changed some of the field's key questions. How can students successfully prepare to work as biologists in such a challenging environment? It also recognized the need to broaden participation in biology to include members of underrepresented groups, and to make scientific knowledge more accessible to audiences both within and outside of the scientific community. All these goals demanded important changes in the teaching and learning of biology. The *Vision and Change* report made recommendations about the (a) core concepts and (b) core competencies that should form the foundation of an undergraduate education in the biological sciences. These core concepts and core competencies are at the center of Lewis & Clark's Biology curriculum. They are:

CORE CONCEPTS:

- Evolution
- Pathways and transformations of energy and matter
- Information flow, exchange, and storage
- Structure and function
- Systems

CORE COMPETENCIES:

- Apply the process of science
- Use quantitative reasoning
- Use modeling and simulation
- Tap into the interdisciplinary nature of science
- Communicate and collaborate with other disciplines
- Understand the relationship between science and society

Biology 110, Biological Investigations, is the introduction to the Biology major. Bio 110 focuses on the core competencies, especially on learning and applying the process of science. In Biology 110, you will learn to formulate questions and pose hypotheses. Using rigorous observational or experimental investigations, you will analyze data quantitatively and interpret your findings to draw conclusions. In Biology 110 you will work collaboratively and learn to communicate your findings in writing and in oral presentations. Different sections of Biology 110 have different conceptual emphases, depending on their professor's area of biological

interest and expertise, but all focus on developing these core competencies. In Biology 110, you will experience the process of science and can determine whether you would like to continue this kind of activity as a Biology major.

Biology 110 is followed by Biology 201 (Biological Core Concepts: Systems) and Biology 202 (Biological Core Concepts: Mechanisms), which introduce students to the five core concepts. In Biology 201, students learn how the core concepts operate at larger scales of biological organization (e.g. the study of behavior, ecology, and evolution). In Biology 202, students encounter these same concepts at smaller scales of biological organization (e.g. the study of biomolecules, cells, heredity, and development).

The Biology major at Lewis & Clark College

Biology 110 is a course for students who are interested in exploring a possible Biology major. It introduces students to the process of scientific investigation as practiced by biologists, and supports students as you engage in this process. At the end of Biology 110, you will probably have a pretty good idea of whether you enjoy thinking like a biologist, and would like to do more.

If so, the next step is to take Biology 201 and 202. These two courses may be taken in any order, as long as students have completed Biology 110. Biology 202 requires concurrent enrollment in Chemistry 120. Biology 201 and 202 do not have laboratory sections, but they include a strong emphasis on the process of science, by examining hypotheses, experiments, and data.

Biology is inherently interdisciplinary. Posing hypotheses about biological processes requires some understanding of the chemical processes governing the behavior of biomolecules and of the molecules that make up Earth's environment. Thus all biology majors take Inorganic Chemistry, Chemistry 110 and 120. A year of Organic Chemistry is strongly encouraged, and is a must for any student planning postgraduate work in the health sciences or graduate work in biology.

Analyzing data means thinking quantitatively. Quantitative reasoning takes many forms, e.g. calculus, statistics, and modeling. Different fields of biology use different forms of quantitative reasoning. Therefore, all Biology majors take at least one semester of mathematics above the level of Math 115 (Elementary Functions, or pre-Calculus). The ideal quantitative reasoning course for Biology majors is Math 123, Calculus and Statistics for Modeling the Life Sciences. As the title makes clear, this course gives students the opportunity to practice several important kinds of quantitative reasoning in the context of biological problems and examples. Other courses often chosen by biology majors are Math 131 (Calculus I), Math 132 (Calculus II), Math 255 (Statistical Concepts and Methods), and Computer Science 171 (Computer Science I). Students are encouraged to take additional coursework in quantitative reasoning beyond the one semester required for the Biology major. It is advisable to take the required course in quantitative reasoning as early as possible, so that this background can be applied to your biology coursework. The first semester of the sophomore year is an ideal semester to enroll in Math 123, Math 131, or CS171.

After completing Biology 110, 201, and 202, and Chemistry 110 and 120, you are ready to take advanced coursework in Biology. Biology majors take at least six courses numbered above Biology 202; at least four of these courses must have a laboratory or field component. Note that some advanced courses require that students complete the Biology quantitative reasoning requirement before they may enroll. Beyond these, there are no limits or restrictions on which advanced courses students may opt to take. Thus you may choose a broad path with coursework from a wide variety of biological subfields, or you may specialize by focusing on courses in cellular and molecular biology, or ecological, evolutionary, and behavioral biology.

As is clear from our focus on research in Biology 110, independent research is an important part of the Biology major. All courses with laboratory or field components involve multi-week independent research projects. In addition, many students opt to earn credit by carrying out practica (Biology 244), independent studies (Biology 499), or senior theses (Biology 495). In a practicum, students work under the close supervision of a faculty member to learn a particular technique or gain experience in research. In independent studies and senior theses, students propose and carry out more independent work (often related to their faculty mentor's research interests).

Students wishing to graduate with honors in biology must have at least a 3.5 GPA in the major and overall, and must carry out a two-semester senior thesis that earns an A or A-.

Here is a typical 4-year-plan for a Biology major. However, there are many other possible pathways. For more information about the Biology major, course options, and policies, see the Biology Department website or the College catalog.

year	**fall**	**spring**
1	Bio 110 Chem 110 Core Elective or GE	Bio 201 Chem 120 Core Elective or GE
2	Bio 202 Math 123 (Chem 210?) Elective or GE	Bio elective Elective or GE (Chem 220?) Elective or GE
3	overseas? (either fall or spring)	Bio elective Bio elective Elective or GE Elective or GE
4	Bio elective Bio elective Elective or GE Elective or GE	Bio elective Elective or GE Elective or GE Elective or GE

BIOLOGY 110: BIOLOGICAL INVESTIGATIONS

Each section of Bio 110 — Biological Investigations — focuses on a different subfield of biology. Instead of expecting all students taking Bio 110 to learn the same biological "facts," ideas, and processes, we ask all students to practice the same skills, while investigating interesting and diverse biological questions. It's not that "facts" are unimportant; they are fundamental to investigating and understanding life. But these facts were all discovered by people using *the process of science*. All sections of Bio 110 are centered around this process.

The Process of Science

OBSERVATIONS
The scientific process begins with an observation that seems interesting, or surprising, or unexpected. The observation might be a pattern in nature, or an association, such as the fact that patients with a particular disease harbor a particular kind of bacteria. Each section of Bio 110 will focus on different kinds of observations.

QUESTIONS
Observations give rise to questions like "why" or "how?" Why does this pattern exist? What natural forces are responsible for the pattern? Does this bacterium cause the disease?

HYPOTHESES
A hypothesis is a potential explanation for the observations, a suggested answer to the question. For a hypothesis to be useful, it must be testable. That is, it must be possible to imagine evidence that would indicate whether the hypothesis is right or wrong.

TESTING
Hypotheses are tested in two main ways. Sometimes a hypothesis is tested by collecting additional observations whose nature can either refute or support the hypothesis. Alternatively, hypotheses can be tested by performing an *experiment*, where the scientist manipulates some factor thought to be responsible for the observation, and compares the manipulated situation to a *control* where the factor has not been altered. Both observational studies and experiments need to be carefully designed in order to rule out potentially erroneous interpretations. In Bio 110 you will learn how to design rigorous investigations. By doing so, we hope you will learn both the power and the limitations of scientific methods in biological explanation, so you'll be able to evaluate the results of other scientific investigations you encounter.

PREDICTIONS
A good test of a scientific hypothesis should make specific (often quantitative) predictions about what outcome should be observed if the hypothesis is correct. If that predicted outcome is not observed, the hypothesis is not supported, and is discarded.

ANALYSIS AND INTERPRETATION
What do your observations tell you about your question? Do they support or contradict your hypothesis? In order to decide, you must analyze the data you collect, comparing the observed outcome with your predictions. Analysis usually involves summarizing the data and creating graphs to display the data. With some kinds of data, it is also necessary to evaluate your confidence in your conclusion, using the tools of statistical inference. Depending on the result of the analysis, the hypothesis might be accepted, or modified, or rejected. All of these outcomes represent progress in understanding the original observation.

COMMUNICATION
A critical part of doing science is communicating effectively with other scientists. In Bio 110 you will spend a lot of time working on ways to communicate your results graphically, in writing, and through oral presentation.

ETHICS
Good science, and good policy informed by science, depends on both the reliability of the scientific process and on the scientist's awareness of other areas of human concern affected by the results. In this course, you'll become familiar with standards regarding the ethics of practicing and communicating science, and many of you will discuss how scientific results may be relevant to social issues.

The philosophy behind Bio 110 is that you learn more effectively by practicing. This goes for biology as much as for playing a musical instrument or a sport. By grounding your learning in authentic research questions, we also hope you will be motivated to practice very hard, so that you will be confident in your abilities when the semester is long past. That is how professional biologists keep up with the incredible change and growth in biological knowledge over their own lifetimes. If you go on to the 200-level biology courses, you'll find that your practice in Bio 110 has prepared you for the task of understanding organisms by integrating ideas from across the spectrum of biological sub-disciplines. And, if you don't intend to major in biology, we are certain that Bio 110 will provide you with critical analytical tools needed in many other fields (e.g. medicine, environmental studies, law). These tools are also essential for interpreting the results of biological research in your daily life. We hope all of you will be prepared to learn what you need to know to evaluate the biology of your future.

Collaborating as a Member of a Group

Much of your work in Bio 110 will be performed as a member of a group. There are many reasons for this. The exchange of ideas among individuals with different perspectives leads to more interesting questions, more creative studies, and more rigorous conclusions. Collaboration allows us to perform more complex studies than we could accomplish alone. Working in a group helps us to develop skills like responsibility, self-awareness, communication, confidence, and sensitivity. Explaining our ideas to others helps us to develop them. In your future career, whatever it is, you will need to collaborate with and communicate with many different kinds of people. Finally, we are social animals; working as a member of a group is more fun than working alone. But for a group to function effectively (and to enjoy the experience), group members need to be responsible to one another. Each member needs to

be adequately prepared for each step of the study. Each member needs to give their best effort to the group. And each member needs to be committed to fostering the success of every member of the group. If you do this, your group will produce a final result that is far more polished and satisfying than you could have produced alone, and you will have a positive experience in the process.

Working in groups means collaborating with individuals you may not know, and who will differ from you in many ways. This experience will be enriching, and it may also be difficult at times. Research shows that when we anticipate interacting with individuals who differ from us, we prepare harder, we anticipate hearing diverse perspectives, and we expect that it will be harder to reach agreement. We think in different ways. And these same studies demonstrate that socially diverse groups (containing diversity of race, ethnicity, gender, and sexual orientation) are more creative and arrive at more innovative solutions to problems (Phillips 2014).

Our goal for Bio 110 is that it will be an inclusive community where individuals will learn from the many different perspectives that come from having different backgrounds and beliefs. We will aim to value the differences in ability, race, ethnicity, religion, gender, sexual orientation, economic circumstances etc. that characterize us. We expect that everyone -- faculty, staff, and students -- will work to create an environment that facilitates inquiry and self-expression, in which everyone is heard, and everyone is respected. We want to foster an environment in which everyone can succeed -- and one that allows us to do the best possible science.

Groups can experience problems when different members devote different amounts of effort to a project. No one likes to feel as though they must do more than their fair share in order for a project to succeed, and no one likes to feel that their contributions are not valued. Group work is successful when group members all understand their responsibilities and work hard to meet them. Effective communication during all phases of a project, from generating hypotheses to dividing up the workload and writing a report, is the most important way to ensure that a group works well together.

To build collaboration skills, Bio 110 will have opportunities for *peer evaluation***:** At several points during the semester, you will assess your group's success at meeting your collective responsibilities, and provide feedback to the different members of your group. The first assessment will be advisory only, to provide feedback and create an opportunity for improvement. Other assessments will influence students' lab grades.

References:
Phillips, K.W. (2014) How Diversity Works. Scientific American 311(4):42-47.

YOUR LABORATORY NOTEBOOK

Each student (or team) is required to keep a notebook of their scientific activities. This notebook must contain a complete record of your work on each investigation you carry out (in the lab, in the field or in the greenhouse). You may use the course manual as your lab notebook, as described below, or your instructor may suggest using an electronic notebook.

Why is a lab notebook important?

It is impossible to over-emphasize how important detailed notes are in the practice of good science. These notes serve as a record of your logic concerning your study as well as a place for your notes and observations. Often it is the "trivial" details you record during an investigation that will allow you to fully interpret your data later. Your lab notebook allows you to figure out why a study turned out as it did. Your ability to correctly and accurately interpret your data will be directly related to how well you use your notebook. Finally, your lab notebook prevents you from unintentionally committing scientific fraud. Our memories are notoriously imprecise. It is very easy to forget exactly how you did a particular step; your notebook will keep you honest.

To guide how much information and detail should be included, use “the two year rule.” That means you should be able to understand your entries and be able to navigate in your notebook at least two years after making the report. If something looks suspicious, write it down; if something looks interesting, write it down; if you have a problem, write it down. The standard by which a lab notebook is judged is whether a person with no previous knowledge of the study would be able to repeat the work and get a similar result by following your records. *Your lab notebook should be complete, clear, and neat.*

Your notebook has two audiences. One audience is you and your partners. Your written records of your project should be sufficiently complete that each member of your team can easily reconstruct what you did and what results you obtained. The details (numbers of samples, experimental conditions, etc.) will be essential when you analyze your results and incorporate the data into a manuscript or an oral presentation. The second audience (in the real world) is any scientist who might wish to try to replicate your work. Other scientists will not understand your shorthand and cannot read between the lines. Therefore, your notebook should contain sufficient detail that someone *not* in your group can make sense of it. (And unless it does, after a few weeks even you won’t remember what your cryptic notes mean!) Keep these audiences in mind as you decide what to include in your notebook.

Your instructor and teaching assistants will judge whether your notebook passes this test. They will check on and grade your notebook from time to time. These notebook checks will contribute to your grade in the course.

What should be in your lab notebook?

1) content that you want to remember, highlights of important material presented during class and from your background readings;

2) flowcharts and/or a protocol for all the investigations you design. For those you do not design yourself, describe any modifications to the protocol you have been given;
3) text accompanying the flowchart or protocol, briefly describing the rationale, significance, and expected products of your study;
4) the details of your preparation for the investigation; a record of all calculations you do, and tables that record the details for all media or solutions, etc. that you make up;
5) for field or greenhouse investigations, the date that each step was performed or that data were collected. The timing of events might be important as you try to interpret your results;
6) a record of all observations you make during the course of the investigation;
7) tables in which you record all the raw data you gather;
8) your brainstorming about hypotheses, data analysis, and interpretation of your data;
9) answers to any questions asked of you in the protocol/handout for that investigation;
10) if applicable, recommendations about what could be done differently to make the study more reliable or efficient when it is repeated.

How to organize your lab notebook

The following instructions assume you will be using a paper notebook. If instead you use an electronic option, the same organizational and content principles should apply.

For a paper notebook, you may dedicate a section of the lab manual to serve as your lab notebook. Add loose leaf pages to this section. Number (by hand) all the pages that you add, in the upper, outermost corner of each page. *Reserve the first two pages for a Table of Contents.*

For each investigation you do, begin on a new page. As you make entries, record investigation headings and subheadings and page numbers in the Table of Contents. You may want to write the bulk of your entries only on the right hand side, using the left page for unorganized notes or to carry out incidental calculations. Record your notes *directly and immediately* into the laboratory notebook as you go along. You should make your observation or write down the data at the moment something happens. *Never use scraps of paper with the intention of transcribing the data into the lab notebook later.* When something goes wrong, do not scribble out or obliterate the useless data. Instead, use a single line to cross it out, and add a brief explanation. Be sure that all quantities are identified and that they carry the proper units. Make sure that your entries are legible and organized. Try to keep notebook pages from becoming cluttered. Keep in mind that the goal for your lab notebook is for it to be used as an unambiguous record of what you did for someone not necessarily familiar with your work.

Here is a student-created lab notebook that is a good example of what your laboratory notebook should look like. Blue text in Arial is guidelines and suggestions. Black text in Arial is an example of what you might write.

Start with the names of your group members and a **table of contents** to make it easy to locate information. Date all entries! The notebook example begins on the next page.

Lab Notebook Project 3

Hermione Granger, Harry Potter, Ron Weasley
Bio 110-01 Group 3
Lewis & Clark College

Table of Contents:

Project background: In this section of the notebook, enter any background information covered in class, esp. notes about any previous research that has been done, and your thoughts about your project plans.

10/28/2021:
Brainstorming ideas for our independent project:

- What are the dominant mushroom species?
- Compare mushroom abundance in presence/absence of ivy?
- Compare mushroom abundance near walking paths compared to deeper in forest?
- How does samara length affect dispersal distance?
- Does rain affect samara dispersal? Do wet samaras fall differently?
- Are deer more abundant where the ivy grows (check for signs of the ivy being eaten)?

We chose to test the hypothesis that the presence of ivy increases mushroom abundance.

Reasoning: Mushrooms prefer cool, dark, and humid environments (we think). Ivy creates these conditions under its foliage.

Experimental Design/Methods: In this section you should include detailed information about lab or field procedures you learned or carried out. You may take photos, and/or draw diagrams and paste those visuals into your notebook. This section should be detailed enough for a student that is NOT in the class to be able to replicate the work at a separate time, just using your notes. You can refer to slides or handouts about protocols or procedures, either with links, by copying and pasting (with attribution), or by re-writing the information in your own words.

We will use the paired experimental plots (ivy-invaded, ivy-removed) created in the L&C forest by L&C staff and students in 2014 (more info in Project 2 handout, linked here). Using these paired plots will allow us to assure that ivy-present and ivy-absent plots are as similar as possible in sunlight, slope, tree cover, etc. We will count the number of individual mushrooms in each of the subplots. We will represent the data as two boxplots, one for each condition (ivy present vs. ivy-removed).

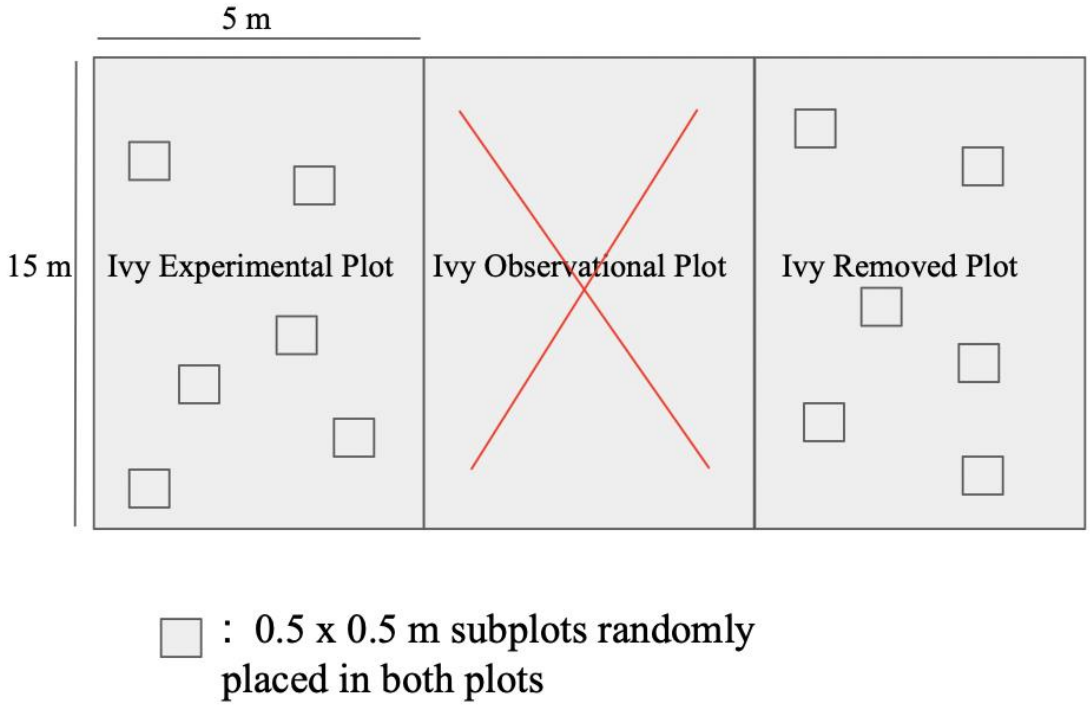
A possible confounded variable is that it will be easier to see mushrooms in the ivy-absent plots than in the ivy-present plots. This could lead us to think there is a difference when in fact there is not. We will need to search carefully. But if we undercount mushrooms in the ivy-present plots, and still find more mushrooms in those plots, it will actually provide stronger support for our hypothesis. Any counting error will result in us having weaker support for our hypothesis instead of wrongly accepting it as correct.

11/2/2021:
We refined our study design and data analysis plan. We decided to census plots 9-13 but not 14-16 (the ones in the cemetery) because plots 9-13 are more accessible and time efficient. We also decided to only count gilled mushrooms. For data analysis, we decided to make an interaction plot as well as a boxplot, so we can more easily show any differences between the paired subplots.

11/4/21:
Today we censused plot 11. In the ivy-present subplot, we found a total of 271 mushrooms, compared to 43 in the ivy-removed subplot. The mushrooms growing in the ivy-removed subplot seemed much larger. It was hard to see past all the ivy leaves, and it's very probable that we missed some. However, we did clear the leaves in each spot that we searched to keep our count as accurate as possible. Censusing this one plot took us the entire class period, so we discussed a revision to our data collection plan that would be more time-efficient.

We decided to sub-sample each subplot instead of trying to count every individual mushroom. We will randomly choose six 0.5m x 0.5m quadrats in each subplot and only count the mushrooms in those. Because we can express the mushroom abundance on a square meter basis, we can still validly compare the counts from plot 11 with the ones that we sub-sample. Subsampling will not only be faster, but it will also allow us to be more meticulous in our counting, since we can focus on small areas rather than the entire plot, which will make it less likely to miss anything. We will use the metal quadrats available in class.

A diagram of our new design:



Data: In this section, you should describe/record any data that you collected in a particular lab session. If you used Google Sheets to record the data, provide a link to the spreadsheet. If the spreadsheet contains data for multiple projects, be sure to explain how to find the specific data within the spreadsheet (name of the file, name of the specific tab, rows and columns where the data is found).

11/9/21:
Today we continued data collection, and censused plots 9,10,12, and 13. We selected the location of the subsamples at random. In the spreadsheet, 0 represents the “ivy absent” condition, and “1” the “ivy present” condition.

Data collected:

plot	condition	mushrooms
9	0	2
9	1	3.3
10	0	2.7
10	1	6
11	0	0.9
11	1	3.6
12	0	1.3
12	1	4.7
13	0	0
13	1	4.7

Or, include a link to a Google Sheets spreadsheet.

Data analysis: In this section, state whether you have run preliminary or final analyses of the data. For example, if you calculated the mean or median to summarize the data for a given day, report that here. Report the software you used for calculations or graphs, the specific calculation you performed, and the results (in a table if complex). If you used R, include the R package you used (e.g. ggplot, or something else) and your code.

11/11/21:
Today we began using R to analyze our data. We summarized the data by calculating the mean, median, and standard deviation of mushroom number in each condition. Then we made an interaction plot so we could clearly see the difference in mushrooms/square meter between the ivy removed and ivy present portions of the same plot. Our code:

```
#read in the data
data.csv<-read.csv("data.csv", header=T)
#check how the variables are coded
str(data)
#make condition a factor variable
data$condition<-as.factor(data$condition)
#calculate mean, median, and standard deviation for each condition
tapply(data$mushrooms, data$condition, mean)
tapply(data$mushrooms, data$condition, sd)
tapply(data$mushrooms, data$condition, median)
#replace "condition 0" and "condition 1" with explanatory labels
data$condition<-factor(data$condition,labels=c("Ivy Removed", "Ivy
Present"))
#make an interaction plot, label the axes, suppress the legend
interaction.plot(data$condition, data$plot, data$mushrooms,
xlab="Condition", ylab="Mushrooms per Square Meter", legend=F)
```

There were many more mushrooms per square meter in the ivy-invaded subplots.
Mean in ivy-removed plots: 1.38 mushrooms/meter squared, standard deviation 1.03
Mean in ivy-present plots: 4.46 mushrooms/meter squared, standard deviation 1.07

Our interaction plot:

We also created a pair of boxplots to summarize the difference between the ivy-present and ivy-removed conditions.
Median of ivy-removed plots: 1.3 mushrooms/square meter
Median of ivy-invaded plots: 4.7 mushrooms/square meter
The boxplot code:

```
boxplot(data.csv$mushrooms~data.csv$condition,names=c("No Ivy",
"Ivy"), xlab="Condition",ylab="Mushrooms per Square Meter")
```

The boxplot:

To strengthen our conclusion that there is a difference between the two conditions, we ran a paired t-test. The code:

```
t.test(data$mushrooms ~ data$condition, paired=T)
```

The t-test output:

```
Paired t-test

data:  data$mushrooms by data$condition
t = -5.5825, df = 4, p-value = 0.005049
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -4.611833 -1.548167
sample estimates:
mean of the differences
                 -3.08
```

The test gave us a p-value of 0.005, meaning the difference is very statistically significant. The t-test also showed that the ivy-present subplots had an average of 3 more mushrooms/square meter than the ivy-removed subplots did.

