INTRODUCTION TO THE SCIENTIFIC METHOD

OBJECTIVE:

In this lab you will be introduced to the scientific method by investigating the effects of drugs (alcohol and caffeine) on the heartbeat rate in *Daphnia magnus.* By the end of this lab you will also learn the basic skills needed to use a dissection microscope.

INTRODUCTION:

Biology is more than the description of life forms. It is a dynamic, and systematic field whose aim is to unravel the mysteries of life and our environment. Throughout history humans have been intrigued with the world around them. They have observed natural phenomena and wondered "why?". All scientists have certain traits in common, whether it was Aristotle 2,000 years ago or today's molecular biologists. Scientists have inquiring minds and great powers of observation, and will use a systematic approach for testing the unknown.

In this course you will have the opportunity to develop your potential skills as a scientist. The laboratory exercises are designed to stimulate your thought, heighten your powers of observation and, introduce you to the scientific method. You may decide to pursue a career in science after this course.

The **scientific method** is neither complicated nor unique to science. It is a powerful tool of logic that can be employed anytime a problem or question about the fundamental nature of something arises. In fact, we all use elements of the scientific method to solve every day problems and questions. We accomplish this so quickly and automatically that we are not conscious of the methodology. In brief, the scientific method consists of **observation**, **prediction**, **testing** and **interpretation**. All research begins with a fundamental **question**.

In this lab we will ask two questions: How is the heartbeat rate affected by caffeine? How is the heartbeat rate affected by alcohol?

OBSERVATIONS:

Observation is the most basic element of the scientific method. All biological knowledge is based on situations in which an individual made an observation of a particular event and recorded that observation. Scientists can rely on just their own sense organs, or they can use technological aids that extend their perceptual limits. These aids may be gel electrophoresis to see protein molecules, microscopes to see the very small and spectrophotometers to observe wavelengths of light.

You will base today's experiment on observations of our lifestyles. You have probably observed that when people drink too much coffee they are often hyperactive. They may be jittery, nervous, and may complain of not being able to relax. On the other hand, when people drink alcohol, their speech often slurs, they lose control of muscular coordination, and their reactions may slow down. Too much alcohol may cause them to pass out and loose consciousness.

HYPOTHESIS:

The next step in the scientific method is to make an “educated guess” or **hypothesis**, based on your observations and your knowledge of the material. A possible hypothesis as the answer to the above question could be that alcohol causes a decrease in heart rate, whereas caffeine causes an accelerated heart rate.

DESIGNING AN EXPERIMENT:

The next step in the scientific method is to design a **controlled experiment.** You will use a living water flea *Daphnia magna* to test your hypothesis. You must identify an organism by its proper scientific name so that other scientists know what you are talking about. Something is not a ”bug” simply because it is small. The advantage of studying *Daphnia* is that it is nearly transparent. You can observe the heart beating, the squeezing action of the intestine, muscular movements and occasionally babies in the brood pouch. Also, because *Daphnia* is a small, aquatic organism, it makes an excellent subject for studying the effects of drugs on the circulation system.

For the design of your experiment, you will record the heartbeat rate after adding drops of increasing concentrations of caffeine, then drops of increasing concentrations of alcohol. You will also perform a **controled** experiment by determining the heart rate of a Daphnia in water.

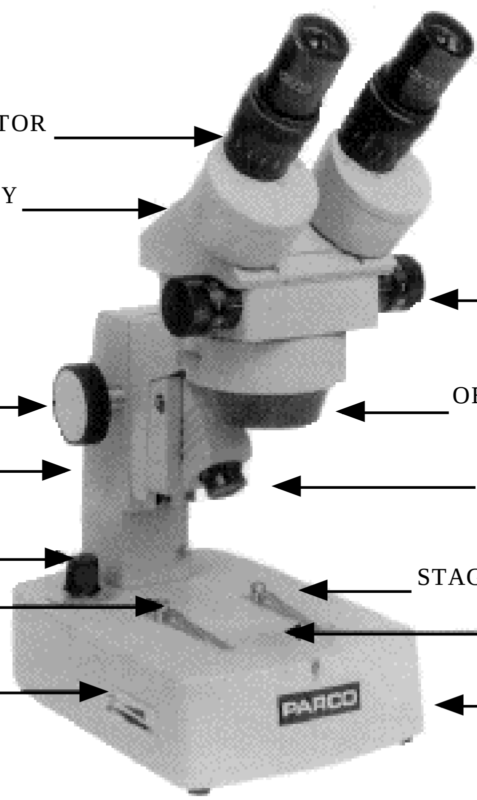
In order to observe water fleas, you will need to examine them at a greater magnification than you could observe with the naked eye. To achieve this magnification you will be using a dissecting microscope. You will need to become familiar with the parts of this microscope.

THE DISSECTION MICROSCOPE:

In todays lab you will be using the dissecting microscope. Despite their sturdy appearance, all microscopes are delicate, precision instruments. They should be handled carefully and with common sense. The following suggestions will help you avoid some common mishaps.

**To avoid dropping the microscope or banging it against a laboratory bench, carry the microscope in an upright position using both hands.
**When carrying the microscope, place one hand on the base and the other hand around the arm
**DO NOT PLACE THE MICROSCOPE IN AN UPSIDE DOWN POSITION. PIECES WILL FALL OUT.
**Keep microscope away from the edge of the bench, particularly when not in use.
**Make sure power cords are out of the way.
**Never force the microscope parts to work.
**Never dismantle the microscope.
**Use lens cleaners and paper both before and after use.
**Take all slides off the stage prior to storage.
**Always store the microscope in low power objective.
**Place cover over the microscope after each use.
**Bring all defective microscopes to your instructors attention.

