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Supplemental Materials

for

The Tigriopus CURE – A Course-Based Undergraduate Research Experience with Concomitant Supplemental Instruction

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Appendix A: Materials for Weeks 1-6 of the Tigriopus CURE

Lab Topic	Item	Number per student group	Total number for a lab of 24 students (6 groups of 4)
Week 1: The Scientific Method			
	Height chart		1
	Yardstick	2	12
Week 2: Literature Review and Critique			
	Copy of the research article	2	12
Week 3: Using the Microscope			
	Dissecting microscope	2	12
	Compound microscope	2	12
	Tigriopus californicus		2 cultures from Carolina Biological Supply Co. (#142366)
	Disposable transfer pipette	4	24
	Petri dish	2	12
	24 well plate	2	12
	Glass slide	4	24
	Cover slip	4	24
	Tetraselmis algae		2 cultures from Carolina Biological Supply Co. (#152610)
Week 4: Dilutions and the Standard Curve			
	Red food coloring		1 stock solution
	Spectrophotometer	1	6
	1ml pipette	2	12
	5ml pipette	2	12
	10ml pipette	2	12
	Wax pencil	1	6
	15ml test tube	10	60
	Spectrophotometer tube	8	48
	Parafilm		
	Test tube rack	1	6
Week 5: Counting Algae			
	Spectrophotometer	1	6
	1ml pipette	2	12
	5ml pipette	2	12
	10ml pipette	2	12
	Wax pencil	1	6
	15ml test tube	10	60
	Spectrophotometer tube	8	48
	Parafilm		
	Test tube rack	1	6
	Hemocytometer with coverslip	1	6
	Micropipette to transfer 10µL volume	1	6
	Tetraselmis stock solution	1	1 (grown in clear carboy)
	Nannochloropsis stock solution	1	1 (grown in clear carboy)
Week 6: Graphing and Data Analysis			
	Access to computer	1	6

*Appendix B: General Supplies for Research Projects for the Tigriopus CURE**

Glassware	Disposables	Equipment	Miscellaneous
• Beakers and flasks of a variety of sizes • Petri Dishes (20-30) • Serological pipettes • Glass slides and cover slips • Test tubes • Spectrophotometer tubes	• Transfer pipettes • 24 well plates • Instant Ocean	• Spectrophotometer (6) • Refractometer (6) • Chicken egg incubators set to a variety of temperatures • Lights with timers to place in incubators • Balances with weigh boats	• Labeling tape • Markers • Parafilm • Test Tube Racks • Carboys filled with 35ppt artificial seawater • Kimwipes

*note that because students will design their own experiments, this list will be modified based on individual student requests

Appendix C: Abridged Laboratory Manual for the Tigriopus CURE

BIO 110: Principles of Biology

Laboratory Manual

Ginger R. Fisher and Thomas McCabe

How to Be Successful In Lab

1. Read the assigned laboratory exercise before coming to class. This is key for the first 6 weeks of lab when you are conducting the pre-designed exercises. Your teaching assistant will assume that you have read the lab exercise and therefore will not spend a great deal of time reviewing the laboratory topics.

2. Participate in the laboratory exercises. The best way for most students to learn is to actually do hands-on work with a subject. Science is one of the few fields in college where hands-on exercises are part of the curriculum, so take advantage of these opportunities. You will be able to better understand the concepts presented in class if you fully participate in lab. In addition, participation in the exercises is part of your overall laboratory grade.

3. Work as a team and share ideas. At the beginning of the semester, you will be placed into lab groups and expected to work together. This means that you will collaborate on ideas and conduct the experiments together. In addition, you will each be assigned specific roles in your group that you must complete. This means that it is not possible for one person to do all the work while the other students watch. All of you should be contributing members of the team and participate (see #2). You will be assessed on the contribution that you, individually, make to your team.

4. Do your own work. While it is important that you work as a team, in the end you are responsible for the material in your lab notebook as well as your understanding of the concepts. So, share ideas and discuss with your lab group, but the answers you put in your lab manual should be your own.

5. Take the lab seriously. We have redesigned the entire laboratory experience to make it an authentic research experience for students where you will be discovering new information in biology (something that no one else yet knows!). Our goal is to introduce you to <u>real</u> science rather than simply have you conduct cookbook experiments that have been done hundreds of times to arrive at a pre-determined result. However, this requires a great deal of effort on your part and you will be expected to contribute in a meaningful fashion.

Safety Guidelines

1. No eating, drinking, or chewing gum in the laboratory. If you bring a drink (such as coffee for those 7AM labs), you may place it outside the door of lab and exit the room to take a drink. You may only leave when the instructor has given you permission.
2. If you spill a chemical, do NOT clean it up. Inform the teaching assistant so that disposal can be handled properly. Be prepared to aid the teaching assistant in cleaning up any mess you may have made.
3. At all times, wear close-toe shoes and wear personal protective equipment (PPE—such as gloves and lab coats) when appropriate. For example, wear gloves whenever handling potentially hazardous materials, such as strong acids, strong bases, biological hazards, and toxicants.
4. Chemical glasses will be worn in the lab whenever working with hazardous substances.
5. Mouth pipetting is **a*bsolutely prohibited***.
6. Use extreme caution when using sharp instruments, such as razor blades.
7. Read labels before handling any chemicals.
8. Wash your hands before you leave the lab at ***any*** time.
9. Locate the nearest fire extinguishers, fire alarms, emergency eye washes, and emergency showers. If necessary to use an eyewash or chemical shower, do so for a minimum of 15 minutes.
10. Keep your lab area clean and well-organized. Coats and unnecessary books should be stored in the drawers.
11. Broken glass should be carefully discarded into the broken glass container in the lab. Sweep up small pieces with a broom and dustpan and discard them into the broken glass container.
12. Assume that any chemicals other than distilled water are toxic and hazardous. Handle them accordingly. Any spills should be ***immediately*** covered with paper towels. Then ask your lab instructor for proper cleanup/discard procedures.
13. Children and pets are ***not*** allowed in the lab due to potential hazards to them and others working in the lab. For service animals please see the school Chemical Hygiene Plan.
14. In the event that additional emergency assistance is needed, see your instructor and/or dial 911
15. Conduct yourself in a professional manner and maintain a safe and studious atmosphere in the lab.
16. Treat all equipment with care. Frequent breakage of equipment may result in an increase of lab fees.
17. When you have finished with the laboratory exercise for the week, clean up all supplies on your table and wipe down the table with cleaner and paper towels. You will not be allowed to leave the lab until you have successfully cleaned your area.
18. If you have any medical conditions, please inform your teaching assistant immediately.
19. It is the responsibility of any person in the laboratory to notify the proper authority in the appropriate hierarchical order, first the laboratory instructor, then the laboratory coordinator or the research advisor, and finally the School Director, if unsafe laboratory conditions exist or if a possible safety concern is present.

I (print name:____) have read the safety rules for the lab and I understand all of the guidelines. I agree to follow these guidelines to help reduce the risks to myself and fellow classmates.

Signature____Date________________

Format of the BIO 110 Lab

PREMISE

Most introductory biology lab courses consist of a number of cookbook-type exercises followed by a limited amount of experimental design. This approach is useful in that it reviews topics that were covered in lecture and provides students with an opportunity to interact with the material again. It also teaches basic techniques and aspects of experimental design. However, this is not a representation of real science and does not allow you to create new scientific knowledge. Instead, you are simply repeating experiments that have already been done thousands of times in universities around the world.

The structure of our BIO 110 lab is designed to provide many of the same benefits of the traditional laboratory experience but also give you a more realistic taste of how science is actually done. You will still be given the opportunity to review lecture material and learn new techniques, but you will also be given an authentic opportunity to design experiments to collect novel data.

OVERVIEW OF LAB STRUCTURE

Labs will be held for three hours. The first 45 minutes of lab will start with a weekly quiz, followed by a recitation section taught by an upper-level undergraduate student with the graduate student TA as the supervisor. This is when you will get the opportunity to review concepts that were discussed in lecture to be confident of your understanding of the material. The recitation sections will NOT be more lectures; instead they will focus on active learning techniques.

After the first 45 minutes, the lab will revert to a more traditional lab structure. For the first 6 weeks the labs will focus on learning specific techniques such as microscopy, dilutions, using a spectrophotometer, using a hemocytometer, etc. When learning these techniques, you will gain experience with the model organism for the course, the marine planktonic copepod *Tigriopus californicus*. You will also be drafting a proposal for the experiments that you will conduct during the later portion of the semester. The lab manual contains background information on the copepods as well as areas of potential research, where the answers to basic questions are still unknown. Then, working in groups, you will choose an area of research, develop a testable hypothesis, and design a series of experiments to test this hypothesis.

During weeks 6-16, you will arrive at lab, participate in the recitation, and then work on your own experiments with *T. californicus*. You will first present to the class your progress to date, any problems that you are having, and any conclusions that you are able to make at that point in your experiments. Every student will have the opportunity to present this information individually. Following the group presentations, you will then continue to work on your experiments. Other students may also arrive to work on their projects as well if they have experiments that they need to assess. The graduate TA will act as a research mentor, providing guidance to each group, as well as any other students who may arrive during the time the TA is scheduled to be in the lab. Your graduate TA will also check your lab notebook each week and assign a grade.

COURSE SCHEDULE

Week	Topic	Assignment due
1	Lab 1: The Scientific Method	
2	Lab2: Literature Review and Critique	Quiz; Lab 1 Homework; **Research Question**
3	Lab 3: Using the Microscope	Quiz; Lab 2 Homework; **Preliminary Proposal**
4	Lab 4: Dilutions and the Standard Curve	Quiz; Lab 3 Homework
5	Lab 5: Counting Algae	Quiz; Lab 4 Homework; **Final Proposal**
6	Lab 6: Graphing and Data Analysis	Quiz; Lab 5 Homework
7	Research Projects	Quiz; Lab 6 Homework
8	Research Projects	Quiz; Lab Notebook 1; Weekly Update
9	Research Projects	Quiz; Lab Notebook 2; Weekly Update
10	SPRING BREAK – NO LABS	
11	Research Projects	Quiz; Lab Notebook3; Weekly Update
12	Research Projects	Quiz; Lab Notebook 4; Weekly Update
13	RESEARCH DAY - NO LABS	
14	Research Projects	Quiz; Lab Notebook 5 Weekly Update
15	Finish Research Projects Statistics Workshop	Quiz, Lab Notebook 6
16	Presentations	**Final Presentations** **Final Lab Report**

GRADING BREAKDOWN

Weekly Quizzes	9%	Proposal Final Draft	15%
Lab Exercises	4%	Weekly Update	8%
Homework Questions	5%	Lab Notebook	10%
Research Question	3%	Final Presentation	16%
Preliminary Proposal	5%	Final Lab Report	25%

ROLES FOR STUDENTS

For this semester, students will work in their groups to design and carry out experiments and write up the results. However, each student in the group will assign himself or herself to a particular role with a specific list of tasks for that role (roles modified from Luckie et al., 2004). This allows each student to work in a group setting, but also requires him or her to have individual responsibilities. In this way, the grade for the laboratory portion of the course will include both individual and group grades. The roles for the semester are outlined below, and each group will be allowed to determine which student takes on which role.

1. Principal Investigator
   a. Organize and schedule members of the group
   b. Do background research
   c. Write the introduction section
   d. Present the introduction

2. Protocol Expert
   a. Write the protocols for the group
   b. Modify protocols as methods change
   c. Write the methods section
   d. Present the methods

3. Data Expert
   a. Create graphs and tables as data are collected
   b. Enter data into combined course file
   c. Write the results section
   d. Present the results

4. Analysis Expert
   a. Analyze the data and summarize existing research as it relates to the new data
   b. Develop new hypotheses based on data
   c. Write the Discussion section
   d. Present the Discussion

An Introduction to Copepods and *Tigriopus californicus*

WHAT IS PLANKTON?

The research system that we will be using in the course is a planktonic organism known as a copepod. **Plankton** are actually a wide group of organisms that are defined by their habit rather than by their actual species. The traditional definition of plankton is an aquatic organism that cannot swim against the current. This means that they are typically small organisms, often in the microscopic range, and they cannot make any progress against the currents in their body of water. Plankton occur in both freshwater and saltwater environments, but we will be examining saltwater plankton in this course.

Plankton are also classified into two large groups (phytoplankton and zooplankton, as shown below) based on the way they obtain their food. **Phytoplankton** are those plankton that can photosynthesize and therefore are considered autotrophs. Some of the more common phytoplankton are algae and diatoms. These are the basis of the food chain in the world's oceans and therefore play a critical role in the survival of life on this planet. Remember that photosynthesis is the process of using solar energy to create carbon-containing compounds, such as glucose. There are so many phytoplankton in the oceans that they create 50% of the carbon-containing compounds on the planet!!

Zooplankton, on the other hand, are the members of the plankton community that obtain their energy by eating other plankton. Some of them eat phytoplankton, and some of them eat other zooplankton. There are a wide variety of these organisms including things such as copepods, which spend their whole life as plankton, and others, such as crab larvae, who are only plankton when they are young. Zooplankton serve as a food source for a wide range of larger organisms such as fish, jellyfish, and even blue whales.

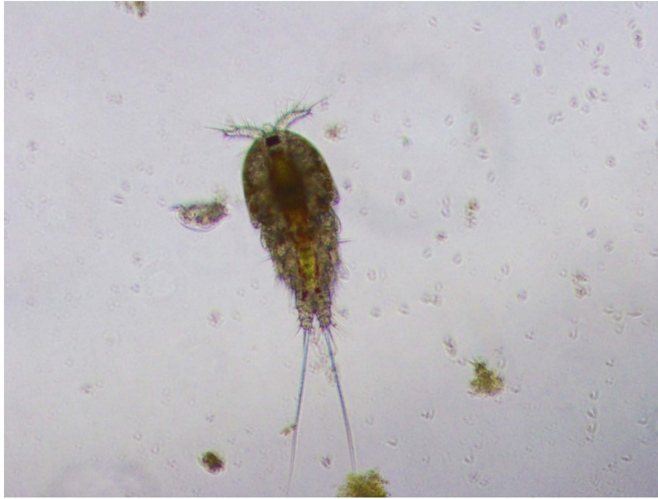
WHAT ARE COPEPODS?

The particular species of plankton that we will be studying this semester is *Tigriopus californicus,* which is a member of the zooplankton and a copepod in the Family Harpactidae. To understand what this means, we must first consider what copepods are. They are small members of the Phylum Arthropoda in the subphylum Crustacea. Hopefully you remember the classification scheme from high school (Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species). You can see the classification information for *T. californicus* below. If we follow this classification scheme from the top to the bottom, we can see that it gives us a clear explanation of just who *T. californicus* is.

Domain: Eukarya
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Maxillopoda
Order: Harpacticoida
Family: Harpacticidae
Genus: *Tigriopus*
Species: *californicus*

Copepods are in the domain Eukarya, which means that they are comprised of eukaryotic cells, just like humans. They are in the Kingdom Animalia, which clearly makes them animals. The Phylum Arthropoda actually translates to “jointed-foot,” and arthropods do, in fact, have legs with joints. The arthropods are the largest group of organisms on the planet, and this group includes insects, crabs, lobsters, spiders, ticks, mites, centipedes, millipedes, beetles, horseshoe crabs and many more. Their uniting characteristic is their **exoskeleton**, which is the external skeleton that makes crab shells so hard to crack. The copepods that you will be examining this semester are arthropods, and do in fact have many joints on their legs as well as an external skeleton. If we continue down the classification scheme, we see that our copepods are in the subphylum Crustacea – these are the arthropods with especially harder outer shells. The Class Maxillopoda are those crustaceans that are typically smaller in size and feed with their modified head appendages called **maxillae**.

We are now to the Order Harpacticoida. All species in this order are copepods, and the majority live on the bottom of the ocean or lake. However, our species, *T. californicus*, lives up in the water column. The harpacticoids are grouped together because they have short first antennae and a major joint halfway down their body, where they are quite flexible. If we continue to look at the taxonomic classification, we will see that currently our species is listed in the Family Harpacticidae, but this is still under debate and at the moment, most members of this family live in freshwater. It is likely, therefore, that *T. californicus* will not remain classified here much longer. Let’s put all of the pieces together: so far, we have a small animal with a hard exoskeleton, jointed legs, eats with its head appendages, and has short first antennae and a major joint in the body. If you look at the images below, this is clearly a good description.



Adult Male *T. californicus*

Adult Female *T. californicus* with egg sac

(Images by G.R. Fisher)

T. CALIFORNICUS HABITAT

As the name implies, members of the species *T. californicus* are commonly found along the coast of California, and are actually found all along the western coast of the United States from the Baja Peninsula to southern Alaska. They are most commonly inhabitants of tide pools along the shoreline. A tide pool is a body of water that remains when the tide has receded. Most tide pools are found on rocky shores, where the rocks form small depressions that hold water (see image below). Tide pools are actually a very stressful environment for aquatic species, which are left stranded in them when the tide goes out. Because tide pools contain a relatively small volume of water, the temperature of the tide pool fluctuates more quickly than the ocean, which can be a stressor for the organisms in the pool. Salinity can also change with evaporation or a sudden rainstorm. Of course, when the tide returns, the temperature and the salinity can change quite quickly again! Many aquatic organisms have evolved the ability to deal with slow changes in environmental conditions, but these quick changes can be more challenging. In addition to the natural stressors in a tide pool, human-caused issues occur as well. Run-off of pesticides and fertilizer from nearby agriculture can change the chemical composition of the tide pools, as can oil spills and any pollutants on the beach. As you might imagine, tide pool organisms must be very resilient and able to deal with a wide range of environmental stressors.

LIFE CYCLE

The life cycle of *T. californicus* is quite complex and involves a number of different stages. If we start at the beginning, we see that females will carry eggs attached to their bodies in an external egg sac. A life cycle stage called the Nauplius will hatch from the egg, looking very different from the adult copepod. The Nauplius will then undergo metamorphosis for a total of 6 different naupliar stages (N-I through N-VI). Each stage lasts about 1-2 days, and the next stage is larger and has an additional segment or two added to the abdomen (it is unclear how many are added at each specific stage). At the end of the last naupliar stage, the N-VI nauplius will metamorphose into the next life cycle stage, the copepodid. This stage looks much more like the adult, but will continue to mature until it reaches the 6th and final stage, which is the adult copepod. Each stage can last from 2-4 days, but this appears to depend on the

environmental conditions, and there is little detail available in the literature on the timing of each stage (Powlik, 1996). At the copepodid matures from one stage to the next, more appendages appear and more body segments are added. By the 5th copepodid stage, it becomes possible to determine the sex of the individual, although Coull (1982) argued that this was distinguishable during the C-IV stage. In particular, males have larger antennules and a larger body size than do females. While much is known about similar species of copepods, we are still lacking a complete description of each stage of the life cycle of *T. californicus* including the specifics of the size and changes that occur during each phase.

MATING BEHAVIOR

Tigriopus californicus exhibits a mating style that is common in the arthropods, but unusual to many humans. Because arthropods have a hard external skeleton, internal fertilization can be an issue. In particular, it can be difficult to deliver sperm to the internal body of the female when she is covered in armor. One solution to this problem is to wait until the female molts. During molting, she will shed the hard exoskeleton, and the new one underneath will be soft for a period of time. For a male, the best strategy then is to find a female that has recently molted. However, such females can be difficult to locate, and it would be a waste of time and energy to constantly swim from one female to another in hopes that she is ready to molt. Instead, males will find a female who is in the later copepodid stages and hold on to her until she is ready to molt. For this reason, when you find copepods in the tide pools, you will find a significant number of coupled pairs with the male grasping the back of the female, as seen in the image below. In many cases, it can be nearly impossible to find a solitary male in a population.

See http://photoity.com/creepy-crawlies-nikon-small-world-competition-2014/
For image of a male grasping an immature female in preparation for mating when she molts to the adult stage.