11/23/21:
After finalizing our graphs, we created an outline for our presentation, and started thinking about what our data means. As there were more mushrooms per square meter in ivy-invaded plots, we wondered if this means that ivy can have positive effects, whereas we had only thought about its invasive properties in a negative way, and whether allowing ivy to grow in certain areas may be beneficial. We discussed how we can be very confident in our results, because of the p-value and how we have a conservative error in the fact that we weren't able to see the mushrooms under the ivy as well in ivy-invaded plots but still found a higher number of mushrooms. We learned that mushrooms don't necessarily prefer cool, moist conditions. Other possible reasons for our findings:

- Other students stepping on the mushrooms more in the non ivy plots?
- Mycorrhizal associations, i.e. mushrooms' underground mycelium connects to roots of green plants and gets nutrients from them. We wondered if the presence of ivy means there are more roots, which might provide more nutrients and lead to a higher abundance of mushrooms.

12/2/21:
We started creating our Google slideshow. First we created an outline of what to put on each slide, then we added the information, photos, and decided who will present each slide. We worked on completing the slides for the rest of class.

Project 3 (link to our Google slides)

HONESTY IN SCIENCE

We live in a world where ethical dilemmas abound, and where people with good lawyers can get away with, well, murder. Even scientists, a group of people once regarded as beyond reproach, are being accused of data fabrication, plagiarism, and the like. This section is intended to be a guide to navigating some of the ethical dilemmas you might encounter in Biology 110 and future Biology courses.

Always check with your instructor to determine whether they have a course policy that is more specific than the guidelines presented here.

Data Fabrication

Students in science classes are sometimes tempted to create or alter their data. The varieties of possible temptations are numerous, ranging from the truly heinously criminal "I didn't have time to do the study, so why not just make up some results?" to the tragic "I wrote my results down on a scrap of paper towel and now I can't find them anywhere and the report's due tomorrow" to the oh-so reasonable sounding "This one data point is way off from the others and I could make a lot more sense out of the results if I just left it out." Is it wrong to succumb to these temptations? Why or why not? How should one deal with these and similar situations?

It is **always** wrong to fabricate data, no matter what the reason. The value of a scientific study depends on the quality of the data that it contains and the care with which the data are interpreted. Making up data makes a study worthless, turning an honest inquiry into an empty exercise.

How then to deal with the situations described above? Obviously, organizing your time so that you can accomplish all the assigned steps is essential. In Bio 110, where investigations are collaborative, it seems unlikely that situation one (running out of time) will even arise, since lab partners will watch over one another's progress. What about situation two (lost data)? To avoid such a crisis, make sure that you always record your data in your lab notebook. If despite these precautions you find yourself in this situation, these solutions, ask your lab instructor for advice.

What about leaving out an outlier? Is it ever okay to leave out a point that doesn't conform to the trend? Well, it <u>is</u> sometimes okay to leave out a point. For example, if you are doing a bacteriological study and you sneeze onto one of the Petri dishes, you can be pretty confident that that dish is going to differ from the non-sneezed-on ones. Throw it out before you even collect the data from it. Explain in your methods that you discarded one plate because it was accidentally contaminated. But what if you had no reason to expect that one of your samples was going to be odd, and then you found that it gave you a weird result? Well, you're stuck with it. Include it, discuss it, speculate about why it was odd, decide to dismiss it if you can make a reasonable case for doing so – **but leave it in your report! Remember that your**

grade does not depend on how "pretty" your data are, but rather on the integrity and the logic, thought, and good sense you apply to their interpretation.

Plagiarism

This term applies broadly to any activity in which a person represents another's work, words, or ideas as their own. The most obvious examples of plagiarism include:

- Taking a sentence or more from a source without putting it in quotes.
- Copying another student's paper (or part of their paper), with or without their permission.
- Submitting work that was written by a paper-writing service.
- Submitting work created, even in part, by using Chat GTP or another AI tool.

But you may not be aware that there are quite a few more varieties of plagiarism, and that these are also unethical. **Plagiarism is considered a very serious breach of academic honesty, and committing plagiarism has serious consequences, so be sure you are thoroughly familiar with its varieties.** Here are some other instances of plagiarism:

1. You take several sentences from a book on invasive species and insert them into a report you are writing, being careful to put them in quotes, and to list the name of the book in your References section. That's okay, isn't it? **NO.** Why not? In college, you are supposed to be learning to do your own thinking. Writing is thinking. Using someone else's ideas, no matter how apt, doesn't demonstrate that you've learned how to do anything but be a parrot. It certainly doesn't demonstrate that you really understand the ideas you're writing about. In most scientific writing, the only time it makes sense to quote a source verbatim is when you want to draw attention to the specific words an author has chosen to use.

2. The Office of Technology Assessment has produced a great reference for your report, Harmful Non-Indigenous Species in the United States. It reads, in part,

> Distinguishing between "good" and "bad" nonindigenous species is not easy. Some species produce both positive and negative consequences, depending on the location and the perceptions of the observers. Purple loosestrife, *Lythrum salicaria*, for example, is an attractive nursery plant but a major wetland weed.

Knowing that you should not quote it word-for-word, you write in your report:

> Telling the difference between "good" and "bad" alien species is difficult. Some species create both beneficial and harmful effects, depending on the place and the viewpoints of the observers. For example, purple loosestrife is a pretty garden plant that is a major weed of wetlands (Office of Technology Assessment, 1993).

Have you solved the problem? No, not in a way that has intellectual integrity. You've demonstrated that you know how to use a thesaurus, but not that you've learned how to

compose original ideas. Paraphrasing such as this, while not technically illegal, doesn't involve thinking. Unless you can express an idea in your own words, neither your instructor nor you can be certain that you understand it. To remove all doubt, digest the information and incorporate it into your paper in your own original way, without using its words or sentence structure. For example, with the above, you might write:

> The effects of alien species often depend on the situation. A species that is considered attractive in a garden may be considered a noxious weed when it occurs in a nature preserve (Office of Technology Assessment, 1993).

Notice that you still need to cite the source of this information, because this information is something you did not know before you read the report.

3. You prompt Chat GPT (or a similar program) to write a paragraph about the different kinds of invasive species, and then paraphrase it for your paper. You do not acknowledge the use of this tool. Here, you have lied about the authorship of the work, and you have squandered the opportunity to learn and develop your own thinking about this topic. Writing is thinking.

4. You can't decide how to interpret your data. Your TA Serena says "It looks to me like there's a negative relationship between the dependent and the independent variables. Look, graph them against each other and see what you see." Well, lo and behold, you see a clear negative relationship. What should you do? In your paper, it is fine to describe this relationship and discuss it. But in the Acknowledgments section, be sure to say "Serena pointed out the relationship between variable x and variable y". If you don't acknowledge Serena's insight, you are committing an act of intellectual dishonesty – you are pretending that her idea was yours. Great scientists don't come up with all their own ideas; honest scientists admit that they don't.

To avoid plagiarizing or paraphrasing accidentally, be careful how you take notes from the sources you read. If while reading the source you write phrases or sentences from the source into your notes, you might reread them later, and think you made them up yourself. If you're copying quotes, put them in quotation marks to remind yourself who the author was (i.e. not you!). Then, when you write your report, read your notes, then close your notebook, and compose from scratch, without having your notes in front of you. Then there will be a much greater chance that the words **and** organization will be your own.

What are the penalties for data fabrication or plagiarism? There are psychic penalties, of course. In addition to those, there are academic penalties, ranging from a reprimand to a failing grade on the assignment or in the course. You will need to appear before the Honor Board to plead your case. The penalties depend on how serious the instructor and the Board judge your offense to be. Because this section on Scientific Honesty is included in your lab manual, which you are expected to have read, little mercy is shown to violators of this policy in Bio 110.

See the Lewis & Clark Academic Integrity Policy (http://college.lclark.edu/student_life/-our-departments-/student-rights-responsibilities/student-code-of-conduct/college-

policies/academic-integrity-policy/) for a description of the policy and how charges that a student has violated it are handled.

In the wider world, published authors are sometimes (rightly) accused of plagiarism, including the use of Chat GTP. Quotes from their books and from their plagiarized sources are printed side-by-side in newspapers. It causes a scandal, brings shame on the guilty author, and has financial repercussions.

TIPS FOR ACADEMIC SUCCESS IN BIO 110 AND BEYOND

The transition from high school to college can be a bumpy one for even the best-prepared student. If you approach your college classes in the same way you approached high school, you're especially likely to experience difficulties. To prevent yourself from struggling, consider the advice found in the sections that follow. In addition, your instructor is an excellent source of information and explanation, so be proactive about asking questions in class and in office hours.

How Much Time Should I Spend on Bio 110?

The faculty expect that for every hour you spend in class, you will spend three additional hours reading, reviewing, completing assignments, and preparing for upcoming classes. A typical course load of 16 credits will therefore involve as much as 48 hours per week outside of class. Face it, you're going to be very busy, and you're normally going to work seven days a week. College is more than a full-time job. If you are working part-time or involved in sports or other extracurriculars, you will need to budget your time very carefully. This section will help you.

Just as important as how much time you spend is WHAT you use that time to do. This section will describe how to get the most out of the time you spend. These ideas come from Dr. Saundra McGuire's 2015 book, Teach Students How to Learn.

Metacognition

The key to academic success can be summed up in one word: *metacognition*. Metacognition refers to thinking about your own thinking. In other words, using metacognition means being consciously aware of your thought processes, and evaluating how well they are serving you as a student. Conscious awareness of your mental processing allows you to monitor your learning, evaluate how successfully you are learning, and accurately judge how well you have learned something. Employing metacognition allows you to evaluate the success of the learning strategies you are using, and informs you about whether it is time to try a new strategy that will make you more successful. Using metacognition will empower you as a student because it gives you the ability to teach yourself what you wish to learn. Sound interesting? Then read on.

Metacognition is especially important in college because college classes come with high expectations for the complexity of the material you will learn and for the depth at which you will learn it. In addition, college students are expected to learn a great deal of material in a short time.

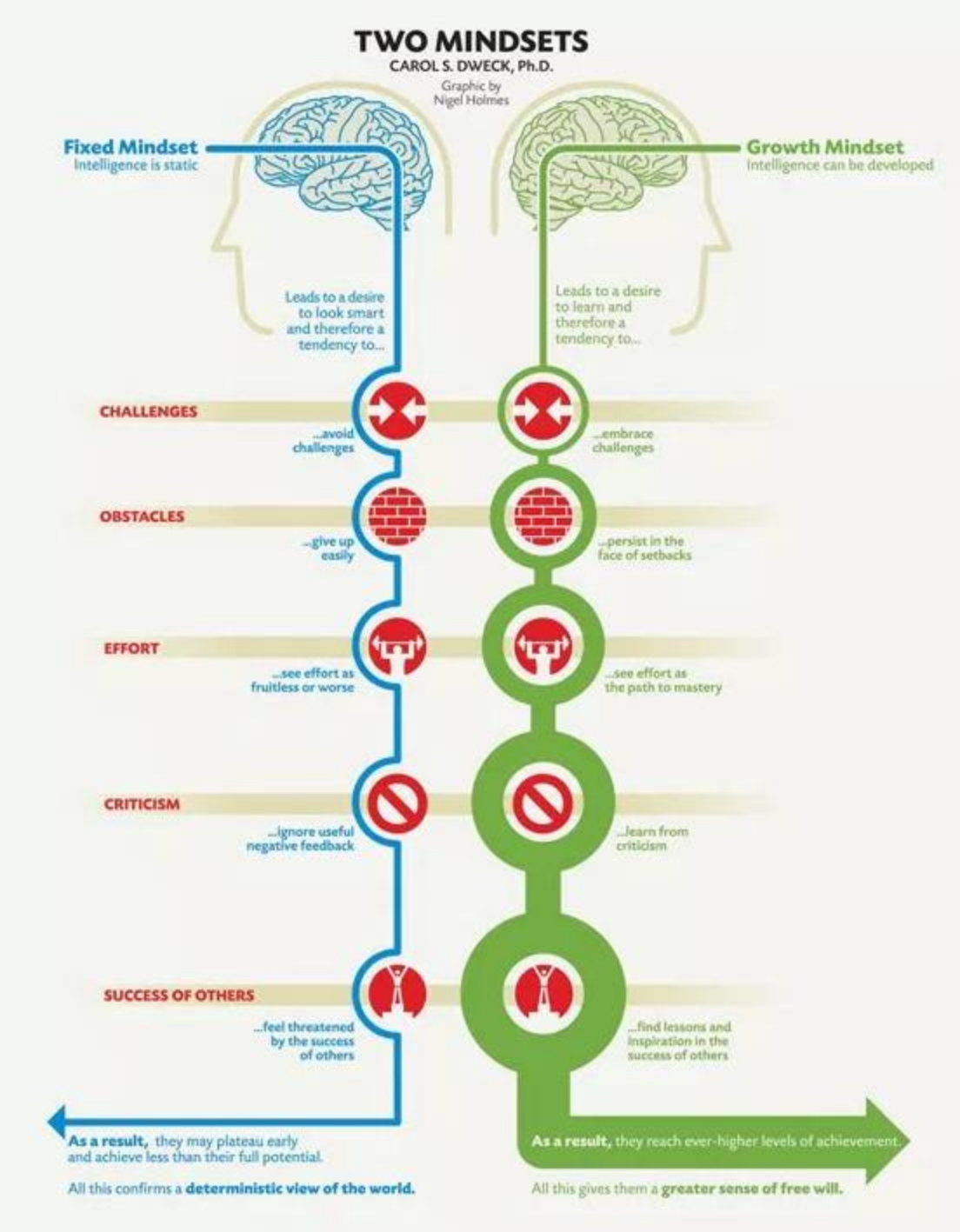
Using metacognition means asking: "what do we mean by 'learn'?" Is there a difference between "studying" and "learning?" Most of us use these words somewhat differently. Many people use "studying" to refer to an activity that has a short-term result ("I studied those definitions for the Bio test last semester, but I've forgotten them."). Learning, on the other hand, is usually used to refer to a longer-term result ("I learned my ABCs in preschool.") As a student, do you spend your time studying or learning? Often, we are merely studying instead of truly learning.

Mindset

Changing from studying to learning requires an understanding of *mindset*. Have you ever believed that you just weren't "good" at something? Maybe you don't feel that you have artistic talent, or musical ability, or athletic grace, or the ability to do math? Carol Dweck, a psychologist at Stanford University, describes this belief as a "fixed" mindset, i.e. the belief that there is a limit to what your brain can learn to do. But Dweck and her colleagues have collected some evidence that everyone can in fact get better at all of these things, given sufficient practice and motivation (Dweck, 2006). Think of the kids who practice skateboard tricks for hours every day after school. What grades would they get on math tests if they devoted a similar amount of time to learning math? Having a "growth" mindset instead of a "fixed" mindset opens the door to greater possibilities for learning.

Prof. Dweck and her colleagues believe that you can change your mindset. To shift to a more growth-oriented mindset, pay attention to your "inner voice." What is it saying? "That was colossally stupid!"? or "I must be really tired. Time to get some sleep and try again in the morning." Recognize that what you believe about your mindset is up to you; you have a choice. When you hear negative self-talk, talk back to that voice with a growth mindset perspective. And when you are faced with choices, ("why work these problems? I'll never be good at math!"), choose the growth mindset option ("The more I try, the harder I work, the better at this I'll get.").

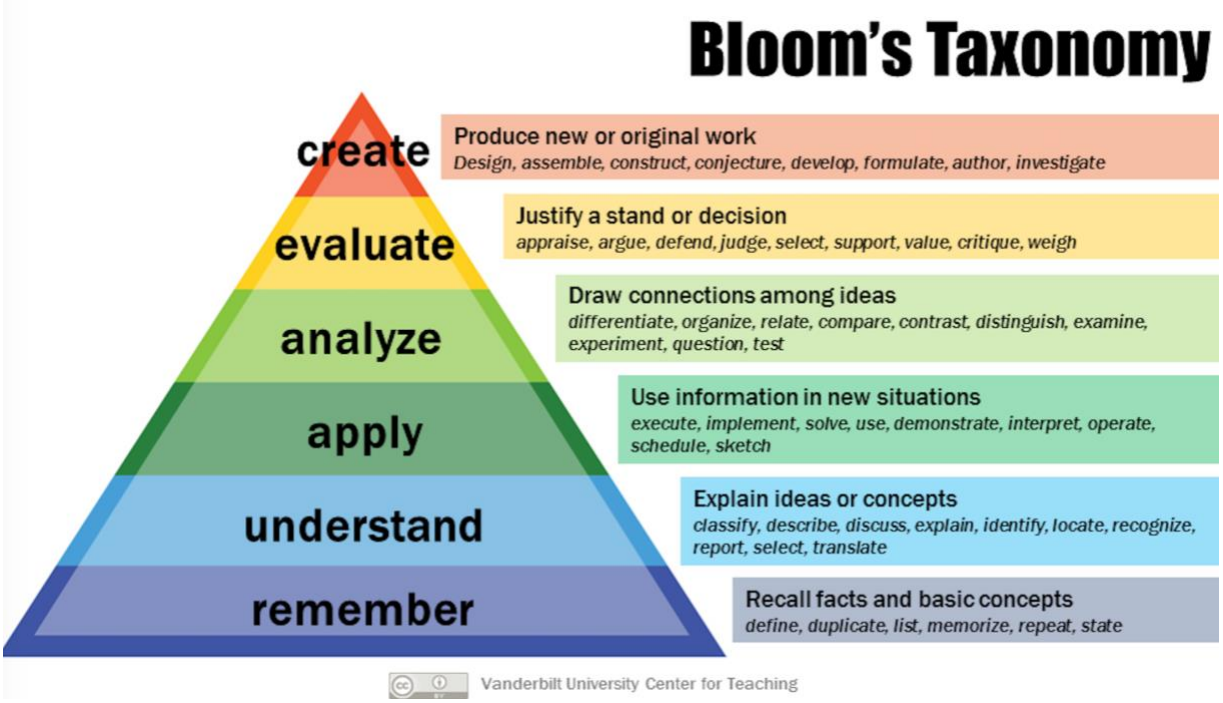
The figure on the next page illustrates the differences between a fixed mindset and a growth mindset.



Bloom's Taxonomy

How do you know when you have actually learned something? Learning requires a level of mastery of the material that goes well beyond being able to remember and restate what you have read in a book or heard in a class. You have perhaps not thought much about "levels of mastery." The diagram below, called Bloom's Taxonomy after its originator, lays out the different levels of mastery of a subject, from simple (at the bottom) to complex (at the top).

Consider which of these levels you needed to work at in order to earn A's or B's in your high school classes. Most studying strategies that you used in high school (reading and underlining, reviewing notes, flashcards, etc.) are helpful for the first level, remembering, and somewhat helpful for the second one, understanding. College classes will expect you to operate at all the higher levels of Bloom's taxonomy, where those study strategies are not sufficient. How can you prepare yourself for applying the material, analyzing it, evaluating it, and creating new knowledge? And how will you know when you are actually prepared to do these things? New metacognitive learning strategies and self-assessment can help you.

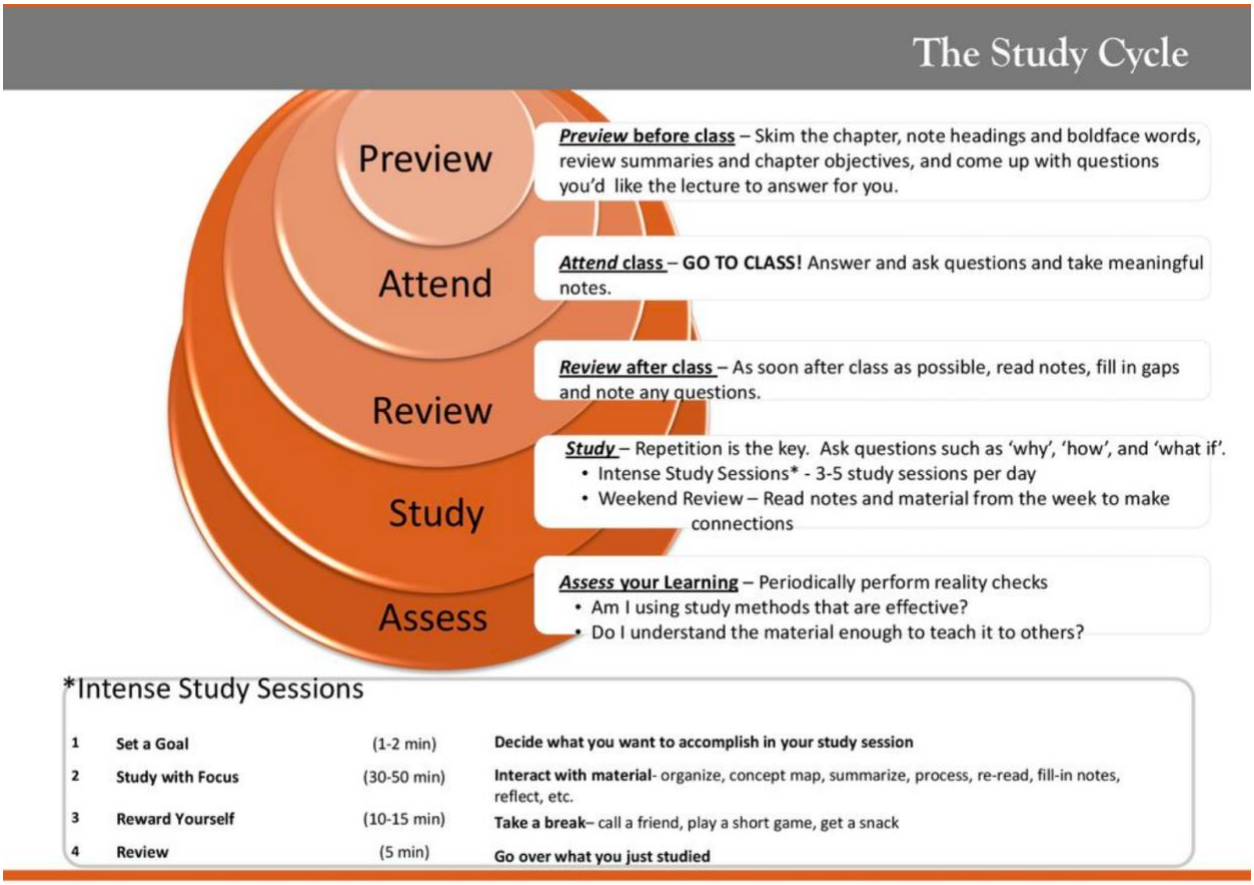


The Study Cycle

To pursue true learning, and to be prepared to operate at all the levels of Bloom's taxonomy, many experts in metacognition recommend that students use the Study Cycle, shown on the next page.

The study cycle has five steps: preview, attend class, review, intense study session, assess. Previewing the reading assignment before class prepares your brain to more effectively absorb the information, and to take better notes. Attending every class is key, because information will be shared there that will not be available in the reading. Taking notes will help you stay alert during class and will help you retrieve information later. Studies have shown that taking notes by hand is a far more effective learning strategy than using a laptop. As soon as possible after class -- the same day is best -- review your notes, fill in missing information that you

were writing too quickly to include, and make notes in the margins about ideas that may not have been clear.



Intense study sessions are a recommended strategy for doing most of your work. They are not just for preparing for an exam. They should be relatively brief (no more than 50 minutes at a time, maybe less if you have a short attention span) because your brain will not be able to stay focused for longer than this. During this time you should be intensely focused on the task at hand; do not listen to music, or have your phone nearby, or allow yourself to be distracted by people or incoming messages. Set a goal for your session, and apply yourself to a specific activity. Maybe it's doing a reading assignment, or working on a problem set, or summarizing a chapter, or outlining a paper draft, etc. When your time is up, give yourself a reward (a snack, a brief walk, a visit with a friend), and then come back and review what you have been doing. If you have been using the session to learn new material, quiz yourself on what you studied. Try to explain it to someone else (even if they are imaginary). Was your work effective? If not, take note and try a new metacognitive strategy next time, e.g. concept mapping (https://learningcenter.unc.edu/tips-and-tools/using-concept-maps/) instead of flashcards. Aim to have several intense study sessions each day, for different classes and/or assignments.

