**Paramecium Behavior: Focus on Experimental Design and Results**

Prior to coming to lab for Week 1, you should print both the lab and the Case Study. Read the entire lab and then, using the introductory information, complete the Case Study questions (provided as a separate handout) and write a 100-200 word summary of what you will be doing in lab the first week (space provided on the Case Study handout).

Prior to coming to lab for Week 2, you need to write a pre-lab summary (100-200 words) and complete a Source Information assignment. See your TA for details about submission of these assignments.

**At the end of the laboratory you will be able to:**
1. identify elements of experimental design in an example case study.
2. describe ways to evaluate online source material.
3. design appropriate experimental procedures that investigate *Paramecium* behavior.
4. collect and analyze data from your group-designed experimental procedures.

**Introduction**

Biology is the science that studies living organisms and their interactions with each other, and their environment. Living organisms have the following features: order; sensitivity; ability to use and transform energy; evolutionary adaptation; growth, development, and reproduction; regulation; and homeostasis, the maintenance of a relatively constant internal environment. While living organisms share basic characteristics, they are also very diverse. Living organisms come in a wide variety of shapes and sizes, and by some estimates there are well over 5 million different organisms on earth!

As you might suspect, biologists study life in a variety of different ways. In our own UVM Biology department, scientists are investigating Chagas disease, fluctuations in mice populations in Vermont, spatial patterns in African savannah communities, signal transduction mechanisms during brain development, or the importance of competition in ecology, as a few representative examples.

These diverse activities share a common desire to understand the natural world through the process of science. In fact, the word *science* comes from the Latin word meaning “to know.” Science is a way of knowing that uses objective information to construct an understanding of the natural world (Moore, 1993). As we have discussed, there is no rigid, single “scientific method,” rather there are common approaches scientists use to study life. Biologists make observations, raise questions, construct hypotheses (possible explanations to explain these observations), conduct experiments to test their hypotheses, interpret results, draw conclusions, and pose further questions.

Each discovery in biology leads to further questions, making scientific inquiry a continuous and self-correcting process. Recently, the development of new techniques and innovative experimental approaches has yielded a phenomenal amount of new knowledge. We must remember that biology is not just a compilation of facts to memorize. It is a creative, imaginative, intuitive, and social endeavor.

The best way to learn how biologists do their work is to do it yourself. Experience is the best teacher! In this lab, you will explore the power of the scientific method to learn about living organisms. You will take the elements of the process of science discussed during lecture, and explored in the pre-lab...
activity, and apply them to an investigation of Paramecium behavior. During the next two weeks you will work your way through every part of the scientific process. Have fun!

**Essential elements of experimental design**

Scientists observe the natural world and pose questions. A possible explanation for a given observation is termed a **hypothesis**. A good hypothesis is in the form of a statement, is testable, and is useful only if data could be collected to reject it. We cannot ever completely prove a hypothesis to be true or false. As a result, a crucial step in the scientific method is to design an experiment that allows us to clearly support or reject the hypothesis. This is one of the biologist's most challenging and creative tasks. Biologists spend considerable time reading the scientific literature and critiquing other experiments before undertaking their own work. In designing a good experiment, scientists must define the variables, outline a procedure, and determine controls.

When designing an experiment to test a hypothesis, it is essential to identify and carefully consider the **variables**. Variables are the factors that may change during an experiment and they must be clearly defined and measurable. Variables fall into one of three categories.

- **Dependent** – This is the variable that the researcher actually measures, counts, or observes. The DEPENDENT variable is what the researcher thinks will change in response to the experimental treatment.
- **Independent** – This is the variable that is intentionally changed by the researcher. An INDEPENDENT variable is selected that the scientist thinks will affect the dependent variable.
- **Controlled** – These variables are held constant between each group. By keeping CONTROLLED variables equal, this helps to prevent these factors from influencing the dependent variable.

The **procedure** refers to the actual method or particular series of steps used to conduct the experiment. When designing the procedure it is important to consider the presence of a control group (where the independent variable is zero or constant), the level of treatment (magnitude of the independent variable), the number of replicates (how many times the experiment will be repeated) and the sample size (what portion of the whole will be tested).