Once the female molts, she will mate with the male, and this will be her only mate for life. She will store the sperm and produce multiple clutches of eggs over her lifetime, but all the sperm will be from the original male. In contrast, once the male releases the female, he will move on and seek another mate. Burton (1985) found that the males will mate with an average of 2.5 females in 72 hours. There are some interesting questions that arise from this type of mating behavior. How does the male choose a particular female? What are the costs of holding on to the female? Is there competition between males for specific mates? We do know that for some species of copepods, the males will choose the oldest females they can find (this reduces the wait time, and he can then mate more often during his lifespan). In addition, males of a similar species of copepod are able to recognize sisters and will avoid mating with them if given a choice. Preliminary evidence from Dr. Fisher's lab here at UNC has indicated that male *T. californicus* will choose an older female will avoid his sibling and mate with a non-sibling. However, these are just preliminary data with small samples sizes.

FEEDING AND DIET

One of the most basic questions about any animal is what do they eat? Strangely enough, this question still remains for *T. californicus*. This is odd because many aquarists actually raise copepods to feed to their fish. What, then, do they feed them? Many aquarists feed their copepods basic fish food and this seems to be quite successful. While this may work for rearing animals to feed to fish, this is clearly not what they eat in their natural environment. Because many researchers work on various species of copepods similar to *T. californicus*, a quick search of the literature will find that each research lab seems to feed their copepods a slightly different food. Most use algae or bacteria (or both), but there is not a standard amount or species recommended for rearing these animals in the lab. Copepods, especially *T. californicus*, have a reputation as generalist feeders, in that they seem to survive on many different food sources. However, there is a paucity of information on which foods result in the best survival rates, growth rates, egg production, etc. Students who have taken this course last year started researching diet and found some interesting data listed below. However, many questions are still left to be investigated.

FINDING MORE INFORMATION

This lab manual was designed to introduce you to the basics of copepods and their lifestyle. With your own research question(s) in mind, you will now need to conduct your own literature search to see what research is out there. On the blackboard site for the lecture portion of the course, you will find a folder entitled "copepod resources". This folder contains quite a few articles to get you started, but is by no means all the information that is available. You and your group are expected to use this information as a **starting point** to conduct your literature searches.

SO MANY QUESTIONS!!!!

For this semester, your group will need to choose a research question that you will spend the majority of the class trying to answer. It is important that you choose your question carefully and consider whether you will be able to gather data to answer this question within the timeframe you are given and with the available supplies. Below, I have listed a summary of the types of data collected by students in the class in the past year. It is very important that you do NOT repeat their exact studies, but instead you can ask questions based on that results they found. These are just some guidelines to get you started as you develop YOUR UNIQUE question to be answered. Below, I have listed just a few of the potential areas where we need more information about *T. californicus*.

1. Diet: The overall results from previous students indicate survival rates are highest when fed the algae Tetraselmis. This algae has been compared to protein powder, Isochrysis, Chlorella and fish food.
    a. Questions that remain.
        i. What is the effect of combination diets? Other algal types? Supplementing diet with amino acids or fats?
        ii. What does each life stage eat? One species of food? A mix?
2. Salinity

a. There have been a number of studies by former students which indicate that survival is highest around 30-35ppt, and lower salinities are more stressful than higher.
b. Studies on reproduction indicated highest levels of reproduction at 35ppt.
c. Questions that remain.
    i. What are the more nuanced effects of salinity (looking at more than survival)?
    ii. How do smaller ranges of salinity affect growth and reproduction?
    iii. How does salinity impact other life stages?
    iv. Does salinity affect growth rate? Mating behavior? Egg production? Hatching rate? Timing of life cycle stages? Sex ratio of offspring?

3. Temperature
    a. More than 30 studies have been done by former students examining the effect of temperature. Most studies were done on adults and looked at survival as their dependent variable. It is consistently clear that 35ºC is fatal to almost all copepods. Many studies also indicate that 30ºC is fatal to a high percentage of copepods. Data are a bit conflicting as temperature declines. The majority of studies indicate that 20-25ºC is optimal range, but some show high survival as low as 10ºC.
    b. Studies on temperature and reproduction show highest reproduction at 25ºC. Reproductive measures included number of eggs and/or number of females mated.
    c. One study indicated higher growth at 20-25ºC.
    d. Questions that remain.
        i. What are the other effects of temperature (looking at more than survival)?
        ii. How do smaller ranges of temperature affect growth and reproduction?
        iii. What are the effects of combined stressors such as increased salinity at slightly higher temps?
        iv. Few studies have looked at life stages other than adults.

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Lab 1: The Scientific Method

Objectives:

At the end of this experiment, students will be able to:

1. Identify components of the experimental design process
2. Critique experiments to determine their validity
3. Design high-quality scientific experiments that include all the necessary components
4. Draw conclusions from data and present alternate hypotheses to explain those data

Introduction

For the laboratory portion of this course, you will be designing and carrying out a series of experiments to discover new information about our model organism, *Tigriopus californicus*. Because you will be spending most of the semester involved in the specifics of experimental design and data analysis, it is crucial that these experiments are well designed and carried out successfully. If the experiment is flawed, the results, too, will be flawed. In today's lab, we will review the basics of the scientific method, specifically elements related to the experimental design process. The first step in the scientific method is to make observations. Interestingly, many people believe that the first step is to make a hypothesis, but no hypothesis can be made without first observing something. In some cases, this can be a physical observation, such as noticing that one type of plant only grows in sunny areas, or that the cells growing in the lab don't grow as well at colder temperatures. Another type of observation that is crucial in how science is actually done is to make an observation based on data published in the scientific literature. Most scientists start by reading journal articles on their topic of interest and collating the information available. From this, they then see where there are areas where more information is needed or questions need to be asked. From this, a scientist can then start the next step in the scientific method, make a hypothesis.

When developing your hypothesis, it is important to consider if it is a valid hypothesis. To be valid, it must be both testable and falsifiable. A testable hypothesis is one that can be tested and measured using the equipment we have today. For example, a hypothesis that states that there are living beings in another solar system is not yet testable. The other important component of a hypothesis is that it must be falsifiable, meaning that one could gather data to disprove the hypothesis. Some things cannot be determined by an experiment, such as any statement that is a judgment or opinion. For example, the hypothesis that pit bulls are evil dogs is not testable or falsifiable. There is no way for us to define evil and, therefore, the hypothesis is not valid.

Once you have formed your hypothesis, the next step in the scientific method is to design and conduct the experiment. The idea behind designing an experiment is to test your hypothesis in a manner that is objective and that will either support or falsify your hypothesis. In order to understand experimental design, let's test the following hypothesis: if roses require sunlight to grow, then they will

grow more slowly if placed in the shade. So, I want to put some roses in the shade and measure how fast they grow. However, there is more to experimental design than just that. First of all, we need a **control group**. This is a group of plants that receive the same conditions as the **experimental group,** except for the experimental treatment. To test my hypothesis, I have taken 40 rose plants, each in separate pots. I have placed 20 in the sun in the greenhouse and 20 in the shade in the greenhouse. Each pot gets the same amount of water and fertilizer, and all are kept at the same temperature. Those in the sun are the control group and those in the shade are the experimental group. If my initial hypothesis were correct, we would expect those roses in the shade to grow slowly while those in the sun to grow more quickly. If this happens, we can conclude that the sun is likely to be the important factor in plant survival since the only difference between the two groups was the amount of sun.

As we consider experimental design, there are a number of terms that need to be clearly defined. The first is the **independent variable**. This is the variable that is being tested or manipulated in the experiment. In our simple experiment, we are testing the effect of the sun on plant growth and we are manipulating the amount of sun available to the plants, so the amount of sunlight is the independent variable. It is critical to have only one independent variable being tested in each experiment. That way, if changes are observed, you can be sure that the one independent variable is the reason for the change. For example, suppose I had some plants that had more water and more sunlight and other plants that had less water and less sunlight. The plants with less water and less sunlight did not grow as well. Was this because they lacked water or light? I cannot answer this question because I tested two independent variables at the same time.

Another variable to consider is the **dependent variable**. This is what is being measured in the experiment. For our study, we are measuring the growth of the plants in response to sunlight, so growth is the dependent variable. One quick way to remember these is to put them into the following sentence – I am testing the effect of the independent variable on the dependent variable. So, in our experiment "I am testing the effect of the amount of sunlight on plant growth". If you reverse them, you can immediately see the problem. "I am testing the effect of plant growth on the amount of sunlight" makes no sense and therefore cannot be correct. In many experiments, researchers will measure more than one dependent variable. For example, I could measure the height of the plants, the number of leaves, the number of flowers and the length of the roots. All of these could be affected by light and would be considered dependent variables. Basically, the dependent variables are the data that you collect in your experiment as your results.

Another important term in experimental design is the **standardized variable.** There are many of these, and these are all the conditions of the experiment that must remain the same for both the control and experimental groups. In our plant growth experiment, all plants must receive the same amount of water, plant food, soil, soil type, humidity and carbon dioxide so that the only thing that differs between the control and experimental groups is the amount of light. All of these other variables are called the standardized variables.

Before we move away from this simple experiment, there is one more thing to consider. Why did I use 20 plants in both the experimental and control groups? Why wasn't one enough? This is

something we call **sample size**, which is simply the number of individuals in each test group. If we had only one plant in the sun and one in the shade, we could have inadvertently chosen plants with very different genetics that would skew our results. By choosing more than one, we reduce the chance that random factors will affect the data. Instead, we will get what is referred to as a representative sample, meaning that the individuals in the experiment represent all of the roses in the world. Determining the sample size for an experiment typically depends on how difficult it is to make the measurements and get the test subjects. For roses, larger sample sizes are not difficult, but if you were working with elephants, the sample sizes would necessarily be much lower.

Now that we have designed and carried out our experiment, the next step in the scientific method is to analyze the data. Using the rose example, we will have growth rates for plants in the sun and plants in the shade. We can calculate an average growth rate for each group and determine if there is a difference. This determination is typically made by using statistical techniques. We will look at this process in more detail in a few weeks in the lab. Once we have our data, we can use this to draw our preliminary conclusions. These are the initial statements to determine if the data support or refute our hypothesis. Finally, we are at the last step in the scientific method, making a new hypothesis based on our results. In some cases, this new hypothesis is an extension of our original one. From our example, we may want to know what level of sun is best for growth. Another type of new hypothesis is what is called an alternate hypothesis. This can be a hypothesis that is based on your data, but provides a different explanation for the results. You will find that in many cases, there is more than one explanation for the data you collect. Additionally, if your hypothesis was not supported, you may want to develop new hypotheses to attempt to explain the phenomenon of interest. What you will discover this semester is that as soon as you collect some data, you will immediately discover new questions that you want to answer. **In this way, science is a continuing process with no definite end.**

Additional Considerations:

Before we move on with the procedures for today, it is important to consider a few more aspects of how science is done. The first consideration is the idea of a **model organism**. If you read the primary literature in biology, you will notice that there are a significant number of studies done on the same organisms (rats, a worm known as *C. elegans,* a plant called Arabidopsis, yeast and *E. coli*, to name a few). These are examples of model organisms, and they have a number of features that make them amenable to research in the laboratory. They often are small in size, have a short life span, a high reproductive rate, an ability to grow well under laboratory conditions, and similar features to other organisms in the wild. Rats are often used as an approximation for mammals, Arabidopsis are used in genetics and other experiments as a model for plants, and *E. coli* is used as a proxy for other species of bacteria. What is learned from these model organisms can then be extrapolated and used as a basis for understanding related species. For the laboratory portion of this course, we will also be using a model organism, the copepod species *Tigriopus californicus*. You will learn a great deal about this organism later in the lab manual and through your research, but we will be using it as a proxy for other planktonic species as well as other marine organisms that live in the intertidal area. The information that you

gather can then be applied to a wide range of species in the oceans and will add to our base of knowledge.

Procedures

EXERCISE 1: Making Observations and Developing Hypotheses

One critical step in the scientific method is to gather information from the literature and use is to develop new and testable hypothesis. This reading of the scientific literature is one means of making observations. Rather than making the observations directly in the lab or field, you are observing what others have done and then building on it. In this exercise, you will need to read the following and create three valid hypotheses based on the information presented. I have started with an example for you to help you understand the process.

1. Almost two thirds of traded goods worldwide are transported by ship (Kumar and Hoffmann, 2002). To ensure ship buoyancy, stability and maneuverability, oceangoing ships need ballast water. Based on an estimation that the world seaborne trade in 2013 amounted to 9.35 billion tons of cargo, the global ballast water discharges in 2013 are estimated to about 3.1 billion tonnes (David, in prep.). There is significant transfer of ballast water between different continents and oceans, and it has been known for decades that ballast water also transports organisms into new ecosystems, where, under favorable conditions, they can become invasive (Carlton, 1985 and Williams et al., 1988). The introduction of invasive aquatic species into new environments has been identified as one of the four greatest threats to the world's oceans. When including terrestrial species, invasive species were identified as key factor in 54% of all known species extinctions as documented in the Red List database maintained by the International Union for Conservation of Nature (Clavero and Garcia-Berthou, 2005). Aquatic invasions are virtually irreversible and, once the newcomers are established, their impacts may also increase in severity over time. The transfer of invasive species does not occur only over larger distances, between continents, but also as a secondary spread in regional seas (David et al., 2013). (This information copied directly from Werschkun et al., 2014)

   Hypothesis 1:

   Ballast water of ships arriving to North America from the Mediterranean will have live species of plankton native to the Mediterranean.

   Hypothesis 2:

   Planktonic organisms can survive transoceanic trips in ballast water

   Hypothesis 3:

   One aquatic invasive species from seas around Europe has been found in the ballast water of ships entering the Great Lakes

2. The mating behavior of male organisms is strongly influenced by the reproductive biology of conspecific females. Of particular importance to the male are the timing of the female's receptivity to mating, her capacity to store sperm (keep sperm viable in her body for prolonged periods of time), and the probability of successful sperm displacement (removing sperm from the female's body) by future matings of the female with other males. Among crustaceans, male mating behavior frequently involves a period of time during which a male clasps a female without actively attempting copulation (the pre-copulatory "passive" phase of Parker, 1970); during this period, which may last a week or more, a male cannot inseminate a female other than the one he is holding. (modified from Burton, 1985).

   Hypothesis 1:

   Hypothesis 2:

   Hypothesis 3:

3. *Tigriopus californicus* is a harpacticoid copepod found in small upper tidepools along rocky areas of the west coast of North America (Ricketts et al, 1968). Occupying this habitat, the copepod is exposed to relatively large and sometimes sudden changes in seawater concentration due to evaporation or dilution of the media (Kontogiannis, 1973). In response to this stress, *Tigriopus* has evolved adaptive mechanisms allowing it to survive over a wide range of salinities. Survival and reproduction have been observed at salinities between 21.2 and 75.3 parts per thousand under laboratory conditions (Huizinga, 1971). (McDonough and Stiffler, 1981).

   Hypothesis 1:

   Hypothesis 2:

   Hypothesis 3:

EXERCISE 2: Determining the Validity of Hypotheses

Read over the following hypotheses to determine if they are testable. If not, explain why not.

1. Glucosamine supplements reduce joint pain in elderly human patients.

2. Retinal cream A reduces the depth of wrinkles around the eyes.

3. Dogs with long hair prefer cooler climates.

4. The higher the intelligence of the horse, the easier it is to train it to jump over obstacles.

5. High-fat, high-sodium diets for humans increase the risk of atherosclerosis (hardening of the arteries).

6. The *Tyrannosaurus rex* could not digest the citric acid in lemons.

EXERCISE 3: Understanding Experimental Design

For each experiment, answer the questions about the experimental design.

1. A researcher is studying the effect of the pesticide Cypermethrin on the survival of juvenile salmon in the laboratory. She has 100 salmon in a solution of 10mg/L of cypermethrin for 96 hours and measures their survival, which was 87%. She then places another 100 salmon in 100mg/L of cypermethrin for 96 hours and measures their survival, which was 52%. She concludes that Cypermethrin levels should be regulated to be less than 10mg/L in waterways where salmon are found.

    Independent variable:

    Dependent variable(s):

What other variables should he standardize?

Control group:

Experimental group(s):

What are the strengths of his experimental design?

What are the weaknesses of his experimental design?

2. A marine biologist wants to examine the effect of salinity on the plankton *Tigriopus californicus*. He takes 100 individuals and places them into a beaker where the salinity is 35 parts per thousand (normal seawater). He then takes another 100 individuals and places them into a beaker where the salinity is 45 parts per thousand. Finally, he takes another 100 individuals and places them into a beaker where the salinity is 25 parts per thousand. After 48 hours, he counts the overall mortality (i.e., death) in each beaker.

   Independent variable:

   Dependent variable(s):

   What variables should he standardize?

   Control group:

Experimental group(s):

What are the strengths of his experimental design?

What are the weaknesses of his experimental design?

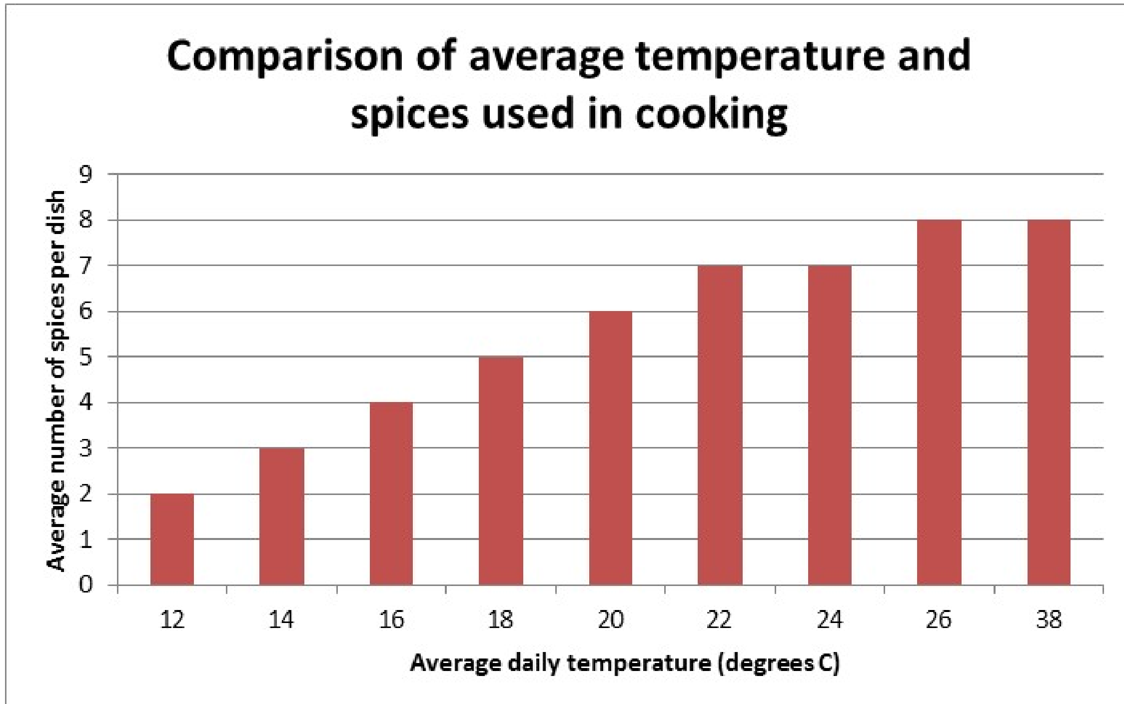
EXERCISE 4: Collecting Data

Create a table to collect the data, and draw a graph of how the data would look if the hypothesis was supported.

1. A researcher hypothesizes that survival rates will decrease when *T. californicus* is exposed to increased salinity. She sets up 3 96-well plates, each of which has 96 copepods in individual wells. These are filled with water at 35 parts per thousand. She then sets up 3 more plates the same way but with water at 45 parts per thousand. At the end of 96 hours, she measures the percent survival in each plate. Create a table for her to collect her data as well as a table for her to summarize her data. Then create a hypothetical graph with summary data that would support her hypothesis.