Office hours

Office hours are times that your professor specifically sets aside to meet with students. There are many reasons to go to office hours: to clear up confusion that occurred in class; to get more information about a professor's expectations for an assignment; to get help with a paper topic or homework; to follow up on interesting information introduced in class; to discuss a class-related concern; to get tips on studying more effectively; to discuss the grade on an exam or paper; or just to get to know your professor better, and to help them get to know you. Professors generally announce their office hours with a sign on their office door, as well as in the course syllabus, and on the course Moodle or Google site, if there is one. Some professors ask that students sign up for a specific appointment time during their office hours, and others have a drop-in policy. Just ask your professor about their policy.

Time management

Successful learning requires time. Planning ahead will assure that you have enough time in your schedule for successful learning to happen. We suggest that you maintain two kinds of calendars.

1. A semester calendar that shows the entire semester on 1-2 pages. Using the syllabi for all of your courses, enter on your calendar all assignment deadlines, exam and quiz dates, etc. Then add in important social events and any travel plans. Update this calendar as new information becomes available. A glance at your semester calendar will show you how much time you have to prepare different assignments and where the time crunches might occur. Knowing about these in advance gives you the opportunity to plan ahead to get started on a project sooner than you might otherwise. This site (https://templates.office.com/en-us/Any-year-custom-calendar-TM33674675) has downloadable calendar templates for any month and year.

2. A weekly calendar. This will be larger and more detailed and will allow you to plan each day's activities on an hourly basis. On these weekly calendars, enter your class meetings, hours for part-time jobs, group study sessions, intense study sessions, social time, club meetings, athletic workouts, music rehearsals, meals, laundry, and sleep time. The heavier your load of classes and other activities, the more valuable it will be to maintain a weekly calendar that reserves time for these essential activities. This site (http://templatelab.com/daily-planner-template/) has downloadable daily planner templates.

Preparing for Exams

Preparing for exams is something to start doing on the very first day of class. If you use successful study strategies throughout the semester, then preparing for exams will not be so stressful and you will be much more successful. Here are some strategies that many students have found successful:

- Use effective strategies from day 1 (i.e. the study cycle);
- Determine what the test will cover. Find out what styles of questions to expect. Multiple-choice? Essay? Problem-solving?
- Organize the information by drawing diagrams, graphs, concept maps, etc.

- Set aside multiple specific sessions for studying particular portions of the material;
- Set aside time to visit your professor's office hours with questions about material you found unclear when reviewing your notes or readings;
- Practice your ability to explain terms, concepts, etc. to others -- even an imaginary audience will be helpful. Include examples that demonstrate how to apply these terms and concepts;
- If your professor provides a sample exam, do not waste time memorizing answers to those specific questions -- they will probably NOT be on the exam. Instead, use the sample exam to get an idea of what sorts of questions to expect and what body of material will be covered;
- Make up your own practice test questions, along with a partner, and "test" each other. Once you have reviewed, this is the single most important thing you can do to prepare.

On the day of the test, read the directions carefully, ask for clarification if anything is confusing, and budget your time. Do the easiest questions first. Reduce stress by breathing deeply and talking (to yourself) about how well you have prepared.

After the exam, look at the answer key if one is provided. Review your exam and pay attention to those questions that you answered well. Think about how you prepared for that material. Look at those questions where you did less well. Reflect on what you could have done differently. Ask to meet with your professor for tips on how to do better next time. If you have not been using metacognitive study strategies, now is the time to begin. In her book, McGuire describes many students who have experienced dramatic upturns in their success on exams after adopting these strategies.

Stress and Learning

College students report experiencing a great deal of stress. Many things generate stress: disappointing academic performance; concern about meeting our expectations for ourselves; concern about meeting the expectations of our peers, our families, and/or our professors; juggling multiple responsibilities; worry about the emotional, financial, or physical well-being of friends and family members; our relationships with peers; financial worries; concern about our futures; world events; and more. Some of these sources of stress can be addressed and reduced. Other things are out of our control, but the stress they generate can be managed.

There are important reasons for students learning to reduce and manage stress. A structure in our brains called the hippocampus plays a role in memory formation. Very simplistically, the hippocampus helps move information from short-term memory to long-term memory. Feeling stressed causes our adrenal glands to produce cortisol. In the presence of high levels of cortisol, the functions of the hippocampus are impaired, which can lead to short-term memory loss and problems with long-term memory retrieval. In other words, stress about learning prevents us from learning!

There are many proven strategies for reducing and managing stress:

- Budget your time well. If you plan ahead, you are less likely to fall behind, or to have insufficient time to do your best work.

- Get between 6-8 hours of sleep a night. Try to get on a regular sleep schedule. You cannot make up for an all-nighter by sleeping 10-12 hours on the weekend.
- Eat adequately and healthily. Eat breakfast in the morning; don't skip lunch. Get enough protein and don't overdo on sugar. Make healthy choices and reward yourself occasionally with snacks, but remember: the three main food groups are NOT chocolate, coffee, and gummy worms.
- Get regular exercise. A PE class is a great way to build regular exercise into your schedule. Or join a varsity sport, sign up for frequent College Outdoors trips, take a dance class, go for walks in Tryon.
- Meditation and/or yoga might be effective tools. The Counseling Center has .mp3 meditation tapes (https://www.lclark.edu/offices/counseling_service/selfhelp/) available for download.
- Positive self-talk is a great way to manage difficult emotions. Remind yourself of your strengths and how you have used them to succeed in the past.
- If your stress is resistant to these strategies, or if you are worried about another student, talk with someone at the Counseling Center, (https://www.lclark.edu/offices/counseling_service/for_students/). One-quarter of all Lewis & Clark students visit the Counseling Center at some time during their four years. There are drop-in hours and appointments.

Some Tips for Reading Assignments and Class Preparation

Planning for the week ahead

Spend some time on Saturday planning for the week ahead. Check your syllabus, or Moodle (depending on your professor's system). What are the week's assignments? What reading do you need to complete? Begin with a quick overview of the reading assignment. If it is a textbook, look at the figures as you skim the text. Pay attention to boldface terms, subheadings, chapter summaries. What are the main concepts that the reading is addressing? Your goal for this preview is to get the big picture.

Eventually you should read the assignment again. Your goal on this second reading is to capture the details. If you have been given questions to answer about the assignment, read with these questions in mind, and answer them as you go along. If the assignment is in a textbook, and completion is the only goal, then you can either re-read now, or else wait until after the class meeting, depending on what works best for you. In this second, more detailed reading, do not skip the figures; in a science chapter they are often the most important parts. Pay attention to how the figures relate to the text. Stop periodically (every 2-3 paragraphs) and try to summarize what you have read, with the book closed. Start a list of questions to ask your instructor, mentor, teaching assistant – or all three. Keep a list of key terms and highlight any that you don't fully understand. You'll also want to ask about these.

During the week

Go to class (be on time)! Take notes as you listen to your instructor and other students. Use paper and pen, not a computer; studies show that students who write by hand take in more information. Make marks in the margins of your notes if you're unclear about the content of

some part of the class. These will be questions you may ask later. However, don't hesitate to ask questions in class. If you're confused, chances are good that your peers are, too. Be sure to participate in discussions. Share your thoughts, your questions, and your opinions. Remember, you're going to learn from both your instructor and your fellow students. Contribute to making the classroom an interactive environment. Before leaving class, be sure to get a copy of any assignments or handouts.

After class you should review your notes. Fill in any gaps. You may need to ask your instructor if you've missed something. Identify any questions you may have. Examine your notes to be certain the major concepts are clear to you. Be sure you can rework example problems without the aid of your notes. If homework problems have been assigned, be certain to review the problems immediately so that you can ask any questions you may have. Try to do each problem as soon as possible. Work with a friend in the class. Working together with a peer is also a good way to review.

References:

Dweck, C. 2006.Mindset: The New Psychology of Success. NY, New York: Random House.

McGuire, S. Y. (2015) Teach Students How to Learn. Sterling, Virginia: Stylus Publishing.

SAFETY ADVICE FOR FIELD AND LABORATORY WORK

Following this advice will help you to stay safe and work efficiently while collecting data.

1. Read the procedure thoroughly before coming to class, and think about how best to prepare for the day's work. If field work is planned, create a list of the supplies you will need. Whether you are doing lab work or field work, make sure you understand the safety hazards involved. Do you know how to handle any waste the study will generate? If you have questions, be sure to ask.
2. Record your measurements and observations directly into your field/laboratory notebook in permanent ink following the guidelines of "The Laboratory Notebook" lab manual section. If doing field work in rainy conditions, use a sheet of Rite-in-the-Rain paper (which your instructor can supply) for your data; write on this paper in pencil.
3. During *field* work,
    a. always take a companion who can go for help if you should twist an ankle, etc. **Never** do fieldwork alone;
    b. wear sturdy shoes, *not sandals*; dress for the weather, and anticipate that it may change;
    c. bring drinking water (and a snack) if you plan to be out for several hours;
    d. use sharp tools like shovels or clippers carefully;
    e. know what poison oak and nettles look like, and stay clear of them;
    f. on narrow, steep, or rocky trails, watch your step;
    g. pay attention to your location and how you got there; carry a map and chart your location on it. Carry a cell phone, but don't rely on it to work if you are in a remote location. Cell phones and GPS systems may not work well under forest cover;
    h. keep an eye on the time so you can get home before dark;
    i. bring all your supplies home with you.
4. During *laboratory* work,
    a. work carefully to avoid accidents;
    b. use only the amount of reagent or lab materials needed;
    c. Dispose of excess liquid reagents and solutions according to directions given by the lab instructor. If you don't know where it goes, ask. Never return reagents to the reagent bottle;
    d. always use deionized water in an experiment, not tap water;
    e. if you need extra equipment, see your instructor in order to obtain it. Do not borrow equipment from other lab benches;
    f. ask your instructor or a TA for help if you need it;
    g. clean your work area before leaving the lab;
    h. when you have finished the experiment, check with your instructor before leaving lab;
    i. For more information please refer to the "Lab Safety" appendix of the lab manual.

DATA ANALYSIS AND INTERPRETATION

Data analysis (including data visualization) is a fundamental skill for conducting and interpreting science. It is also important for being a responsible global citizen, because it enables you to assess when data are being used appropriately, and when they are being misused. Here we provide a general introduction to data analysis and interpretation, including the role of statistical analysis, in scientific inquiry.

There are typically three ways of analyzing data collected in a scientific study. One of these is to summarize the data and to present them in a way that facilitates their interpretation. Summarizing data often requires the use of **descriptive statistics.** These calculations reveal tendencies in the results (such as the average value found for a particular variable or the degree of variability in the observations). Another important step is to graph the data, so that any patterns can be seen visually. A third step is to use the data to draw a conclusion about the question being asked and the hypotheses being tested. Depending on the type of study conducted and the sort of data collected, **inferential statistics** are sometimes used to help determine if treatment level had an effect on one or more dependent variables.

The purpose of this section is to help you understand these steps and to carry them out properly. In the section that follows, you will learn about a powerful computer programming language, R, in which you will carry out your analyses.

Categories of data

A study generates "raw" data: the actual measurements collected by an observer. Interpreting these raw data requires summarizing them in some way so that any patterns can be recognized. Different categories of data are summarized in different ways. Here are some important data categories.

Independent and dependent variables

When scientists design studies, they are frequently interested in looking at how different treatments or conditions affect some property of a group of objects. They refer to the treatments/conditions and to the properties they are measuring as **variables** – i.e. things whose value can vary. There are two kinds of variables—an **independent** variable and one or more **dependent** variables. The scientist hypothesizes that the independent variable, which always has more than one level, is responsible for some kind of effect on the property of the objects (the dependent variable).

Here are some examples. Suppose you are interested in whether students' exam scores are affected by the number of hours of sleep they get the night before an exam. "Hours of sleep" and "exam score" are **variables**. You hypothesize that students' exam scores depend on their sleep level. Thus, the dependent variable is the students' exam scores, and the independent variable is the hours of sleep they got. Or, perhaps you are interested in how the temperature of a biochemical reaction affects the amount of product produced. Temperature is the independent variable, and the amount of product is the dependent variable. Or consider a clinical trial in which doctors treat cancer patients with one of three different forms of

chemotherapy, to determine which one is most effective. In this example, the independent variable is chemotherapy treatment type (there are three levels), and the dependent variable is the rate of cure. In any study you do, be sure you understand what the variables are, which ones are independent, and which ones are dependent, because these categories affect how you will analyze the data.

A **data point** (or datum) is an individual value of a variable (e.g. Mark's exam score, the temperature of an individual reaction, whether the patient is cured or not), and **data** are a group of values of a variable, e.g. all the exam scores for a class, the amount of product for all of the reaction trials, the cure rate of all the patients in the study.

Categorical and continuous variables

Both kinds of variables (dependent and independent) can also be classified as either categorical or continuous. When data values fall into one of two or more distinct groups or categories, they are said to be **categorical** (nominal means the same thing). In the first example described above, if the researcher created two groups of students, one group sleeping for 8 hours and another group sleeping for 4 hours, the independent variable, hours of sleep, would be categorical. Or the researcher might opt to compare first-year students with seniors: year in school is another categorical independent variable. But if the researcher asked each student how many hours of sleep they got the night before the exam, and a student could answer any number of hours, the independent variable would be **continuous**. In the other examples, amount of product produced by a reaction would be a continuous dependent variable. Whether an individual patient is cured or not is a categorical dependent variable.

Summarizing Data with Descriptive Statistics

Suppose you conducted the study described above, comparing the exam scores of groups that got 4 vs. 8 hours of sleep. A friend asks what you found. How would you describe your results? It would be useful to give a "typical" exam score for each of the sleep groups. Your friend might also want to know how much exam scores differed *among* students within each category: did everyone in the "high sleep" category outscore everyone in the "low sleep" category? Or was there a lot of overlap in the scores of the two groups? To describe the results of your study, you need a **measure of central tendency** and with a **measure of variability** for each group. These measures are **descriptive statistics** -- ways to summarize data. All of us have used these measures many times, perhaps without even knowing it.

Measures of Central Tendency

The most appropriate measure of central tendency depends on the *distribution* of the data values. If the data values have a "normal" distribution, i.e. when plotted as a histogram they form a roughly bell-shaped curve, as in Fig. 1a below, the **mean** (5.5 in this example) -- which lies at or near the peak of the distribution -- is an appropriate measure of the data's central tendency. Sometimes the distribution of data values is not "normal" at all, as in Fig. 1b. In this case the mean (2.0 in this example) is a poor measure of the data's central tendency; most of the nests had only 1 egg. For data that are not normally-distributed, a much more accurate measure of central tendency is the **median** (the middle value when the data values are

arranged in order from smallest to largest). When the distribution of data is not known, the median is a safer choice.

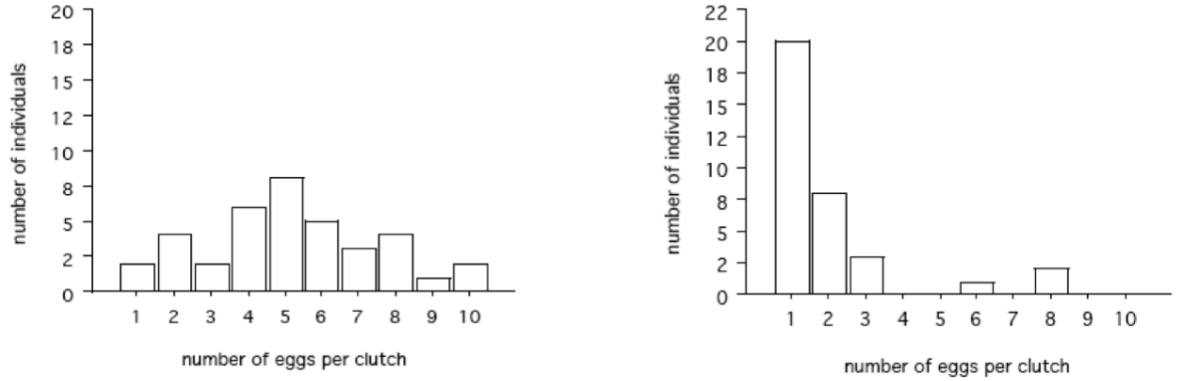


Fig. 1. Distribution of individual bird nests containing different numbers of eggs. a) (left) the distribution is approximately normal, with a peak at 5 eggs. b) (right) a distinctly non-normal distribution, with 1 egg the median clutch size.

1) **arithmetic mean** —The mean is popularly referred to as the *average*. Symbolized by $\bar{x}$ (pronounced "ecks-bar"), it is calculated by summing the data values and dividing the sum by the number of different values, n, the sample size. The formula for the mean is:

$$\bar{x} = \frac{\sum_{i=1}^{i=n} x_i}{n}$$

In this formula, x_i = each data point and n = the number of data values in the data set. The symbol Σ means the sum of all the individual data values that follow the symbol.

2) **median**—The median is determined by arranging all of the values in a data set in order, from smallest to largest. The median is the value in the middle of this range (or the mean of the two middle values, if there are two instead of one). Therefore, 50% of the data values lie above the median, and 50% lie below.

Measures of Variability

There are several different ways to express the amount of variability in a data set. The first four are designed for data that are normally-distributed, while the final one can be used for any kind of data.

1) **variance**—Variance is a measure of the average amount by which a data value deviates from the mean. The more the data points differ from each other, the larger the variance. Variance is often symbolized by the Greek letter σ^2 (pronounced "sigma squared"). The formula for the variance is:

$$\sigma^2 = \sum_{i=n}^{i=1} \frac{(\bar{x} - x_i)^2}{n-1}$$

In this formula, x_i = each data point, $\bar{x}$ = the mean, and n = the sample size.

2) **standard deviation**—the standard deviation of a data set (symbolized as σ and abbreviated s.d. or SD) is the square root of the variance. Standard deviation (and standard error, see below) are the measures of variability most often used in written and graphic presentation of data. In the text of scientific papers, authors will often describe data by stating the mean ± the standard deviation and the "sample size," that is, the number of values in the data set (abbreviated n or N). For example, a typical sentence in a report might read "The mean exam score for the high sleep group was 87 (s.d. = 5; n = 25)."

3) **standard error**- Standard error measures the precision of an estimate of the mean. The standard error of a mean is the standard deviation divided by the square root of the sample size. A mean accompanied by +/- 2 standard errors is nearly identical to the 95% confidence interval (see below).

4) **confidence intervals**– Sometimes means are accompanied by "95% confidence intervals" or "99% confidence intervals." Confidence intervals, as the name implies, are measures of one's confidence that a sample mean represents the "true" population mean. There is a 95% probability, for example, that the "true" mean lies within the 95% confidence interval of the sample mean. The size of a confidence interval is based on the number of samples and on the amount of variability among them. Lower variability and larger sample sizes lead to smaller confidence intervals. A rough rule of thumb is that if the confidence intervals of two bars do not overlap, then the means can be considered to be *significantly* different. The meaning of "significantly" will be explained in the section on Inferential Statistics.

Standard deviation, standard error, and confidence intervals are compared in Fig. 2.

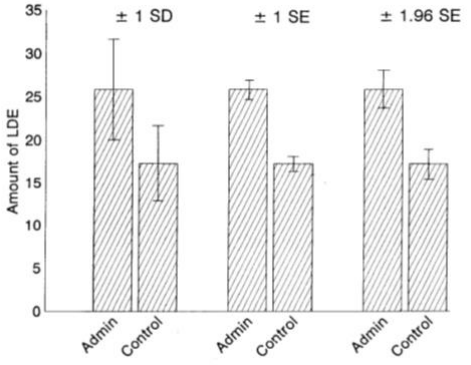


Fig. 2. Each pair of bars summarizes exactly the same data. In each pair, the mean for the Admin group is 25.8 and the mean for the control group is 17.3. The first pair of bars displays ± 1 standard deviation (SD) of the means, the second pair illustrates ± 1 standard error (SE) of the means, and the third pair represents the 95% confidence intervals of the means. Reprinted from Streiner (1996).

5) **quartiles or percentiles**— When data are not normally-distributed, quartiles or percentiles (these words are synonyms) are used to express the amount of variability in

the data. The median represents the middle value in a ranked list of data values; it is equivalent to the second quartile or the 50th percentile. The first quartile (the 25th percentile) is the value that separates the lowest 25% of the values from the upper 75%; the 3rd quartile (the 75th percentile) is the value that separates the lowest 75% of the data values from the upper 25%. The interquartile range is the range of values between the 1st and 3rd quartiles. The more variability there is in the data values, the larger the interquartile range.

There is an important point to remember about variability. For some kinds of data, especially data collected in a laboratory setting, variability suggests that a process is not being carried out consistently. For other kinds of data, especially those collected outside of controlled laboratory conditions, variability is a fundamental, expected aspect of nature—no two students, and no two square meters of forest, are exactly alike. You will need to interpret the variability in your data in a way that is appropriate to your study.

Visualizing Data

In addition to summarizing your data, another essential step in data analysis is to examine your results visually, as a graph. In fact, graphing your data should be the first step in your analysis. A graph will help you determine what your data mean by illustrating any patterns in the data. This section contains some basic principles and methods of data visualization (i.e. graphing).

The type of graph you should create is dictated by the type of data you have (see "Categories of Data," pp. 27-28). If the independent variable in your study is continuous, you should use a scatterplot. If the independent variable is categorical, then you can either use:

- a scatterplot (if you want to show all of your individual datapoints on the graph),
- a bargraph (which shows the means for each category, a suitable format for data values that are normally-distributed), or
- a boxplot (which shows medians, and is appropriate for any data distribution).

The sections that follow describe the details of these three commonly-used graph types.

Scatterplots:

Scatterplots are used when the independent variable is either categorical or continuous, and you wish to represent each individual data value instead of a summary of the values. Figure 3 shows a scatterplot for a study of the relationship between hours of sleep and exam score, with each point representing a different participant in the study. In this instance, the independent variable is continuous.

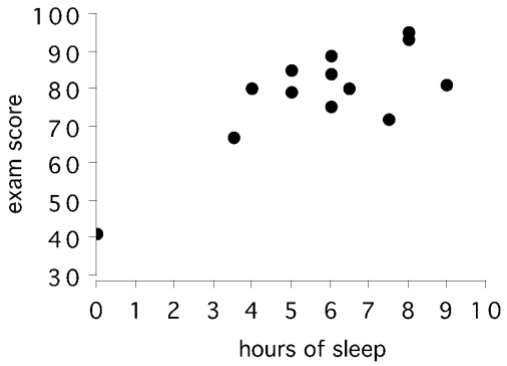


Fig. 3. Exam scores for Bio 110 students vs. hours of sleep before the exam. N=13.

Scatterplots can also be used when the independent variable is categorical. They are a good choice if each different treatment group has only a few data values. Fig. 4 clearly illustrates how the scores of the two sleep groups differ.

Notice that in both scatterplot versions, continuous and categorical, both axes are clearly labelled. The type of label that is appropriate depends on the study. Axis labels should state the variable you are plotting (e.g. exam score) and the **units** of measurement (e.g. hours). If the scatterplot is complicated, with more than one type of symbol or color, it should have a **legend** (also known as a key) to explain what the symbols or colors indicate.

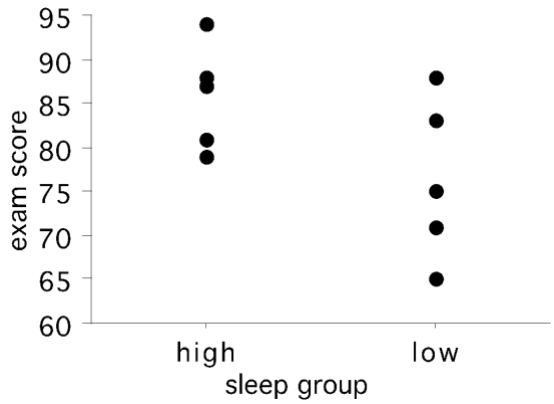


Fig. 4. Exam scores for two groups of students who varied in the number of hours of sleep they had the night before an exam. “High” group received 8 hrs. sleep, “low” group received 4 hrs. N = 5 for each of the two groups.

Boxplots:

When the independent variable is categorical, you are comparing two or more categories, and there are too many data points to plot each one individually, boxplots and bargraphs are the appropriate options. Because boxplots (also called box-and-whisker plots) display medians rather than means, there are no restrictions on the kinds of data (normally-distributed or not) for which they can be used. Thus boxplots are an all-purpose or default graph style.

In Fig. 5 on the next page, the two horizontal bars represent the median exam score for each group. Each box encloses the interquartile range of the data values (the bottom of the box is

the first quartile, while the top of the box is the third quartile). The "whiskers" extend to the maximum and minimum data values for each group. (Some boxplots show extreme data values as individual points falling outside the whiskers.) Note that the boxes and whiskers are not symmetrical about the median; this indicates that the data values are not normally distributed.

