

Exercise 1  
**An Introduction to Scientific Methodology**

*Substitute the exercise that follows for Part 1.3 Designing an Experiment  
in Lab Topic 1: Scientific Investigations*

**Run-down of the assignment:**

**In class:**

Read the entire lab contained in the two files `Exercise_1_Scient_Invest.pdf` and `Exercise 1 Supplemental.pdf`. The supplemental file serves to replace Exercise 1.3 in the Lab exercise entitled **Lab Topic 1: Scientific Investigations** (`Exercise_1_Scient_Invest.pdf`). The supplemental file contains several pre-lab questions on page 1s-3 that you should answer prior to class (see below). These are for discussion purposes. The questions on pages 1-20 to 1-23 in `Exercise_1_Scient_Invest.pdf` should be reviewed and answered on your own in preparation for the **quiz on Friday**.

**After class:** Answer and submit all questions found in the **Practicing Experimental Design** section (pages 1-23 to 1-27 in the file, `Exercise_1_Scient_Invest.pdf`; please type out your answers) and questions 1 through 4 on page 1s-6 in the section entitled **Content Questions: Analyzing your results** in the file `Exercise 1 Supplemental` (see below). Turn these in on Friday.

### Exercise 1.3

#### A. Introduction to the specific problem

Life responds to its immediate environment in order to enhance its survival. For example, plants move toward light and many grow upwards, away from gravity. Animals often make complex responses to environmental stimuli that include locating food, migrating to more suitable habitats, moving away from light, and seeking mates. Many organisms have developed a variety of sensory organs that allow them to discern significant environmental cues and respond accordingly. You are probably familiar with the ways macroscopic organisms sense their environment. They use their sensory organs to see, smell, hear, taste, or touch their environment. But how does a microscopic organism, one that doesn't have eyes or a nose or ears, sense its environment? In today's exercise we will look at whether a single celled organism, *Paramecium* (Figure 1), can respond to external stimuli such as food, light, magnetism, heat and so forth.



**Figure 1.** A scanning electron micrograph image of *Paramecium*. *Paramecium* is a ciliated protozoan covered with hairlike projections known as cilia. The cilia beat as a means of locomotion. Each cilia is a complete motile apparatus containing a complex arrangement of microtubules, linking proteins, and the motor protein known as ciliary dynein. These work together to bend the cilia.

## **B. Discussion questions**

1. List some of the more common sensory responses that humans make, such as responding to food aromas, listening to music, and so forth. To which do you think a single-celled organisms could respond?
2. How does the ciliate's lack of the complex sensory organs found in higher animals influence the complexity of stimuli to which they are able to respond?
3. Once a signal is received, how do you imagine the information is transmitted to the cilia to have them speed up, slow down, or reverse direction?

## **C. Constructing an hypothesis and a set of predictions**

*Observation:* Paramecium are not uniformly distributed in a pond. They tend to be found near vegetative material or near the bottom of the pond. Since they feed on decaying matter and bacteria this seems to make sense. But given your answers above, how do you think this uneven distribution comes about? This question will form the basis for your hypothesis, that is, a possible explanation for an observed phenomena.

*Constructing a hypothesis:* What are the possible explanations for the phenomena described above? (a simple statement as to how they 'know' where to go)

*Method for testing your hypothesis:* On the next few pages, we present a detailed procedure for testing your hypothesis. Briefly, a very small glass tube will be used to make a chamber, called the chemokinesis testing apparatus, to hold *Paramecium*. Each end of the chamber will consist of an agarose plug, which may or may not also contain a bacterial food source (see Figure 2 below).

*Stating your predictions:* Above you proposed an hypothesis about why *Paramecia* are not randomly distributed in a pond. Your hypothesis will lead to certain **predictions** when you use the chemokinesis testing apparatus. Based on your hypothesis, what are your **predictions** about the distribution of *Paramecium* in the testing device under the following situations?

If plain agar plugs are at both ends of the chamber.

If a plain agar plug is at one end, and an agar plug containing bacteria at the other.

Any experimental manipulation should be compared to some other group (presumably not manipulated). In the above experiment which is the control and which is the experiment?

#### D. Testing your hypothesis: The experiment

**Objective:** To describe and analyze adaptive sensory mechanisms in ciliates.

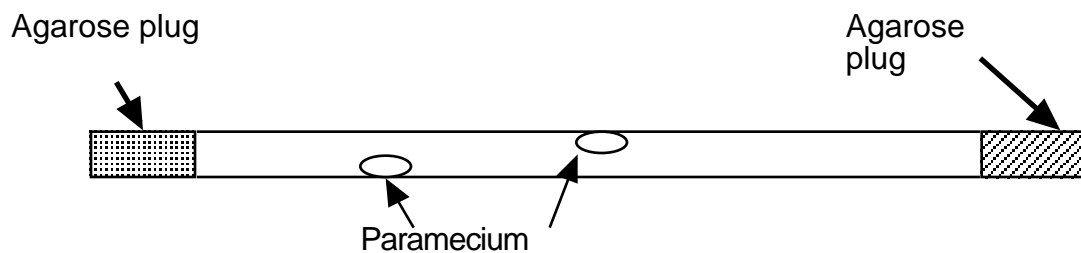
##### Materials:

Stereoscopic microscopes (12)	12 black sharpies
deionized water (500 mLs)	100 $\mu$ L capillary pipettes (1 container)
1 box of Pasteur pipettes	12 tan bulbs for Pasteur pipettes
6 P200 pipetmen	3 boxes of yellow tips
24 nine-hole depression slides (preferably sterile)	3 files
3:1:1 resting solution (3 mM HEPES (pH 7.2), 1 mM CaCl <sub>2</sub> , 1 mM KCl)	
3% Agarose plates made using 3:1:1 resting solution	
3% Agarose plates made using 3:1:1 resting solution + <i>Klebsiella</i> inoculated Wheatgrass (50% 3:1:1)	
Culture: <i>Paramecium tetraurelia</i> washed once in 3:1:1 resting solution	

##### Procedure for constructing a testing unit:

1. Obtain capillary pipettes of sufficient size to comfortably hold *Paramecium* cells (so they can turn around). A capillary pipette of 100 $\mu$ L size should be sufficient. You may find that breaking the pipette in half will work better. Use a file to score the pipette, wet the score mark with a wet finger and break along the score. Try to keep all experimental pipette chambers at about the same size.
2. Remove a small aliquot of *Paramecium* cells and place them in one of the wells of a 9-hole depression slide. Observe the *Paramecium* and determine whether 100  $\mu$ L will provide a reasonable number of cells to work with (between 20-100 cells). How can you determine this? What would you do if there are too many cells in 100  $\mu$ L to work with comfortably? (If you decide to dilute the cells you should do so in 3:1:1 Resting solution; see above for composition).  
*Food for thought:* How would you concentrate the *Paramecium* if there are too few per 100  $\mu$ L?

3. Add about 20-100 *Paramecium* cells in a sufficient volume to completely fill the capillary pipette. Are air bubbles of concern? What should you do if you see air bubbles? Consult with either the TA or the professor if you are unsure.
4. On the back table will be two types of agarose plates. **Plate A** contains 1% agarose in 3:1:1 resting solution (3 mM HEPES Buffer; 1 mM KCl; and 1 mM CaCl<sub>2</sub>). **Plate B** contains the same with the addition of bacterial cells (*Klebsiella*). These will be used later to test if *Paramecium* can respond to the presence of food.
5. Take the capillary pipette and stab it into plate A to plug up one end of the pipette. Once you have plugged one end, you should use a thin wire or pipette tip to push that end in further so as to create a space behind it. This will allow you to add the second plug without losing the first plug (it will push the first end back to where it was approximately).
6. You will now plug up the other end of the capillary pipette by inverting **Plate A** and firmly pressing it on the capillary pipette to plug up the hole (see Figure 2). Your *Paramecium* are now completely sealed (you hope). You may have to experiment in order to find the best way with which to seal the second pipette end.



**Figure 2.** The chemokinesis testing apparatus. The *Paramecium* will be contained within the pipette by the two agarose plugs at either end. A trick to try - after putting one plug in, push it into the pipette using the pipette tip or a thin wire. Now when you insert the second plug it will not push the first all the way out of the pipette.

**NOTE: You will have plugged both ends of the pipette with the same material (plate A). Would you expect to see a difference in the distribution of *Paramecium* cells within the testing apparatus? Why or why not?**

7. Place an inverted petri dish upon the stage. This will provide insulation from the warm stage on your microscope. Place the capillary pipette upon the inverted dish and observe it under the dissecting microscope.
8. Divide the pipette into two equal sized sections using a sharpie. Let the pipette rest horizontally for 15 minutes and then carefully observe how many cells are in each section. Record your data.
9. Your next assignment will be to determine if the *Paramecium* cells can sense a food source and selectively orient themselves so that they are near it or away from it. Set up an experiment that will allow you to test this hypothesis.
10. An important concept in science is that experiments should be reproducible. What does that mean and why is it important? You should repeat your experiment at least three times.

11. *Food for thought:* What other materials or physical parameters could you test to see if *Paramecium* might respond to them? How might you sort out which are interesting to try and which are not?

### E. Content Questions

**Analyzing your results.** (Submit your type-written answers to the following questions.)

1. The data you have collected should be summarized. Why? In what ways can you think to summarize your data?
2. The mean and the standard deviation are useful summary statistics for scientific data. Consult Pechenik for instructions on calculating these statistics. Summarize your data as a mean and standard deviation.
3. Record your results in a table form on a separate piece of paper. Remember, a table has a title and footnotes but no legend. Exercise 1.4 has some very good advice on how to construct a table. You may also want to refer to Pechenik for additional guidelines.
4. Briefly describe your analysis of the results. Do you think your results are significant? How could you rule out a series of "lucky or unlucky breaks"? In order to draw conclusions from data you often have to do additional statistics. For this exercise we are only asking that you perform the summary statistics described above. If you were to perform the kind of statistics appropriate for this kind of data, you would perform a *Chi-square test*. This test is described in the appendix, *Chi-square test primer* and will be used in a later experiment.

### F. Your assignment.

1. You will be responsible for answering the questions 1 through 4 above and the questions (1 through 5) found on pages 1-23 (starting with **Practicing Experimental Design**) to 1-27 in *Lab Topic 1: Scientific Investigations*.

**Please type out your answers to this part and number them as they are numbered in the lab manual.**

### References

Material was borrowed from the following source:

Leick, V. 1997. Chemosensory and Behavioral Adaptation. *in* "**Explore the World Using Protozoa**" ed. by Anderson, O. R. and Druger, M. Arlington: National Science Teachers Association and the Society of Protozoologists.