EXERCISE 5: Interpreting the Data

1. The data shown below were collected to test the following hypothesis: foods in warmer countries have more spices to prevent bacterial growth (Billing and Sherman 1998). (Many spices have mild antibiotic properties). Examine the data, and answer the questions below

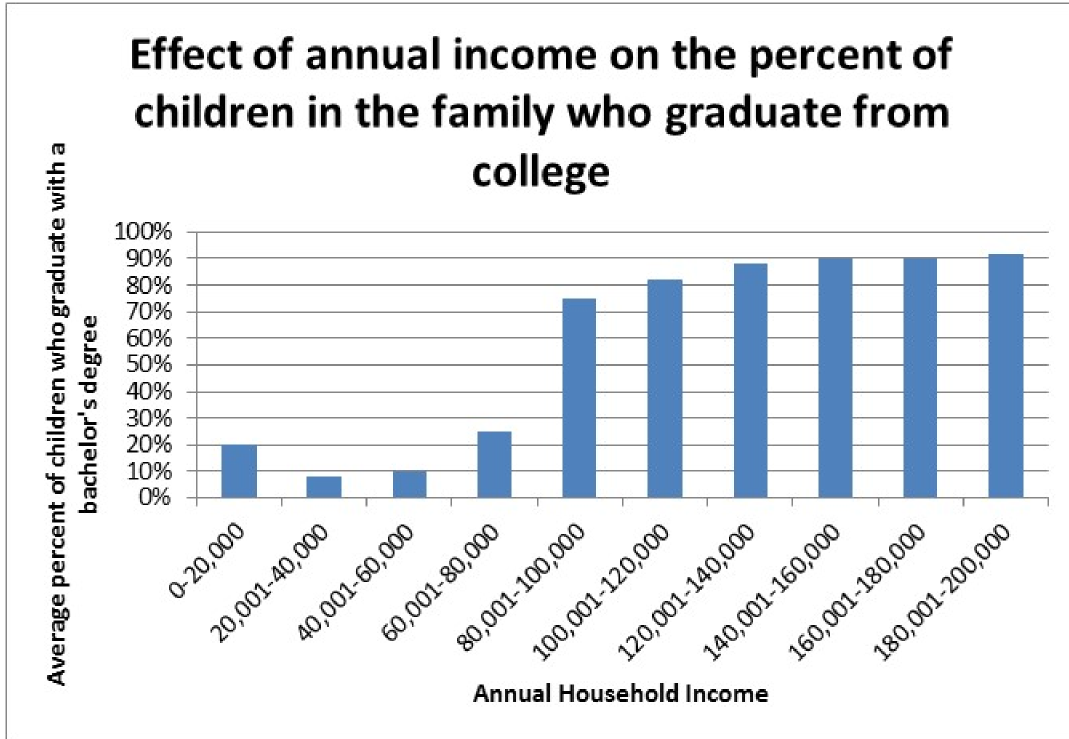


Do the data support the hypothesis? Why or why not?

List two other hypotheses that you could develop based on the data shown.

What other conclusions can you draw from these data?

2. The data shown below were collected to test the following hypothesis: families with higher annual income will have more children who complete an undergraduate education. Examine the data, and answer the questions below.



Do the data support the hypothesis? Why or why not?

List two other hypotheses that you could develop based on the data shown.

What other conclusions can you draw from these data?

Exercise 6: Conduct an Experiment

<u>Conduct an experiment to test the following hypothesis: If the length of the femur is correlated to height, then we can use femur length to predict overall height in college students.</u>

This type of inference is used widely in paleontology, when it is rare to have an entire intact skeleton. Instead, individual bones may be recovered and used to infer information about the individual. In this experiment, you will determine if femur length is a good proxy for overall height of an individual.

Protocol

1. In your groups, assign each member to one of the roles for the semester (these roles will only be for today). For more information on the roles, refer to the section entitled "Format of the BIO 110 Lab". All students in the group should be involved in each role.
2. The PI will read over the provided background information and summarize it.
3. The Protocol Expert will write up the methods/protocol that you will follow. Be sure to pay attention to detail, and be specific.
4. The Data Expert will then design the tables for data collection and record the results.
5. The entire group will carry out the experiment that you have designed.
6. The Analyst will summarize the data and relate it to the original hypothesis.

Protocol:

Tables for Data Collection:

Summary and Analysis of the Data:

Questions for Exercise 6

1. Did your results support your hypothesis? Why, or why not?

2. Some paleontologists use the following equation to determine height: Femur length (in cm) x 2.6 + 65 = height (cm). Complete this equation for three of the individuals in your group, and write the results below.

3. Based on your data from question #2, does this equation work? If not, why do you think it was not successful for you?

4. In your experiment, what was the independent variable?

5. In your experiment, what was the dependent variable?

6. What were the factors of your experimental design that were problematic or needed refinement?

Homework

1. Explain the advantages and disadvantages of using a model organism (include at least 3 of each).

2. A researcher was testing the hypothesis that students in graduate school have higher GPAs than students working on undergraduate degrees. She looked at the transcripts of 10,000 undergraduate students and 10,000 graduate students at universities all across the country. She included students from a wide range of university types (state schools, liberal arts schools, ivy league colleges, etc.) and was careful to balance the genders of the students so that she had nearly equal numbers of males and females. She found that the average GPA of an undergraduate student was 2.9 and the average GPA of a graduate student was 3.6. She concluded that her data support her hypothesis, and she argues that the reason for this difference is grade inflation (teachers giving higher grades) in grad school. Explain the strengths and weaknesses of her experiment and her conclusions (give at least 2 of each).

3. From question 2, develop an alternate reason that we see this discrepancy between undergraduate and graduate GPAs.

4. You are designing an experiment to test the following hypothesis: Temperature affects the ability of students to think clearly, and, therefore, students in a hotter room will have lower exam scores than students in a cooler room. For your experiment, list the following
   Sample size

   Independent variable

   Dependent variable

   Control group

   Experimental group

   Standardized variables

   How will you ensure that you have a random sample?

5. Now describe the methodology you will use to test the hypothesis from question #4

Lab 2: Literature Review and Critique

<u>Before coming to lab this week</u>, read over the article entitled "Copper tolerance of the life history stages of the splashpool copepod *Tigriopus californicus* (Copepoda, Harpacticoida)" that is included here as well as posted in blackboard for you. Make sure to print it out and bring it to your lab session this week. The quiz this week will be partially based on the information in the paper listed above.

Objectives

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

1. Identify the differences between popular literature and primary literature in biology
2. Summarize the information presented in an article from the primary literature
3. Critique the experimental design in a research article from the primary literature
4. Develop new hypotheses based on information present in a research article from the primary literature

Introduction

When most people think of biology research, they think of scientists conducting research experiments in the laboratory or in a field setting. While this is the way in which science is conducted, this view of biology is missing a vital component. One of the most critical aspects of biology is writing and publishing the results of one's experiments so that others can learn from them and build on the new knowledge that has been created. For scientists, there are a number of different literary venues in which to present your data. We can quickly divide these literary venues into two different groups: the popular literature (intended for the non-science audience) and the primary literature (where original science research is presented for the first time and is intended for a scientific audience). The popular literature includes books, such as those written by Stephen Hawking and Stephen J. Gould. Also included in the popular literature would be some of the more general magazines such as *Scientific American* and *Discover Magazine*. These are great resources for the general public, and they present science in a more approachable context for many individuals. However, the literature that is the foundation for science and biology is the primary literature.

The best and most common examples of primary literature are the peer-reviewed journals that contain articles with original research. The key idea behind the primary literature is the peer-reviewed concept. This means that every article in that journal is first reviewed by other scientists in the field before it can be published. For many journals, the acceptance rate of an article on its first attempt is around 50%. This means that one half of the articles are rejected by these reviewers and sent back to the author for revisions. Unlike traditional editing, this type of review means that the author may have

to go back and conduct more experiments, reanalyze the data, or do more research on the background of the article to make it acceptable. The idea behind peer review is that each article has been carefully vetted to ensure the quality of the experimental design, data analysis, and strength of the conclusions. Peer review is one of the cornerstones of scientific literature.

While many students struggle initially when reading the primary literature, you will find that it becomes easier and easier the more you read and the more science you learn in your classes. It is essential to develop the ability to read and critique the primary literature in order to be successful in science. The information presented in research articles is used in a variety of different ways by scientists. Many start with reading the literature to become familiar with a new concept, a new model organism, or a new idea in their field. As you will see this semester, scientists also read over the literature in order to determine what types of questions still need to be answered and then use this information to develop new hypotheses. The research that is published also presents details on new methods and techniques to answer research questions and is a valuable resource for new methodology. Throughout the course of the semester, you will be reading the primary literature to help you develop your background knowledge, form a new hypothesis, and formulate the methods you will use to answer your new questions. To get you started on this process, this laboratory exercise will focus on just one article from the primary literature for you to summarize, analyze, and critique.

Before you can begin, it is important to understand the individual parts of a standard research article and what should be included in each component. In order, research articles typically have an Abstract, Introduction, Methods, Results, Discussion and Literature Cited section. The Abstract is a summary of the entire article and provides a broad sweep of the methods, results, and discussion. This is a good place to start when reading an article because it gives you the overview before you dive into the details. The Introduction is the next section, and this is designed to provide the background information on the topic and set up the reason for the experiment that was conducted. This is also where you find the hypothesis for the experiment. Following the introduction is the Methods, which is a detailed description of what was done. The idea behind the methods is that it should provide you with enough detail to replicate the experiment on your own if you so choose. This will be an area for you to read carefully, as it provides the information about the experimental design for you to determine if it was a well-designed experiment. It also contains techniques and methods that you may be interested in using in your own work. The Results section follows the methods and contains the results obtained in the experiment. You will notice that the results rarely, if ever, contain raw data – which are the individual measurements. Instead, the results are summarized and analyzed using statistical approaches before publication. There are some exceptions to this (such as DNA sequences, or images), but in the work that we will do in this lab, it is expected that your results sections will not contain raw data. The final prose section of the article is the Discussion. This is where the data are placed into context to determine if they support the hypothesis, explain why the data were obtained, and relate this to other experiments on similar topics. Please note that the Discussion will not be a restatement of the results, but rather an interpretation of them. The final portion of the journal article will be the Literature Cited, which contains a detailed list of every source that the author used in writing the article. This section is often ignored by students, but in fact it includes some very critical information. This is where you can find other research articles on the topic you are reading about, find other authors who are doing similar

work, and find the references for some of the methods that the author used. Be sure to look at this section closely when reading a journal article. Also, when you are writing your report, this section will be where you place all of your source information that you cite in your report.

Procedure

1. Each lab group will count off into fours. Then all the ones will meet together, as will the twos, threes, etc.
2. For those of you who are “1’s”.
    a. Create a concept map of the topics in the introduction
3. For those of you who are “2’s”
    a. Create cartoons to explain the methods
    b. May need multiple drawings
    c. Be sure to include the specific details on the drawings
    d. Cannot be a list
4. For those of you who are “3’s”
    a. Analyze and redraw the figures in your own way
5. For those of you who are “4’s”
    a. Summarize the author’s conclusions in outline style format
6. Once each group has completed their assignment, you will return to your original lab group. Each individual will then explain what they found to the rest of the group.
7. Following the discussion, you will then critique the article

Introduction

a. What were the best aspects of this section?

b. What were the worst aspects of this section? (saying that it was too complicated is not a good answer)

Methods

a. What were the best aspects of this section?

b. What were the worst aspects of this section?

Results

a. What were the best aspects of this section?

b. What were the worst aspects of this section?

Discussion

a. What were the best aspects of this section?

b. What were the worst aspects of this section?

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Homework

Read the article "Effects of photoperiod on egg production and hatching success, naupliar and copepodite development, adult sex ratio and life expectancy of the tropical calanoid copepod *Acartia sinjiensis*" by Thomas Camus and Chaoshu Zeng, and use it to answer the following questions.

1. List four reasons why the authors feel that copepods would be a good food source for fish in hatcheries.

2. Describe how the copepods were fed and how their food source was maintained for the experiment. Be specific.

3. What is the significance of testing the effects of photoperiod? (i.e. why bother doing this particular experiment)

4. Explain how egg hatching success was determined. What are the strengths and weaknesses of this particular technique?

5. In your own words, summarize the results shown in figure 1. What are the strengths and weaknesses of this particular figure?

6. Explain the different effects of photoperiod on egg hatching success, egg production rate and life expectancy.

7. Based on what you read in this article, what photoperiod would you recommend to raise the largest number of copepods? Why did you choose this particular photoperiod?

8. Describe the three biggest strengths of the paper.

9. Describe the three biggest weaknesses of the paper. How would *you* address these weaknesses if you were on the research team with Camus and Zeng?

10. List three things that you could take from this paper to use in your own research for this semester.

Lab 3: Using the Microscope

by Thomas McCabe

Objectives:

At the end of this experiment, students will be able to:

1. Resolve images using both the compound and dissecting microscopes
2. Troubleshoot resolution issues with either scope
3. Learn and practice basic maintenance and use of compound and dissecting microscopes

The Dissecting Microscope (aka the Stereoscope)

Procedure

Exercise 1: Manipulating Copepods

Your first task will be to practice manipulating your copepods: transferring them between containers, picking out individuals, and in general gently handling them to prevent injury! Just in the same way that an injured athlete cannot perform as well as a healthy athlete, your copepods will not act 'normal' through your experiment if they are injured. Even small variations because of injury can create *experimental error*, which can completely invalidate our results!

1. Obtain three disposable pipettes
2. Leave one pipette aside; use scissors to cut a section off of the tip of the remaining two pipettes at about 0.5 inches and 1 inch
3. Obtain a beaker with a small amount (30ml) of culture media to work with
4. Your TA will provide you with a number of different container sizes that you may work with over the semester; your TA will also provide you with a container of copepods
5. Pour your copepods on to the petri dish provided—make sure <u>*all*</u> of them get out of the container! Use a clean disposable pipette and fresh media to wash the walls of the container down into the dish. You will leave your copepods in this dish for the remainder of the period.
   **Make sure to keep the dish off of the microscope when not in use! The light will cause the temperature to rise and some water to evaporate (increasing the salinity)—your goal is to keep the copepods as happy as possible while working with them. Work fast and clean!!
6. One partner will go first and the second one will follow. Give each other *constructive feedback* while they are practicing. Does it look like they are comfortable? Could they hold the pipette or the container in a better way?
7. Practice sorting out a group of 10 copepods into INDIVIDUAL containers. Make sure to have each of these containers labelled 1-10 (with a sharpie or tape). Note: We do not have a set protocol for you to do this, your job is to figure out what works best for you.
   -Try using the different sizes of pipettes to pick up a single or multiple copepods at a time.
   -Watch how much media you draw up with the copepod; will all of it fit in the next container?
   -Consider whether you should add media into the container *first*? Or put the copepod in a small amount of media in the container and add extra media after?
   -You may or may not need the microscope to see them at this point. If you do, try a number of different magnifications to figure out what settings help you see them best.
8. Don't forget, when you are done, your partner gets to take a turn handling the copepods!

Exercise 2: Identifying Life Stages of Copepods

In a previous lab, you were shown a general scheme for copepod development. We will use that image (and any images that you have found in your literature search) to pick out the various life stages of *Tigriopus californicus*.

See http://www.imas.utas.edu.au/zooplankton/image-key/copepoda for a general life cycle diagram for Calanoid copepods, a similar order of copepods, but not exactly the same as our species.

1. Use the microscope to zoom onto one of the copepods you put in individual containers.
2. Use the diagram above and the descriptions of the life stages on page **11** to identify whether you have a nauplius, copepodid, or adult. KEEP YOUR ANSWERS HIDDEN FROM YOUR PARTNER. Write your guesses on the space next to the diagram above.
3. You may need to use some of the fine tools provided to ***gently*** move the copepods into a position that is easier to visualize
4. Today we will not ask you to identify the particular molt of each of these, but you should be able to start distinguishing between the major life stages
5. Your partner should have a go after your turn; when they are done, compare your guesses. If you do not agree on some, revisit those containers and discuss why you chose one stage over another.
6. Make sure to separate out one individual from each stage—if you cannot find one of them, return to your petri dish to separate out the missing stage

Exercise 3: Sexing Copepods

The last major skill you will practice on the compound scope today is determining the sex of ADULT copepods. This will be important for keeping males and females segregated (when possible) to prevent mating or to explore questions about either sex individually.

1. Separate the containers that you determined were adults from Exercise 2
2. Use the microscope and tools to determine whether each is a male or female copepod—you can do the same game as the last exercise where you make a guess and your partner makes one after and you compare notes.
3. You will need to separate out 3 of each sex; don't forget you may need to return to your petri dish for more adults to work with.
4. Below are some representations of a general male (LEFT) and female (RIGHT) body type. Use these to help you sex your copepods.

5. Write down some rules for telling them apart: do they have the same body parts/plan? Are they the same size? Is there any one distinguishing characteristic for them?

Sex Identification Criteria:

6. When you have finished sorting your copepods and writing down your identification criteria, ask your TA to check your work

Once you are done with these activities:

1. Move all separated copepods back to the main petri dish—the other group will use them next
2. Clean up any spills or messes at the station you were working at
3. Switch places with the other group when they are ready

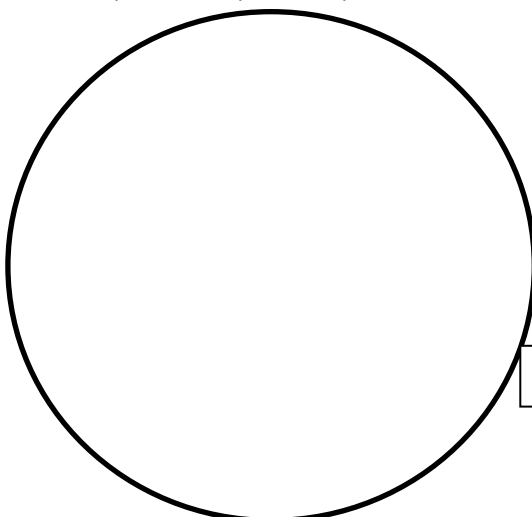
The Compound Microscope

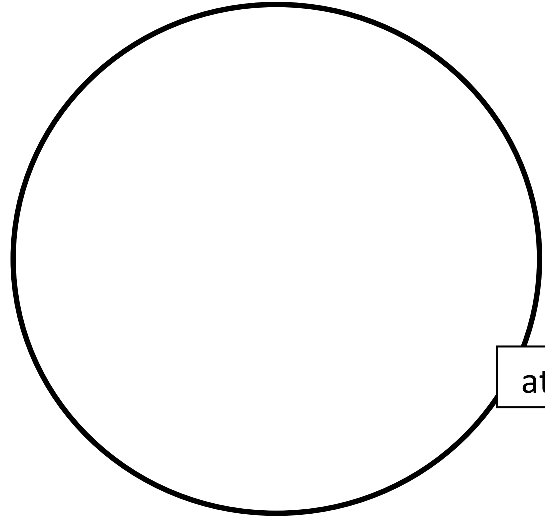
Procedure

Exercise 1: Examining Nauplii

For some of you, identifying and describing the various life cycle stages of *T. californicus* will be a major focus of your semester project. For others, this may not be as important, but you will need to be able to check how quickly your copepods are developing by looking at their current naupliar stage.

1. Draw a picture of your nauplius in view at 100X magnification (that is, right now using the 10X objective). D





2. Using the nosepiece, move to the 40X objective
3. Do not forget to adjust your light at this point: first adjust the iris diaphragm in the condenser by moving the lever on the condenser back and forth till you get a crisp image; THEN, adjust the brightness of the light as needed
4. Draw what you see at 40X. Again, draw your picture relative to the field of view

   Compare and contrast what you see at either magnification—what changes between the two powers?