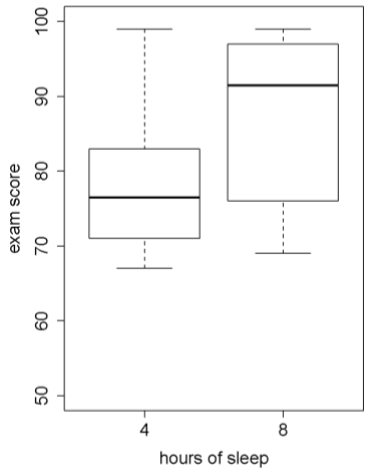


Fig. 5. Boxplot of exam scores for two groups of 25 students each. One group slept for 4 hrs before the exam, while the other slept for 8 hrs.

Bargraphs:

With a categorical independent variable having two or more levels, and too many data points to plot individually, bar graphs are another option, but only if the data values in each category are approximately normally-distributed. This is because the bars represent means, and means are an accurate representation of central tendency only if the data values are normally-distributed. In Figure 6, the two bars represent the mean exam scores for the two sleep groups.

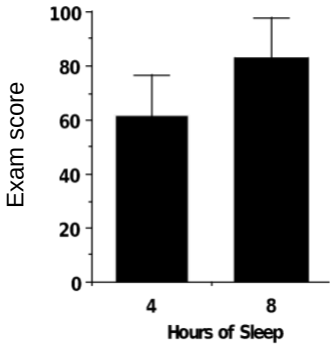


Fig. 6. Exam scores (means + 1 standard deviation) for two groups of students who varied in the number of hours of sleep they had the night before an exam. N = 25 for each group.

Given that drawing conclusions about the results requires considering the variability within each group as well as the differences in the means, bar graphs should have "**error bars"** that represent the variability in the data. (Note: the use of the word "error" does not imply experimental error, but rather error in our estimate of the "true" mean.) Error bars are thin vertical lines that extend above (and sometimes below) the top of each bar. Error bars may be used to display standard deviations, standard errors, or confidence intervals, so it is always

important to explain in a figure's caption what these bars represent. Large error bars indicate that there is a lot of variability in the data values, leading to uncertainty about the value of the true mean. The existence of these bars helps you draw conclusions about the importance of a particular-sized difference between two means.

Sometimes a study has several different independent variables. For example, in the study whose results are illustrated in Figure 7, enzyme activity was measured in three groups of 10 patients each, both before they received an experimental drug (either of two different doses, or a placebo), as well as afterward. One independent variable is treatment group; the other independent variable is timing (before vs. after drug administration). These results can be illustrated in a variant of a bar graph called a clustered bar graph. Note that in this case there needs to be a "legend" (also known as a "key") to explain what the different colored bars represent.

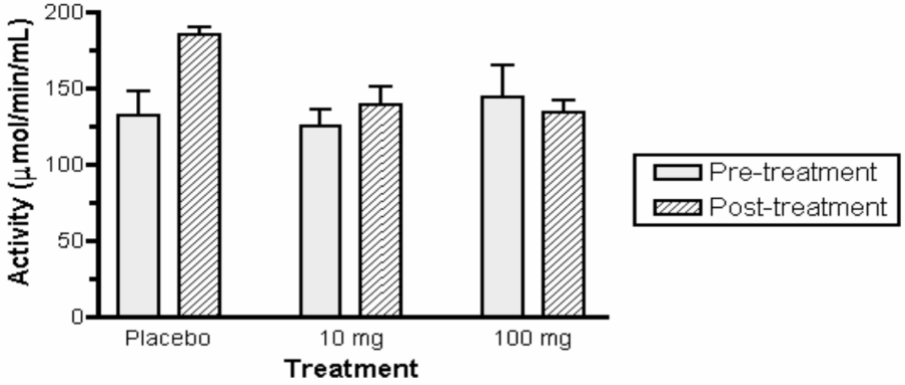


Fig. 7. Enzyme activity before and after treatment with the experimental drug. N=10 for each category.

Other Important Aspects Of Figures:

A figure should be self-explanatory. For this reason, figures have two parts—a) the figure itself, including graphics, axis labels, (possibly) a figure key or legend, and b) the figure caption, an explanation of what the figure is showing. By reading the figure caption and legend or key, if any, a reader should be able to understand the results of your study.

The **caption** of a figure (sometimes confusingly called the figure **legend**) appears *under* the graphic portion. It has two components; a title, and a description of how the experiment was done and what various symbols mean.
Note that in a written report there is no heading or title at the *top* of the graph. However, in oral presentations using slides, it is typical to leave off the caption and to put a brief, large title *above* the graph.

Some things to consider when writing a figure caption/legend:

- The ***title*** of the figure should capture the point of the figure, the conclusion, what the data show.
- The title is followed by a description of the experiment. Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods are described in the Methods section, not in a figure legend.

- A method that is unique to one of several experiments may be reported in a legend only if the discussion is very brief (one or two sentences).
- Define all symbols used in the figure and define all abbreviations that are not defined in the text.
- Do not use the figure legend to interpret the data.
- Do not use the figure legend to describe things that are obvious from the figure itself, such as the names of the axes. Instead, provide information about how the data were collected.
- If there are different samples graphed on the same figure, each should be clearly identified in the figure legend.

There are many more complex ways to present data that are complicated and involve multiple variables. However, a good rule of thumb is: always use the simplest possible figure style that is appropriate for your study. Simplicity aids understanding.

Presenting Data in Tables

Some kinds of data presentation do not involve looking for a visual pattern. In these (rare) cases, data may be presented in the form of a table. Tables are most often used to present data that are qualitative rather than quantitative, as in the example shown below.

Table 4. The effect of medium and temperature on color development in *Serratia marcescens* colonies.

Medium	**Temperature**		
	4°C[1]	22°C	37°C
Minimal Salts + glucose	no growth	pink 61	colorless
Minimal salts + succinate	no growth	pink 2	colorless
Peptone-glycerol	no growth	pink 7	colorless
L-agar	no growth	red 2	colorless
Trypticase-soy	no growth	brown 2	colorless

1 Numbers refer to paint color chip designations.

Notice that the table caption is at the top of the table, not at the bottom (as in figures). When making tables:

- Give each table a number, but number tables separately from figures.
- Make sure that all columns of a table are clearly labeled.
- Try to avoid large tables, as no one will read through them. Perhaps you can present the information better in several smaller tables.
- Do not present raw data and expect your reader to do the arithmetic. While a spreadsheet has rows and columns, it is not a table.

Comparing groups

Scientists often want to compare two or more groups in order to test a hypothesis. For example, suppose you want to compare the length of *Chlamydomonas* flagellae under two conditions. (*Chlamydomonas* is a unicellular alga used as a model organism.) To compare groups, you need repeated measurements for each group. In this example, you need measurements of the flagellar length for multiple individual cells of *Chlamydomonas* for each condition. Then you can compare the central tendency for each group.

For example, suppose you found that under condition A, the *Chlamydomonas* cells had a mean flagellar length of 8.2 micrometers (μm), while the mean length for condition B was 10.4 μm. How can you best communicate this difference? You *could* simply subtract 8.2 from 10.4 and state that "Flagellar length in condition B was 2.2 μm longer than in condition A." But if the actual means for the two conditions were 160 μm and 162.2 μm, a difference of 2.2 μm would be trivial. So reporting this "raw" difference is not very informative.

A better comparison is the *ratio* of the two means. In this example, the mean flagellar length in condition B, 10.4 μm, is 1.3 times higher (or 130% higher) than the mean flagellar length in condition A (10.4/8.2 = 1.3). This is a much more meaningful way to describe the difference. There are yet other ways to make a useful comparison between two values; think about what some of them might be.

While the approaches just described can help a reader ascribe meaning to the differences found between two or more sets of observations, it should be obvious that this remains a very subjective assessment. It should also be apparent that differences between groups will almost always be found. For example, *simply based on chance*, it seems very unlikely that we would generate *the exact same* mean if we measured the flagellae of 10 *Chlamydomonas* cells from each of two different conditions (even if the conditions had no effect on flagellar length). Different cells will differ for many different reasons. Here we face a challenge that all scientists face: given that we expect *some* level of difference between our sample groups, how can we know what level of difference we can expect to occur by chance alone? Clearly, we need an objective way to judge whether the differences are meaningful.

Inferential Statistics

Probability theory gives us this objective approach. Based on the null hypothesis that any differences observed between two groups of data values are simply a product of chance, inferential statistics examines the way that individual observations vary from one another. From patterns of variance within the groups and between the groups, it is possible to compute a probability, or *p value*, that the observed differences are due to chance alone. Computing this probability is called carrying out a *statistical test*. We use a conservative approach to statistical testing, rejecting the idea that there is an association between variables, or that the treatment had an effect, unless the test reveals that there is only one chance in 20 (i.e., a probability of 1/20 or $p = 0.05$) that the result might have arisen simply by chance. If we find that this probability is above 0.05, scientists generally reject the hypothesis that the variables are associated, or that the treatment had an effect.

Just as with graphing, the kind of statistical test we perform depends on the nature of the data we have collected. We use a different test depending on whether the independent and dependent variables in our study are continuous or categorical. Here are some commonly-used tests.

Both Independent And Dependent Variables Are Continuous:

When both variables are continuous, we are generally hypothesizing that there is some *association* between the value of the independent variable and the value of the dependent variable. The likelihood that an apparent association is due to chance can be investigated with a regression analysis.

The values of the two variables are plotted as a scatterplot (see Fig. 3). The points form a cloud that may indicate a pattern of association. To better see this pattern, we can generate a "line of best fit" through the points. This line, by definition, is the one that minimizes the distance between each point and the line. A linear regression analysis can calculate the parameters for the equation $y = mx + b$, where m = the slope of the line ($\Delta y/\Delta x$) and b = the y-intercept. This line describes how the value of the dependent variable changes with respect to changes in the value of the independent variable.

A linear regression analysis can also tell you whether the observed relationship between the two variables is more than mere coincidence, because it assigns a probability (from 0 to 1) to the statistical hypothesis that the slope of this best-fit line is different from 0. (A line with a slope of zero is flat, indicative of no association.) You will find the procedure for carrying out a regression analysis in one of the R tutorials.

Independent Variable Is Categorical, Dependent Variable Is Continuous:

Comparing two or more groups involves calculating the median or mean values for each group. But the size of the difference between the means does not tell us whether the two groups are truly different. Instead, we need to compare the difference between the means or medians to the spread or variation in the data values found within each group. The larger the between-group difference relative to the within-group variation, the less likely it is that the observed difference between groups is due to chance alone.

For example, consider the following exam scores, earned by two groups of 10 students who each got different amounts of sleep:

8 Hrs Sleep	**4 Hrs Sleep**
93	88
91	83
90	82
89	81
89	79
87	77
88	76
83	75
83	74

The means for these two groups are:
8 Hrs: $\bar{x}$ = 87.6 4 Hrs: $\bar{x}$ = 78.7

Based on the nearly 9-point difference between the means, you might be tempted to conclude that the amount of sleep students received truly affected their exam scores. This conclusion is supported by Fig. 8 on the next page, a scatterplot that shows that most of the scores for the 4 hr sleep group are lower than most of those for the 8 hour sleep group.

83 72

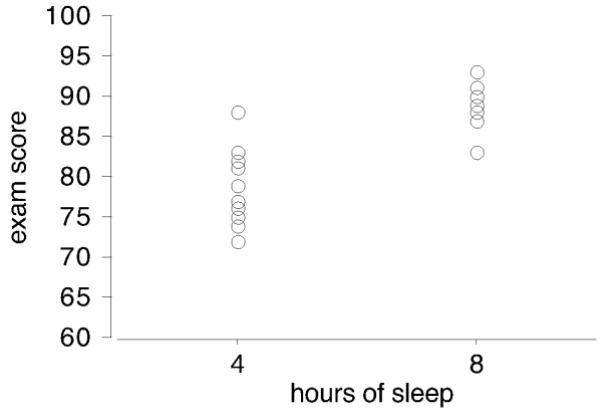


Fig. 8. Exam scores for two groups of 10 students each. One group slept for 4 hrs before the exam, while the other slept for 8 hrs.

But what if you obtained the following data for the two groups instead?

8 Hrs Sleep	**4 Hrs Sleep**
99	99
97	90
97	83
95	81
93	80
90	73
85	73
76	71
75	70
69	67

Notice that the means for the two groups are still different by nearly 9 points:
8 Hrs: $\bar{x}$ = 87.6 4 Hrs: $\bar{x}$ = 78.7
But now the variation around each means is much greater, with scores from both groups much more interspersed (Fig. 9).

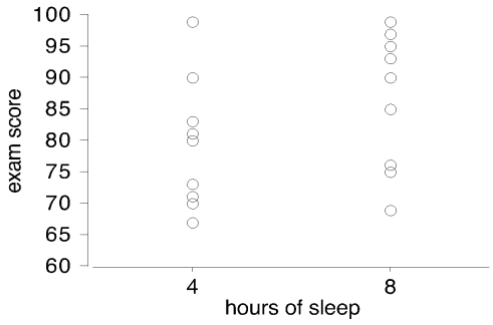


Fig. 9. Exam scores for two groups of 10 students each. One group slept for 4 hrs before the exam, while the other slept for 8 hrs.

For these data, there is little support for the hypothesis that hours of sleep affected exam scores. This is why we cannot simply look at the mean values for different groups and decide

whether the treatment level had an effect. Instead, for situations where the independent variable is categorical, the data values are normally-distributed, and two groups are being compared, we should conduct a t-test. If more than two groups are being compared, an Analysis of Variance (ANOVA) is used. And if the data values are not normally-distributed, and we wish to compare two groups, we can use a Mann-Whitney (Wilcoxon) test. You will find the procedures for carrying out these tests within the R tutorials.

Depending on the nature of your data (i.e. if your sample size is large enough and your results suggest a trend), it may be appropriate to use a statistical test on your results. Your instructor and teaching assistants can help you decide whether a statistical analysis is an appropriate complement to your graphical analysis. To learn more about inferential statistics, take Math 123: Calculus and Statistics for Modeling the Life Sciences.

R, A Powerful Programming Language for Data Display and Analysis

There are many different software packages available for data analysis. For example, you have probably used Excel for simple computations and graphing. All of these packages must be purchased, many are quite expensive, and all have limits to their functionality. In Bio 110 you will learn to use the programming language R. R is a powerful, sophisticated open-source statistics and graphing programming language. R is free and there are virtually no limits on what you can do with it. Users all over the world are constantly developing new R code to carry out sophisticated new analyses, and sharing it. While R is a bit challenging to learn, once you develop some basic skills, you will find yourself using it in many of your courses. In addition, the ability to program in R is an important and sought-after real world skill. In Bio 110 you will learn the fundamentals of R, and after that you can expand your range of R abilities on your own.

Eventually you will want to install a copy of R on your own laptop computer. But for now, because it is so easy to use, you will use R via R Studio Cloud. You do not need to sign up for an account in order to use it.

There are many sites available that can help you build you R skills. Here's one useful one: https://ourcodingclub.github.io/tutorials/intro-to-r/. Another easy-to-use site is Quick-R.

References

Streiner, D. L. 1996. Maintaining standards: differences between standard deviation and standard error, and when to use each. Canadian Journal of Psychiatry 41(8): 498-502.

COMMUNICATING THE RESULTS OF SCIENTIFIC INVESTIGATIONS

A critical part of the scientific process is communicating the findings. In this section, we describe three ways scientists convey ideas to their colleagues: research manuscripts, posters, and oral presentations. Each has distinct forms and conventions, but also similarities. In this manual we describe each type of communication and their conventions. As in all courses, it is important that you check your instructor's expectations.

The Research Manuscript/Scientific Paper

A research manuscript is a formal way for working scientists to report the results of original investigations in a public and permanent fashion. Research manuscripts appear as articles in scientific journals published in print and online. Due to the importance of this "primary literature" (see Appendix 3) in informing new investigations, articles published in journals undergo rigorous peer review for clarity, accuracy and importance. We want you, as a working scientist, to understand this process.

A major goal of BIO110 is to help you hone your skills in reporting scientific results in written form. In BIO110, we have incorporated a series of writing assignments that build to a written manuscript in the style of a primary research article submitted to a scientific journal. You will practice writing in a format that many of you will use as future scientists.

To ensure that every student gets feedback on the basics of writing a manuscript, there will be individual assignments first. However, professional scientific writing is often a collaborative exercise. To model this, your writing project in BIO110 will be collaborative as well. Your group members can and should provide editing input on the section you write, as you should for them. To do this effectively you will need to complete work early enough to allow time for feedback prior to submission in class.

General Advice When Writing A Scientific Paper

The rules of good scientific writing are not so different from good writing in any other field. The purpose of a scientific paper is similar to that of other academic writing. It is a narrative of your investigations and an argument about their meaning. In many ways, the principles of scientific writing are the same as academic writing in other disciplines:

- **Language:** You need to be articulate. Scientists, however, make very precise use of words, so it is particularly important that you develop a scientific vocabulary that will allow your meaning to be clear. Scientific writing is often described as concise and non-ornamental. This does not mean it has to be boring.
- **Organization:** You will need to organize your ideas into paragraphs that start with clear topic sentences. Logic is especially important in scientific writing, so you will need to use some tool (an outline?) to organize your ideas.

- **Spelling:** You need to spell correctly and to be grammatically correct. Take advantage of spell checkers and grammar checkers and fix the problems that the software identifies for you.
- **Structure:** Scientists use a particular format to organize their papers (discussed in detail below).

The instructions below explain how to write a scientific paper. We will evaluate your papers based upon points emphasized in this manual. In addition, the best way to learn about the correct format is to look at papers published in the *primary* scientific literature. There are many scientific journals in the Watzek Library (or accessed online through the library). Some are general science journals like *Science, Nature,* and *The Proceedings of the National Academy of Sciences of the U.S.A.* Others are more specialized journals focusing on a particular area of biology, such as *Cell, Genetics, Ecology,* and *Evolution.* Perusing any of them will give you a good idea of what is typical, although *Science* and *Nature* typically publish articles in a very condensed format, so the other journals listed would be a better place to look for examples of how your own manuscript should look.

Organization of a Scientific Paper

Scientific papers usually contain these sections in this order: Title, Abstract, Introduction, Methods, Results, Discussion, Acknowledgments, and References.

Title

The title tells what the paper is about, so the best time to determine it is after you have completed your paper. A title should be informative, specific and concise. Since you are not writing a murder mystery, it is all right to tell the "ending" in the title. A strong title includes the objective and primary conclusions of the investigation in a concise manner. It is often this information that helps readers decide if the paper is something they want to read.

Under the title, place the author(s)' names and "professional address," which here is your specific course and laboratory section. This information should either be placed alone on a title page, or at the top of the first page in order to save paper (ask your instructor).

1. Choose an appropriate title that is clear and concise but gives some insight into the aim of your experiment.
2. Avoid trivial titles like "Our Lab Report" or "Biology Lab Report #1" or "Mutant Investigation."
3. To see some models for what titles of scientific papers look like, check out an issue of a scientific journal in the Watzek Library, such as those mentioned above.

Below are examples of titles that: (1) are Weak, because they tell the reader very little about the investigation; (2) are Better, because they give specific information about the type of investigation, though they fail to inform the reader about the nature of the results; and (3) are Strong, because they indicate the objective and primary conclusions of the investigation in a concise manner.

Weak: "Lab 2 - Plant phenotypes"
Better: "Growth and phenotypic variation in *Solidago gigantea*"

Strong: “Heritability in *Solidago gigantea* is lower for growth than for leaf size”

Weak: “Lab 1: Heart Lab”
Better: “The function of frog hearts”
Strong: “Epinephrine Increases the Strength but not the Rate of Contraction of Frog Hearts”

Weak: “Lab 1: Growth curves”
Better: “Growing fibroblast cells in culture”
Strong: “Lower serum concentration inhibits fibroblast cell growth in vitro”

Abstract

The Abstract is a short summary of your paper, designed to help readers decide whether to read the entire paper. An Abstract includes a brief introduction to the problem being studied, a brief statement of how the study was conducted, a brief summary of the major results, and a brief statement of the significance of those results.

1. Ideally there is one sentence in the abstract representing each section of your paper (e.g. 1-2 sentences summarizing intro & research question; one sentence summarizing methods; one sentence summarizing results; one sentence summarizing discussion/conclusions).
2. It should be short and to the point—only one paragraph and no more than ~250 words. Make every word count, so you can convey the most information in these few words.
3. It should be informative—what was the purpose of your study, how did you carry it out, and what are your main conclusions?
4. The Abstract should be written *last*. You cannot summarize a written work you have not yet composed.

Here is an example of a strong abstract:

> Fluoxetine (Prozac) is a frequently prescribed antidepressant, identified as a selective serotonin reuptake inhibitor. Prozac’s function as an SSRI leads to the popular belief that its antidepressant mechanism is related simply to an increased serotonin level in the synapse, due to the blockage of the serotonin reuptake pump. However, while some previous research has suggested that Prozac acts as an agonist, other research has suggested that Prozac also acts as an antagonist of 5HT2C receptors, a function apparently contradictory to its role as an SSRI. We sought to further elucidate Prozac’s effect on 5HT2C receptors in the crayfish neuromuscular junction. To determine if Prozac acts as an antagonist of 5HT2C receptors in crayfish *Procambrius clarkii* neuromuscular junctions, we compared excitatory postsynaptic potential (EPSP) amplitudes of control/5HT treatments, and control/Prozac and 5HT. Our results suggest that Fluoxetine does indeed act as an antagonist of 5HT2C receptors in the crayfish neuromuscular junction.

Introduction

The introduction should briefly describe all the background information necessary for the reader to understand what question you are asking and **why** this is an interesting problem. It must also make clear what your specific testable hypothesis was. A good introduction will mention the major issues that will be considered in the Discussion section. For that reason, it may be helpful to write it, or at least to revise it, after finishing the other sections.

Assume that the reader is at least moderately familiar with the general subject of the paper. It may be useful to imagine other students in the class as your *audience*. There are many ways to organize an introduction, depending on its length and audience, but one principle is to begin by giving your reader some background and then developing the general importance of your question. For example, just saying that you want to test the effect of pH on enzyme activity is insufficient. A reader will want to know *why* you want to test this. Next, move to the ideas leading to your specific study and provide a clear rationale for the experimental question. If you are using a particular experimental method, provide a rationale for having selected it. If your study tests a particular hypothesis, you might end your introduction by stating your hypothesis (with justification, of course) and describing in the most general terms how your investigation addressed it. For example, are most enzymes sensitive to pH? Why? What pHs will you test and why? What is the logic behind your hypothesis? Finally, be sure that any sources you use to develop your background section are cited. These will normally be textbook references or published peer-reviewed scholarly articles. Much self-published web content is not peer reviewed and may be unacceptable. Please check with your instructor if you are not sure about the validity of a web site source. Note: Wikipedia sites with substantial cited sources are usually acceptable.

1. Raise the context for the question you're asking. Why is the area of research interesting or important?
2. **State your question.** Explain to the reader why the question itself is important, why the reader should care.
3. Clearly **state the hypothesis**—an informed "best guess" answer to the question. Provide a logical explanation about why that hypothesis is reasonable. This **justification** must be grounded in existing knowledge, with reference to existing literature. In some cases multiple competing hypotheses may be reasonable. Explain and justify each of these.
4. Explain what **existing knowledge** has helped you to generate your hypothesis (-es). What have others found? Cite at least 2-3 primary sources. See Appendix 3 for guidelines to finding and employing references.
5. Provide a skeletal **outline of your experimental design**, and explain the logic of how the experiment tests the hypothesis. In other words, explain HOW you will answer the question.
6. Include a statement of what you **predict** you will see in the results (generally) if your hypothesis is true.
7. By the end of this section the reader should know why your study is important and what hypothesis you are testing or question you are asking, they will understand the basic experimental design by which you test your hypothesis, and they will have a description of the outcomes that would indicate support for the hypothesis. At the end

of the Introduction, state (or re-state) your question before you launch into a more detailed explanation of your experimental approach.

Here are two examples of strong Introductions:

Example 1 (ecology):
As urbanization pushes human settlements into natural ecosystems, we need novel paradigms to understand the ecological dynamics of these heterogeneous habitats and to quantify the impact of human activities on biological processes. Urban landscapes can be complex matrices of disparate factors that both contribute to disturbance, such as chemical pollution, and nurture biodiversity, such as well-designed parks. This complexity makes it difficult to tease out the ecologically relevant impact of urban disturbances (McDonnell & Pickett 1990) and evaluate the quality of ecosystem dynamics at local sites (Sadler et al 2006). To simplify these assessments, research in urban ecology employs the "gradient paradigm" which examines the variation in factors related to human disturbance with distance from a city center to less densely populated suburban and rural areas (Austin 1987).