Following the development of a hypothesis and a set of methods, biologists make **predictions** about the effect of the independent variable on the dependent variable. Predictions can be expressed in the form of if/then statements. (General form: If the independent variable is X and an experiment is conducted in which X is varied, then the dependent variables will be affected in this way.) In addition, predictions are useful for evaluating the experimental results. If the results do not match your prediction then the hypothesis is rejected. If the results match, then the hypothesis is supported.

**Bibliography**

Before conducting experiments to address a question, it is important that scientists look for information already known so their investigations can build upon pre-existing knowledge. Therefore, you will need to collect background information about *Paramecium* before making observations about the organism, and designing an experiment that investigates the response of *Paramecium* to its environment.

Spend some time on the internet looking for information about *Paramecium*. Consider the following questions as you search:

- What is a *Paramecium*?
- What does the organism look like?
- Where does a *Paramecium* live?
- What does a *Paramecium* eat?
- Do these organisms move? If so, how?
- How do they respond to their environment? (e.g. chemicals, temperature, pH)

Choose a browser to use. What search term(s) or phrase(s) did you use? Search at least twice to compare the results. Write your search terms or phrases below.

1. 

2. 

Then, in the space below, summarize the answers to the above questions and indicate where you found the information (webpage address OR location of website).
What additional information did your classmates discover?

When gathering background information, it is important to consider the reliability of the sources you are using for your information. Spend time in your group discussing the answers to the following questions and write down your responses in the space provided.

1. How reliable is the information you wrote down for the above questions?
2. Are some websites more reliable than others?
3. What factors might help you judge the reliability of the internet sites you visited for information?
Investigating *Paramecium* Behavior: Designing Your Own Experiments – Week 1

1. **Make Observations**

You will be working in groups of two (2). First, familiarize yourself with the function of the two different types of microscopes you have for use. After that, you will prepare slides to observe *Paramecium*. You should take your time watching them, and noting their behavior. How do they move? Do they interact with each other? Get to know them! The techniques you will use to observe the *Paramecium* are outlined below – please read through them ALL (including the “important details” list at the end) before you begin anything!

**Stereo (dissecting) microscopes** are used to examine surface features of solid objects, and illuminate with reflected light, not transmitted light. They magnify objects between 10x and 75x, and provide a 3D image to the viewer. Typically, the magnification is a zoom type that allows smooth adjustment of any magnification within the range available.

**Compound microscopes** are used to view very small objects, and are capable of magnifications up to 1000X. They utilize light that is transmitted through the specimen to illuminate the interior. Compound microscopes must be used with glass slides and cover slips over the specimens to be viewed. The slide is moved around under the lens by a finely adjustable mechanical stage, and the magnification is changed by selecting a particular objective lens.

The following procedures are a general guide to how you can observe the *Paramecium* provided. These techniques can be modified as you see fit when you become more familiar with the process, and your needs change for the experiments you design.

**To view *Paramecium***:

Clean a glass slide using a lab wipe and a little 70% ethanol from the squirt bottle. Take a Pasteur pipet (and bulb) and your slide, to the rack containing the *Paramecium* culture tube. Give the culture tube a light shake, and using the pipet, place a small amount of the culture on the slide. Use your stereo microscope to observe the *Paramecium*.

**For a closer look**:

Place a coverslip on the drop of *Paramecium* on your slide. Wipe up any excess liquid that spills out from under the coverslip with a lab wipe. Make sure that your compound microscope is adjusted to the lowest power (4x) and place the slide on the stage. Using both course and fine focus adjustments, focus the scope on the specimen being careful that the coverslip does not contact the objective lens (focus too close)! Use the mechanical stage to scan the slide and look for *Paramecium*. When you have focused on the organisms, you can switch to a higher magnification for an even closer look. Be aware that as you increase magnification, your field of view (area you can see) becomes smaller, and the *Paramecium* may not stand still for your viewing pleasure! After you switch magnifications you should only need to use the fine focus adjustment – no more coarse focus adjustments!

**To expose *Paramecium* to chemical solutions**:

To obtain solutions to experiment with, you will use a mechanical pipette (or pipettor) to take a small aliquot (a 0.5 ml sample) from the stock bottle, and place it into a new clean micro centrifuge tube. Be sure that you use a new, clean tip on the pipettor every time you put it into a stock bottle! You do not
want to cross contaminate solutions! The pipettor should be adjusted to deliver 500 µl, or 0.5 ml, which should be plenty of solution for your experiments.