Final Note: while we are here, we want to point out one more special thing about the compound microscope. Use the knobs hanging off of the stage to move the stage left and right. Now do this while looking in the ocular lenses. What you will notice is that the image travels opposite to the direction the slide does—slide moves to the left, image moves right. The same opposite movement will happen with up and down movement of the stage. Because of how we are looking at the specimen and the way the scope is built we get an image that is upside down *and* backward from the actual specimen. You will notice that the dissection scope is not the same so be careful not to get disoriented when switching between the two!

Exercise 2: Examining Algae
VERY SOON you will have to prepare food for your copepods and will need to be able to visualize individual cells of algae to be able to count them. Today we are providing you with a sample of a variety of different algae to visualize.

1. Obtain a glass slide and coverslip.
2. Use the windex and kimwipes provided to clean your slide and coverslip. Make sure to avoid making marks on the glass while handling them. Make sure they are completely dry before placing a specimen down.
3. Place slide on a paper towel on your table.
4. Place a SMALL drop of algae onto the middle of the slide.
5. Look at the diagram below. While holding the coverslip on its sides, place one edge of the coverslip onto the slide next to the algae drop.
6. Let the rest of the coverslip fall onto the slide, covering the algae drop. You may have air bubbles, but DO NOT press on the coverslip to try and push them out—you will squish your specimen!!
   -Generally, if you are making bubbles under your coverslip you may be letting it fall from too high. To fix this, tilt the coverslip closer to the slide before you drop it. Also, if your specimen solution has bubbles in it to begin with you will probably just trap them, make sure there are none on the specimen to begin with. Use a probe to move or pop the bubbles before continuing.
   -If you need more practice, use a clean coverslip and slide with drops of water before moving to the algae.
7. Follow the steps we have previously discussed to bring the algae into focus at 400X magnification. It is especially important that you use the FINE FOCUS when at the highest magnification to avoid running the objective into the slide.
8. Note changes between views as you move between the 10X and 40X objectives:
   What changes about the image as you move between these two magnifications (for example, number of algae cells in view)? Describe your observations using the terms we discussed in the introduction.

What do we sacrifice by moving to a higher magnification? In other words, what are some pros and cons of using higher magnification?

Once you are done with these activities:

1. Move all separated copepods back to the main petri dish—the other group will use them next
2. Clean up any spills or messes at the station you were working at
3. Clean the slides and coverslips you used; water and soap from the dispenser is enough. BE CAREFUL NOT SMASH THE COVERSLIPS WITH YOUR FINGERS!
4. Switch places with the other group when they are ready

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Homework

1) On a separate sheet of paper, rewrite the functions of all of the parts of the microscope in your own words. You will be expected to name all parts of both microscopes and describe their functions.

2) Explain why the microscope has both a fine and course focus knob. Why have both if they both do the same thing?

3) Of course, we focus on the way that you carry a microscope because they are expensive pieces of equipment that we want to keep safe. However, from the exercises we did in the lab, why else should we care about the way that we transport the microscopes? Hint: Think about the compound microscope.

4) Below is an image taken with a microscope. Describe at least two issues with the quality of the image AND describe what you would do to fix these issues.

5) Below is a picture taken by a copepod researcher. Using the terms you learned in this lab, explain whether you think this will be a useful picture for this researcher to publish in a paper. Also explain your thinking: *why* do you think this is a useful or useless image? Consider the experimental questions that this research could be answering using this picture.

Lab 4: Dilutions and the Standard Curve

Objectives:

At the end of this experiment, students will be able to:

1. Carry out a serial dilution
2. Use a spectrophotometer and dilution techniques to create a standard curve
3. Correctly measure fluids using a pipette
4. Explain why standard curves are important in biological research

Introduction

When using solutions in biological research, it is often critical to be able to make the correct concentration from a stock solution. For example, this semester, you may need to make dilutions of algal solutions in order to feed the plankton. Alternatively, you may need to dilute specific chemical solutions if you are examining the effects of particular chemicals (such as pesticides) on the plankton. To do these dilutions, you first need to know a few terms. A **stock solution** is the concentrated solution which is being diluted. From this, you need to make a **working solution**, which is the diluted solution that you want to use. When doing dilutions, you will use a **diluent**, which is the fluid you use for diluting. In this lab, the diluent will be water. The most common way to create solutions of the desired concentration, is to use the following equation

$$(C1)(V1) = (C2)(V2)$$

C1 is the stock concentration
C2 is the desired concentration
V1 is the unknown volume
V2 is the desired volume

In order to do this, you will need to solve for V1

$$(V1) = \frac{(C2)(V2)}{(C1)}$$

Let's look at an example:

To prepare 50ml of 50% hydrochloric acid from an 85% stock solution

$$(V1) = \frac{(50\%)(50ml)}{(85\%)}$$

$$(V1) = 29.4$$

So, you take 29.4ml of the 85% solution and bring the total volume up to 50 ml.

Now is a good time to check yourself. How will you prepare 100ml of a 20% solution of sodium chloride from a 75% stock solution?

C1 =

C2 =

V2 =

Use these values to solve for V1

$$(V1) = \frac{(C2)(V2)}{(C1)}$$

What is your final solution to the problem?

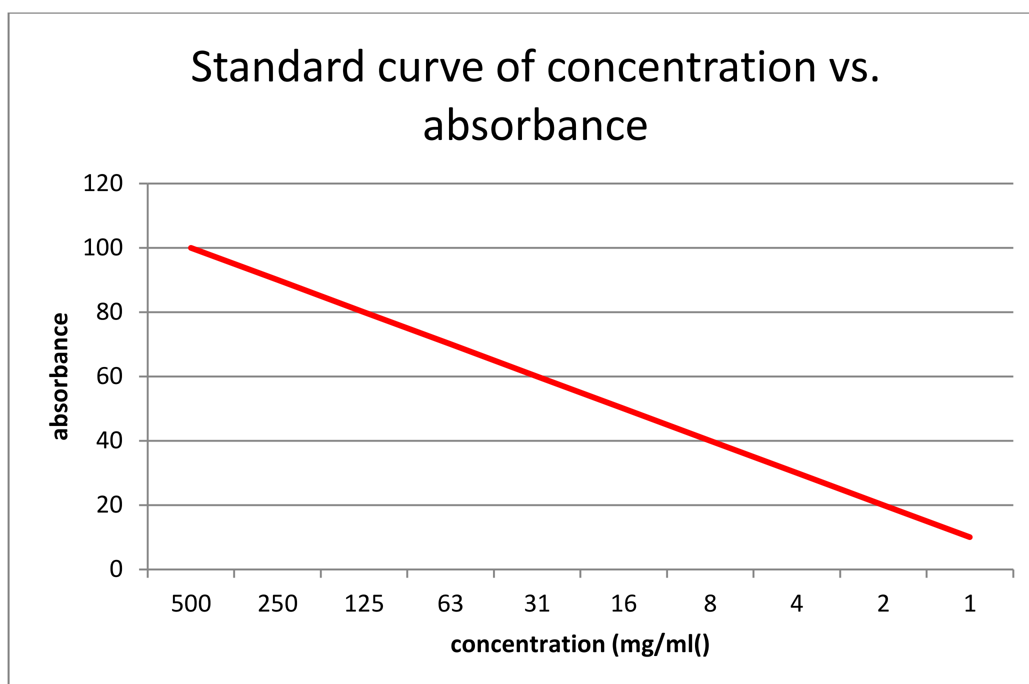
Another option when making dilutions is to determine the **dilution factor**, which is a number that describes the strength of the dilution. For example, a dilution factor of 10 means a 1:10 dilution of the stock solution. To calculate the dilution factor, you take the volume of the stock solution that you are going to use and divide this by the total volume of the entire solution. For example, if you are doing a 1:10 dilution, you would take 1ml of your stock solution and add 9ml of water for a total volume of 10ml. Therefore, you would have a dilution factor of 10. In many cases, a dilution factor of 10 or 2 is used to dilute a stock solution and create a series of less concentrated solutions. This process is referred to as making **serial dilutions**. A serial dilution is a sequential set of dilutions, where each solution acts as the stock for the next solution. Essentially, it is just the process of making dilutions with the same dilution factor over and over.

To carry out a 1:10 serial dilution, assume that you start with 11ml of your stock solution. To make a 1:10 dilution, take 1ml of your stock and add 9ml of water. This will give you a new stock solution that has been diluted by a dilution factor of 10. You now take 1ml of this new stock solution and add 9ml of water. This gives you yet another new stock solution that has been diluted by a dilution factor of 10. This process is repeated until you reach the total dilution you were looking for.

Once you have made your dilutions, it is useful to create a standard curve to help you keep track of the amount of a particular substance in a solution. A standard curve is a graph with the concentration of the solution on the X-axis and a different measurement on the Y-axis. This different measurement is one that is more easily measured than the concentration. For example, assume you have a sample of algae, and you want to know how much algae is in the sample. There are ways to actually measure this under the microscope (as we will see next week), but this is time consuming and difficult. You could have thousands of algal cells in one drop of water and counting them is nearly impossible!! If, instead, you have a standard curve, you can use the graph to determine the algal concentration. See the graph below for an example.

For this semester, the measurement that we will use on our standard curve is the amount of light that is absorbed by the solution, referred to as the **absorbance**. The more algae that are in the solution, the

more light that will be absorbed. An easy way to measure absorbance is to use a machine known as a **spectrophotometer**. This machine shines a light on the sample, measuring the amount of light that is absorbed and the amount that is transmitted through the sample to the other side. The advantage of using the spectrophotometer is that we can place a sample in the machine, record the absorbance, and then use the standard curve to determine the actual algal concentration of our sample. This is much easier than actually counting the individual algal cells. However, it is important to remember that if your absorbance is beyond the scale of the graph (for example in the graph below if your absorbance is 150), you cannot then use that to determine your concentration. A standard curve is only accurate for those data that fall within it.



Procedure

Exercise 1: How to use a volumetric pipette

In order to perform serial dilutions of a stock solution, you must be able to accurately withdraw specific volumes from the stock solution with a pipette. The pipette works in similar fashion to a straw that allows fluid to be sucked up in one end. (NEVER put your mouth on the pipette!!!) You will use a pipette pump to suck up the correct amount of fluid and then push that fluid back down the pipette into a new solution. Today, you will start by learning how to use the pipette.

1. Obtain a weigh boat.
2. Obtain a 5ml pipette.
3. Obtain the appropriate pipette pump for the 5ml pipette. Pipette pumps differ in size and are designed for specific pipettes, so be sure you have the right one.

4. Place the proper pipette pump on the top of the pipette. Do NOT push on the end of the pipette, as this is an easy way to stab yourself in the hand with glass.
5. Place the weigh boat on the scale and tare (or zero) it.
6. Measure out exactly 1ml of water into the weigh boat, and weigh it.
7. Record your results below and check with your TA to test your accuracy.
   Weight of 1ml of water:

8. Repeat the process three times to check your accuracy. Record your results below. If your weights are not similar, repeat until you can accurately measure 1ml of water. Each member of the group must complete this process individually.

Weight of 1ml of water

Attempt #	Weight
1	
2	
3	

Exercise 2: Carry out a 1:10 serial dilution and create a standard curve

Now that you have learned how to accurately pipette, you will practice conducting serial dilutions and making a standard curve. The stock solution that you will be working with is Allura Red AC, also known as FD&C Red 40, or red food coloring.

1. Start by labeling your test tubes with a wax pencil. Label one tube “stock” and label the rest 1-7.
2. Measure out 5.5ml of the stock solution into the test tube labeled “stock”.
3. Using a 1ml pipette, take 0.5ml of the stock solution and add it to test tube #1.
4. Using a 5ml pipette, take 4.5ml of the water solution and add it to test tube #1. **Mix carefully**.
5. Now, use a 1ml pipette to take 0.5ml of the solution in test tube #1 and add it to test tube #2.
6. Add 4.5ml of water to test tube #2, and **mix carefully**.
7. Repeat until you have done all of the dilutions.
   Why did you start with 5.5ml of the stock solution rather than 5ml?

Now that you have your dilutions, you can measure their absorbance in the spectrophotometer. First, however, you will need to zero the spectrophotometer.

1. Turn on the spectrophotometer and allow to warm up for 15 minutes.
2. Set the wavelength on the spectrophotometer to __________ nm. Why did you choose that wavelength?

3. Be sure the lever at the bottom is set to the correct filter range
 (Be sure you check with your TA before proceeding).
4. Using the knob on the left, set the absorbance reading to infinity.
5. Now, obtain a clean spectrophotometer tube and fill it 2/3 full with distilled water.
6. Cover the top with parafilm, and stretch the parafilm across to make a tight seal.
7. Wipe the spectrophotometer tube with a Kimwipe, and place it into the sample holder.
8. Using the knob on the right, set the absorbance to 0.
 You have now told the machine that a solution with no pigments is essentially the control and that this is what "no pigment" looks like.
9. You are now ready to read your samples. Start with the stock solution, and use it to fill a spectrophotometer tube 2/3 full.
10. Cover the top with parafilm, and stretch the parafilm across to make a tight seal.
11. Wipe the spectrophotometer tube with a Kimwipe, and place it into the sample holder.
12. Record the absorbance on the table below.
13. Repeat this process for all of your diluted samples.

Tube Label	Dilution from stock	Absorbance
Stock	0	
1	1:10	
2	1:100	
3		
4		
5		
6		
7		

Using the information from the above table, create a standard curve using excel. Be sure to print it out and attach it to your lab exercise.

Exercise 3: Carry out a 1:2 serial dilution and create a standard curve

1. Start by labeling your test tubes with a wax pencil. Label one tube "stock" and label the rest 1-7.
2. Measure out 7.5ml of the stock solution into the test tube labeled "stock".
3. Using a 5ml pipette, take 2.5ml of the stock solution, and add it to test tube #1.

4. Using a 5ml pipette, take 2.5ml of the water solution, and add it to test tube #1. **Mix carefully**.
5. Now, use a 5ml pipette to take 2.5m of the solution in test tube #1, and add it to test tube #2.
6. Add 2.5ml of water to test tube #2, and mix carefully.
7. Repeat until you have done all of the dilutions.

You are then ready to use the spec 20 to create a standard curve, as you did for the 1:10 serial dilutions. Use the data gathered to fill out the table below.

Tube Label	Dilution from stock	Absorbance
Stock	0	
1	1:2	
2		
3		
4		
5		
6		
7		

Using the information from the above table, create a standard curve using excel. Be sure to print it out and attach it to your lab exercise.

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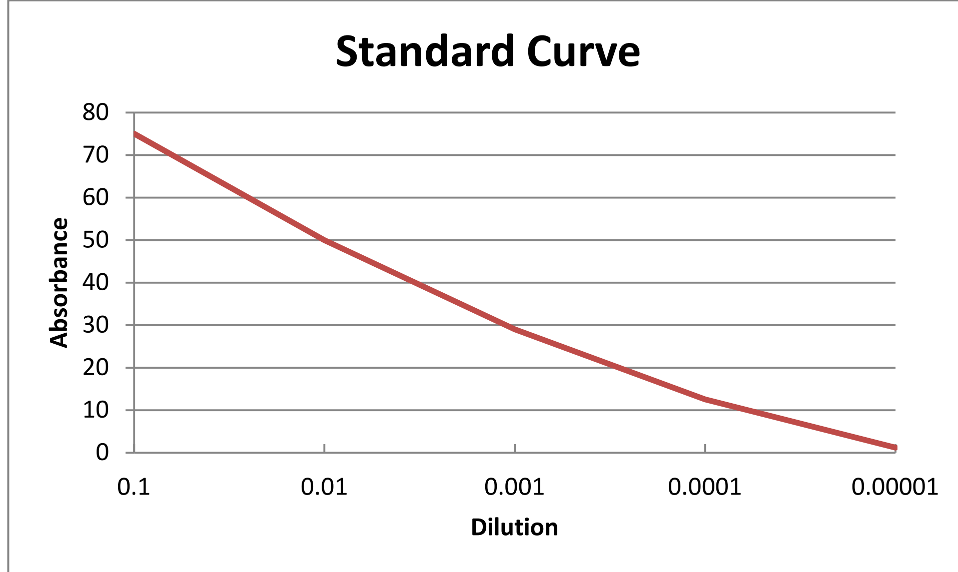
Homework

Use the graph below to answer the following questions:

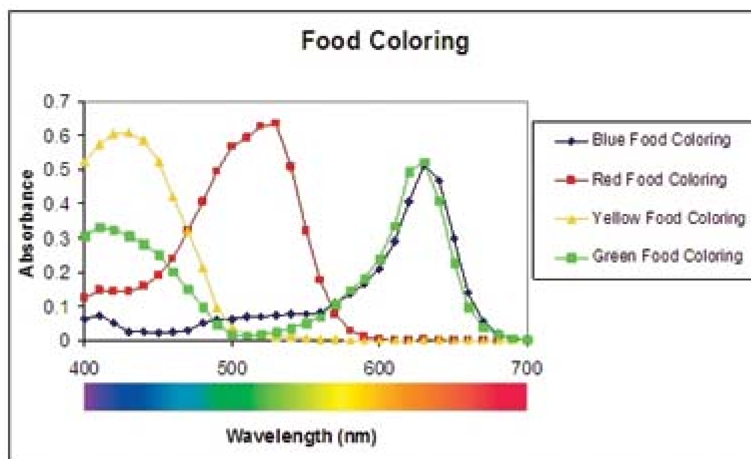


1. What type of serial dilution was conducted? i.e. 1:2, 1:5, 1:10, etc. How do you know?

2. A researcher using the same solution in the graph above recorded an absorbance of 50nm. What was the dilution of her stock solution?

3. Write or draw out how to make a 1:5 serial dilution of a stock solution. You will need to dilute it 4 times.

4. A researcher was measuring the absorbance of yellow food coloring. What wavelength should he use on the spectrophotometer? Why?



(Taken from Orvis et al, 2007)

5. You are asked by your TA to pipette 1ml of water into a test tube. Which pipette should you use – 1ml, 2ml, 5ml, or 10ml. Why?

6. **In your own words,** explain how a standard curve is created and what purpose it serves.

7. When making Coca-Cola, the absorbance of the coke is measured in the clear plastic bottles for quality control. For each run of 1,000,000 bottles, the absorbance of 100 is measured. Explain how this is a measurement of quality control.

Lab 5: Counting Algae
Spec20, Hemocytometer, and Standard Curves

Objectives

At the end of this experiment, students will be able to:

1. Describe the various methods for counting cells
2. Use a hemocytometer to determine algal concentration
3. Use data from a spectrophotometer to create a standard curve showing algal concentration
4. Dilute an algal stock concentration

Introduction

The purpose of this laboratory exercise relates directly to the experiments you will be conducting throughout the semester. In today's lab, you will learn how to determine the concentration of algae in a solution of saltwater. This is critical for working with plankton, as the algae are the food source that they need in order to survive. If your algae are not concentrated enough, the plankton will starve and will die in large numbers. If your algae are too concentrated, this can affect the swimming and breathing ability of the plankton. Therefore, it is important to know just how many algal cells are in the solution that you are using to feed the plankton.

There are a few different methods for counting the number of cells in a solution. One option is to use a machine known as a cell counter, which optically scans your sample and uses an algorithm to identify cells vs. debris and count the number of cells. There are various types of cell counters from flow cytometers to coulters, but they all have the same advantages and disadvantages. The advantages are that they may be able to sort the cells by size into different containers and can allow you to add fluorescent tags to particular cell types. However, although these instruments are powerful tools, they are typically quite expensive and require more training to operate. In addition, they are often specific to mammalian cells, so they are not as accurate at measuring algal cells.