Researchers then seek to relate this gradient of anthropogenic disturbance to easily measured indicators. These indicators can include populations or communities that are especially sensitive to various manifestations of human activity, such as soil compaction or the amount of paved area. Noss (1990) suggests that the organisms be widespread, manageable to sample, and responsive to environmental changes (Figure 1).

Previous research has identified bird and butterfly populations as reliable biological indicators that are remarkably sensitive to gradients of urbanization (Blair 1999). These models, however, respond to large-scale changes in the landscape, not small-scale changes that could impact micro-sites in vegetation or soil characteristics. Soil arthropods may fill this need by serving as ubiquitous, minimally motile, and easy-to-collect indicators of local site variables. Additionally, the abundance and community structure of these arthropods has important ecological implications; soil organisms influence the rate of litter decomposition (Seastedt 1983) and balance of soil nutrients (Blair et al 1992). Some soil arthropod species graze on bacteria and fungus, impacting microbial communities (Dress & Boerner 2003).

Based on comparisons between sites with distinct natural histories, soil organisms do respond to dramatic disturbances, such as intense grazing (Mikola et al 2001; Clapperton et al 2002) and fire (Dress & Boerner 2004). Clapperton et al (2002) also concluded that Acarina, a group that dominates most samples across a wide range of environments (Dress & Boerner 2003; Cedpeda-Pizarro & Whitford 1989 and others), are especially sensitive to soil disturbance. Less research, however, has investigated the soil community's responses to finer-scale variation like that associated with urban landscapes.

To determine if soil microarthropods can be used as an accurate biological indicator, this study examined their abundance and group diversity along a transect from the urban middle to the rural edge of a college campus in Grinnell, Iowa. We collected soil arthropod samples and assessed relevant site and landscape variables along the Prairie Walk, a kilometer long strip of prairie plantings.

The study investigates the following questions:

Does the Prairie Walk accurately represent an urban-rural gradient? Do site variables, such as soil compaction, organic matter, moisture, and temperature, and landscape variables, such as impervious surfaces, correlate with distance from the campus?

Do site and landscape variables—including soil compaction, soil moisture, soil temperature, soil organic matter, and proportion of landscape covered by impervious surfaces—reflect the abundance and diversity of soil microarthropods?

Do the abundance and diversity of biotic communities below ground, at the surface, and in the air correspond to an urban-rural gradient?

We anticipated that the sites furthest from campus would be subjected to less intense human disturbance and would exhibit greater percent soil organic matter and moisture and lower soil compaction, soil temperature, and surrounding impervious surfaces. Vegetation height may correlate with higher soil moisture and lower soil temperature because the leaves would shade the soil surface. Most likely, the most abundant and diverse soil microarthropod communities would inhabit these sites on the rural end of the gradient (Figure 2). We expected the arthropod communities both below and above ground to respond similarly to the urban-rural gradient.

Example 2 (neurobiology):
The knowledge of how nerves function is imperative to understand bodily functions in any animal. Many chemicals are involved in the nervous system and most of them have multiple effects. For example, 5-HT, one of the most abundant neurotransmitters in the nervous system, has been shown to increase excitatory postsynaptic potential (EPSP) amplitudes in the crayfish neuromuscular junction (Dropic et al. 2005 and Etzkorn et al. 2006). The exact mechanism behind this is unknown, but the increase in EPSP amplitudes could be due to one of two intracellular calcium release receptors—that of either IP3 or ryanodine (Mattson et al. 2000). Past research has also suggested a link between the effects of IP3 inhibitors and 5-HT on EPSP amplitudes (Dropic et al. 2005). 2-APB is known to be an IP3 inhibitor but may affect EPSP amplitudes through mechanisms other than an IP3-induced Ca2+ release (Dropic et al. 2005). Through application of the IP3 inhibitor 2-APB, we aim to determine whether 5-HT affects EPSP amplitudes through IP3-induced Ca2+ release and if 2-APB and 5-HT have an unknown combined effect on EPSPs. This research is important because understanding the effects of 5-HT and the mechanisms through which it affects EPSPs will lead to a greater understanding of many neurological functions and how they occur.

We hypothesized that the application of an IP3 inhibitor, 2-APB, will negate the effects of 5-HT on EPSP amplitudes. We already know that 5-HT causes an increase in EPSP amplitude (Etzkorn et. al. 2006), however, we wanted to know the combined effects of 5-HT and 2-APB. Our data supported our hypotheses that 5-HT works through an IP3-induced Ca2+ release and that 2-APB decreases EPSP amplitudes when 5-HT has previously been applied to the preparation.

Methods

This section should carefully explain how the research was done. This section describes what you did, where, when, and for how long. This section is detailed so that anyone wishing to confirm your results could repeat the experiment as you did it. If you have used a published protocol or one provided to you, you can summarize and refer to it without repeating it in detail. However, be sure to include enough information that a reader could get a general sense of what you did without looking up each of your sources. You'll also need to include any modifications you made to the published protocol. Keep in mind that this section is written in prose, just like all the other sections of your paper.

Methods sections should be organized by procedures rather than by chronology. Use subheadings for different procedures. Describe the key details of each technique rather than writing a blow-by-blow description of all the steps you did in order. This organization is quite different than the set of instructions you may have received as a handout, which is usually chronological. The handout is a protocol, not a methods section.

1. The Methods section should be written in the past tense. For clarity and conciseness, use the active voice.
2. Do *not* write lists of materials you used, nor write the Methods as a protocol (i.e., in cookbook fashion). Methods should be written in a narrative fashion.
3. Methods sections always describe solutions and reactions in terms of the final concentration of the compound or drug in the reaction; expressed as % (vol:vol) or mM or mg/ml (weight/vol). This is because others will do different sized reactions, and it is not the volume but the *concentration* of the components that is important in the reaction. Thus do not use any volume units in your methods section.
4. If you develop your own technique, explain it in sufficient detail that another person could replicate your work.
5. If you are doing a field study, indicate the location of the study and the dates on which it was carried out (since this may be important to the results). Do not do this for a laboratory study.
6. The Methods should include a description of any statistical tests you employed.
7. Include references when referring to published protocols or methods. You may cite course handouts for any techniques that you performed as described therein.
8. Do not present your Methods as a diary or the materials as a list. Write in complete sentences and organized paragraphs.

Here are some excerpts of strong and weak methods:

Weak:

“We measured growth rates and wrote down the data.” How did you measure growth rates? You don’t need to tell the reader that you recorded the data or entered it into the computer.

“The data were entered into Excel for manipulation.” Likewise, graphical or spreadsheet programs do not need to be mentioned; however, statistical programs and statistical tests should be mentioned.

“The cells were washed in saline and re-suspended in medium.” What solution did you use to wash the cells?

"On the first lab day, we extracted the DNA and froze it. Then, the next week, we ran the DNA on an agarose gel." The methods section is not a diary of your lab work. Describe what you did concisely and in a logical order. It needn't be the exact order you did it in, unless that is critical.

Strong:
"We used R software to perform a t-test to determine whether mean photosynthetic rates differed between the two light environments."

“We prepared 5 solutions ranging in concentration from .0050M to .010M, by serially diluting a solution of .050M silver nitrate.”

“We pelleted cells at 5000 x g for 5 minutes and then washed them in 0.05 M NaCl. Cells were pelleted again and re-suspended at a concentration of 108 cells per ml in peptone-glycerol broth.”

Here are some examples of strong Methods sections:

Methods Example 1 (insect biology)

Sexual Size Dimorphism Assay

To investigate sexual size dimorphism of *Callosobruchus maculatus*, we conducted a factorial experiment (two strains x two hosts) using Yemen and Burkina Faso strains of beetles placed with the natal hosts *Vigna radiata* (mung beans), and *Vigna unguiculata* (black-eyed peas). We created four 96 well plates with each of the four strain x host combinations: Yemen on mung, Yemen on black-eyed peas, Burkina Faso on mung, and Burkina Faso on Black-eyed peas. We isolated virgin beetles by placing each bean in its own separate well, then monitored the trays for emerging beetles. This incubation took place at 27℃ in 70% humidity. Once the beetles hatched, we removed them from the wells and used them to set up the oviposition preference experiment. We avoided females that had emerged with males in the same cell. We massed all beetles on an Ohaus scale, then placed ten pairs in plates that contained ten mung beans and ten black-eyed peas each. We incubated the plates for 72 hours at 27℃ in 70% humidity. After seven days, we used a microscope to count the number of eggs laid on each bean type. We then photographed each beetle and used ImageJ to measure the elytron length.

Statistical Analysis

We conducted a statistical analysis on 34 of the 41 plates (no eggs were laid on 7 of the plates). We calculated the proportion of eggs laid (by females) on mung beans per plate. We used two three-way ANOVAs to investigate strain, natal host, sex, and their interactions. We performed a two-way ANOVA for the effects of natal host and strain on oviposition preference. We performed all statistical analyses in R.

Methods Example 2 (cell biology)

Samples

For both the quantitative cell count and the turbidometric mass determination, the lab instructor supplied samples of the same 24-hour old stock culture of *Serratia marcescens*.

Quantitative Cell Count of *S. marcescens*

We serially diluted an aliquot of the stock culture of *S. marcescens* (approximately 109 cells/mL) in sterile saline solution to generate three solutions with dilution factors (Fd) of 105, 106 and 107. Using a flame sterilized glass spreader, we spread 100 µL of each solution onto sterilized peptone/glycerol agar plates, and incubated the plates for a 40 h period at 30°C, at which point we counted visible colonies. We used plates yielding colony forming unit (CFU) counts between 25 and 250 to determine viable cell counts of the original undiluted culture (CFU mL-1).

Turbidometric Mass Determination of *S. marcescens*

We serially diluted an aliquot of the stock culture of *S. marcescens* (approximately 109 cells/mL) in sterile saline solution to generate four solutions with dilution factors (Fd) of 1.25, 2.5, 5, and 10. We then measured the absorption of these four solutions and an aliquot of the original culture at 550 nm using a Cary 50 spectrophotometer using a 1 cm path length plastic cuvette.

Results

The Results section should summarize your findings and present your data, usually in the form of one or more figures and/or tables, for the reader to evaluate. (See the section on Data Analysis and Interpretation for detailed instructions about the preparation of figures and tables.) The Results section includes not only a description of the results, but sometimes includes a brief reiteration of the question and the experimental design, especially if the project addressed multiple questions and multiple experiments. If several experiments were done, it is easiest to explain each one separately. In each case, start your paragraph by reminding your reader what the question was. It is also helpful to provide a brief explanation of what you expected to find, if your hypothesis was correct; including these predictions can increase the clarity of your paper a great deal. Finally, you should summarize, in words, what the actual data show.

Graphs and tables help the reader understand complicated data more easily than a written description. The same data should not be presented in two different forms (e.g., a table should not contain data also summarized in a graph), so decide which format best informs your reader. Be sure to refer the reader to the tables and figures that contain the data that support your statement of what the data show -you want to point to the evidence for your statement. The text should tell the reader the important point trends shown on the graphs or tables. Finally, remember that scientists always analyze data quantitatively. For example, it is not enough to say that a rate increased over time. How much did it increase? Did it double? Triple? Finally, you should not write a lengthy explanation of why your results do or do not fit your expectations. Save this for the discussion.

1. Describe the results of your experiment. Focus on the general trends (the take-home message). You might also include some very specific information (any interesting parts). Observations made while conducting your experiment are useful here, too, if those observations help you make sense of the results (or give you reason to be skeptical of them).
2. Use tables and figures to illustrate key points. These should summarize and display the overall trends, too. See the section on Data Analysis and Interpretation for more information, or see any primary literature source for examples.
3. Number tables and figures in the order in which you refer to them in the text. Refer to each figure and table in the text.
4. Make sure each table or figure has a caption/legend that explains it. Legends are placed at the top of a table, but at the bottom of a figure.
5. Do not include the raw data in either paragraph or image form; only summary data belong in the manuscript. Summary data include things like means, variances, total counts, etc.
6. Include the results of any statistical analyses. Include the value of the test statistic itself, the degrees of freedom, and the associated p-value.
7. In this section, do not make any attempt to explain your data. Save explanations for the Discussion.

Examples of strong Results sections

Results Example 1 (cell biology):
Our chemotaxis assay quantified the difference in motility between juvenile and adult unc-60 mutants by measuring the ability to detect and move towards an *E. coli* food source. Both wild type juveniles and wild type adults were motile and exhibited a similar rate of chemotaxis (Fig.1, $t = -1.1$, d.f. = 58, $p > 0.05$). The unc-60 juvenile worms possessed some motility, and the adults did not move at all. Our study shows that the unc-60 mutation becomes more debilitating with age, as 19% of the juvenile mutants were able to move to the *E. coli* ring, while 100% of the adult mutants remained completely paralyzed (Fig. 1 $t = -16.45$, d.f. = 58, $p < 0.05$). We also found that the unc-60 juveniles exhibit less motility (19%) than the wild type juveniles (30%) (Fig. 1, $t = -3.5$, d.f. = 58, $p < 0.05$).

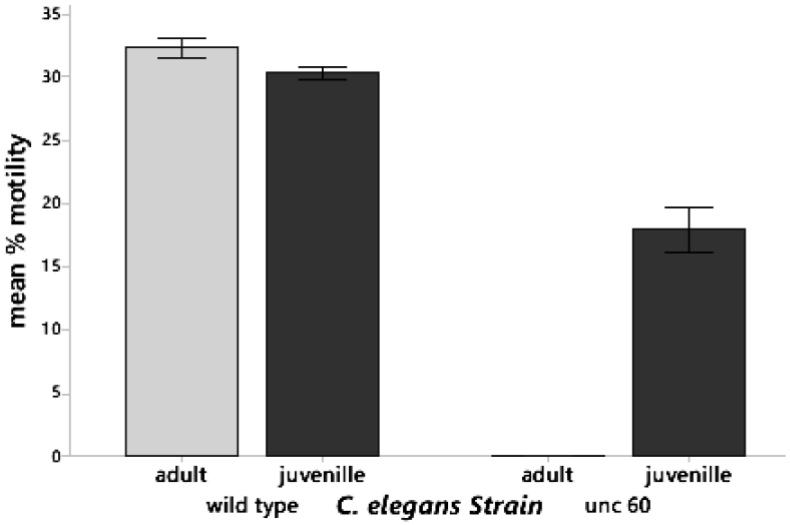


Figure 1. Unc-60 adult mutants are immotile compared to wild type. Unc 60 juveniles are shown to be motile and can chemotax (19%), while adults were completely immotile ($t = -16.45$, $p<0.05$). Wild type juveniles and adults are fully motile and maintain similar levels of motility (30% and 34% respectively, $t = -1.1$, $p>0.05$). Motility was determined as presence in the *E. coli* perimeter. Error bars represent SE. N equals 30 in all cases.

Results Example 2

Adapted from Pasachnik, S. and G.R. Ruthig. 2004. Versatility of Habitat Use in Three Sympatric Species of Plethodontid Salamanders. Journal of Herpetology 38: 434-437.

After accounting for the block effect, the species-treatment interaction was not significant (Table 1). However, both species and treatment effects were significant (Table 1). A post hoc Scheffe test determined that *E. cirrigera* had a significantly higher gain in mass than *P. cinereus* when all three habitat treatments were combined (Fig. 1, d.f. = 7, $p<0.05$). An additional Scheffe test determined that all individuals (regardless of species) within the stream treatment gained significantly less mass than individuals in either the bank or forest treatments (fig. 1, d.f. = 14, $p<0.05$).

Continued on next page…

TABLE 1. ANOVA results for the change in mass/initial mass data. Change in mass is the difference in mass from the beginning of the experiment to the end of the experiment. Species include *Desmognathus fiscus, Eurycea cirrigera, and Plethodon cinereus.* Treatments include stream, stream bank, and forest.

	df	Mean	*F*	*P*
MODEL	17	0.027	2.87	0.001
BLOCK	9	0.024	2.16	0.016
SPECIES	2	0.063	6.72	0.002
TREATMENT	2	0.059	6.32	0.003
SPECIES × TREATMENT	4	0.008	0.86	0.492

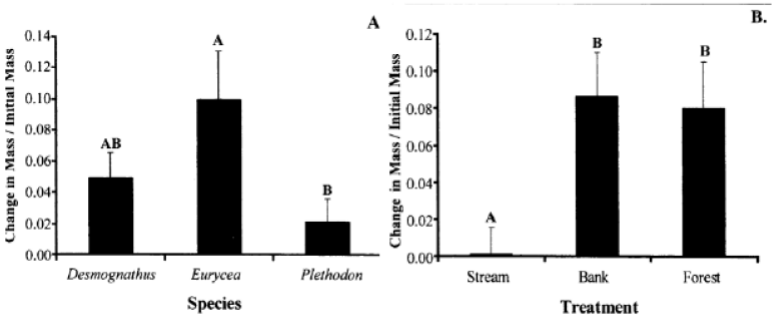


Figure 1. (A) Species performance, independent of habitat treatment for *Desmognathus fuscus, Eurycea cirrigera, and Plethodon cinereus*. (B) Treatment performance, independent of species, in the stream, bank, and forest habitat simulations. Differing letters depict significant differences. Error bars represent standard error of the mean. N ranges from 6-10.

Discussion

This is the section of your manuscript in which you interpret your results, in essay form. Proceed in this section from the specifics of your study to the general question that motivated the study (just the opposite from the Introduction). This is where you compare your results to the expected outcome, specifically whether or not your data support or refute your hypotheses, and explain why you think you got the results you did, and what you think they mean. In this course, the Discussion should NOT include an analysis of sources of error, and should consider instead the rigor of the experimental design. All experiments have error in them; that is why we test duplicate samples and analyze data using statistics. Generally, your results are much less affected by experimental error than you might think, and you should focus your discussion on trying to explain the *biological significance* of your data. "Our experiment did not work" is not an interpretation; indeed, it's a misunderstanding. It implies that if you didn't get what you expected, something went wrong with the experiment. It could be equally true that your expectations were incorrect. In fact, you're not doing an experiment if you know the outcome before you do it; it's an exercise. So it's okay, and common in science, to not get the results you expected. Your job is to think about why and if there is a biological meaning to the results you did get.

You may develop a further hypothesis as a result of analyzing your data. The Discussion is the place to state that hypothesis and to suggest new experiments that you could do to test it. This section requires that you take some intellectual risks, propose some ideas. You do not have to be right; usually there is no one right answer. You should just try to be internally consistent,

and logical in your thinking about the problem. Remember, very often it is when we do not get the results we expect that we learn the most in science.

1. Briefly remind the reader of your question, the basics of your study design and your predictions. Then restate what you found, in general terms.
2. Explain what your results mean with respect to the question, and how those results relate to your hypothesis or goals and defend that explanation. Offer an explanation and rationale for your findings. Do not simply say "We found (or didn't find) what we expected." Explain whether or not (and in what way) your data supported (or refuted) your hypotheses. This should include references to other studies that showed similar or different results. Include references that help support your explanations.
3. The explanation should rest on biological principles, not on mistakes you may have made. Take your results at face value. If they are reliable and real, what do they indicate about how nature works?
4. Quantitatively compare your results with other studies, if possible.
5. Discuss the relevance of your results and their interpretation to the larger questions that motivated the study.
6. End the discussion with a summary, the "take home lesson" that you want your reader to remember about your work; indicate interesting future directions. What would you do next, if you had more time? What mysteries remain, and how might you go about solving them? Don't just suggest that you might repeat your experiment with a larger sample size or more time. Move beyond the work you've done to work that will clarify (not just expand) the knowledge you've just generated.
7. [NOTE: There are two competing aims in thinking about experimental results that are often at odds with each other. The first is to trust that the data you get are *real* and that they offer real insight into the workings of the natural world. Treat them as such. Do not be dismissive of the results you get because you don't happen to like them—this would mean that your work and your insights are shaped by ideology, not by rigorous and unbiased analysis of the data as they are. The second is to always maintain a scientifically-minded skepticism. If the results match your predictions, maybe your hypothesis is correct, but maybe some other mechanism could have produced the same results. If your results don't match your predictions, maybe human error is to blame, but maybe your hypothesis is false after all, or maybe a general truth doesn't apply in the case at hand. We expect you to straddle this fence—to discuss your data first with trust and then with skepticism—and then to inform the reader which side of the argument you find more convincing (and why).]

Tips on common errors in discussion sections:

- Do not conclude that because your hypothesis wasn't supported, you made a mistake. "Negative" results can be important too, since they may suggest that your hypothesis was incorrect. What would be the benefit of testing hypotheses, if you could never reject them? If you did make an error somewhere in your investigation, acknowledge it and move on.
- Do not omit or minimize discussion of findings that you did not expect. Such results are often the most interesting.

- Do not center your discussion on a proposal to repeat your experiment with a larger sample size. This is not as interesting as suggestions for new investigations that arise from your findings.
- Do not end the paper with the phrase, ". . . but of course more work needs to be done." Describe what kind of work would be the most interesting extensions of the study and why.

Acknowledgements

In this section, thank any persons who contributed any significant help during the study. Such contributions include help in experimental design, collection of data, preparation of graphs, drawings or the manuscript, critiquing a draft of the manuscript, and financial or physical support of the work. Always acknowledge your partners in group projects!

References

You will undoubtedly get ideas from other sources when you are designing your experiments, supporting your hypothesis, or interpreting your results. In science, you should not use another's words directly. Instead, restate the idea in your own words and then cite the source. List only the papers or other publications that were directly cited in your paper. A References section is not a bibliography. Citing a paper means you read it — reading the abstract is not sufficient, unless specifically allowed by your instructor.

To see how to cite references, see Appendix 4. A note of caution: websites are often a good place to get ideas, but when it comes time to write your manuscripts you should cite published textbooks, professional reviews, or peer reviewed primary research papers. Some websites such as nih.gov are permissible-ask your instructor for guidance.

1. Make sure you back up all statements with citations from the primary literature. The "educated guess" that is your hypothesis and the context of your results both stand on the back of research that has come before yours, and that research must be acknowledged.
2. Plan on using at least two primary sources to back up the contentions you make in your Introduction. Additional reference to the literature may aid the strength of your Discussion as well.
3. When citing a paper within the text, follow each sentence that refers to it with the author's last name(s) and the year of publication in parentheses.
4. Do *not* include quotes in your papers; *always* put the information in your own words.
5. The References section at the end of the paper should include the full title of the article and the journal each reference came from (or the book), plus the author's names, the date of publication, and the issue and page numbers. See Appendix 4 for instructions on how to construct proper citations.

Hints for success:

1. Start with a detailed outline: To write concisely requires clear logic and organization. This is best accomplished by beginning with an outline. The outline will make your organizational logic transparent.

To illustrate how to do this, here is an example of what the first paragraph of an introduction might look like in outline form. Note that the topic sentence is presented at the beginning of the paragraph. There are three major points in the paragraph (each is numbered) and these are supported by cited data. The final point represents the logical conclusion of the argument made in this paragraph.

Introduction

A. While much has been learned regarding assembly of lysosomes in *S. cerevisiae*, likely that lysosome assembly is more complex in multicellular animals.

1. Genetic studies in yeast have led to identification of > 60 genes that function in assembly of lysosomes (Bryant and Stevens, 1998).
   a. Many of the genes encode SNAREs, Rabs, and components of protein coats
   b. many of these genes are functionally conserved
2. lysosomes in multicellular animals = functionally more diverse than yeast lysosomes.
   a. Melanosomes are lysosomes that contain color pigments
   b. T-lymphocyte lytic granules are secreted lysosomes that initiate immune response (Blott and Griffiths, 2002).
3. Recent genetic studies in mice and *Drosophila* have identified genes necessary for the formation of lysosomes that are not found in yeast.
   a. *hook* gene in *Drosophila* is involved in endocytosis and transfer of material to the lysosome (Sunio et al. 1999).
   b. The HPS1 gene encodes a novel protein necessary for the assembly of lysosomes (Dell'Angelica et al. 2000).
4. Therefore, to fully understand the mechanisms that control lysosome assembly in multicellular animals, we cannot rely entirely upon what is being discovered in unicellular organisms like yeast.

2. **Manage your time well.** To make sure you have enough time to write your report, start thinking about the data over the weekend following their collection. Then go see your instructor if you feel stuck or confused. We are always happy to help you think about data! We can help you clarify your thinking, but we can't think for you, so invest the time necessary to reflect on your data first and then use us as a resource.

3. Draft, edit, revise. Don't expect to write a good lab report in your first draft. It doesn't happen that way in professional science, and it won't happen that way in this course. Put a draft together. Have each of your group members read and critique the work as a whole. Meet to discuss your comments and plan revisions. Then rewrite it taking into account all good suggestions.

4. A few writing tips:

- *For this course, always write in the active, not the passive voice*.
- In the Methods section, use the past tense, not the present tense.
- Describe results in the present tense. You did the experiment in the past, but the results are true in the present as well as in the past.