To observe the Paramecium with the chemicals, you should only use the stereo microscope and a slide with no coverslip. You will place a small amount of the Paramecium culture on a slide as you did before using a Pasteur pipet. You want to be able to add a small drop of chemical solution to the drop of Paramecium, and observe their behavior. To add chemical solutions to the slide, use the pipettor to add small drops as needed. If you are careful, you can watch the Paramecium under the microscope as you drop the solutions onto the slide. You may notice that when you add the drop, a lot of turbulence is created, and the Paramecium are tumbled around. You can avoid this if, instead of a round drop of Paramecium culture on the slide, you make a line of culture solution on your slide. You can then add chemical solutions to one end of the line and not disrupt the Paramecium as much. You can also place a drop of chemical solution on the slide right next to the Paramecium, and then connect the drops carefully with the tip of a pipettor.

Important Details!

- Please use one glass Pasteur pipet for transferring Paramecium for your experiments, and then dispose of it appropriately. Keep it clean between uses so that you do not contaminate the tubes of Paramecium culture. If you do need a new pipet though, get one.
- Be sure to use clean tips on the pipettor, and dispose of the dirty tips appropriately.
- Keep all the solution bottles, and culture tubes closed between usage.
- Wipe up any liquids you spill on the microscope stage using the lab wipes, and dispose of them appropriately.
- Clean your slides with 70% ethanol between uses, and finally at the sink when you are done with them.
- Dispose of everything, except glass pipets, in the waste bins provided. Waste glass pipets must be disposed of in the glass bin provided.

2. Develop Hypotheses
Discuss your observations of Paramecium behavior with your lab partner. Based on your observations, come up with TWO hypotheses about Paramecium behavior in response to environmental stimuli. RECORD both hypotheses on the Lab Report page.

3. Present your observations with your peers
Biologists share their findings with the scientific community. There are many ways to communicate—giving talks or presenting posters at professional meetings, participating in conferences, and writing papers for journals. In your group, put together a short oral summary that includes both your observations and your hypothesis. Share this summary with the class during group discussion time.

4. Modify hypothesis
Choose one of your two hypotheses that you will test in week #2. Modify this hypothesis, if needed, based on any new information you learned about Paramecium behavior during class discussion time.
Investigating *Paramecium* Behavior: Designing Your Own Experiments – Week 2

5. **Design an Experiment**
Design an experiment to test your hypothesis and write your detailed methods on the lab report page for Week 2. You will have available all the solutions and equipment that were available the first week. **Requests for something specific (within reason) for your experiment during Week #2 must be told to your TA before you leave the first week of lab, and we will try to accommodate you.** Consider what kind of data can be collected. For instance, can you quantify *Paramecium* behavior in some way? In addition, consider the following elements of experimental design and identify them in your methods section, if appropriate: dependent variable, independent variable, controlled variable(s), number of replicates, and magnitude of treatment. Prepare a table or chart to organize your data. Remember that a good experiment can be repeated.

Once you have drafted a method for testing your hypothesis, check the design with your TA.

6. **Make a Prediction**
Make a prediction based on your hypothesis and experimental design. Phrase your prediction in an if/then form and record it on the Lab Report page.

7. **Collect Data**
Conduct your experiment following the procedure that you developed. Collect data from each test and record it in the table or chart you prepared in the last step. Record the results of all trials on the Lab Report page—do not eliminate any data!

8. **Analyze Results**
Examine your data and consider various ways to present the results. Some suggestions for data analysis include: calculating an average for each condition, providing a range for each condition, graphing the results, or preparing a summary chart. Use extra paper or a computer to assemble your results.

9. **Draw Conclusions**
Based on your analysis of the results, reconsider your original hypothesis and prediction. Do you accept or reject your hypothesis? Explain why on the Lab Report page.
Observations and methods:

Hypothesis:

Peer observations:

Modified hypothesis:
Experimental Design:

Description –

Dependent variable –

Independent variable –

Controlled variable(s) –

Number of replicates –

Sample size –

Data collection (table or graph):
Analyze results (written description):

Draw conclusions:

Do you accept or reject your hypothesis? Why?