At the opposite end of the technology spectrum, one method for counting cells is simply to place one drop on a slide and count the cells that are present. This drop technique works because one drop of seawater is approximately equal to 0.02ml. So if you find one algal cell in a drop of the sample, you can multiply to estimate the density at 50 cells/ml or 50,000 cells/L. Clearly there are some issues with this technique, the first of which is that you only get a rough estimate. Not all drops are the same, and, therefore, not all drops are exactly 0.02ml. In addition, there are often too many cells in the drop to count them accurately. Some may not be in the focal plane and therefore will be missed. The drop technique is often only used in field research when other methods are not available or when only a rough estimate is needed.

The most common methods for measuring algae are various types of counting chambers. One chamber is called the Sedgewick Rafter and is essentially a larger microscope slide with a cemented wall in the center to form a chamber. The chamber is 50mm long x 20mm wide and is 1mm deep; the

bottom of the chamber is marked with a grid of 1000x1mm squares. The researcher places the algal solution into the chamber and then adds the coverslip so that the volume in the chamber is precisely 1ml. The number of cells in a grid of the chamber is counted, and then the total number in the chamber can be estimated. This method is very effective for larger species of algae as well as for when you are attempting to process a large number of samples quickly. However, it can only be used with a dissecting microscope or a compound scope on the lowest power and therefore is not accurate for smaller algal species. In addition, if the sample is highly concentrated, it can be impossible to count all of the cells.

The counting method that you will learn how to use today is a type of counting chamber known as a hemocytometer. Although these were originally designed for counting blood cells, they are surprisingly accurate for counting algae as well. A hemocytometer is a thick glass slide with two counting chambers on it, each chamber holds $0.9mm^3$ ($9x10^{-4}$ml) when the coverslip is placed on top. Each chamber also has a specific grid etched into the glass on the bottom. The grid is shown below.

Notice that the grid has squares of various sizes. This allows you to choose where to count the cells depending on their size and density and gives you more flexibility than the other counting chambers. The size of the hemocytometer and the grids also allow you to use a higher power for microscopy and therefore count smaller cells. The specifics on how to use this instrument will be described in the exercises for this laboratory.

The goal for today's laboratory is to learn how to count algae with a hemocytometer and to use that information to create a standard curve. Essentially, you will be diluting a stock solution of algae in a serial dilution and then counting the number of algae in each dilution. You will use the spectrophotometer to measure the absorbance of each dilution as well. Finally, you will create a standard curve with the concentration of the algae on the X-axis and the absorbance on the Y-axis. This way, whenever you need to know the concentration of the algae in the future (such as when you are feeding the plankton), you can simply measure absorbance and use the standard curve to determine the concentration. This will be much easier than using the hemocytometer each time.

Procedure

Exercise 1: Serial dilution of the algae

1. You will need to do a serial dilution of the stock algae concentration. Your TA will let you know which dilution you will be doing. Because you are already familiar with this technique from the last lab, you will be responsible for writing your own protocol. Remember that you will need enough in each dilution to later read them in the spec 20 (at LEAST 5ml).
2. Write your protocol here in great detail. Include all steps you will need to do. Last week's laboratory exercise will help you with this. Have your TA check your work before you actually do the diluting.

PROTOCOL:

Exercise 2: Use the hemocytometer to count the algal concentration of each dilution

1. Collect a hemocytometer and coverslip from your TA. Please be very careful with these, as BOTH the hemocytometer and the coverslip are specially made and are expensive. Do NOT break the coverslip – treat it with great care!!!! Also, NEVER throw the coverslip away‼ Instead, rinse them for use next time.
2. Clean the hemocytometer and coverslip with lens paper – NOT Kimwipes, but lens paper.
3. Add the coverslip to the hemocytometer
4. Transfer 10µL of your stock solution into the chamber at the sample introduction point. Your TA will show you how to use a micropipette to measure out 10µL accurately.
5. Steps 3 and 4 are reversed
6. Place the hemocytometer onto the compound microscope at the LOWEST magnification and bring the grid and algae into focus.
7. Increase the magnification on the microscope very slowly. Note that the hemocytometer is much thicker than an ordinary slide, so you must only use the fine focus knobs to focus.
8. Count the total number of algal cells in the center counting area. This is the area with 25 squares in the very center of the hemocytometer counting grid.
    a. Some counting rules: Some cells will lie on the edges of the square. Only count those cells that touch the top and right sides of the edges. If any part of the cell touches the bottom or left, do NOT count it. This way, each square will be counted with the same method and no algal cell will be counted twice.

9. Now, take the number of cells you counted, and determine how many cells/ml are in your sample.
    a. Concentration of sample = (number of cells counted/volume counted)
    b. Because you counted the 25 larger squares, you counted a total volume of 100 nanoliters or 10^{-4} milliliters.
    c. Let's look at an example. Assume that you counted 187 cells.
       $187/10^{-4}$ml = 1,870,000 cells per ml
    d. If the squares that you counted were different, your total volume is different. The information below can be used if you counted different squares.

ilution	Cell concentration	Absorbance
Stock		

Exercise 3: Measure the absorbance of all dilutions in the spectrophotometer

1. Now that you have performed the dilutions, you need to measure their absorbance in the spectrophotometer.
2. Set the wavelength to 430nm.
3. Using the knob on the left, set the absorbance reading to infinity.
4. Obtain a clean spectrophotometer tube, and fill it 2/3 full with distilled water.
5. Cover the top with parafilm, and stretch the parafilm across to make a tight seal.
6. Wipe the spectrophotometer tube with a Kimwipe, and place it into the sample holder.
7. Using the knob on the right, set the absorbance to 0.
8. Now, you are ready to read your samples. Start with the stock solution, and use it to fill a spectrophotometer tube 2/3 full.
9. Cover the top with parafilm, and stretch the parafilm across to make a tight seal.
10. Wipe the spectrophotometer tube with a Kimwipe, and place it into the sample holder.

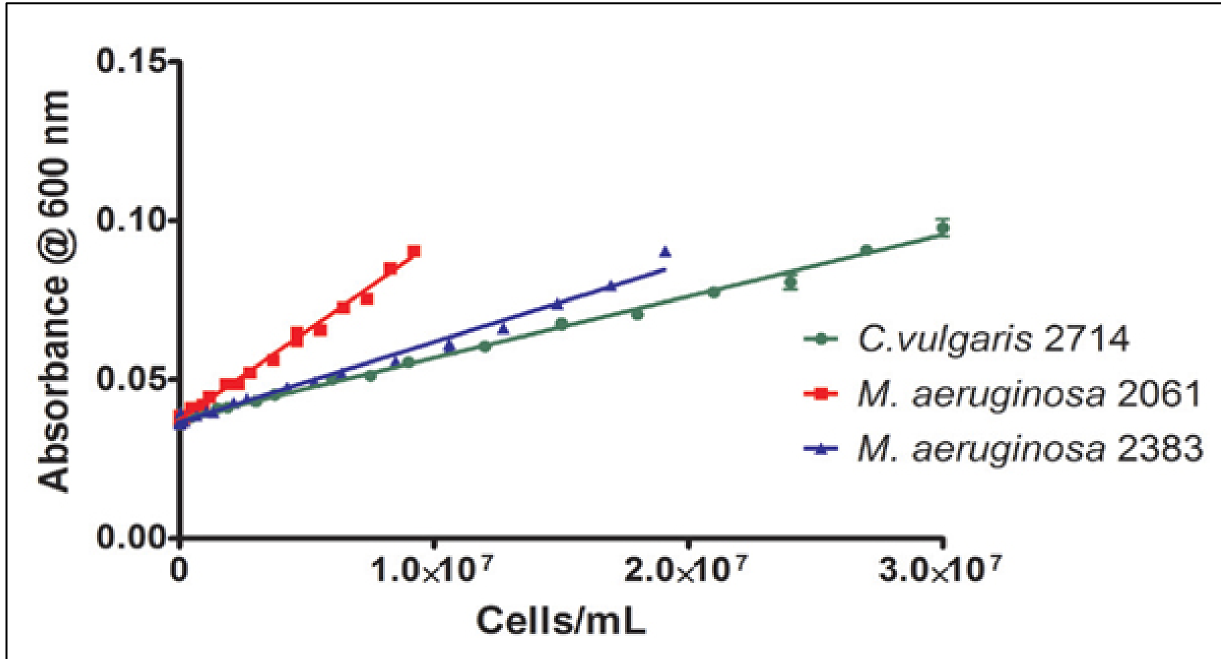
11. Record the absorbance in the table above.
12. Repeat this process for all of your diluted samples. Be sure to enter your results in the table above.

Exercise 4: Create the standard curve

1. Using the data from your results table, create a standard curve in excel. The concentration should be on the X-axis, and the absorbance should be on the Y-axis. Be sure to save your graph and print it out to attach to your laboratory notebook for future reference.

Homework

Use the graph below to answer the following questions



(Taken from Held, 2011)

1. Explain the basic trends you see from this graph. Why don't all three species of algae have the same absorbance at the same concentration?

2. A researcher measured the absorbance of a sample of *C. vulgaris* and found it to be 0.05. What is the concentration of the algae in that sample?

3. You used the hemocytometer to count algal cells, but found a very high concentration. To save time, you only counted the number of cells in ONE of the squares in the center (1 of the 25 squares). You found 165 cells. What is the total concentration of cells in your sample (per ml)?

4. What is wrong with the technique you used in question 3 (only counting one square)? What should you have done instead?

5. Assume that you have a total concentration of algal cells of 150 cells/ml. How many cells should you find in one drop if you used the drop technique? Show your work.

6. What are two areas for error when using a hemocytometer?

7. The algae that you used for this experiment are grown in the lab in large containers under grow lights. Students at the beginning of the week and at the end of the week were comparing their data. The students at the beginning of the week found that their <u>stock solutions</u> had a smaller absorbance than those at the end of the week. What has happened?

Lab 6: Graphing and Data Analysis

Objectives

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

1. Summarize data using descriptive statistics
2. Carry out a t test to compare means
3. Graph data in proper format

Background

Throughout this semester, you will be collecting a large amount of data that you will need to analyze and present. At first glance, a long list of numbers can be overwhelming when you are trying to determine if they support or refute your hypothesis. However, in this laboratory exercise, we will look at how to summarize your data, how to analyze it to draw conclusions about your hypothesis, and how to present these data to others.

We will first look at how to summarize your data using descriptive statistics. The best way to do this is to use a simple example. First, assume that you work for the Pepsi Co. and you are in charge of making sure that all of the bottles are truly filled with 20 fluid oz. How can you determine if this is true? You could measure every bottle of Pepsi as it leaves the bottling plant, but this will greatly slow production and your bosses will fire you. The best idea would be to take a subset of the bottles and weigh them so that you can determine if they are 20 oz. If they are, then you can assume that the rest are 20 oz. as well. The first point to consider is how many bottles to take (your sample size). You want enough to get a realistic idea of what the bottles weigh, but not so many that it takes too much time or money. For the bottles, you can easily measure 100 without slowing down the system, so you decide to measure 100 bottles. Therefore you have a sample size of 100.

The next issue is which 100 bottles to sample. Do you want to take the first 100 produced in the morning? Probably not, because the process could change as the machines warm up, and the amount in each bottle could be different. You want to take bottles at random times throughout the day to give you a more accurate picture. Therefore, you are taking a **random sample** of 100 bottles. Now, imagine that you have collected and measured your 100 bottles, and you have a list of 100 weights in front of you. What is the next step?

You will now calculate your first descriptive statistic, the **average**, also called the **mean**. You simply add up all of the weights and divided by the sample size – in this case 100. You now have the average weight of the Pepsi bottles. For our example, let's assume that the average is 19.3 oz. Is this good enough? It gives you an idea of what the average bottle weighs, but it doesn't tell you how far some of the others are from average. Are all the bottles close to 20 oz., or are some at 15 and some at 25? You could get the same average either way. Although the average value is important, it is also crucial to know how much the weights vary around the mean.

There are a few different ways to measure variation in a sample. The most common is to calculate the **standard deviation**. This is a value that represents the precision in your data, which is the amount of variation present with repeated measurements. If all of your bottles were between 19.9 and 20.1, the standard deviation would be quite low. However, if your bottles were between 14 and 26, your standard deviation would be quite high. You can use Microsoft Excel to calculate standard deviation (see below for how to do this), and let's assume that you did this for your soda bottles and found a standard deviation of 2.16. This is a nice number, but what does it really tell us? By the definition of standard deviation, it means that 66.7% of the samples should fall within one standard deviation of the average, and 95% should fall within two standard deviations of the average. So, if I added one standard deviation to the average and then subtracted one standard deviation from the mean, 66.7% of my bottle weights should be within those values. Let's actually do this.

To add one standard deviation: 19.3 + 2.16 = 21.46
To subtract one deviation: 19.3 – 2.16 = 17.14
So, 66.7% of our bottles weigh between 17.14 and 21.46 oz.

To add two standard deviations: 19.3 + 4.32 = 23.62
To subtract two standard deviations: 19.3-4.32 = 14.98
So, 95% of our bottles weigh between 14.98 and 23.62 oz.

You can now go to your boss and say that the Pepsi bottles come off the line at an average weight of 19.3 oz. with a standard deviation of 2.16 oz. He or she can then decide if the machinery needs to be adjusted to make each bottle weigh closer to 20 oz. However, there is another question you need to answer, and that is: what is the accuracy of the bottle weight?

Accuracy in this sense refers to how close a measurement is to a true value of the mean. The true value in this case would be 20 oz. Our last measurement, the standard deviation, was a measure of precision (how close the results were to each other), and this new measurement, the **standard error**, is a measure of how accurate the results are to the true mean.

When we calculated the standard deviation, we were determining how precise the values were, but we did not take into account the accuracy. For our soda bottles, a standard deviation of 0.02 would mean that all of the bottles were very close to the same weight, but if the mean was 14 oz., our bottles are not at all accurate in their weight. So, we need a measurement of accuracy; this is where **standard error** comes into play**.** You can use Microsoft Excel to calculate standard error (see below for how to do this), and let's assume that you did this for your soda bottles and found a standard error of 0.68. You can now report to your boss that you have an average bottle weight of 19.3 with a standard error of 0.68. You can use the standard error to calculate the confidence intervals and therefore state that you are 95% confident that 67% of your samples should fall within one standard error above and below the mean. In addition, you are 95% confident that 95% of your samples fall within two standard errors above and below the mean. You can now present these data to your boss for him or her to determine if this is accurate enough.

You have to answer one more question for the boss before you can go home for the day. She now wants to know if the Pepsi bottles and the Mountain Dew bottles have the same average weight.

You already know about mean and standard error, so let's assume that the average weight of the Mountain Dew bottles is 21.2 and the standard error is 0.24. There appears to be a difference between the two, but how can you be sure? There are a number of different statistical techniques one could use, but we will focus on only one for this course, the **Student's t-test**. This is a method for comparing two sets of data to determine if the means are the same or are significantly different from one another. So, is the difference between the two means due to random chance or because there is a true difference? For this test, we start with the **null hypothesis**, which states that the two means are not significantly different from one another. In our example, the null hypothesis would state that the average weight of the Pepsi bottles coming off the line is equal to the average weight of the Mt. Dew bottles coming off the line. Our **alternative hypothesis** would state that the two average weights are *not* equal. We then use Microsoft Excel to conduct a t-test and will be given a **t-statistic** and a ***p*-value**. The t-statistic reflects a ratio with the observed difference on the top of the ratio and the difference expected by chance on the bottom of the ratio.

$$\text{T-statistic} = \frac{\text{observed difference}}{\text{Difference expected by chance alone}}$$

The larger the t-statistic, the larger the difference between the two sets of data and the more likely that this difference is real and not just due to chance. So, how big does the t-statistic need to be for us to be sure that there is truly a difference between the two sets of data? This is what the ***p*-value** will tell us. The *p*-value is the estimated probability of rejecting the null hypothesis when that hypothesis is true. So, if the *p*-value is 0.1, there is a 10% probability of saying that the means are different when they are actually the same. For most researchers, this value is too high to make this kind of mistake. Traditionally, you are looking for a *p*-value less than 0.05. This would mean that there is only a 5% chance of saying that the means are different when in reality they are not. Another way to say this is that 95% of the time, if you find this t-statistic, the means are truly different, and this level of error is acceptable to most scientists. **When you conduct your studies, if your *p*-value is less than 0.05, you can state that the means of the two groups are significantly different**. If we look at our example of soda bottles, if the *p*-value is 0.21, we can state that the means are NOT significantly different, and therefore, the Mt. Dew and Pepsi bottles are the same average weight as they come off the line.

Now that you understand descriptive statistics and the t-test, you are ready to present the data to your audience. Although most students typically think of presenting data in graphical or tabular format, sometimes the best option is just to present it in the text. For our soda bottle example, one can easily just state that "a t-test indicated that there was no significant difference between the weight of the Mt. Dew and Pepsi bottles." Perhaps no more information is needed, based on the audience for your data. However, in many cases, a table or graph may be more appropriate. A **table** is useful for presenting data in columns when you want your reader to be able to see the detailed numbers. Tables are not the best way to show trends or relationships between variables, as these are clearer in **graphs**.

For example if you wanted to show the change in growth rate over time, a graph would be better. However, if you wanted to show the number of students who earned each grade (A-F) on a particular exam, a table would be more appropriate.

Once you have decided how you are going to present your data, you need to be sure to follow the basic guidelines for constructing tables and graphs. Tables must have a legend at the very top (what you might consider the title), and each legend starts with the number of the table. Within a lab report, tables and figures are numbered in the order in which they appear, but tables and graphs are numbered separately. The legend of the table should explain the information found in the table and a BRIEF bit of the methodology that was used to obtain the table data. Below the legend, the structure of the table begins, with an appropriate column heading for each column. Under each column heading are the data you want to present. Finally, at the bottom of the table are any footnotes that you need to explain abbreviations in the table. The following is an example of a table constructed in the proper format:

Table 1: Survival rate of female *T. californicus* in increasing salinity. Survival was measured after 96 hours of exposure to the individual salinity condition. Animals were considered dead when no visible movement was observed in response to touch with a metal probe.

Salinity	Average Percent Survival	Standard deviation	N (sample size)
25 ppt	92	1.4	75
30 ppt	90	0.5	75
35 ppt	88	3.2	73*
40 ppt	72	3.5	75

*Sample size smaller due to error in sex identification

Graphs and images that you may want to include are both often referred to as Figures. Unlike tables, the figure legend for a graph is placed at the bottom of the graph. Each axis must be clearly labeled and include the units of measurement. The axis labels need to be in a large enough font to be read easily. Many graphs will contain a key as an insert to describe what each symbol means, but this is not always necessary. One of the biggest mistakes students make is in deciding what type of graph to use. **Line graphs** are used to show changes over time for a particular group when data are **continuous**. **Bar graphs**, or columns, are used to compare different groups in a **category**. **Pie charts** are less common and are typically used when attempting to show how a whole is divided (e.g., how an animal spends its time in a 24 hour period). Below is an example of a bar graph in the proper format.