The Scientific Poster

Professional scientists regularly present the results of their work at local, national, and international meetings. At most scientific meetings, posters are the primary way that scientists share information about their work. The poster, although a smaller unit than the published journal article, is a fully professional entity, and almost always the first form in which a scientist's story is made public. It is also the most egalitarian form of presentation in that tenured researchers and students alike use it. Its principal advantage is that it promotes extensive two-way communication between the presenter and the audience. Not only are results and conclusions presented to an audience, but also the audience provides the scientist with ideas that help in planning future studies.

A poster is a visual way of presenting scientific results. A good poster is virtually self-explanatory; it will contain the elements of a paper (Title, Abstract, Introduction, Methods, Results, Discussion, Conclusions, and References), but it is a distinct form in which different elements are emphasized, each in its own panel. There are several examples of research posters distributed around the Bio/Psych and Bodine buildings. Look them over. If you still have questions or are unclear about the elements and structure of posters, talk to your instructor.

You can find basic guidelines for making a poster on the **Conference Posters** page within the Opportunities in Science at Lewis & Clark webpage:
https://college.lclark.edu/science/opportunities/rogers/students/posters/

A more comprehensive guide for making scientific posters has been authored by Prof. Sharon Torigoe, of the Biology Department and Biochemistry and Molecular Biology Program. This document can be accessed here:
https://currentprotocols.onlinelibrary.wiley.com/doi/full/10.1002/cpet.18

A short set of guidelines for how to make a poster can be found below.

Organization of a Scientific Poster

- *Title and Author Panel:* The title should be descriptive but short, in boldface letters 1.5 inches high. The authors' names and institutional affiliations may be somewhat smaller.
- *Abstract:* A short (50-100 word) summary of your research. It should be completely self-contained (i.e., independent of the rest of the poster). This is the one portion of a poster that is commonly published.
- *Introduction:* Introduce the topic of the work, briefly summarize any relevant background information, including a short review of the work of other investigators, and succinctly state your objectives or hypothesis.
- *Methods:* Unless the primary focus of the poster is the novelty of its experimental methods, this section should be kept to a minimum. There must, however, be sufficient detail to permit the reader to understand what was done and evaluate the appropriateness of the experimental design and technique. Include relevant methods within the text associated with figures in the Results section, and don't include a

separate Methods section. Additional methodological details may be included in figure legends when necessary. Use bulleted lists of procedures; sketches, figures, diagrams, or photos of equipment; and a listing of conditions. If detailed materials and methods are required, they may be appended in smaller type.

- *Results:* This is a summarized report of your observations, not your interpretation of the results. Present your results in a logical sequence, not the sequence in which they were obtained. Remember that this is primarily a visual, rather than verbal, presentation. Graphical representation of data is almost always more effective than tables or text. Use text only to explicate the figures and, if necessary, to make transitions between figures. Number all figures and tables consecutively (e.g., Fig. 1, Fig. 2, Table 1, Table 2, etc.). Raw data should be included only when absolutely necessary; if in doubt, ask your instructor.
- *Discussion:* Analyze and discuss your findings, though less expansively than in a paper. You may use summaries such as numbered or bulleted items. Point out the general meaning and importance of your results, and relate them to those of other investigators (be sure to cite their work appropriately). You should also include a description of further work that could be done in this area.
- *Conclusions:* This includes a few brief and concise statements summarizing your work.
- *References:* List sources that you cited in your poster.
- *Acknowledgments:* Acknowledge funding sources, individuals, facilities, and personal conversations that aided you in your research.

Oral Presentations

The goal of an oral presentation is to convey to your audience a clear, easy-to-understand synopsis of your project: your research question, your results, your conclusion, its significance. People reading a written report can reread a section they don't understand, but this is not an option in an oral report--it has to be crystal clear the first (and only) time through. The best way to ensure that you communicate well with your audience is first, to **plan** what you're going to say and write it out in outline form, and second, to **practice it**. As you plan your presentation, try to imagine yourself as an audience member who knows little or nothing about your study. What information do you need to convey? Make every effort to make it easy for your audience to comprehend and remember the main points of your talk.

One way to ensure that your audience will remember your main result is to include it in the title. Repetition is also important, both within the structure of the talk and in reinforcing the main points on a slide with what you say. In the words of an old adage, "tell 'em what you're gonna tell 'em, then tell 'em, then tell 'em what you told 'em."

Several software packages provide easy ways to format slides for presentations, such as Powerpoint, Google Slides or Prezi. Whichever package you use, make sure that your slides help you communicate effectively with your audience, and are not distracting. Use the simplest possible background. Leave out sounds and cute animations. Slides should reinforce what you are saying, and not draw your audience's attention away from you, the speaker. So keep each slide simple, with just a few lines of text. Bullets are preferable to complete sentences. Think about what kinds of visuals will best help an audience understand your study. Slides are most useful for showing figures (e.g. of results) or photos (e.g. of important organisms, or site characteristics) -- a good rule of thumb is to have no more than one slide/minute of presentation. That gives the speaker plenty of time to thoroughly explain each slide.

Class presentations are typically limited to **12-15 minutes**, including time for questions.

Organization Of An Oral Presentation

Your audience needs to know four things:

- what question you investigated and why,
- how you did the study,
- what data you obtained, and
- what you think your data mean.

The Question

A clear and focused introduction will be essential in making your presentation interesting and understandable to your audience. In this section, explain what question you asked and *why* you asked it. Take the time to develop some background information first, to provide a context for your work. Remember, you are speaking to an audience that has not been thinking about your problem, so it's important to explain clearly why you decided to study what you did. Once the overall problem is clear, be sure that you clearly state what *specific testable hypothesis* your research project addressed. Justify the hypothesis you provide. Your best

guess answer to the question you pose is not pulled out of the blue, but based on existing knowledge formed from your own experience and from what you've read or learned elsewhere. In other words, explain how you came to your hypothesis and why it seems reasonable. What predictions does your hypothesis make?

The Design of the Study

How did you go about answering your question? What procedures did you use, what did you measure and how? What controls did you use and why? Present your methods conceptually, diagrammatically, rather than as a list of text. We need to understand the *design* of your experiment. Don't bog down your presentation with small details; focus on the big picture. For example, we need to know the final concentrations of chemicals, but not how many mls of solution X you mixed with solution Y. Be sure to include a rationale of **why** you chose your particular design. Diagrams are often very helpful in this section. If you include a diagram of your design, walk your audience through it.

The Results

Present your results in summary form so that any trends (or lack thereof) are readily apparent. Use figures with clear axis labels, points and fonts that are big enough to see from a distance. Instead of a figure caption, include a large title over each figure that states its take-home message, to reinforce your points. Be sure to explain each figure slowly and carefully, starting with an explanation of what the x- and y-axes represent, and what each point or bar represents. Point to particular features of the figure that you want to emphasize, so everyone can see what you're talking about. Don't forget to explain the control data! No experimental data is interpretable without control data.

Interpretation and Conclusions

In discussing your results, share with your audience the logic you used to reach your conclusions and why you rejected alternative explanations. Do your data allow you to formulate a definitive answer to your question? If so, what is it? Were you able to disprove any of your hypotheses? Explain why you rejected alternative explanations. Were your data equivocal? If so, what more would you like to know before drawing a firm conclusion? Did your results surprise you? If so, in what way? Can you speculate about an explanation? Explain any skepticism you have that causes you to make your conclusions cautiously. As you provide this information, you will inevitably discuss some of the problems you encountered in your research. However, balance this with a discussion of possible solutions. Also, point out some of the new questions your research project may have generated. By presenting the "difficulties" in a positive light, you provide a way for the audience to take part in the thought process behind your research. To conclude, summarize the overall message you wish your audience to take away. This might be a reiteration of what your data suggest, or it might be a cautionary tale about experimental design. Do, however, end with optimism: your aim is to encourage the ongoing acquisition of new knowledge!

References

If you use any resources other than course handouts for images/ideas, place the citation for these on the bottom of the slide where they are shown.

Acknowledgments

Acknowledge the people who assisted you by stating who they were and what kinds of contributions they made.

Ask for Questions

The final step in any presentation is to ask your audience if they have questions. Allow at least 10 seconds for them to formulate and ask their questions. In case their question couldn't be heard by everyone in the room, repeat the question before you answer it. Be prepared to think on your feet. Base your answers on the data or your background research, or answer by explaining what you observed or thought as you conducted the experiment. Do not be afraid to say you don't know (although you should still propose likely or possible answers in that case).

Effective Presentation Style

In an oral presentation, just as in a written report, your style affects how well your audience understands you. Once you have carefully planned what to say, and created effective slides to support your presentation, it is essential to **practice**. Do not read your talk from your slides -- slides should not be a substitute for your speaking notes, or for practicing your talk. Practice will help you to speak clearly and fluently and will assure that your talk is neither too long nor too short. If you opt to make up your talk "on the fly," the added pressure of being in front of a group while you're trying to explain complex ideas can reduce you to incoherent babbling.

Speak loudly, clearly, distinctly, and slowly. Make eye contact with audience members. Don't stand in front of your slides and block your audience's view. Display tables or figures long enough for your audience to read them, and be sure to explain how they are set up and what you think they show. When giving a group presentation, it is essential for all presenters to be equally-prepared and to have agreed in advance on the roles each will play. Practicing a group presentation is essential for assuring that all of the parts of the presentation are well-coordinated.

For an excellent overview of how to prepare and deliver a scientific presentation watch https://www.youtube.com/watch?v=Hp7Id3Yb9XQ

Audience responsibilities

Members of the audience have an obligation to:

1. pay attention, and not talk to one another during presentations.
2. stay mentally engaged and be prepared to evaluate the validity of the presenters' conclusions.
3. ask questions.
4. recognize your colleagues' work with applause!

Appendix 1: Why We Use Animals In Teaching Biology

Biology is the study of the unity and diversity of life. The unity derives from the fact that all species on earth are related by descent and share many basic features. One only has to consider DNA, ATP, or plasma membranes to appreciate the fact that all life is built on some common forms and processes. Because of these basic similarities, we have learned a great deal about our own biology by studying non-human organisms, from *E. coli* bacteria to *Drosophila* to laboratory mice. When one considers the diversity of life, however, it becomes clear that each species is unique, and there is a limit to how much one can learn about one species by studying another. We learn more about ourselves by studying frogs than by studying *Drososphila*, still more by studying mammals, and still more by studying primates.

Biologists, as all human beings, should share a deep respect for all life, and avoid unnecessary death of living things. As biologists, it is important to balance this respect with a consideration of the benefits of learning things that cannot be learned in any other way except through studying animals. We have a responsibility to articulate the reasons for using animals in biology classes and to provide clear policies for their humane and thoughtful treatment. Students should be aware of the potential for misuse of animals, and should be able to articulate the reasons to use animals in our classes.

We recognize that some students have deeply-felt reservations about using live or sacrificed animals. There are no required courses in our curriculum that use vertebrates in this way. While many careers in biology, including medicine, are incompatible with a moral objection to animal research, biology is a tremendously diverse field, and there are many kinds of investigation that do not require using animals, or that study those animals non-invasively.

So why use animals in any of our courses? First, a selfish reason: our health depends on it. If you are not already convinced of this, a quick scan through the statements of the professional associations listed below will reveal the magnitude of our debt to nonhuman animals used in research. The discoveries in medical science are directly dependent on the research conducted using nonhuman animals. And this research is directly dependent on the training that those interested in pursuing a career in medicine must have. Simply put, the goals of humanity have been advanced by this discovery-research-education linkage.

Three reasons why we use animals in teaching biology:

1) Animals are needed for students to develop the skills necessary to conduct animal research, which has been the basis for almost all of the important discoveries in biomedicine. If we fail to educate, train and excite tomorrow's biologists, our future understanding of organismal function is in jeopardy. The number of up-to-date laboratories which teach new and advanced techniques is decreasing because of cost and the difficulty of animal use. If we do not teach the necessary skills, then proper or "humane" treatment of animals will be more difficult to realize. Moreover, training is necessary so that future experiments using animals are of the highest quality and do reveal the secrets about how animals "work."

2) Animals are necessary to demonstrate the difference between natural biological variability and measurement error. Working with real organisms in real investigations brings one face-to-

face with complex biological phenomena that cannot be experienced in books and models. This is critical if we are to teach critical thinking and foster creativity in the design and execution of scientific experiments. Textbooks, lectures, and simulations relay the final general principles, but seldom discuss in detail how these discoveries have been made. The excitement and frustration that accompany the discovery of new information can only be experienced by direct involvement (i.e. using all available senses). The realization that textbook relationships are not simple functions, but have variation due to the organism and measurement technique is essential.

3) Animals give students a much-needed appreciation for the diversity of life. Most animals are very different from ourselves. We are the exception, not the rule, with respect to many aspects of animal function. If we never understand how animals respond to their environment, then how can we ever know if they are being treated "humanely" or what "humane" even means? Paradise for a human will certainly kill an Antarctic fish, whereas we would feel quite uncomfortable living in the hypoxic mud of some amphibians. Students must experience animal diversity with respect to function.

Why not use computer simulations instead of animals?
• Computer simulations are not a substitute for using animals in teaching for the same reason they are not a substitute for animal research: simulations are simplifications that contain only what we already know, not what we have yet to discover.
• We use computer simulations to supplement the novel, exciting discoveries that emerge from investigations of real animals. Computer simulations are best at presenting a simple, clearly understandable model of structure and function.
• The greatest shortcoming of computer simulations is that, taken alone, they falsely represent biology as a static field where the facts are already known, and where there is nothing more to discover.
• Computer models cannot enable students to experience the excitement of studying real animals as complex integrated systems, where the unexpected can lead to profound discoveries.

It is the responsibility of all those concerned with animal welfare --professional biologists, students, and animal rights activists -- to know what policies and procedures are advocated by those societies most directly involved in working with animals. Here is some relevant information regarding the use of animals in teaching and research:
• Federation of American Societies for Experimental Biology, https://faseb.org/Science-Policy-and-Advocacy/Science-Policy-Research-Issues/Animals-in-Research-and-Education/Teaching-Advocacy-Material
• American Physiological Society, https://www.the-aps.org/career/policy-advocacy/animal-research?SSO=Y
• National Academy of Sciences, A guide for the care and use of laboratory animals, https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
• Foundation for Biomedical Research, https://fbresearch.org/biomedical-research/

Appendix 2: Lab Safety

Any laboratory contains hazards. It is important to develop an awareness of these hazards and an ability to deal with them. Some of the dangers of particular concern are:
cuts and related injuries;
chemical toxicity;
chemicals getting in your eyes.

Most incidents occur because of lack of attention, care, knowledge or a combination of these factors. The responsibility for safety ultimately rests on you. Whenever you are in the lab you must be careful, patient, informed, and considerate of others. Above all, you need to know and follow the laboratory safety rules. Your cooperation and adherence to these rules is expected at all times. Please ask questions about points you do not understand. The best pieces of advice we can give you are to remember to always use common sense in lab, err on the side of caution.

Laboratory Safety Rules

1. Your clothing should be comfortable older clothes that you can tolerate damaging. Clothing should essentially cover all of your body and should not have loose floppy sleeves or other appendages that can get in the way, catch fire easily, or catch on an apparatus. Shorts, open-toed shoes, sandals, skirts that do not go below your knees, and shirts or blouses that do not cover your entire front are discouraged.
2. If you have long hair, tie it back.
3. Eating, drinking, and smoking are strictly prohibited. Food and drinks may be stored in the hall outside the lab.
4. Practical jokes, boisterous conduct, and excessive noise are prohibited.
5. Cell phones and other electronic gadgets are distractions and should be turned off before you enter the lab.
6. Treat all chemicals in the lab as though they are hazardous unless you are instructed otherwise.
7. Never taste anything. Never directly smell the source of any vapor or gas; instead use a cupped hand to bring a small amount of air to your nose. Do not inhale these vapors, but take in only enough to detect an odor if one exists.
8. Perform no unauthorized experiments.
9. Clean up all broken glassware immediately and dispose of the pieces in the “Broken Glass” box.
10. Avoid rubbing or touching your eyes unless you know your hands are clean.
11. Keep solid matter not soluble in water (notably paper, towels, broken glass, etc.) out of sinks at all times to minimize the chance of plugging drains.
12. Notify the instructor or TA immediately in case of any accident or injury, no matter how slight.

13. Observe all the special precautions cited in each experiment.
14. Know the location and proper use of eye wash stations, safety showers, fire blankets and first aid kit. Also, make sure you know the locations of the exits for the lab.
15. Keep backpacks and personal items (except for lab notebooks) in the laboratory cubbies.
16. Wash your hands before leaving the laboratory.

Other considerations

Selection of Chemicals
Some of the investigations you may work on or design may include the use of chemicals. The faculty member in charge of the lab will train you in the proper handling of any chemicals and must approve the selection of any chemical substance for a reaction or process not already specified by standard procedures.

Personal Protective Equipment
Wear gloves for handling of all chemicals that may cause irritation, allergic sensitization or skin absorption of toxic chemicals. The faculty member in charge must check the material safety data sheet (MSDS) for the chemical to ensure that the type of glove material used is protective for the substance.

Lab Safety Equipment

Listed below are several safety and emergency items generally found in each lab. Familiarize yourself with their location before beginning any lab work.

First-Aid Kit
Even minor injuries should be reported to the lab instructor. First-aid kits are located in each lab in a drawer or cabinet marked with a green cross.

Eye Wash Fountain
These fountains are useful for extended irrigation of eyes that have received a chemical burn. Eye wash fountains are available at the sinks of every lab. If you get a chemical in your eyes, it is imperative that you wash your eyes immediately.

Safety Shower
In the event of a serious chemical spill, where much of the body and/or face is involved, safety showers are available. The safety showers in the Biology department are located in Bodine 201 and Red Lab. To operate the shower, pull the lever and water will flow from the showerhead until the lever is returned to the off position. To ensure that all traces of the chemical have been removed it will be necessary to remove all contaminated clothing.

Fire Extinguisher
Fire extinguishers are located in each lab and the hallways. Before beginning work, be sure to note the location of the nearest fire extinguisher. To operate a fire extinguisher, remove the

safety pin, aim the nozzle at the base of the fire, and squeeze the handle to discharge the chemical flame retardant.

Fire Blanket
There are fire blankets in every lab to smother flames if clothing catches fire.

Waste Disposal
a) The lab instructor will provide instructions about proper disposal of waste generated in the lab. No chemical should be disposed of down the sink or in waste containers without approval.
b) Dispose of biohazardous materials (anything that has been in contact with living organisms, such as broth cultures, petri plates, plastic pipette tips, etc.) in autoclave waste containers or treat them with bleach before disposal.
c) Receptacles for broken glass and "sharps" are provided in each lab. Do not dispose of broken glass or "sharps" in the general trash.

Spill and Leak Procedures
a) For biological spills, notify the lab instructor.
b) Very small spills of low-toxicity chemicals can be absorbed with paper towels and allowed to evaporate under the hood, after which the towel may be disposed of.
c) Larger spills: Evacuate lab and notify the lab instructor.

In An Emergency

1. If you have a medical condition that could cause a problem during lab (*e.g.*, diabetes or epilepsy), let your lab instructor know about it (and possibly your lab partners as well).
2. All personal injuries that occur in the laboratory, however slight, must be promptly reported to the faculty member in charge, or, if he/she is not available, to some other member of the faculty. In any case, the first thing to do is to yell loudly that an accident has occurred. The injured person must go or be taken to the Health Center or hospital immediately.
3. If a person has come in contact with an irritant chemical, thoroughly wash the exposed area. If it is a case of major exposure (*i.e.*, splashed over a large part of their body), take exposed clothing off immediately. Embarrassment is a small price to pay to avoid permanent disfigurement from chemical burns, severe poisoning, or death. Go under the safety shower and wash your entire body thoroughly. Any affected clothing should be washed in the sink. If it is a chemical base spill, wash at least five minutes. Wash the face and eyes thoroughly. Use the eyewash if there is a possibility of chemical contact with the eyes.
4. If a person has chemicals in their eye(s), get them to the eye wash station as quickly as possible. If the person wears contact lenses, they need to be removed immediately. Hold their head down and help open their eyes as far as possible. Be certain to wash under the eyelids and remove all foreign matter from the eye. Rinse for 15 minutes. For some

chemicals a matter of seconds can make the difference between losing or keeping your vision.

5. If a person is on fire, pull their head down away from any flame and fumes, drag them to the safety shower and activate it to douse the fire. If something is on fire, clear the area and carefully use a fire extinguisher. If the fire is too strong, pull the fire alarm and evacuate the building.

Appendix 3: Primary Literature: What it is, How to Find it, and How to Read it

In BIO 110 you will write a paper that reports the results of your independent investigation in the style of a professional publication primary data paper in science. We will ask you to cite 2-3 *primary sources* in your paper. This section is intended to teach you a little more about what is meant by primary sources, how to find primary literature, and how to read and interpret this style of paper. Also, having a better understanding of what primary literature is should help you as you structure and write your paper.

What is primary literature?

As you explore the scientific literature, it is important to appreciate its different forms:

- The **primary literature** consists of articles written by scientists for scientific magazines, called "journals." These "journal articles" are where scientists present new scientific data. They have been reviewed by peers before being published. The peers who review primary literature papers are scientific experts in the field of focus of the paper. Specific journals focus on particular fields of research. The focus of some journals (*e.g. Science, Nature, Proceedings of the National Academy of Sciences*) is broader than that of others (*e.g. Genetics, Animal Behavior, Ecology, Molecular Biology and Evolution, Journal of Arachnology*). Primary literature is the central venue in which scientific knowledge is archived and accessed by scientists. It is the mechanism that allows scientific progress to occur on a broad scale. You probably have little experience with the primary literature, and one of our goals is to help you learn to use it; it is a wonderful resource.

- The **secondary literature** is a "digest" of the primary literature, written by individuals other than the authors of the original studies. It is variable in form and quality.
    - **Review papers:** typically written by scientists who are experts in the field, these papers do not necessarily present new data or analyses, but rather synthesize the current state of understanding of a scientific issue or field. They are written for an audience of trained scientists. They are peer reviewed, but their goal is different from that of a primary data paper. They can be very valuable for getting an understanding of the "state of the art" of a particular field.
    - **Lay literature:** refers to articles that review current knowledge or new discoveries but are written in more generalized language that is accessible to non-scientists. They are typically not peer-reviewed. Articles in *Scientific American*, the *New York Times Science* section, and *Discover* magazine fall into this category. These should not be cited when writing a scientific manuscript, because their information has not been fact-checked.
    - **Websites:** refers to information found on the Internet. Websites can be great resources but are not peer-reviewed, so they should be selected critically and used carefully. Listing an author, having a date, including sources for the

information on the site, and having correct grammar and spelling are some signs that a website is reliable. The .edu and .gov domains, which are reserved for colleges/universities and governmental organizations, are usually reliable. More information about evaluating websites can be found on this website: https://advice.writing.utoronto.ca/researching/research-using-internet/

Ask your professor if it is appropriate to use secondary literature to inform your investigations, and what kind of secondary literature is acceptable.

The Watzek Library has many resources (including librarian Parvaneh Abbaspour, website https://library.lclark.edu/parvaneh) to help you learn to use the scientific literature.

How do I find primary literature?

If you are a scientist publishing a paper it is essential that you have done a thorough literature search on a subject. The amount of primary literature available can make this task overwhelming. Thankfully, there are many databases of primary literature and review papers for different academic disciplines that allow thorough searching for literature that is relevant to specific research questions. Searching with either key words or authors will allow you to find papers reporting relevant articles. The databases vary in how thorough they are with respect to inclusion of the content of different journals and how far back they go in time. Below is a description of two databases that are available to you at Lewis & Clark College.

1. BIOSIS Previews (Web of Science)
The Web of Science is an excellent place to start a literature search. It contains information for an enormous and inclusive set of scientific journals. Access to the Web of Science requires a subscription (very expensive), but Lewis & Clark has one. To use it you must work through a Lewis & Clark portal with your username and password. If you are on the Lewis & Clark network, access is automatic.

To access the Web of Science, go to Watzek Library's main page, and select **Research Guides by Subject** under the **Research** header on the top left menu bar of the page. Select **Biology** within the **Subject Guides** section. Then Select **Article Databases** and scroll down until you find **BIOSIS Previews** (**Web of Science)**. The window will tell you it is starting a new session. The college has a subscription that only allows a finite number of sessions to occur at the same time; however, that limit is rarely reached.

The Web of Science has an easy user interface. Video tutorials for the Web of Science can be found on YouTube:
https://www.youtube.com/playlist?list=PLM1kuGdwRdGkBjxnVPR0PL5Y2zDuw9d1s

The Web of Science Search Tips video link is below:
https://www.youtube.com/watch?v=xwhy0JBHlMw&list=PLM1kuGdwRdGkBjxnVPR0PL5Y2zDuw9d1s&index=8&t=0s

You can search for either specific key words or for particular authors.