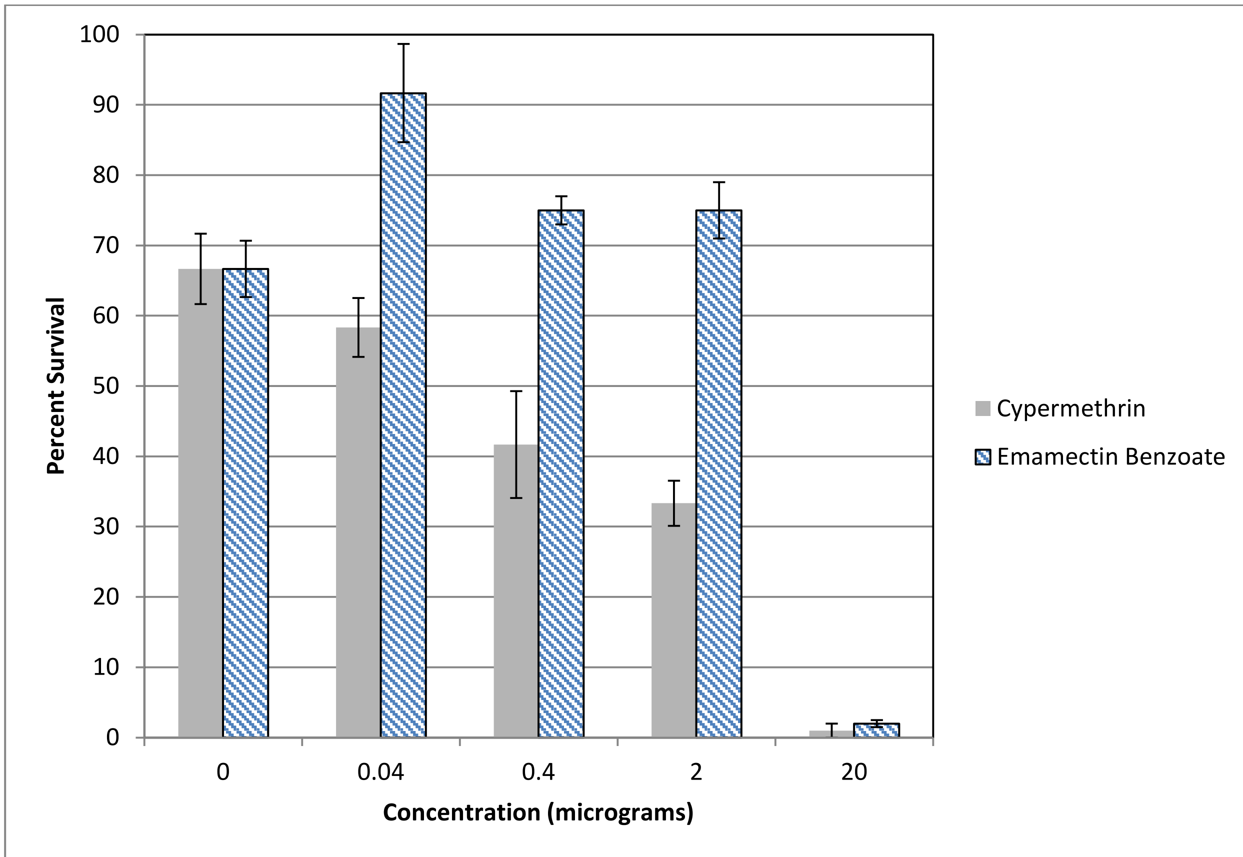


Figure 1: Survival of female *T. californicus* after 96 hours of exposure to common pesticides, either cypermethrin or emamectin benzoate. N=36 for each pesticide concentration.

Procedure

Exercise 1: Analyzing the Data

For today's lab, you will be examining data from actual experiments conducted in the Fisher lab here at UNC. The hypothesis for the experiment is that the older life cycle stages will be more resistant to the effects of the pesticide cypermethrin. This pesticide is commonly used in agriculture to kill a wide variety of insect pests (which are also arthropods). However, it is a non-specific pesticide and, therefore, kills many different arthropods including copepods. Although it is a pesticide approved for use on land, much of it can run off into the oceans, and there has been very little testing to determine its effects on aquatic species. Therefore, this study was conducted to look at these effects, especially on the younger, potentially more vulnerable life cycle stages. The life cycle stages examined were the naupliar and adult stages. For the experiment, nauplii were placed individually into wells of a 24 well plate. This means that each plate had 24 nauplii and each Nauplius was placed in its own well. The wells were filled with a mixture of seawater and cypermethrin, and the survival of each Nauplius was recorded after 48 hours. This was repeated for each life cycle stage, and then the entire experiment was repeated three times. The **raw data** are shown below (0 = dead, 1 = live):

Nauplius	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Well #	Alive or Dead	Alive or Dead	Alive or Dead	Alive or Dead
1	1	1	0	0
2	0	0	0	1
3	1	0	0	0
4	1	1	1	0
5	0	0	1	0
6	0	0	1	1
7	0	0	1	0
8	1	0	0	0
9	0	1	0	1
10	0	1	1	0
11	0	0	0	1
12	1	0	1	0
13	1	0	0	0
14	1	0	0	0
15	0	1	0	1
16	0	1	1	0
17	0	1	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	1	1
21	0	0	0	0
22	1	1	0	0
23	0	1	0	0
24	1	0	0	1

Adult	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Well #	Alive or Dead	Alive or Dead	Alive or Dead	Alive or Dead
1	1	0	0	0
2	1	1	0	1
3	1	1	1	1
4	0	0	1	1
5	0	0	1	1
6	0	1	1	0
7	1	1	1	1
8	1	1	1	1
9	1	1	0	1
10	1	0	1	1
11	1	1	1	0
12	0	1	1	1
13	1	1	0	1
14	1	0	1	1
15	0	1	0	1
16	1	1	1	0
17	1	1	1	0
18	0	1	0	1
19	1	0	0	1
20	1	1	1	0
21	1	0	1	0
22	0	1	1	1
23	1	1	1	1
24	0	1	1	1

*There are a couple more things to consider before you process any of the data. The first is **copy errors**. You could go line by line for each of the above tables and enter them into Excel by hand. However, you are a human being, and we all make mistakes. Even if you only make a mistake in entering one out of a hundred boxes, there are 288 data points above so that means that 2 or 3 of the above data points would be wrong. That doesn't sound like a lot but it could be enough to change the results of your statistical analysis. If your error rate were only to go up to three out of a hundred than 8 or 9 of them would be recorded wrong. So whenever possible use a computer to copy raw data points like that, Excel will get that data copied correctly 100% of the time.
The second thing to consider is to use your RAW data for all of your calculations. We've discussed the importance of large sample sizes. If you were to calculate the proportions, averages or standard error of each experiment (like you will below) then run a t-test on those instead of your raw data; then you just reduced your sample size from 96 for each group to 4. That kind of systemic error virtually ensures that your results will not be statistically significant.
Finally, use Excel to perform all of these calculations. You could use a calculator for some of this but then you're entering data by hand (remember copy errors?). In addition, Excel is a very common program that you will use over and over again. You may not know all the ins and outs of

it right now, but the more contact you have with it the sooner you will. It's like when you first started using Word or any other program; until you had a certain amount of experience with it some things were difficult. When you get used to using Excel these things will be much easier, make it do all the hard work for you!

Step 1: Enter your data into an Excel File

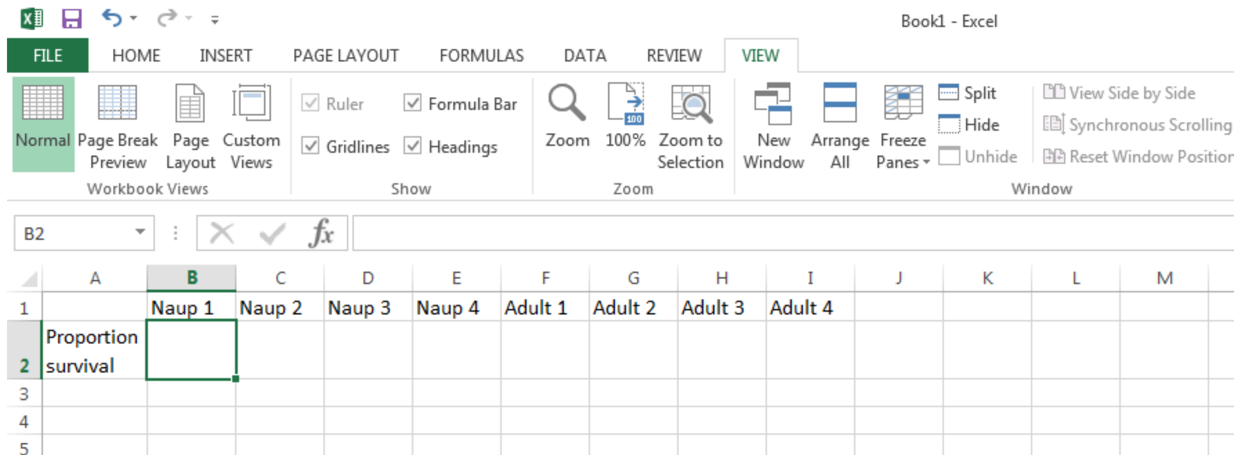
Step 2: Calculate the proportion (or fraction) of organisms that survive in each experiment. Some have been calculated for you to get you started

	Naup 1	Naup. 2	Naup. 3	Naup. 4	Adult 1	Adult 2	Adult 3	Adult 4
Proportion survival	0.375	0.375			0.667	0.708		

Step 3: Calculate averages and standard error for each age group

To calculate standard error in excel: There is no formula for standard error in excel, so you must create it. Essentially, you use the standard deviation formula and then divide this by the square root of the sample size.

a. First enter your data in excel so that they look like this:



b. In a cell below your data, type =(STDEV(B2:E2))/(SQRT(COUNT(B2:E2))) and hit enter

c. CAUTION: The equation above will work if your data are in cells B2 to E2. Be sure to change the cells to fit where you typed your data!!

d. Your TA will help you with this process the first time.

	Nauplius	Adult
Average		
Standard Error		

Step 4: Conduct a t-test to compare each life cycle stage to the other. To do this, go to the following link http://www.graphpad.com/quickcalcs/ttest1.cfm and follow the directions provided. Your TA will help you with this process the first time through.

	Nauplius vs. adult
T-statistic	
p-value	
Significant difference (yes or no)	

Does your t-test indicate a significant difference between the two life cycle stages?

How do you know the difference is (or is not) significant?

Putting it all together now:
First, restate the original hypothesis for this experiment.

Now, do the results of the t-test support your hypothesis? If yes, why do you think this is so? If not, why not?

When conducting research, you can have a t-test that shows a significant difference and still NOT support your hypothesis? Explain how this could occur.

Step 4: Present your data in graphical form. Print out and attach your graph to this sheet.

Homework

Now that you understand the concepts, you will need to see if you can do them on your own for homework. For the data below, the hypothesis was that female adult copepods are less tolerant of the pesticide cypermethrin than are male adult copepods. Here are the data: (1=Live, 0=Dead)

Females	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Well #	Alive or Dead	Alive or Dead	Alive or Dead	Alive or Dead
1	1	1	0	0
2	0	0	1	0
3	0	0	0	0
4	0	0	0	1
5	1	1	0	1
6	0	0	1	0
7	1	0	0	0
8	1	0	0	1
9	1	0	1	0
10	0	1	0	0
11	0	1	0	1
12	1	0	1	1
13	1	0	0	0
14	0	0	0	0
15	1	1	0	0
16	0	0	1	1
17	1	1	1	0
18	0	0	0	1
19	0	1	0	0
20	0	0	0	0
21	1	0	1	0
22	0	0	0	1
23	0	1	0	0
24	1	0	1	0

Males	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Well #	Alive or Dead	Alive or Dead	Alive or Dead	Alive or Dead
1	1	0	0	1
2	1	0	1	1
3	1	1	0	0
4	0	1	0	0
5	0	1	0	0
6	1	1	1	0
7	1	1	1	1
8	0	0	1	1
9	1	0	1	1
10	1	1	1	1
11	1	1	0	1
12	0	0	0	1
13	0	1	1	1
14	1	1	1	1
15	0	0	0	1
16	1	1	1	1
17	1	1	1	1
18	0	1	1	0
19	1	1	0	1
20	0	0	0	1
21	0	0	1	1
22	1	1	1	1
23	1	1	1	0
24	1	1	1	0

1. What is the proportion of each gender that survived?

2. What is the average proportion survival for females and the standard error?

3. What is the average proportion survival for males and the standard error?

4. Carry out a t-test to compare the survival of each gender. Show the results below, including both the t-statistic and the *p*-value.

5. Is there a significant difference between the two means? How do you know?

6. Was the hypothesis supported? Explain your answer.

7. Create a table to present your data. Be sure it is in the proper format. You can create it electronically and attach it to the homework that you hand in.

8. Crate a graph to present your data. Be sure it is the proper format. You can create it electronically and attach it to the homework that you hand in.

9. If you were presenting your data in a scientific journal article, would you use a table or a graph? Explain your answer.

10. A researcher hypothesizes that pesticide exposure will be harmful to copepods. She finds that the mortality rate of her pesticide treatment is an average of 26.3% and the mortality rate of her control group is 56.5%. She conducts a t test and her p value is 0.002. Are her results significantly different in the two groups and do her results support her hypothesis?

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Guidelines for the Research Question

Overview

The purpose of the research question is to get you started on the process of defining your ideas and narrowing down your area for experimentation. As a group, you should discuss which variables of the copepods are the most interesting to you all and from there, which aspects of these you may want to investigate. At this point, be sure you are reading over the primary literature on the copepods that has been provided on blackboard for you. This will give you the necessary background information to develop your ideas and also ensure that you are not asking a question that has always been answered.

Once your group has decided on the specific aspects that you would like to investigate, you can write your research question. For this, each group will need to turn in a single page including the following information:

1. Team Names & Team Roles (look in your lab manual for the four possible roles and responsibilities, you are not signing your life away, but think about which role you would like to do for the project--if you need help deciding, you can always your TA)

2. The specific question you have about the *Tigriopus californicus* that we will be working with. This doesn't have to be a fully formed hypotheses, but you should have developed a general question that you will later form hypotheses from. Look back at some of the exercises from our scientific method lab from this week, if you need any help thinking about what research questions look like. Your TA will discuss this question with your group in lab to help you flesh out your idea, but **you must come with a question to begin with!** Do not forget, you will get a grade for this assignment--it is 3% of your grade!

3. An explanation of how you came up with your question: write one paragraph about how you came to your research question. You should think about things like: what in the intro material caught your eye? Why did you choose one topic over another? Why do you think your research question needs to be answered? Thinking about why you chose a particular research topic will be critical for writing your introduction and connecting your project to the greater scientific community in your discussion.

Rubric

	Points Possible	Points Earned
Group members and roles clearly identified	__10___	_____
Research question clearly stated	__40___	_____
Explanation of your question, what biology is relevant?	__40___	_____
Statement of expected results (what you think will happen)	__10___	_____

Guidelines for the Preliminary Proposal

Overview

The function of the preliminary proposal is to start the research process by identifying your research questions, your methods, and the materials that you will need. It should contain the following sections:

Research Question

This section should include a statement of the research question and a brief bit of background on the specifics of that question. For example, if your question concerns the diet of *T. californicus*, then you should include information from other researchers that tested different diets as well as some information on the food choice you are considering. This is also where you will need to include your hypothesis and/or predictions based on your research question.

Methods

This section needs to be as specific as possible at this point and include how you plan to answer your research question. Be sure to include sample size, number of replications, dependent, independent, and standardized variables, and the details of how you will conduct your experiments. This section must also include an overall timeline.

Materials

This can be a list of the materials you will need to conduct the methods you have described. This is a key component, as it allows the laboratory staff to compile some of what you need and will help you determine if your methods are feasible. Remember that you will need to consider supplies for keeping the animals alive and growing their food (often algae), as well as any supplies for actually conducting your experiments.

References

You will need to include all of the references that you cited, and these must be in the proper format. You must have at least 3 primary research article references in the proposal. Do not list any references that are not cited in the paper, and do not cite any that are not listed in the references!

Rubric

	Points Possible	Points Earned
Research question clearly stated	__15___	______
Enough background information to support the research question	__15___	______
Experimental design will answer research question	__15___	______
Clear independent, dependent, and standardized variables	__15___	______
Timeline present and adequate	__15___	______
Materials list is well detailed, accounting for all rearing conditions	__18___	______
Correct use of references and references in proper format	__7____	______

Guidelines for the Final Proposal

Overview

The final proposal is a much more detailed version of the preliminary proposal. (Note that the rubric is different!!!) It must include exactly what you are planning to do and have support from the primary literature. It should have the following sections:

Title Page and Authors

The title should be concise but also needs to be specific enough to convey the information in the proposal. For example "temperature and copepods" is too vague but "the effect of changing temperature from 10-20 degrees daily on the survival, growth rate, and egg production of all life cycle stages of the copepod *Tigriopus californicus*" is too long!

Introduction

This is where you want to bring in the background information (from the primary literature) for your research topic and explain your specific research question. You will need to explain a bit about copepods and then the research that has been conducted on the variable you are testing. You also need to explain why any of this matters – i.e., why bother to answer the question you are asking. If this is well-written, it can serve as the guide for the introduction section of your final lab report as well.

Methods

In this section, you will need to lay out exactly what you plan to do and when. This should be done in a clear and concise manner. First, give an overview of the methods you will use and then provide the specific protocols for the experiments you plan to conduct. You must overlay these planned experiments on a timeline so that each member of the group knows what you will be doing and when. Be sure to plan for large enough sample sizes and adequate replication of each experiment. Also include time for when mistakes occur or things go wrong (as they definitely will). It is probable that you will have to alter your methods as you go along, as this is how science actually works, but you want to include as many specifics as possible at the very beginning so that you can anticipate any potential problems in your design.

Materials

This needs to be an extremely detailed list of items and supplies that you will need to conduct the experiments that you have outlined in the methods section. When most scientists conduct research, this list of materials is used to determine the budget for the project when asking for a grant. Therefore, it is imperative that you include all possible items that you will need. One way to do this is to think through each step of each protocol that you will carry out and list what you will need. Then, determine how many times you plan to conduct each protocol so that you know exactly how many of each item you need. Again, plan for things to break or that you will need to repeat some experiments, so add that into your materials list as well.
In addition, you will need to fill out the Experimental Equipment Request on the following page.

References

You will need to include all of the references that you cited, and these must be in the proper format. You must have at least 5 primary research article references in the proposal. Do not list any references that are not cited in the paper, and do not cite any that are not listed in the references!

Experimental Equipment Request

(must be handed in with final proposal: anything NOT on this list will not be provided for you)

Names of all Group Members

Time of lab and TA Name

Research Question/Hypothesis

Independent variable

Dependent variable(s)

Number of copepods and type needed (for example, 45 females with egg sacs)

Number of plates needed (with explanation)

Amount of seawater needed

Amount and type of algae needed

Comprehensive list of all other supplies needed

Draw out experimental setup. Include all plates and the number of copepods in each. Also diagram locations for each and under what conditions each plate will be kept.

Rubric for the Final Proposal

Although the proposal is a group grade, it is expected that the Principle Investigator and Analysis Expert will focus on the literature review and citation sections and the Protocol Expert and Data Expert will focus on the methods, and timeline sections. All group members should focus on the grammar, writing clarity, and all other sections.