Web of Science easily allows searching for what research has been done since a paper was published, i.e.by finding papers that cite the original article. This is the "Web" in Web of science. It allows both forward (who has cited a paper?) and backward searching (what papers did the original paper cite?). Doing forward searches allows you to find the most recent, or "cutting edge" research on a topic.

Once you find articles you would like to read, if Lewis & Clark's library has a subscription to the journal, then you can select **get item** and you will be lead through a series of selections that allows you to download a pdf of the article or to read it online as html. If Lewis & Clark does not have the article, you can request a copy of it through interlibrary loan (ILL). It typically takes 2-5 days to receive an article through ILL. The library also holds print collections of a large set of journals and you can physically go and find a paper in the library.

2. MEDLINE PubMed
PubMed is a publicly available, free database of primary literature created by the National Institute of Health's (NIH) National Center for Biotechnology Information (NCBI).

To find PubMed, simply Google the name. You can search by topics, articles or journals. For a short tutorial go to:
https://www.nlm.nih.gov/bsd/disted/pubmedtutorial/cover.html

Even though the name of this database is PubMed, the inclusion of articles goes well beyond medicine. It is a great place to start if you do not have access to the Web of Science.

How do I read primary literature?

Primary literature in science should reflect the core of scientific inquiry, which is to ask questions and answer them with data. Framing questions that can be empirically answered is a central skill in scientific training, and one of the hardest to learn. Learning to read and write scientific papers is a great way to build competence in the art of asking scientific questions. It takes practice to be able to read primary literature and extract from it what scientific questions were being asked and the types of data that were used to answer them.

Note that the data in a paper should be objective. There should be enough information for you to be able to evaluate whether or not the data were collected by unbiased methods. The interpretation of the data by the authors is simply interpretation. It is your job as a reader to think independently about the interpretation and come to your own conclusions. The results presented usually inspire new questions that others in the scientific community will pursue. This is how science progresses.

There is a specific format for primary literature in science. These manuscripts/papers/articles have the following sections:

Abstract - brief summary of paper, including central results.

Introduction - broad context and background necessary for understanding goals of paper. Clearly states the central question and goals of the paper.

Methods - how the data were collected and analyzed - in sufficient detail to allow others to be able to *reproduce* results.

Results -Summary of results, usually includes tables and figures. The text describes patterns in the data and refers to tables and figures. Results of statistical tests are also included here.

Discussion (merged with results in some manuscripts) - synthetic interpretation of the data and summary of general relevance of the results.

Biology Department Emeritus Professor Deborah Lycan wrote a guide for how to read primary literature, included below with her permission to reprint.

The goal:
"Read not to contradict and confute, nor to believe and take for granted...but to weigh and consider." Francis Bacon

Your role in reading the primary literature is to independently evaluate the *data*. Do you agree with the author's interpretation? Practicing scientists often get ideas for their own experiments from reading the published data from a different perspective. Perhaps the authors have been blindsided by their own particular history, and cannot see some alternative interpretation of their data. To be an independent evaluator, you must be careful not to be influenced by the authors' viewpoint. Consequently, I try to avoid the abstract and discussion section until I have thoroughly evaluated the data from my own point of view.

How to:
The following is a synopsis of how I approach reading and analyzing a scientific paper.

Step1: Read the introduction section of the paper.
After reading this you should be able to write down the answers to the following questions.
a. What is the question that the authors seek to address in the paper?
b. Why did they want to address this question? What is the background that led up to this paper and why is this work important for us to understand?
c. What do they claim they found out?

Step 2: Read the Results section.
The Result section will be organized around specific questions, and the experiments will be designed to answer those questions. Skip the methods section for right now. You can refer back to the methods as you examine the data for each experiment.

- Examine each figure in turn. For each figure you should be able to identify what question was addressed in this experiment. What was the hypothesis? What result did they expect if their hypothesis was correct?

- Your next job is to understand how the experiment was done. If you do not understand the method from the figure legend, look it up in the methods section. If you still do not understand the method, look it up in your textbook to get some background.
- Your next job is to understand the controls. Experimental data only have meaning in the context of control data. The controls are what allow you to exclude other interpretations of the data. What are the controls in this experiment? Why are they included? What do the data on the controls tell you?
- Now you can examine the data for the experimental question. Try to interpret this data without looking up the authors' interpretation in the discussion. What do you think the data mean? Later, when you are all done, you can read the Discussion and see whether you agree with the authors' interpretation of their data or not. If you read the Discussion first, it is very difficult to have an independent view of the data.

After reading the Results section. you should be able to answer the following questions about each figure:
1. What was the question?
2. How did they do the experiment?
3. What is your interpretation of the control data?
4. What do the experimental data show? Are there alternative interpretations?

Step 3: Read the Discussion
Do step 3 only after you have struggled to do step 2. Otherwise you bypass the intellectual effort to think independently and you lose much of the opportunity to learn. Once you have read the discussion, you should be able to:
1. Summarize the evidence they marshal for their particular interpretation of the data.
2. What other papers have cited results that support or refute their view of the process they are studying?
3. What questions remain to be addressed? How will they likely be approached?

Appendix 4: Conventions for Biology References

In-Text Citations
Cite references in one of two ways in the text of your paper:

1. Mention the authors' names as part of your sentence followed by the year of publication in parentheses. When there are three or more authors, give the first author's last name, followed by "et al." (Latin for "and others"):
"Morris et al. (2018) described the function and regulation of GLO-1 in lysosome-related organelle biogenesis."

2. Place authors' names and the year of publication in parentheses following ideas or results from the article:
"The seed banks of restored forests contain few tree or shrub species (Clements and Bierzychudek 2017)."

3. If you are citing a webpage with no author or date, use the organization sponsoring the website as the author, and "n.d." to represent "no date":
"The dominant tree species in Pacific Northwest forests are Douglas fir, Western hemlock, and Western cedar (U.S.F.S., n.d.)."

Reference List
List references alphabetically according to the first author's surname. Standards for reference sections vary among journals, primarily in details of punctuation. Note that Investigations reference style is the same as APA style, making it possible to take advantage of computerized formatting of your references. Please use the appropriate form for each type of reference below:

Journal Article

Author(s). (Year). Title. Journal, Volume (issue): pages.
Hamling, K.R., Tobias, Z.J.C., Weissman, T.A. 2015. Mapping the development of cerebellar Purkinje cells in zebrafish. Developmental Neurobiology 75(11):1174-88.

If you cite more than one paper by the same author(s), the papers should be listed chronologically (earliest first).
If a paper has more than five authors, use "et al." after listing the first five.

Book Chapter of Edited Volumes

Author(s). (Year). Title of chapter. In: Editors names (Eds.), Title of book (pages). Place of Publication: Publisher.
Boyer L.V., Binford G.J., Degan J.A. (2016) Spider bites. In: Auerbach P.S., Cushing T.A., & Harris N.S. (Eds.), Auerbach's Wilderness Medicine, 7th edition (pp. 993-1016). Amsterdam, Elsevier Press.

Book

Author(s). (Year). Title. Place of Publication: Publisher.
Atkins, P.W., de Paula, J.C. and Keeler, J. (2018) Physical Chemistry, 11th Edition, Oxford, Oxford University Press.

Web-pages (use critically!)

Author, A. (date). Title of document [Format description]. Retrieved from http://URL
Date accessed.
The format description in brackets is used only when the format is something out of the ordinary, such as a blog post or lecture notes; otherwise, it's not necessary.
Harr, J. (2002, October 29). Plants of Cedar Creek – Asteraceae. Retrieved from http://cedarcreek.umn.edu/plants/narratives/asteraceae.html 7 January, 2004

Unpublished documents:

Author, A. (date) Title of document. Type of document. If no author(s) are listed, used "anon" for "Anonymous"
Anon. 2023. Biological Investigations: A Handbook for Bio 110 and Beyond. Unpublished, Lewis & Clark College.
Bierzychudek, P. 2023. Project Two handout. Unpublished, Lewis & Clark College.

Appendix 5: Principles of Microscopy

Introduction

The microscope has become one of the biologist's most commonly used research tools. A wide variety of microscopes and associated techniques are currently available that enable one to see cellular detail and microstructure. These instruments have undergone significant modifications and improvements since Leeuwenhoek invented his simple microscopes. Depending upon the principle involved in magnification, microscopes can be grouped into one of two categories - light and electron microscopes--depending on the form of electromagnetic radiation used to "illuminate" the object being viewed. Light microscopes can be modified in various ways to emphasize and visualize particular features of cells; such modified microscopes include bright-field, dark-field, fluorescence, differential interference contrast, and phase-contrast instruments. Transmission electron microscopes are used to study internal features of cells while scanning electron microscopes reveal their surface features.

Each type of microscope possesses an inherent lower limit of visibility: that is, one can see and resolve the details of objects bigger than a certain size but not objects smaller than that minimum size. For example, an ordinary light (bright-field) microscope can be employed to observe objects with dimensions larger than approximately 0.2 micrometers. However, if an electron microscope is used to examine specimens, the sizes of objects which can be observed range down to 1 or 2 nanometers.

Units Of Measure

In biology, as well as in other branches of science, several common metric units are used to express the dimensions of microscopic objects under study. In the metric system length is measured in meters and in the various subdivisions of meters, including centimeters (abbreviated cm = 10^{-2} meters), millimeters (mm = 10^{-3} m), micrometers (µm =10^{-6} m), and nanometers (nm =10^{-9} m). Some terms you may encounter in older books and journals (or hear from older faculty members) such as microns (identical to µm), millimicrons (identical to nm), and Angstroms (= 0.1 nm) are no longer in common usage. The units most commonly used to describe cells and their components are micrometers and nanometers. The relationships of these various units to one another, as well as to other components of the metric system and equivalent values of the English system, are given in Table 1.

TABLE 1 Some Metric Units and Useful Equivalents

Unit	*Symbol*	*Equivalents*[a]
meter	m	1 m = 39.37 in.
centimeter	cm	1 cm = 10^{-2} m = 0.39 in.
micrometer	μm	1 μm = 10^{-6} m = 0.39 × 10^{-4} in.
nanometer	nm	1 nm = 10^{-9} m = 0.39 × 10^{-7} in.
Angstrom	Å	1 Å = 10^{-10} m = 0.39 × 10^{-8} in.
liter	ℓ or L	1 ℓ = 1 dm^3 = 0.001 m^3 = 1.06 qt
milliliter	ml or mL	1 mL = 0.001 ℓ = 0.001 qt
degree Celsius	°C	1°C = 1 K = 1.8°F
		0°C = 273.15 K = 32°F

[a] English equivalents are approximate.

Optical fundamentals

To understand microscopy, you need to be aware of certain fundamental properties of light and optics. With this foundation, explanations of many of the problems encountered in using a microscope will become more readily apparent.

Properties of light

Light is transmitted from one point to another with a velocity of approximately 186,300 miles or 3 x 10^{10} centimeters per second. What we call light colloquially is the visible part of the electromagnetic spectrum that includes visible light, infrared radiation, ultraviolet radiation, X-rays, gamma radiation, etc. According to modern physics, all electromagnetic radiation has both wavelike properties and particle-like properties, but it is the wavelike properties that are important for microscopy and we will consider only those.

Characteristics of electromagnetic waves:

Amplitude.

Electromagnetic waves can be described in terms of their amplitude, frequency, and wavelength. (Fig. 1). This is readily seen by an analogy between a light wave and a skip rope. When two people pull on the rope and stretch it tight, a position of equilibrium is established. Now if the rope is shaken at the ends, a wavelike effect is produced. The vertical distance between the crest of one wave and the trough of the next wave is defined as the wave's amplitude (Figure 1).

Frequency.

This property of a light wave refers to the number of vibrations that occur in one second. Specifically, frequency is the number of times a wave crest or trough passes a particular point per second (See Figure 1). If two people take a rope and shake it, the frequency of the wave can be regulated by the speed with which the rope is shaken.

Wavelength.
A property of waves inversely associated with frequency is the wavelength. A simple definition of wavelength is the distance between two corresponding points on a wave, that is, the distance between two successive peaks or crests.

The wavelengths of electromagnetic radiation that make up the so-called visible spectrum range from approximately 400 to 700 nm. The colors we see result from a combination of factors including amplitude, frequency, and wavelength. Waves possessing lengths either less than or greater than the limits of the visible spectrum also exist. Ultraviolet "light" rays, for example, have dimensions ranging from approximately 100 to 385 nm, while the wavelengths for infrared light are greater than those of visible light.

The resolving power of a microscope -- its ability to reveal the fine details of a specimen -- depends on wavelength of light used. As a general rule, the shorter the wavelength of light used, the greater is the resolving power. Thus, with ultraviolet light as the source of illumination, finer details can be seen in specimens than with visible light.

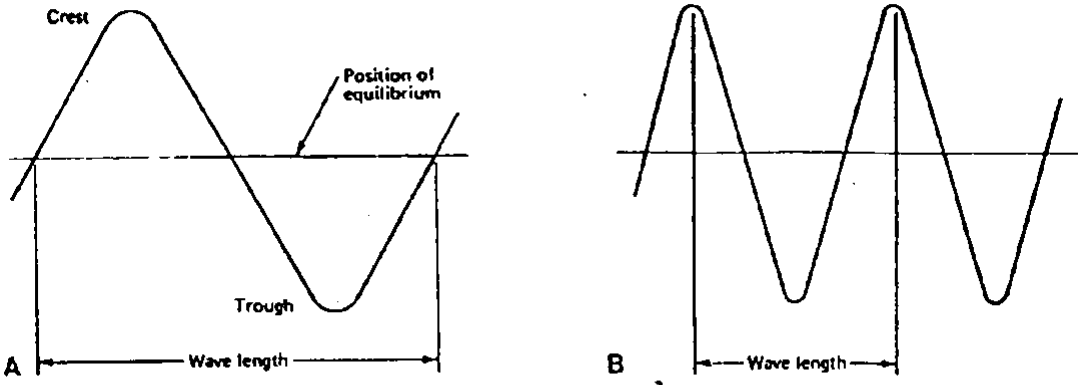


FIG. 1 Properties of light waves. A. The anatomy of a wavelength. Note the position of equilibrium. B. A representation of frequency.

Resolving Power of Microscopes

Resolving power is defined as the ability to distinguish clearly two points or objects. This feature is largely determined by the wavelength of the light source (with a shorter wavelength providing finer detail) and the angular aperture of the lens system being used. The resolution is also affected by the refractive index of the medium through which light passes before entering the microscope objective. The relationship of these factors is expressed in the combined formula:

$$D = \frac{0.612\lambda}{N (\sin \text{ø})}$$

In the formula the variables are defined as follows:

D = minimum distance between two points that allows them to be seen as distinct from one another.

0.612 = Mathematical constant. This number is an inherent property of glass lenses.

λ = Wavelength of illuminating source.
Sin ø = Sine of the angle of the illuminating cone as it passes into the objective lens.
N = Index of refraction of medium through which the illuminating source is passing, sometimes also called the "refractive index".

Numerical Aperture (NA) = N sin ø. The denominator of this equation is a characteristic of the objective lens and is called the numerical aperture. Sin ø is the trigonometric sine of half the angle formed by light rays (in the shape of the cone) coming from the condenser and passing through the specimen." Figure 2 diagrammatically depicts the explanation of numerical aperture. The angular aperture, ø, depends on the width of the objective lens and its distance from the specimen. Moving the objective closer to the specimen increases ø, and hence sin ø, reduces D and thus increases the resolution. Intuitively, you can see that increasing ø allows a greater fraction of the light emanating from the specimen to enter the objective lens, and hence increases the amount of information about the object that can be obtained. Values for NA are engraved on the barrel of objectives and are used to determine the maximum resolution obtainable.

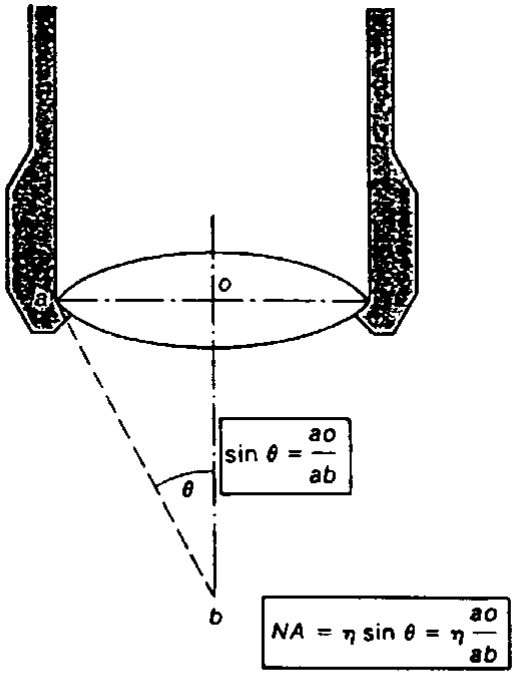


Figure 2 A diagrammatic representation of numerical aperture. (NA).

Oil Immersion:
One way to increase resolving power is to increase NA. Using a media with a higher refractive index, N, such as oil, will increase N sin ø and hence resolution.

N=Index of Refraction = Speed of light in vacuum
Speed of light in medium tested

Examples of indices of refraction in common substances:

vacuum	= 1.0 (by definition)
air	= 1.00003 (for all practical purposes this is the same as vacuum).
oil	= 1.55
water	= 1.33

An intuitive explanation of the improvement in resolution using oil is that a medium with a higher refractive index than air, if placed between the specimen and the objective lens, will "bend" more of the light emanating from the specimen such that it goes into the lens (Fig. 3).

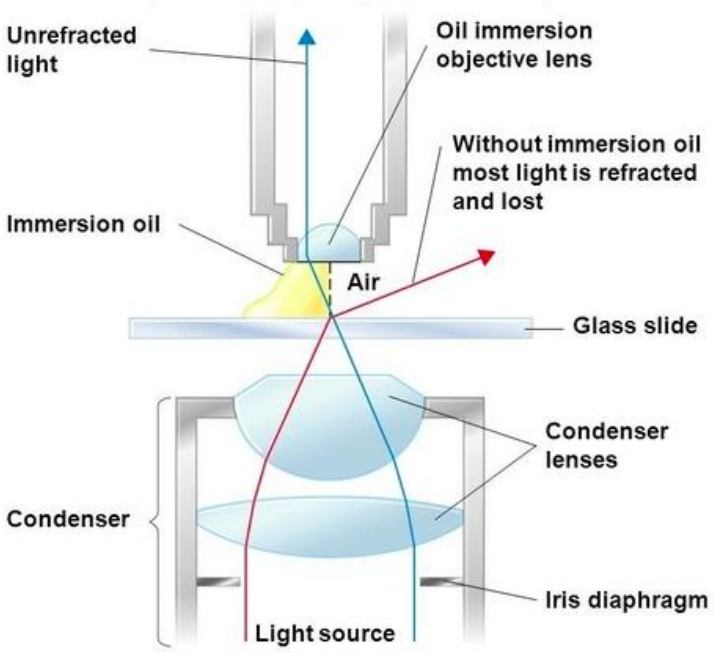


Figure 3. Comparison between the effects of immersion oil and air on the passage of light rays from a specimen to the front lens of the oil immersion objective.

LIGHT MICROSCOPES

Bright-Field Microscopes

The original microscope was a "simple" microscope, because it contained only one lens held in a frame-like device. Similar devices have been in use for centuries as magnifiers or reading glasses. Unfortunately, the effectiveness of the simple microscope is restricted because of its limited magnification and it has been supplanted by the compound microscope in most branches of science and in industry.

The ordinary compound microscope consists of a series of optical lenses rather than just one. These lenses are attached to mechanical adjustment parts and supportive structures that allow them to be maneuvered and focused on the sample; the whole arrangement is the microscope. The optical lenses include the ocular (or eyepiece), several (often 3 or 4) different objective lenses (usually just called the "objectives") with different magnifying powers, and the substage condenser. The coarse and fine focus knobs, the condenser adjustment knob, and

the iris diaphragm lever make up the major mechanical parts required for operation of the instrument. The various components of the scope are held in position by, or are contained within, supportive structures such as the base, arm, pillar, body tube (barrel), and revolving nosepiece. Several of these microscope components are shown in the diagrams in Appendix Vb. The function of these parts is discussed here.

Ocular or Eyepiece

A short tube generally containing two lenses, the ocular fits into the upper portion of the microscope's body tube. Several different types of eyepieces can be used to examine specimens. The specific type used generally depends upon the objective lenses on the instrument. The magnifying power of the ocular usually is engraved on it. Common magnifications for oculars include 1X, 2X, 5X, 10X, and 15X. The functions of eyepieces generally are to magnify the image produced by the objective and to correct certain aberrations or distortions associated with the objective. The microscopes that you will be using have only one type of ocular lens, but a variety of other oculars are available to the microscopist for different purposes.

Objectives

Objectives are the most important of the optical parts, primarily because they predominantly control the quality of the image seen by the observer. Three major types of objectives are in use: achromatic, apochromatic, and fluorite. The first of these objective types is the least expensive and simplest in construction. The latter two are more expensive and are used in more critical types of work, as they have been optically corrected to overcome the major defects of glass objectives.

Most laboratory instruments are equipped with three objectives that have different magnifying powers: the low-power, high-power (or high-dry), and the oil immersion lenses. Sometimes a fourth, intermediate-power, dry objective is also added. The individual objectives commonly can be distinguished from one another on the basis of their respective lengths (low-power is the shortest, while the oil-immersion lens is the longest).

The primary functions of the objective lenses include: (1) gathering or concentrating the light rays coming from the specimen being viewed; (2) forming the image of the specimen; and (3) magnifying this image. Several important properties of a microscope are largely determined by the objectives. One of these is resolving power (RP) or resolution as described above.

Most modern-day microscopes are parfocal. Stated simply, this means that once the microscope is focused, it will stay in focus without major focusing adjustments when you alter the magnification by changing from one objective to another. Thus, if a higher magnification is needed during the course of examining a specimen, you would just rotate the desired higher objective into place and make some minor focusing adjustment to bring the specimen into view.

While there are some advantages to using oil for viewing samples, there are also disadvantages--it’s messy, and hard to remove from slides and lenses (and clothes). Thus you need to be careful when using an oil immersion lens. People using this objective for the first time have a tendency to use large quantities of oil, creating a massive oil slick. One good, small drop of this medium usually is sufficient. One hazard of using oil is that the oil can get

on objectives that are not intended for use with oil and damage them or degrade their image. Thus, you must take care to clean up well after you use an oil immersion lens. Using lens paper, clean the slide and oil immersion lens well, and be sure to remove any residual oil from other parts of the microscope, including the stage, condenser, other objectives, etc.

The Condenser, Iris Diaphragm, And Mirror

A condenser is found under the microscope stage between the source of illumination and the specimen or object to be viewed. It focuses the light directly onto the specimen and maximizes the amount of light that falls on the objective lens; thus the condenser is important for obtaining a bright image. Since microscopic examination of specimens using either high-power or oil-immersion objectives requires adequate illumination, the condenser is an essential part of the modern microscope. Because it is usually below the microscope stage, this component frequently is called a substage condenser.

Occasionally, too much light may pass through the specimen and into the objective lens, significantly decreasing the contrast of the specimen. Microscope condensers generally are equipped with an iris diaphragm with t shutter to increase or decrease the amount of light entering the condenser. When unstained material, such as living protozoa or hanging drop preparations of bacteria, is to be examined, the opening of the iris diaphragm generally is reduced. This component is regulated by the iris diaphragm lever. Many newer and inexpensive microscopes are equipped with fixed condensers and iris diaphragms, regulated for general use.

An adjustable mirror can be used with microscopes to reflect light up into the instrument's condenser, thus aiding in the illumination of a specimen. Today, however, most compound microscopes do not use a mirror, but a rheostat that can control the light intensity.

Sample Calculation to determine the resolution of a light microscope:

If visible light of the shortest wavelength is used for illumination so that $\lambda = 450$ nm, and air is the medium between the microscope objective and the sample, calculate D given that the maximum angular aperture for the best objective lens is 70º (thus Sin ø = 0.94), then

$$D = \frac{(0.612)(450\text{ nm})}{1.0\ (.94)}$$

$$D = \frac{275\text{ nm}}{.94} = 292\text{ nm or } 0.29\ \mu\text{m}$$

Therefore two points (or two structures within a cell) must be no closer than 0.29 μm or they cannot be resolved as distinct points.

Problem: How much improvement in resolution will you get if you use oil immersion?

Appendix 6: Use and Care of the Olympus Microscope

INTRODUCTION: Please read these procedures carefully so that you can use your microscope most effectively. If you have questions or difficulties, obtain help from your laboratory instructor or TA. Microscopes are expensive and delicate instruments--the ones you're using cost about $2500 each--and it's important that you use them carefully and knowledgeably. Proper use will provide you with the clearest, most usable images and will keep the microscopes functioning well for you and other students.