	Points Possible	Points Earned
Title clear and concise	__ 3___	_____
Included enough information from primary literature	__25___	_____
Research question clear	__ 5___	_____
Clear rationale for the study	__ 5___	_____
Methods clearly outlined with specifics	__20___	_____
Methods designed to answer the research question	__10___	_____
Timeline clearly outlined and realistic	__10___	_____
Research cited in the proper format, with proper # of references	__ 5___	_____
Spelling and grammar	__ 5___	_____
Clarity of the writing	__8___	_____
Accurately completed equipment request form	__4___	_____

Guidelines for the Weekly Update

Overview

Each week, one member of your group will provide an oral presentation on the research conducted the previous week. Because there will be eight opportunities for weekly updates, each group member will be responsible for 2 weekly updates. Students who use a Powerpoint presentation for their weekly update are usually more successful. The weekly update must include the following:

1. Details of the research carried out over the past week
2. Problems encountered during the past week
3. Problems solved during the past week
4. A summary of the data obtained over the past week
5. Next steps planned in the process
6. How the activities of the past week relate to the overall research question

Rubric for the Weekly Update

Presentation Style	Points Possible	Points Earned
Limited use of notes	__5___	_____
Good volume and pace	__5___	_____
Clearly practiced, good flow of speech	__5___	_____
Presentation Content		
Details of research conducted to date	_15____	_____
Problems encountered/solved this past week	_15____	_____
Data summary to date	_15____	_____
Next Steps	__20___	_____
Relate activities to overall research questions	__20___	_____

Guidelines for the Lab Notebook

(modified from Susana Karen Gomez and Scott Franklin)

Overview

You will work as 3-to-4-member teams, both in the exercises and the investigations. **However, you will each keep your own notebook.** If you want to be successful at doing lab work, it is very important that you keep a detailed notebook. Your lab notebook will be graded each week during lab, so you must keep it up to date. Here are some guidelines for your lab notebook:

1. The notebook must indicate **exactly what you did**, **when you did it, why you did it, the results, and your interpretation**. If what you did varied from the normal protocol in any way, it must be indicated in your lab notebook. If something unusual happened to a particular sample, you must record that. Although it is important to minimize the number of mistakes in your lab procedures, they will occur occasionally. If you record the mistake, it may be possible to adjust for it later and still have useful data. If you make a mistake, write it down and, before you go further, talk to your instructor to see if there is anything you should change before you complete the procedure. Documentation of observations and results is a crucial and often overlooked part of any scientific study. First-time researchers often take note-keeping for granted, and vastly important information is recorded sloppily, hastily, or is indecipherable to other researchers. Important information can be forgotten ("Oh, I'll go back and fill that in later") or lost, and the long-term value of the research is greatly diminished.
2. Recording why you did the experiment and what your interpretation was will save you time when you are planning for and conducting your next experiment.
3. Write in <u>pen</u>. If you make a mistake or do not do something as you planned, cross it out once with ink.
4. **Write everything down <u>as it occurs</u> directly into your lab notebook.** Do not write on separate pieces of paper and then recopy your notes into your lab book. It is not important for your lab notebook to be extremely neat. Because you will be using it at the bench, you will get buffer or other reagents on it occasionally. This is acceptable. The lab notebook should be neat and clear enough that somebody else can read your lab notebook and make sense of it.
5. Make sure to date each page and put a page number on it.
6. Include a table of contents at the beginning of your lab notebook (leave two blank pages for this).
7. Include all of your calculations (even simple ones).
8. Include all of your graphs, figures, tables, and statistical analyses. If you have done these using Microsoft Excel or other software, print out a copy of the document and tape it neatly into your lab notebook.

Rubric for the Lab Notebook

Lab Notebook is complete – 100%

Lab Notebook is missing pieces – 50%

Lab Notebook is missing critical information and is not adequate *or* has not been completed – 0%

Guidelines for the Presentation

Overview

At the end of the semester, each group will give a professional presentation of their research project. You will be required to make PowerPoint slides that present your material, and the presentations will be given in the same format as a scientific meeting. Each person must present their portion of the material (see below) as part of the group presentation. The time frame for each presentation is 12-15 minutes with 3-4 minutes for questions. As is the case at a real scientific conference, you will not be allowed to go over the time limit. At 14 minutes, your TA will warn you of the time and at 15 minutes you will be cut off, regardless of whether or not you are finished. You will receive a total grade for the presentation, but 80% of that grade will be your individual contribution and 20% will be a group grade. The individual grade will be based on your presentation style as well as the section of the presentation that was your primary responsibility as outlined below:

Principle Investigator- Introduction
Protocol Expert- Methods
Data Expert - Results
Analysis Expert – Discussion

Required Components

Introduction

This is where you want to bring in the background information (from the primary literature) for your research topic and explain your specific research question. You will need to explain the research that has been conducted on the variable you are testing in similar species or in similar situations. You also need to explain why any of this matters. Remember that everyone in the room will already know the basics of copepods, so you don't need to go over that information – this introduction should be specific to YOUR research project.

Methods

You will need to clearly and concisely describe the methods that you used throughout the project to obtain your data. This section needs to provide enough detail that your audience can understand what you have done, but does not need to be at the same level of detail that you will put in your written lab report. Any new protocols that you developed or issues that you had to deal with should be discussed here. Also, be sure to include any shortcomings with the methods that may affect your data and the strength of your conclusions.

Results

This is where you will need to show your summarized data and explain what you found. Be sure not to include all of the raw data, but only the summary and the statistical analysis. This section needs to be clear and concise with enough information that your audience can understand how your data do (or do not) support your hypothesis. When you have finished presenting your results, your audience should be able to decide if they feel you have enough data to support your conclusions.

Discussion

In this section, you need to put all of the previous information together and explain your conclusions. Did you answer your research question? Did your results support your hypothesis? The key part of the discussion is then to explain WHY you found the results that you found. You cannot simply restate the results in the discussion;

instead you must provide an explanation for these results. You will also need to draw on relevant information in the literature to help you explain your data and provide support for you conclusions.

Rubric for the Individual Portion of the Presentation

(80 pts)

Presentation Style (25)	Points Possible	Points Earned
Limited use of notes	5	
Good volume and pace	5	
Good flow of speaking, clearly practiced	5	
Speaker addresses audience	5	
Speaks with enthusiasm and interest	5	
Content and Organization (55)		
Good subject coverage, speaker knowledgeable	10	
Logical flow of topic, good transitions	6	
Provided sufficient detail	10	
Explanation was clear	10	
Covered all required areas of topic	14	
Slides in proper format	5	

Rubric for the Group Presentation Grade

(20 points)

	Points Possible	Points Earned
Quality of presentation overall	5	
Cohesiveness of presentation between sections	5	
Able to answer questions	5	
PowerPoint slides of high quality with no errors	5	

Guidelines for the Final Lab Report

Overview

Each group will write up one combined lab report based on their research project. This will be in the format of a scientific research journal article. You will receive a total grade for the lab report, but 80% of that grade will be your individual contribution and 20% will be a group grade. The individual grade will be based on your writing style as well as the content of the section that was your primary responsibility as outlined below:

Principle Investigator- Introduction
Protocol Expert- Methods
Data Expert - Results
Analysis Expert – Discussion

Required Components

<u>Abstract</u>

This is a brief summary of the entire lab report and should be no more than 250 words. You must **very concisely** summarize the background info, the methods, the results and the conclusions. Be sure to read abstracts on other scientific papers to get a feel for what this includes.

<u>Introduction</u>

This is where you want to bring in the background information (from the primary literature) for your research topic and explain your specific research question. You will need to explain the research that has been conducted on the variable you are testing in similar species or in similar situations. You also need to explain why any of this matters – i.e., why bother to answer the question you are asking. Remember that everyone in the room will already know the basics of copepods, so you don't need to go over that information – this introduction should be specific to YOUR research project.

<u>Methods</u>

You will need to clearly and concisely describe the methods that you used throughout the project to obtain your data. This section needs to provide enough detail that your audience can understand what you have done, and so that anyone reading your report would be able to conduct the exact same experiment in the exact same way. This is important to be able to show that your results are reproducible. Any new protocols that you developed or issues that you had to deal with should be discussed here. Also, be sure to include any shortcomings with the methods that may affect your data and the strength of your conclusions.

<u>Results</u>

This is where you show your summarized data and explain what you found. Be sure not to include all of the raw data, but only the summary and the statistical analysis. This section needs to be clear and concise with enough information that your audience can understand how your data do (or do not) support your hypothesis. When you have finished presenting your results, your audience should be able to decide if they feel you have enough data to support your conclusions. Be sure all tables and figures are in the proper format.

<u>Discussion</u>

In this section, you need to put all of the previous information together and explain your conclusions. Did you answer your research question? Did your results support your hypothesis? The key part of the discussion is then to explain WHY you found the results that you found. You cannot simply restate the results in the discussion; instead you must provide an explanation for these results. You will also need to draw on relevant information in the primary literature to help you explain your data and provide support for you conclusions.

Continued on next page….

References

You will need to include all of the references that you cited, and these must be in the proper format. You must have at least 7 primary research article references in the proposal. Do not list any references that are not cited in the paper, and do not cite any that are not listed in the references!

Lab Report Rubric

	Points Possible	Points Earned
TITLE (3 points)		
Clear and to the point	__3__	_____
ABSTRACT (5 points)		
Accurately summarizes all of the information	__5___	_____
INTRODUCTION (20 points)		
Brought in relevant background information	__6__	_____
Explained rationale for conducting the study	__3__	_____
Adequate review of the primary literature	__7__	_____
Stated hypothesis/purpose of the study	__2__	_____
METHODS (20 points)		
Included all procedures	__8__	_____
Provided enough details but also concise	__10_	_____
RESULTS (20 points)		
Results summarized in writing	__6__	_____
Made references to Tables and/or Figures	__6__	_____
Tables and Figures clearly present the data	__4__	_____
Tables and Figures in proper format	__4__	_____
DISCUSSION (20 points)		
Interpreted results and related to primary literature	__12__	____
Explain/speculate on sources of error	__3__	_____
Place results in larger conceptual context	__3__	_____
Ideas for future experiments	__2__	_____
OVERALL QUALITY (12 points)		
Organization, Clarity and Flow	__4__	_____
Grammar and spelling	__4__	_____
Proper literature cited	__4__	_____

Literature Cited

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Appendix D: Faculty Instructions for Weeks 1-6

Week 1: **The Scientific Method.** Because this is a dry lab and the start of the semester, minimal preparation is needed. Students will focus on the scientific method and the components of a strong experimental design. The reading prompts are included in the laboratory manual (see Appendix C) and questions are designed to aid the students in creating and evaluating various aspects of experimental design (hypotheses, data collection and data interpretation). They will the conduct a small observational experiment to determine if there is a correlation between femur length and overall height. For this experiment, you will need to have meter sticks to measure femur length and a height chart on the wall to measure overall height.

During this lab session, students will also be assigned into lab groups for the semester, will need to think about their individual roles, and start considering their research question. The instructor will need to guide students to some extent at this point, but it is critical that the question is designed by the students and not the instructor. There are instructions and a rubric for the research question provided in the laboratory manual and in Appendix H.

Week 2: Literature Review and Critique. The focus of this lab is on reading and analyzing the primary literature. The students will need to read the article before class, so be sure it is available to them before the laboratory session. During the lab exercise, students in each lab group will be assigned a number (1-4). All students with the same number will get together to analyze a specific section of the research article and then will return to their lab group at the end of the session to report back. In this way, each student must take ownership of the material and be able to present what they have learned to their lab groups. The instructor needs to allow for free discussion of each article section and then guide the discussion where necessary.

Between this lab session and the next, students will need to write their preliminary proposal for their research project. It is important that the instructor work with each group this week to help develop their ideas and aid in the design of their experiments. There are instructions and a rubric for the preliminary proposal provided in the laboratory manual and in Appendix H.

Week 3: Using the Microscope. This lab has two central themes for students – how to use the microscope and an introduction to the research organism (*Tigriopus californicus).* The goal is to provide instruction on both the compound and dissecting microscopes and use the copepod as the model organism so that students can become familiar with both in one laboratory session. Students will also be expected to sex the copepods and identify different life cycle stages, so faculty will need to examine the animals ahead of time to familiarize themselves with these organisms. The laboratory manual provides basic diagrams of the life cycle stages and information on the differences between the sexes.

Week 4: Dilutions and the Standard Curve. The goal for this week is that students will learn how to carry out dilutions and then use their dilutions to create a standard curve. These skills are critical, as students will need to feed algae to the copepods, be able to dilute the algae, and use a standard curve to determine the overall algal concentration. For this week's lab, students will first learn how to use a volumetric pipette by pipetting the same amount of water multiple times and weighing the water to determine accuracy. It can be more interesting to students if this is set up as a friendly competition between lab groups for fastest or most accurate pipetting. Once they understand the basics of pipetting, students will carry out a 1:10 and then a 1:2 serial dilution of a stock solution of red food coloring. This is made by mixing food coloring and water until the solution is a dark red so that as the students dilute the solution, they can use the spectrophotometer to measure the color of each dilution. It is very important that instructors review how to use the spectrophotometer, as this is typically a new piece of equipment for students and can cause confusion. Because there are significant differences between different types of spectrophotometers, it is recommended that the instructor consult the manual for his/her specific model.

During this laboratory session, the instructor should be providing feedback on the preliminary proposal to each student group. This is a critical step in the process and allows students the opportunity to discuss the project with the instructor and refine/correct flaws in their preliminary proposal. It is also important that the instructor discuss the materials requests and remind students that they must accurately and completely fill out their equipment request form so that the instructor can gather all of the necessary supplies.

Week 5: Counting Algae. The focus of this lab is to teach students how to use a hemocytometer to count the number of algal cells and then create a standard curve for future reference. The lab manual includes directions on how to use a hemocytometer, and it is recommended that the instructor review these before the lab to become familiar with this procedure. At the end of the laboratory session, each student lab group should have created a standard curve that indicates the absorbance values associated with specific concentrations of algae. Because this information will be needed throughout the semester, the instructor should combine all of the raw data to create a more accurate standard curve for students to use.

Instructors will need to collect the students' final proposals during this week's laboratory sessions and then compile a comprehensive list of supplies needed to gather them during week 6 so that all materials will be available for students in week 7.

Week 6: Graphing and Data Analysis. The goal of this lab session is to introduce students to the basics of creating tables and graphs as well as teaching students how to use descriptive statistics and carry out a basic t-test. For the course in which this laboratory was conducted, students were not required to have any prior statistical knowledge, so the laboratory manual is very general. If this lab is used in a course where a statistical course was a prerequisite, this laboratory session may not be as applicable and more advanced statistical techniques could be discussed.

Appendix E: Faculty Instructions for the Authentic Research Experience

Because students will design and carry out their own research projects, faculty will need to be flexible in providing supplies and equipment. During the first 6 weeks of the session, when students are carrying out the more proscribed labs, the faculty member will need to culture algae and copepods for student experiments.

Culturing Algae

For these experiments, you will need two different species of algae, *Tetraselmis* (item #152610) and *Nannochloropsis* (item #153220) both of which can be ordered from Carolina Biological Supply. Please note that *Tetraselmis* is a saltwater organism and *Nannochloropsis* inhabits freshwater. The authors typically order 10 vials of each type of algae and then add each to a 20L clear carboys filled with either artificial seawater (Instant Ocean) at a salinity of 35ppt or tap water. The carboys need to be placed under a full spectrum light bank on at 12:12 light: dark cycle with 1 Tbsp of Miracle-Gro added initially to each carboy to increase growth rate. Algal growth will be slow for the first 1-2 weeks and then there will be a significant increase in population growth rate. Once the population is growing steadily, indicated by a dark green color throughout the carboy, it is important to subculture to prevent a population crash. Depending on the growth rate, the authors typically remove 1/3 of the solution every 2-3 weeks and replace this with 35ppt seawater (*Tetraselmis)* or tap water (*Nannochlorpsis)* to keep the population in the exponential growth phase. We also recommend that practitioners start a small subculture of each algal type in a 2L Erlenmeyer flask under the same conditions. This small subculture can be used in case of a population crash in the larger stock carboy.

Culturing Copepods

All *Tigriopus californicus* can be purchased from Carolina Biology Supply (item #142366). Upon arrival, place some copepods in 500ml Erlenmeyer flasks which must be put into an incubator set at 20ºC on a 12:12 light:dark cycle. The flasks should be filled with a combination of both types of the above algal solutions. To ensure adequate food, the solution should be at least light green in color. Aerate the flasks very slightly using an air pump and tubing with no airstone. Place the tubing for the air supply at the very top of the flask, barely into the water, to prevent creating a current in the water which will cause the copepods to be swept into it. These flasks will be as the supplies for the students to select their research copepods.
In addition to the research supplies, it is recommended that you create a stock supply of copepods to prevent the need for reordering throughout the semester. To do this, set up at 10gal aquarium filled with seawater and a mixture of the algal species. The water should be light to medium green in color to ensure adequate food. Aerate the tank with an air pump and tubing, again with no need for an airstone, although in the large volume of water, an airstone does not seem to disrupt the copepods. Add copepods to the tank and periodically check the water to ensure adequate algae. Supplemental feeding with a small amount of fish flakes can be done if the algal population seems low.

Supplies for Student Experiments

There is a great deal of variability in the supplies that will be needed for student experiments. Some of this variability can be reduced by providing guidance as the students design their experiments. For example, if students are interested in the effects of temperature on reproductive success, the instructor could indicate which temperatures will be available, based on information from the literature. However, it is critical to allow as much variability as possible so that students can truly design their own experiments and experience the scientific process as realistically as possible. When the students submit their final proposal, they are also required to submit a materials list that includes all of the supplies they will need, as well as an estimate of the number of copepods they will require. This is handed in 2 weeks before the experiments will start, allowing time for the instructor to collect the information and organize any necessary supplies.
For the work presented in the article, students housed their copepods individually in 24 well plates (ordered from VWR) for the course of their experiments, and copepods were kept in an incubator at 20ºC with a 12:12 light:dark cycle, unless the students were testing the effects of temperature or light cycle.

Other recommended supplies:

Chicken egg incubators (Little Giant still air incubator)
Timers for lights
Lights
Artificial seawater of varying salinities

A variety of chemicals if students are interested in toxicity studies
Plastic transfer pipettes
Dissecting microscopes
Reticule on microscope to measure size of copepods
Camera on microscope to record life cycle stages

Appendix F: Examples of Student Research Questions

In order to assist faculty as they help students design their research project, we have included a list of examples of student hypotheses and the types of data students have collected to address these hypotheses. Please note that many of these questions were examined by multiple groups over time.

Research Question	Data Collected
Does salinity affect overall egg production?	Exposed mated pairs to multiple salinity levels and measured egg production after mating
Does salinity affect sex ratio of offspring?	Exposed gravid females to multiple salinity levels, allowed eggs to hatch and mature until sex could be determined
What is the effect of temperature on growth rate? This was examined for both sexes and multiple life stages	Exposed various sexes/life stages to multiple temperatures and measured rate of growth over a defined period of time
Do males prefer older females?	Provided males with two females of differing sizes and observed mating to determine preference
Is oil pollution toxic to copepods?	Exposed adult females to environmentally relevant levels of motor oil and measured survival
Is cypermethrin toxic to copepods?	Exposed mated pairs to environmentally relevant levels of the pesticide cypermethrin and measured survival
Does increased temperature decrease survival in copepods?	Placed copepods in incubators at higher temperatures and measured overall survival (this was done by multiple groups at multiple temperatures and with various life cycle stages)
What is the effect of supplemental fish food on copepods?	Provided gravid females with regular algal diet and supplemented with fish food and measured survival
Does food with high fatty acid content increase egg production?	Provided mated pairs with fish foods that were low or high in fatty acid content and measured number of eggs produced
Do copepods prefer *Tetraselmis* or *Nannochloropsis*?	Provided adult males with each algal food source individually and measured overall survival

Appendix G: Sample Student Laboratory Report

Tigriopus Californicus: Survival Regarding Algae Based Diet

Research Question/Introduction

T. Californicus is a zooplankton copepod species, which has no known specific or exact diet but has the unique ability to adapt to the nutrients provided in distressed and ever changing environments. They play a critical role in the ocean (basis of the food chain) and "create fifty percent of the carbon containing compounds on the planet" (Fisher, McCabe 2015) and are essential to sustaining the ecosystem of the ocean. By finding a diet which increases these organisms' survival rate, we can in turn ensure the sustainability of the population, along with other species within the ocean. Thus, the research question we would like to address is: does a specific type of algae promote/ hinder the survival rate of the *T. Californicus*?

Through research, we found a study conducted by the Oxford Journal testing various species of algae on the survival rate of the *T. Californicus*. K. Suchy, J. Dower, A. Sastri and M. Niel discovered through their experiment that "Chitobiase-based productivity measurements demonstrate that a single species algal diet of the diatom *Thalassiosira weissflogii* was the best item for *T. Californicus*" (Suchy, Dower, Sastri, Niel 2013). The same study also found that *dinoflagellate Amphidnium carterae* algae caused the copepods to spend more time in early developmental stages which hindered transition into further life phases. This study provides information relevant to our topic because it highlights information regarding species of algae that could promote or hinder survival. As stated, "the use of the *chitobiase* method in a lab setting demonstrates the potential utility and sensitivity of this approach for field studies examining the impact and significance of short term shifts in food quality on entire crustacean zooplankton communities" (Suchy et al,2013), demonstrating the importance of a high quality algae diet.

In another article, information was found through an experiment conducted by three scientists Twombly, Clancy and Burns regarding two species of algae which were tested and compared to determine the "life consequences of food quality" (Twombly et al,1998) on the Copepod species *Boeckella Triarticulata.* The two species of algae tested were a high quality algae *Cryptomonas sp* and a combination of *Cryptomonas sp* and a low quality algae *Anabaena flos-aquae.* Based on the results collected, Twombly, Clancy, and Burns discovered that the combination of algae effected growth and development resulting in older and smaller metamorphus stages and slower growth rates. Research also found that this diet had no effect on survival rates. This study relates to our own experiment because it offers us a more in depth understanding of the effects diet has on copepods, and the possible consequences of a poor diet.

In the research article by J.M Conde-Porcuna, E. Ramos-Rodrguiez and C. Perez-Martinez it states that "several laboratory studies have shown that the limitation of Nitrogen and Potassium reduces the quality of algae as food for *Daphnia*" (Conde, et al 2002). This relates to our research question in that if may foreshadow that algae may not be a sustainable source of nutrients for our *T. Californicus*. If this conclusion is accurate, it is necessary to have a control group that is not algae based in our own research. (Group three, fish food).

In an additional journal on copepod diet, an experiment was conducted testing the survival rate of a community of *T. Californicus* when given either a single or mixed species of a phytoplankton diet. Based on the results of the experiment, the survival rates of the *T. Californicus* were higher when fed the diatom *Thalassiosria Weissflogii* and natural food assemblage as compared to the Dinoflagellate *Amphidinium Carterae* and any mixed diets. This study will benefit us in our research providing that it will support our idea that specific types of algae will have different effects on the survival rate amongst the *T. Californicus* species.

Based on the research stated above we hypothesized that the saltwater algae would promote the greatest survival rate amongst the copepods. Given that the copepods originate from a saltwater environment, we predicted the freshwater algae would not be a sufficient food source. In addition, given that the copepods were placed in a saltwater solution; the freshwater algae may have struggled to survive within the high salinity, and therefore may not have provided the copepods with necessary nutrients. Lastly, we also predicted that since the copepods natural food source is algae based, the fish food would not provide them with the sufficient means to survive.

Methods:

Trial Overview:

To test the effects of specific types of algae on the survival rate of *T. Californicus* copepods we placed 27copepods in three separate well plates (9 each, one per slot) and fed the copepods in well plate one (group one) regular fish food called Tetra Color, the copepods in well plate two (group two) were fed algae A which was the saltwater algae Chlorella, and the copepods in well plate three (group three) were fed algae B which was the saltwater algae Tetraselmis. Our independent variable was the food source we fed the copepods and our dependent variable was the survival rate. To feed our copepods each time we used a 1:2 dilution factor to calculate the 1:32 dilution ratio. To make sure that the cell count of algae was between 10,000-40,000 we used a Spectrophotometer to measure this value. We completed this process for both fresh water and salt-water algae. To prepare the control group's food, we also created a fish food stock solution using a 1:10 dilution factor to reach a 1:100 dilution. For the first week of every trial we began by placing one milliliter of seawater into the specified well plate slots, and then placed nine copepods into each of these slots. Once the copepods were placed, the lids of each well plate were labeled based upon their assigned variable. The copepods were then fed half a milliliter of their designated food source. Once fed, all three well plates were placed into the twenty-degree Celsius incubator until the following week. During the beginning of the second week of each trial, the well plates were removed from the incubator and observed. The survival rate of the copepods in each well plate was then recorded and all deceased copepods were removed. At the end of each trial, we collected the final survival rates, and removed all copepods (dead or alive) and cleaned each well plate.

Dilutions:

To begin the dilution of algae, we started with nine milliliters of stock solution for both the freshwater and saltwater algae. Using a micropipette six milliliters of stock solution was transferred to a test tube. We then removed three milliliters from the initial six-milliliter test tube, placed it within another test tube, and then added three milliliters of distilled water to that test tube creating a 1:2 dilution. Three milliliters were removed from the 1:2 dilution and were placed into a separate test tube where three millimeters of distilled water was added, creating a 1:4 dilution. This same process was repeated until we reached a 1:32 dilution. Using six milliliters of

distilled water to set the absorbance, the Spectrophotometer was calibrated to zero using a 435 wavelength. The 1:32 dilution was then placed into the Spectrophotometer to calculate cell count per parts per thousand.

In order to determine which dilution of fish food would be optimal for the survival of our copepods we had to conduct a pilot test. After we dissolved one gram of fish food into five milliliters of distilled water, we diluted that solution to obtain a 1:10, 1:100 and a 1: 1,000 dilution. We then fed three separate copepods (one in each well plate slot) half a milliliter of their designated dilutions (1:10, 1:100, and 1: 1,000). We then checked on them the next week to determine which dilution was a sufficient food source. It was then observed that the copepod in the 1:10 dilution was dead, and the copepods in the 1:100 and 1: 1,000 dilution were alive. We viewed the 1:100 copepod as most active and chose to use that dilution in our experiment. To create the fish food stock solution, we took one hundred milliliters of distilled water and placed it into a beaker. We then measured out one gram of Tetra color fish food using a weighing boat and the electronic scale provided. We then placed that into the one hundred milliliters of distilled water and began to stir until fully dissolved. To create the 1:100 dilution to feed the copepods, we began with four and a half milliliters of distilled water in a test tube, and then added half a milliliter of the stock solution to that test tube, creating our 1:10 dilution. From this test tube, half a milliliter was removed using a micropipette, which was then added to four and half milliliters of distilled water in a separate test tube, this gave us our 1:100 dilution.

Separating/ removing Copepods:

To retrieve each copepod used within our experiment, we removed a large amount of the copepod stock provided and placed it into a beaker. A small pipet was used to remove individual copepods from the beaker to place them within the designated well plate slots. Nine copepods were placed in each well plate (three in every other row), and labeled (group A, group B, or group C). Each time data was collected (every seven days), the deceased copepods were removed from their well slots using a small pipette and were disposed of. After the duration of each trial, all remaining copepods were also removed and disposed of.

Short Comings:

There were several short comings that may have affected our data. One possible shortcoming was the shortage of a sufficient amount of copepods to satisfy our original sample size. Once copepods were available, the group had difficulty counting, separating, and determining non-activity versus death among them. Another issue we encountered was the uncontrollable reproduction of males and females, which resulted in inconsistent data resulting in many Nauplius, since we did not separate the copepods by gender.

Results:

As shown in figure two our P- Value of .002460 represents a significant difference amongst our data as a whole. However, there was no significant difference between the Control group and the Seawater. A significant difference was found between the Freshwater and both of our other variables (figure two). The p-value for our control group and the freshwater was <.01 and the p-value for our Freshwater and Seawater was <.05 noting a significant difference. This difference can also be noted in figure one by observing the average survival rate amongst the groups. The Freshwater algae had a much lower average survival rate of .665. Seawater had an average survival rate of .853 and the control group had an average survival rate of .91275. Figure three shows the survival rate of the copepods each week of each trial to show the variance throughout the experiment in more explicit data.

The data collected in our experiment supports and hinders our hypothesis. Our hypothesis was supported since we predicted that Seawater algae would have the greatest survival rate and in comparison to Freshwater algae, it did. We were also correct when we hypothesized that freshwater algae would have the lowest survival rate, which it did. However, our hypothesis was refuted because we predicted that the control group would have a lower survival rate than the Seawater algae and have little effect on the copepods as a whole but it proved to have the highest survival rate among our variables.

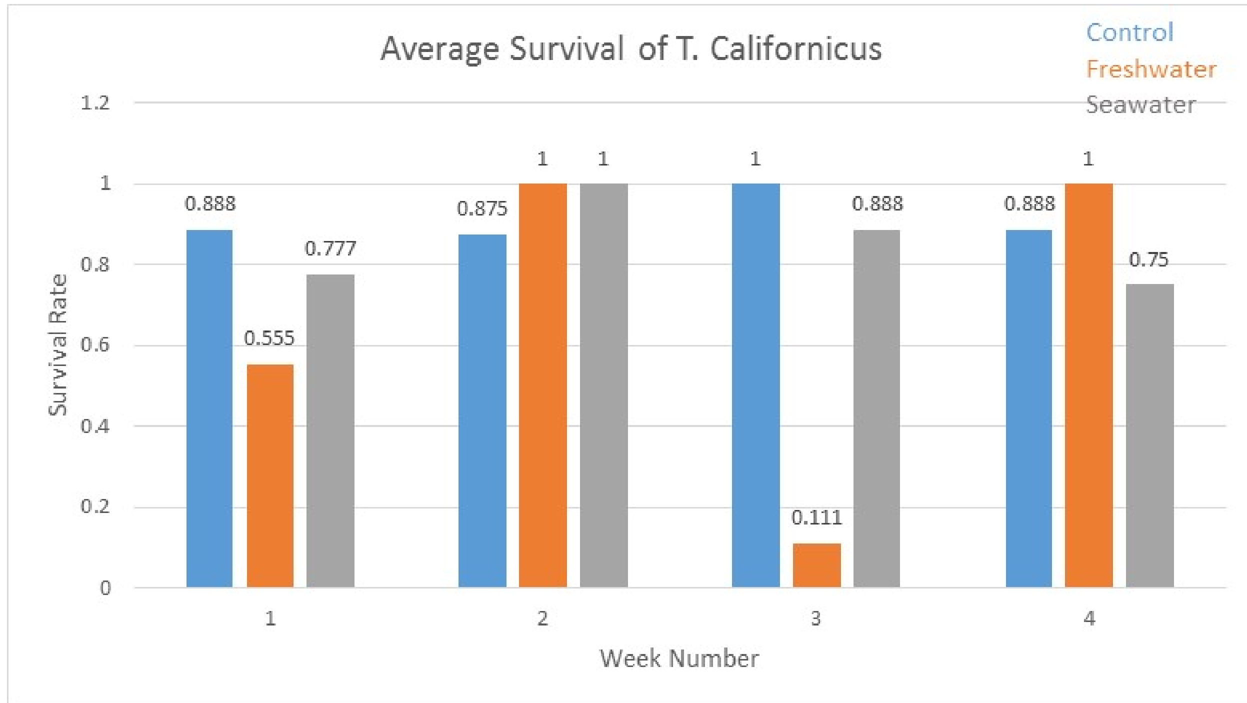

Figure one. Average survival rate of T. Californicus per week.

M1: Control Group M2: Freshwater M3: Seawater	Std. Dev. M1: .8165 M2: 2.3094 M3: .8165	Variance M1: .6667 M2: 5.333 M3: .6667
M1: Control Group M2: Freshwater M3: Seawater ■ M1 vs M2 P<.01 ■ M1 vs M2 nonsignificant ■ M2 vs M3 P<.05	Std. Error M1: .4082 M2: 1.1547 M3: .4082	P- Value .002460

Figure two. Statistical analysis/ summary of the date.

Trial One

Control Group: Freshwater: Seawater:

Well Plate Number	Alive or Dead (Week one)	Alive or Dead (Week two)	Well Plate Number	Alive or Dead (Week one)	Alive or Dead (Wee k two)	Well Plate Number	Alive or Dead (Wee k one)	Alive or dead (Week two)
1	alive	alive	1	alive	alive	1	dead	
2	alive	alive	2	dead		2	alive	Alive
3	alive	alive	3	alive	alive	3	alive	Alive
4	alive	alive	4	alive	alive	4	dead	
5	alive	alive	5	dead		5	alive	Alive
6	alive	alive	6	dead		6	alive	Alive
7	alive	alive	7	dead		7	alive	Alive
8	inconsistent		8	alive	alive	8	alive	Alive
9	alive	inconsistent	9	alive	alive	9	alive	Alive
Total	8/9	7/8	Total	5/9	5/5	Total	7/9	7/7

Trial Two

Control Group: Freshwater: Seawater:

Well Plate Number	Alive or Dead (Week one)	Alive or Dead (Week two)	Well Plate Number	Alive or Dead (Week one)	Alive or Dead (Week two)	Well Plate Number	Alive or Dead (Week one)	Alive or Dead (Week two)
1	alive	alive	1	dead		1	alive	Alive
2	alive	alive	2	dead		2	dead	
3	alive	alive	3	dead		3	alive	alive
4	alive	Alive but inconsistent	4	dead		4	alive	alive
5	alive	dead	5	alive	alive	5	alive	dead
6	alive	alive	6	dead		6	alive	alive
7	alive	alive	7	dead		7	alive	alive
8	alive	alive	8	dead		8	alive	alive
9	alive	Alive but inconsistent	9	dead		9	alive	dead
Total	9/9	8/9	Total	1/9	1/1	Total	8/9	6/8

Figure three. Weekly data collection for trials.

Discussion:

We originally hypothesized that the salt-water algae would provide the most sustainable source of food for our copepods. However when comparing the results we found that our control group, the fish food substance, provided the lowest mortality rate of our copepods. Because our results showed that the fish food was the most sustainable food source this could become a problem. In the experiment conducted by M. Koski and M. Klein Breteler (2003) they highlight the importance of cell count within the provided species of algae. While conducting our trials we observed a lower presence of the freshwater algae based on color, murkiness, and absorbency. Based on these factors we can conclude that the saltwater algae and the fish food had a higher concentration thus a higher cell count, and therefore more nutrients for our copepods. This is supported in Koski and Breteler's experiment since in their research, higher concentrations of the algae provided a lower mortality rate in comparison to lower concentrations. In another article by P. Thor, he discusses that in their experiment the algae did not provide the proper nutrients due to "the lack of longer chain fatty acids" (2002). This could have led to the algae in general not providing sufficient nutrients for our copepods. The freshwater algae could have provided the highest mortality rate due to the saltwater environment the copepods were housed in.

The saltwater that the freshwater algae was placed in could have shocked the algae and could have been toxic, in turn killing the algae and decreasing the nutritional composition of it as a food source.

Sources of Error:

Throughout our trials, numerous factors could have affected our results. One of which being the fact that there were already copepods in the algae stock solution. Since the copepods were already exposed to a certain type of algae they may been shocked when their food supply was changed dramatically. There were also Nauplius within the well slots where an original adult female were present, which led to inconsistent data. Towards the end when we were collecting new copepods to observe there were only copepods in their early developmental stages available for use. This limited variance within the sample and limited sufficient resources.

Future Experiments:

For future experiments, we would like to test a brand new variation of food sources, such as fish food, other types of algae, and other factors. We would also like to increase our sample size and trial length dramatically, whcih would provide us with more accurate results that would allow us to draw more precise and specific conclusions based on our results. We would also like to have access to freshwater solution and freshwater copepods to limit sources of error on future experiments that we found in our current experiment.

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Appendix H: Modified Version of AAC&U's VALUE Rubrics

Inquiry & Written Communication Rubric

	Capstone (3 pts.)	Milestone (2 pts.)	Benchmark (1 pt.)
Topic Selection	Identifies a focused and manageable topic that ***addresses potentially significant yet previously less-explored*** aspects of the topic	Identifies a topic that, while manageable, is too narrowly focused and leaves out relevant aspects of the topic (i.e., topic is not appropriately addressed)	Identifies a topic that is far too general, inappropriate for the structure of the CURE, and/or is not feasible to conduct given available resources
Existing Knowledge, Research, and/or Views	Synthesizes in-depth information from relevant sources representing various points of view/approaches	Presents information from relevant sources (rather than synthesizes), and the information presented is not comprehensive	Presents information from irrelevant sources representing limited points of view/approaches (i.e., incomprehensive info)
Design Process	All elements of the methodology are skillfully developed and appropriate for the proposed experiment(s)	Critical elements of the methodology are missing, incorrectly developed, unfocused, and/or inappropriate	Inquiry design demonstrates a misunderstanding or lack of methodology appropriate for the proposed work
Analysis	Organizes and synthesizes evidence to reveal insightful patterns, differences, or similarities related to the team's central question/hypothesis	Organizes evidence to reveal insightful patterns, differences, or similarities related to the team's central question/hypothesis; ***little or no synthesis is present***	Lists evidence, but it is not organized and/or is unrelated to the team's central question and hypothesis
Conclusions	States a conclusion that is a logical extrapolation from the inquiry findings. ***Conclusion should be comprehensive***.	States a conclusion that is focused ***solely*** on the inquiry findings. The conclusion arises specifically from and responds specifically to the inquiry findings.	States a conclusion that is ambiguous, vague, illogical, unsupported, or (in general) poorly developed
Limitations and Implications	Insightfully discusses in detail relevant and supported limitations and implications	Presents relevant and supported limitations and implications; ***limited discussion***	Presents limitations and implications, but they are possibly irrelevant and unsupported
Context/Purpose for Writing	Report is appropriately targeted to a scientific audience	Report is targeted to a scientific audience but exhibits instances in which non-scientific conventions (e.g., language that is too colloquial) are used	Report demonstrates minimal attention to audience, and, as such, is not appropriate given the assigned task
Syntax/Mechanics	Demonstrates expert command of language that skillfully communicates meaning to readers with clarity and fluency; document is virtually error-free	Uses straightforward language that generally conveys meaning to readers. Document contains only a few syntax/mechanics errors	Use of language is crude and/or sometimes impedes meaning because of a high number of syntax/mechanics errors

Quantitative Literacy Rubric

	Capstone (3 pts.)	Milestone (2 pts.)	Benchmark (1 pt.)
Interpretation *Ability to explain information presented in mathematical forms (e.g., equations, graphs, tables)*	Provides accurate explanations of information presented in mathematical forms. Makes appropriate inferences based on that information. *For example, accurately explains the trend data shown in a graph and makes reasonable predictions regarding what the data suggest about future events***.**	Provides somewhat accurate explanations of information presented in mathematical forms, but occasionally makes minor errors related to computations or units. *For example, accurately explains trend data shown in a graph, but may miscalculate average/error*.	Attempts to explain information presented in mathematical forms, but draws incorrect conclusions about what the information means. ***OR*** No interpretation is provided.
Representation *Ability to convert relevant information into various mathematical forms*	Skillfully converts relevant information (e.g., data) into insightful mathematical portrayals in a way that contributes to a further or deeper understanding	Competently converts relevant information into an appropriate and desired mathematical portrayal	Completes conversion of information, but the resulting mathematical portrayal is inaccurate, inappropriate, and/or incomplete
Calculation	Calculations attempted are essentially all successful and sufficiently comprehensive to solve the problem. Calculations are also presented elegantly (clearly, concisely, etc.).	Calculations attempted are essentially all successful, but are not presented elegantly and/or are "incomplete" in minor aspects (e.g., no post-hoc tests corresponding to an ANOVA)	Calculations are attempted but are both unsuccessful and are not comprehensive
Application/Analysis *Ability to make judgments and draw appropriate conclusions based on the quantitative analysis of data, while recognizing the limits of this analysis*	Uses the quantitative analysis of data as the basis for deep and thoughtful judgments, drawing insightful, carefully qualified conclusions from this work	Uses the quantitative analysis of data as the basis for competent judgements, drawing plausible conclusions from this work	Uses the quantitative analysis of data as the basis for tentative, basic judgments, although is hesitant or uncertain about drawing conclusions from this work
Assumptions	Explicitly describes assumptions and limitations and ***provides a rationale for why they are appropriate***; shows awareness that confidence in final conclusions is limited by the accuracy of assumptions	Explicitly describes assumptions; limited or no rationale is provided as to why these assumptions are appropriate given the context of the team's research	Assumptions and limitations are listed, but no "interpretation" is provided ***OR*** No assumptions are discussed
Communication	Uses quantitative information in connection with the argument or purpose of the work, presents it in an effective format, and explicates it with consistently high quality.	Uses quantitative info, but does not effectively connect it to the argument/purpose of the work	Presents an argument for which quantitative evidence is pertinent, but does not provide adequate explicit numerical support. (May use quasi-quantitative words such as "many," "few," "increasing," "small," and the like in place of actual quantities.)