(I) SETTING THE MICROSCOPE FOR BRIGHTFIELD ILLUMINATION:

(A) Be sure all glass surfaces in the light path are clean. For cleaning lenses see Parts IIIA and IIIB2, below. DO NOT use Kimwipes on microscopic lenses.

(B) Turn on the microscope light, using the sliding control lever on the lower right side of the microscope.

(C) Place a specimen slide on the microscope stage, coverslip on top.

(D) Using a low power (i.e. 4X or 10X) objective, coarsely focus on the specimen.

(E) The eyepieces on a binocular microscope should always be adjusted to your interpupillary distance. The distance is read on a scale on the binocular observation tube and remains the same for you, regardless of which microscope is used.

(F) Hold the knurled dovetail slides on the right and left eyepiece tubes with both hands and push the tubes together, or pull them apart, whichever is required to achieve perfect binocular vision when you look through the eyepieces with both eyes. Memorize your interpupillary distance setting for future use, so that you don't have to waste time in readjusting the distance between the eyepieces every time someone else uses, and changes, the microscope.

(G) Move the tube length adjustment ring on the right eyepiece tube to the zero position.

(H) Look at the image through the right eyepiece with your right eye (have your left eye closed) and focus on the specimen with the coarse and fine adjustment knobs.

(I) Looking at the image through the left eyepiece with your left eye (have your right eye closed), rotate the tube length adjustment ring on the left eyepiece to focus on the specimen, without using the coarse and fine adjustment knobs.

(J) Raise the substage condenser to its maximum height. If there is a granular (grainy) appearance to your image, lower (or raise) the condenser slightly to remove it. If you're using a low magnification objective (4X and 10X), your field of view may not be uniformly lit with the condenser raised all the way and you may see brighter and darker regions. If this happens, slightly lower the condenser until the field seems uniformly bright.

(K) Swing the desired objective into the line of vision.

(L) Adjust the light intensity with the sliding control lever (see Part "IIB").

(M) Adjust the aperture iris diaphragm. Slowly open (or close) the iris diaphragm of the condenser until it is near the middle setting.

Basic Theory: Provided it is smaller than the aperture of the objective, the iris diaphragm of the condenser determines resolution and contrast of the microscope image. *The iris diaphragm must not be used for control of image brightness* (see Part "IIB"). The iris diaphragm should be adjusted such that the back lens of the objective is 2/3 to 3/4 illuminated (step "I" above). Too small an aperture setting produces a contrasty image, with diffraction patterns; too large an aperture produces a glary, washed out image. Note that closing the iris diaphragm increases the contrast, but beyond a certain point (less than 2/3 of the objective aperture), the resolving power of the objective is rapidly reduced.

(II) GENERAL PROCEDURES:

(A) Choice of magnification and precautions in focusing: At the beginning of an examination, always use the low power 4X objective. This permits coverage of the widest field and is thus best suited for scanning. It is also easier to find the plane of best focus. Then, gradually work through the higher power objectives, finally using the oil immersion objective if necessary. Always use the fine adjustment focus with the high power objectives (40X and 100X) to avoid running the objective into the slide and damaging either or both. To prevent damage to the slide, it is always best to focus away from the specimen rather than towards it when using high power objectives. See also Part "IIE."

Better microscopes are parfocal, that is, the specimen should be in approximate focus despite the objective used, so that with the specimen in focus with one objective only slight adjustment with the fine focus knob is necessary to bring the specimen into focus with another objective. Never force an objective into position because you can damage it or the microscope body (the slide and/or preparation may be too thick to use with that particular lens; use a lower power objective). If you are having trouble, ask a TA or your instructor-they may be able to see a solution for you.

(B) Controlling light intensity: Light intensity should be varied with the rheostat located on the lower right side of the base. Light intensity should not be controlled (1) by varying the substage condenser iris diaphragm, or (2) by lowering the condenser.

(C) Tension adjustment of coarse adjustment knobs: Although it's possible to adjust the tension of the coarse focus knobs, it's usually better to leave this alone. However, if the adjustment is not comfortable for you, there is a tension adjustment ring next to the right-hand coarse adjustment knob. With this device the tension of the coarse adjustment is freely adjustable for either heavy or light movement depending on your preference. The arrow mark indicates increase in adjustment tension. Do not loosen the tension adjustment ring too much, because this may cause the stage to drop or the fine adjustment knob to slip. Never rotate the

right and left coarse adjustment knobs in the opposite directions simultaneously; you'll strip the internal gears and make it impossible to focus the microscope.

(D) Automatic pre-focusing lever: This lever, located next to the left-hand coarse adjustment knob, is provided to prevent accidental contact between specimen and objective as well as to simplify coarse focusing. The lever can be locked after you have coarsely focused the objective in order to prevent further upward travel of the stage, and automatically provides a limiting stop if the stage is lowered and then raised again. The automatic prefocusing lever restricts coarse but not fine focusing.

(III) SPECIAL PROCEDURES:

(A) Cleaning lenses: Clean the objectives while they are attached to the microscope. Do not attempt to remove the objectives from the revolving nosepiece! There's too much risk of your dropping and damaging them or getting dirt inside the objective.

Dirty objectives are especially undesirable because they degrade the image and make it hard for you to make out detail (a dirty objective is similar to having grease smeared on your glasses; suddenly everything seems fuzzy). Remove excess dirt from lenses with an air blower. If that fails to remove visible dirt, use a camel hair brush or a brush made from lens tissue folded several times and torn in half to gently dislodge the dirt. If necessary, use lens tissue and wipe the surface with delicate circular motions. Exhaling gently on the surface to be cleaned, before wiping, may help. Do not use solvents unless absolutely essential; they dissolve the glues used to hold objectives together (see Part IIIB2).

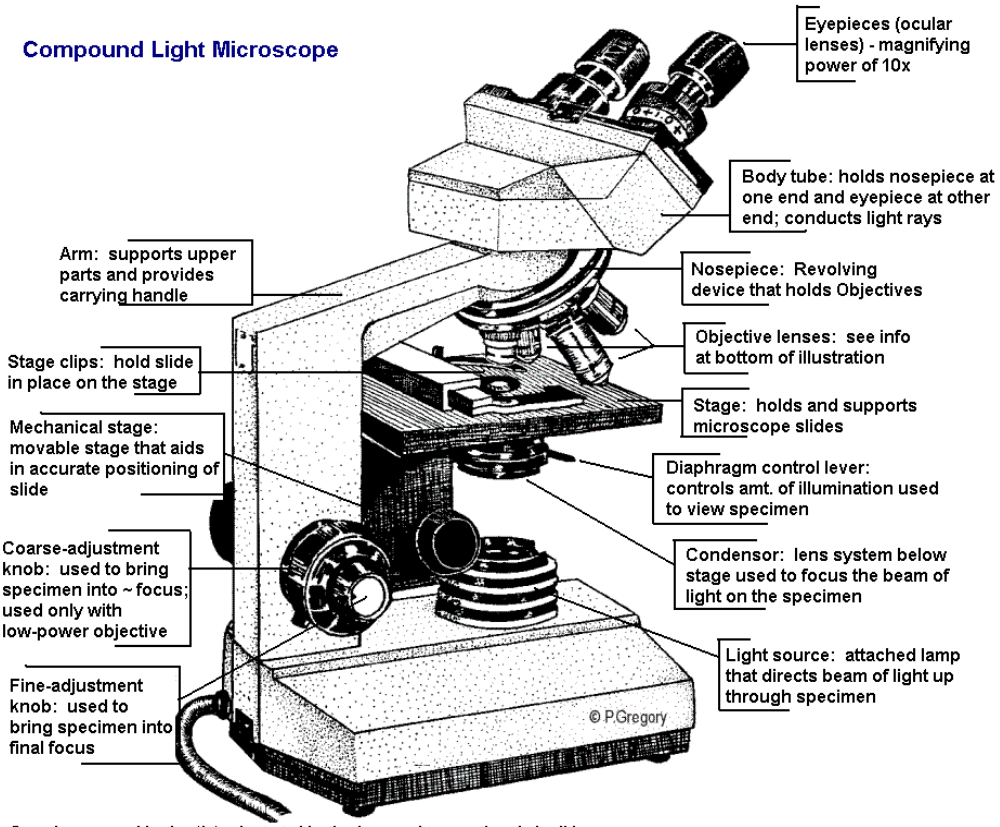
Some diffraction patterns visible in the light path may not be due to dirt on the lenses but rather to a feature of the human eye called mouches volantes ("flying flies"), which resemble small worms swimming through your field of view. These are especially evident on specimens with a wide, clear background, where you may see strange moving irregular objects. These are the shadows of inhomogeneities in the aqueous humor of your eye, which become visible whenever the exit pupil of the microscope becomes very small (i.e., if the iris diaphragm of the condenser is closed down too far or if a high-power objective is used). Just learn to ignore them.

(B) Using the oil immersion objective:
(1) Procedure: Focus your microcope on cells using the 40x high dry objective. Swing the 40x objective out of the way and place a small drop of immersion oil onto the slide when the cone of light is focussed on the slide. Then carefully swing the 100x objective into position. at the small bright spot of light just vertically below the objective. Focus carefully with the fine adjustment only. The objective should not touch the slide; the focal length of all lenses is greater than the thickness of coverslips so the lens will come into focus *before* it touches the coverslip.

(2) Cleaning: When finished with the oil immersion objective, lower the stage again as much as possible. With lens tissue remove excess oil from the objective. If necessary, put a very small drop of xylene (alcohol should not be used) on new lens tissue and lightly wipe the

surface of the lens. Excess xylene (or other solvent) will weaken the cement holding the lens elements in place. Also thoroughly clean the slide by wiping with lens tissue and, if necessary, clean with alcohol or xylene (excess xylene will dissolve mountant holding on the cover glass). Do not use the high dry objective until you have cleaned the slide, to prevent transferring oil onto a non-oil immersion objective.

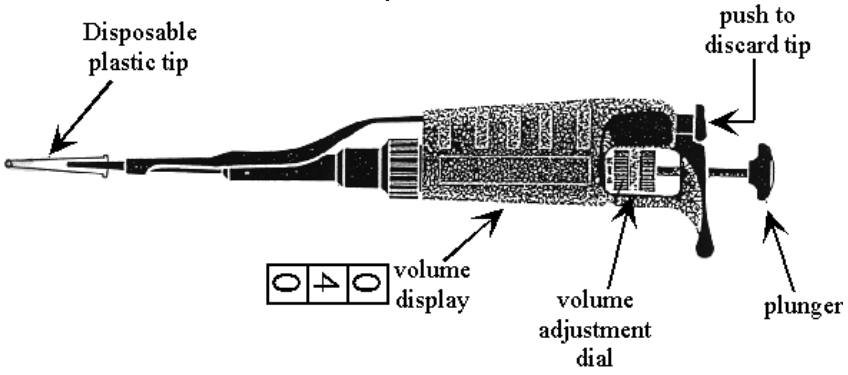
(3) Precautions: Oil immersion and high dry objectives, because of their extremely small working distances, should never be used with thick whole mount preparations or unusually thick slides. Don't put oil on the condenser lens. If you switch to the high dry objective from an oil immersion objective, first clean the immersion oil from the slide.



Scanning power objective (4x): shortest objective lens used to scan the whole slide
Low-power objective (10x): lens next in length (longer than scanning) used to view specimen in greater detail
High-power objective (40x): lens next in length (longer than low-power) used to view specimen in even greater detail
Oil immersion objective (100x): used in conjunction with immersion oil to view specimen in greatest detail

Appendix 7: Use of Micropipettors

The micropipettor is used to measure small volumes (<1 ml) of solutions in conjunction with disposable (often sterile) plastic tips. Micropipettors are graduated in microliters (1000µl=1 ml). The micropipettors you will use come in three sizes: the P20 is for measuring volumes between 1-20µl and the P200 is for 20-200µl and a P100 is for 200-1000µl. Illustrated below is a P200 micropipettor set at 40 µl. The scale is read from the top to bottom; thus 040 is 40 µl. On the P20 scale, the third number from the top is red and represents the place after the decimal point. Thus 040 on a P20 scale is 4.0 µl.



DIRECTIONS FOR USING THE MICROPIPETTOR:

1. Set the desired volume by turning the volume adjustment dial. DO NOT set the P20 to volumes above 20µl or the P200 to volumes below 20µl or above 200µl.
2. Place a tip on the discharge end of the pipettor. **DON'T USE THE MICROPIPETTOR WITHOUT A PLASTIC TIP**. If your sample needs to be kept sterile, do not allow the tip to touch anything (including your hands).
3. The plunger will stop at two different positions when it is compressed. The point at which you first feel resistance is the level of compression you should use when you are filling the pipettor tip with the solution to be transferred. The second stopping point, reached by compressing the plunger past the point of initial resistance, is used for expelling solutions from the tip. At the second point the plunger cannot be further compressed; it contacts the body of the pipettor. Practice compressing the plunger to each of these stopping points.
4. Compress the plunger to the first stop and insert the tip into the solution.
5. Carefully release plunger. NOTE: Release the plunger slowly and allow the tip to fill completely before you remove it from the solution. This will prevent your drawing air into the tip and thus measuring an inaccurate volume of solution. Only remove the tip when the pipettor has finished filling the tip.
6. Discharge the solution into the appropriate container by compressing the plunger. Compress the plunger to the point of initial resistance, wait one second, and then continue pressing the plunger as far as it will go in order to discharge all of the solution. To ensure complete discharge, touch the pipette tip either to the edge of the tube or just into the solution to allow capillary action to empty the tip.
7. Remove tip by pressing down on the tip discarder.

REMEMBER TO CHANGE TIPS BETWEEN SOLUTIONS TO AVOID MIXING OR CONTAMINATING THE REAGENT!

Appendix 8: Some useful R commands

Note: it might not work to copy and paste these commands into the R console; sometimes R does not recognize the symbols, especially quotation marks, properly. It is safer to type them in yourself.

In these examples, certain words such as "data," "depvariable," "indvariable", etc. will refer to *any* dataset or dependent or independent variables. These "placeholders" are printed in blue ink. Be sure to replace these placeholders with whatever names you are using for your own dataset and variables. Go back to the tutorials for more information if needed.

To import a datafile that you have created in Excel or Google Sheets and stored on your desktop in .csv format:

From R Studio Cloud, go to the Files tab in the lower-right-hand window and select "upload." Click "browse," to browse your desktop files. Open the one you wish to import. Then read the file into your R session with this command:

```
data<-read.csv('/cloud/project/data.csv', header=T)
```

Commands for working with data files and variables (from Lesson One):

To assign a set of numerical values to a variable:

```
depvariable<-c(4,3,6,2,4,3,5,4)
```

To see a printout of what a variable or data table contains:

```
depvariable
```

or

```
data
```

To see the data stored in an individual variable from a larger datafile:

```
data$depvariable
```

To see the "structure" of a data file (e.g. what each variable's type is: factor, numeric, etc.):

```
str(data)
```

To change a numeric variable to a factor variable:

```
data$indvariable<-as.factor(data$indvariable)
```

Commands for working with measures of central tendency (from Lesson Two):

To compute the mean of a variable's values:

```
mean(data$depvariable)
```

To compute the median of a variable's values:

```
median(data$depvariable)
```

To display a histogram of values of a variable:

```
hist(data$depvariable)
```

Commands for working with measures of variability (from Lesson Three):

To compute separate functions (such as mean or var) of a first, numeric variable that is divided into different categories according to the value of a second, factor variable:

```
tapply(data$numericvariable, data$factorvariable, mean)
```

To compute the variance of a variable:

```
var(data$depvariable)
```

To compute the standard deviation of a variable:

```
sd(data$depvariable)
```

To compute the standard error of a variable (**requires installing the package "plotrix"):**

```
std.error(data$depvariable)
```

To use a factor variable to extract a subset of the values of a numeric variable, using indexing:

```
data$numericvariable[data$factorvariable==value]
```

To find the 95% confidence interval of the mean of a variable, look at the output from a t-test:

```
t.test(data$depvariable)
```

Commands for making boxplots and modifying their properties (from Lesson Four) :

To make a boxplot of the values of a dependent, numeric variable as a function of the values of an independent, factor variable:

```
plot(data$indvariable,data$depvariable)
```

Note: if both variables are numeric, the plot command will create a scatterplot instead of a boxplot.

To make a boxplot and force R to treat the independent variable as a factor

```
boxplot(data$depvariable ~ data$indvariable)
```

To add axis labels to the plot:

```
plot(data$indvariable,data$depvariable, xlab="the name of your independent variable", ylab="the name of your dependent variable")
```

To also replace numerical values of a factor variable with names of the categories (e.g. "male", "female" instead of 1, 2) on the axes:

```
plot(data$indvariable,data$depvariable, names=c("female", "male"),xlab="the name of your independent variable", ylab="the name of your dependent variable")
```

To also change the range of values on the y-axis:

```
plot(data$indvariable,data$depvariable, names=c("female", "male"),xlab="the name of your independent variable", ylab="the name of your dependent variable", ylim=c(lowerlimit, upperlimit))
```

Commands for making scatterplots and modifying more properties (from Lesson Five):

To make a scatterplot of a dependent variable as a function of an independent variable (when both variables are numeric):

```
plot(data$indvariable, data$depvariable)
```

To make the points blue (or any other color you wish):

```
plot(data$indvariable, data$depvariable, col="blue")
```

To also make the points closed rather than open circles (or any other shape you wish):

```
plot(data$indvariable, data$depvariable, col="blue", pch=16)
```

To also make the points twice as large (or any other expansion you wish):

```
plot(data$indvariable, data$depvariable, col="blue", pch=16, cex=2)
```

For cases when the independent variable is actually a factor, and has only 2 or 3 values, you may wish to space the groups of points more aesthetically. Try changing the x-axis limits to accomplish this (see code from lesson four).

When the categories are coded numerically, but you wish to replace these values with words (see lesson four), you may also want to suppress the printing of the numbers. To do so, include this code within the plot command:

```
xaxt="n"
```

Once the default axis is suppressed, you can add your own x axis, after you have created your graph. This syntax is for placing your labels at the old numerical positions 1 and 2 on the x-axis:

```
axis(side, at=1:2,labels=c('category1name', 'category2name', etc.))
```

Commands for making bar graphs (from Lesson Six):

Note: To use the barplot2 command, you will first need to install the "gplots" package. To compute standard errors, you will need to install "plotrix."

To subset values of one variable into a group defined by its members having a particular value of a second, categorical variable, compute the means (or standard deviation of each subset, and store them:

```
means<-tapply(data$var1, data$catvariable, mean)
firstgroup<-subset(data$var1, data$treatment=="A")
```

To make a bar graph showing means of two different subsetted groups:

```
barplot2(means, beside=T)
```

To add standard error bars representing 2 standard deviations to this barplot, create a new variable (`sds`) containing the standard errors of each group, and add these statements within the parentheses of the barplot2 command:

```
plot.ci=T, ci.u=means+sds, ci.l=means-sds
```

To make a clustered barplot (e.g. comparing the values of some dependent variable for different treatments in different years, you would first need to create a table of the different means you wish to plot:

```
means<-tapply(data$var1, list(data$treatment, data$year), mean)
```

And you need a similar table of whatever measure of variation you wish to plot:

```
errors<-tapply(data$var1,list(data$treatment,data$year),sd)
```

To make the clustered barplot:

```
barplot2(means, plot.ci=T, ci.u=means+errors, ci.l=means-errors, beside=T)
```

If you would like to reverse which independent variables are used for the clustering, use the transpose command:'

```
barplot2(t(means), plot.ci=T, ci.u=t(means+errors), ci.l=t(means-errors), beside=T)
```

To add a legend to the topleft corner (or any corner you wish) of a clustered barplot, first create the graph, then run this command:

```
legend('topleft',legend=c('Year 1','Year 2','Year 3'),
fill=c('color1', 'color2','color3'))
```

Commands for performing statistical tests (from Lesson Seven):

To perform a regression analysis to assess the association between a numeric independent variable and a numeric dependent variable:

```
mymodelname<-lm(data$depvariable~data$indvariable)
```

To create a scatterplot that shows the best-fit line superimposed on the points:

```
plot(data$indvariable,data$depvariable)
abline(mymodelname)
```

To see the values of the slope and y-intercept for this line:

```
mymodelname
```

To statistically test the hypothesis that the slope is not significantly different from zero:

```
summary(mymodelname)
```

To perform a t-test of the hypothesis that the data values of two groups are not different from each other, and the data are roughly normally distributed:
If the data are in a single variable, with the groups classified by values of a different variable:

```
t.test(data$depvariable ~ data$indvariable)
```

If the data from each group are in a different variable:

```
t.test(data$variable1, data$variable2)
```

To perform a Wilcoxon test of the hypothesis that the data values of two groups are not different from each other, because the data are not normally distributed:

```
wilcox.test(data$depvariable~data$indvariable)
```

To perform a one-way analysis of variance (i.e. only one independent variable):
(note: be sure that your independent variable is coded as a factor variable)

```
myANOVAmodelname<-aov(data$depvariable~data$indvariable)
```

To see the output from the ANOVA

```
summary(myANOVAmodelname)
```

When comparing more than two groups, to perform a Tukey's test that computes pairwise comparisons for the groups:

```
TukeyHSD(myANOVAmodelname)
```

To perform a two-way ANOVA (two independent variables, both must be factor variables):

```
myANOVAmodelname<-
aov(data$depvariable~data$indvariable1*data$indvariable2)
```

Useful R commands for situations not covered by the tutorials

Note: this is generic code. You must substitute your own data file and variable names and your own desired label names, color choices, line widths, etc.

To make an interaction plot with lines connecting the two members of a set of paired samples:

Requires a categorical independent variable, a continuous dependent variable, and a variable that identifies the different sample numbers, showing what values are paired with each other.

To make the basic plot, be sure that the independent variable is a factor, then execute:

```
interaction.plot(data$indvar, data$samplenum, data$depvar)
```

To suppress the legend, which is often awkward and unnecessary:

```
interaction.plot(data$indvar, data$samplenum,data$depvar,
      legend=F)
```

To keep the legend but change the title of the legend box:

```
interaction.plot(data$indvar, data$samplenum, data$depvar,
trace.label="sample number")
```

To label the individual conditions, execute this code before making the plot:

```
data$indvar<-factor(data$indvar,labels=c("condition 1",
"condition 2"))
```

To add axis labels, alter line type, width, or make each of 8 (or other number) lines a separate color:

```
interaction.plot(data$indvar, data$samplenum, data$depvar,
trace.label="sample number", ylim=c(0,12),xlab= "name of ind
var", ylab="name of dep var", col=rainbow(8), lty=1,lwd=2)
```

<u>To make a clustered boxplot:</u>

For situations where there are two independent variables, 1 and 2.

To make the basic plot (reverse the order of indvar1 and indvar2 to change the clustering):

```
boxplot(data$depvar ~ data$indvar1 + data$indvar2)
```

To use color to distinguish different levels of one of the independent variables:

```
cols<-c("color1", "color2")
boxplot(data$depvar ~ data$indvar1 + data$indvar2, col=cols)
```

To control the spacing of the boxes on the x-axis to create a gap between the first 2 and the second 2 boxplots:

```
boxplot(data$depvar ~ data$indvar1 + data$indvar2, col=cols,
      at=c(1,2,4,5))
```

To label each x-axis box and make the labels vertical instead of horizontal so that they fit:

```
boxplot(data$depvar ~ data$indvar1 + data$indvar2, col=cols)
     at=c(1,2,4,5), names=c("name1", "name2", "name3","name4"),
     las=2)
```

To add a legend for what the colors represent, execute this command after making the plot:

```
legend("topright",legend=c("level1","level2"),fill=cols)
```

To make a scatterplot where the point colors reflect different levels of a categorical variable:

First make sure the categorical variable is a factor variable.

To choose the colors to use (one color for each level):

```
cols<-c("green", "blue", "red")
```

To associate the different colors with the different levels of the categorical variable, in order:

```
col=cols[data$catvar]
```

To create the scatterplot with filled circles of different colors:

```
plot(data$depvar ~ data$indvar, pch=16, col=cols[data$catvar])
```

To add a legend:

```
legend("topright",legend=c("level 1","level 2","level 3"),
     pch=16, col=cols)
```

To perform an unpaired t-test:

```
t.test(data$depvar ~ data$indvar)
```

To perform a paired t-test:

R needs to "know" which sample is paired with which. Make sure your data file has one independent variable representing the treatment, and another to identify the plot number (each of which contains a treatment subplot and a control subplot). R will assume that the first row where "indvar = 0" should be paired with the first row where "indvar = 1." If data for the two paired subplots are in consecutive rows of your data file, this will be the case. But to be sure, you might sort the data before performing the test:

```
data<-data[order(data$indvar, data$plotnum),]
```

Then, the code for the paired t-test is:

```
t.test(data$depvar ~ data$indvar, paired=T)
```