PARTS OF THE DISSECTING MICROSCOPE:



Oculars: The oculars or eyepiece are the lenses you look through. As with a pair of binoculars, you can adjust the 2 oculars to match the distance between you eyes. Oculars of different microscopes may have different magnifications; usually the magnification is imprinted on the ocular.

Dioptor: The diopters are used to focus the oculars in conjunction with each other to give equal sharpness for both eyes.

Body: The body is the portion of the microscope between the upper optical element and the objective.

Zoom Control: The zoom knob will allow you to change the amount of magnification needed.

Objective: The objective is the lower component of the optical system. Responsible for initial magnification and resolution.

Focus Knob: Use the foucs knob to bring the sample or specimen in focus. The body will move up or down to bring the specimen into focus.

Stage: The stage is the rectangular platform of the microscope where the specimen to be viewed is placed.

Stage Clips: These clips will hold the slide in place if needed.

Arm: The is the frame of the microscope that supports all component parts above the base. This is also used as a "handle" to hold the microscope.

Base: The base is the stand that rests on the table surface and supports the instrument. This also is another "handle" when transporting the microscope.

Variable Intensity Switch: This is the means for controlling the amount of light that is used to illuminate your specimen.

SHOULD YOU WEAR YOUR GLASSES WHEN USING A MICROSCOPE?

Yes and no. If you are nearsighted or farsighted, there is no need to wear your glasses. Remember your are focusing the microscope to your eyesight and this will compensate for any "human" focusing problems. If you have an astigmatism, wear your glasses since the microscope will not correct for this problem.

FOCUSING OF THE DISSECTING MICROSCOPE:

The whole point in using a stereoscopic microscope is that, when properly adjusted, the observer sees the object in three dimension and can perceive heights and depths on the specimen.

To achieve these desirable height /depth effects the images coming from the binocular eyepieces must be "fused" into a single image by the observer. Using the microscope is like using a pair of binoculars. This requires some practice and careful setting of the binocular body. Remember to use both eyes!! Use these steps to focus the microscope:

1) Move the eyepiece tubes in and out to find the place where the distance between the eyepieces match the distance between the pupils of your eyes. When these distances are equal or match each other, one central image will be seen.

2) Set the microscope magnification to the highest power by turning the zoom control counter clockwise.

3) Focus sharply on the specimen.

4) Set to the lowest magnification by turning the zoom control clockwise. *Do not touch the focusing control.*

5) Looking with the right eye through the right ocular, turn the eyepiece's diopter adjustment ring until the image is precisely in focus.

6) Do the same with the left ocular and the left side eyepiece.

7) The focused microscope image should be uniformly sharp throughout the zoom range and need no further focusing.

Now have some fun and take a look at some of the objects that were provided for you to observe under the dissecting microscope.

THE DAPHNIA EXPERIMENT

PROCEDURE:

1) Capture a live *Daphnia* and place it in a depression slide with a drop of pond water from the culture jar. Next observe the anatomy of the *Daphnia* (a chart can be found on the cart). The most obvious structure is the **eye**. The **brain** is the light colored organ lying above the eye. Two pairs of **antennae** protrude from the head. These are used for locomotion and to sense the environment. Inside the **exoskeleton** are 5 pairs of **legs**. Comb-like **gills** are attached to some of the legs. When the legs kick forward they bring a stream of water across the gills and wash bits of food up to the **mouth**, which lies just beneath the beak. From the mouth the **esophagus** runs up into the head and then down into the body, where it widens into the **stomach**, which connects to the **intestine**. The **heart** lies in the upper part of the *Daphnia*. In females a large **brood chamber** is located behind the heart. Usually it will contain eggs, but occasionally a fortunate student will find it filled with baby *Daphnia*.

2) Begin to count the number of heartbeats for 15 seconds. The rate in a healthy *Daphnia* will be very rapid around 2-5 beats per second. Record your data respectfully on the Data Collection sheet provided at the end of this lab. This will be the blank on your sheet. First label the drop contents followed by the percent of that drug and then the number of beats that occurred during a 15 second time period. Then calculate the beats per minute. The simplest way to do this is to use ratios. For example, if you count 10 beats in 15 seconds the calculation is as follows: **X**/60=10/15 There for **X**=600/15, or 40 beats/minute. In other words you multiply the number of beats by 60 seconds. Take that number and divide by 15 seconds. This will give you the number of beats that occur in 1 minute. Now remove the water by placing the edge of a tissue (kimwipe) in the depression of the slide and wicking the water away.

3) Place 1 drop of a 0.5% caffeine solution on the *Daphnia.* Wait 1 minute and then begin to count the heart beats. Remember to record your results on the data sheet.

4) Using the same procedure, monitor the effects of 1.0% and 2.0% caffeine solutions. Be sure to remove the drug prior to testing the other drugs. Record your results on the data sheet.

5) After completing the caffeine series, try the second set of drugs. This time use varied concentrations of ethanol instead of caffeine. Again record your results on your data sheet.

6) Having administered all of the drugs, it is time to return your test subject (*Daphnia)* to the recovery tank located on the cart.

Even if you performed all of your experiments very carefully, you cannot be certain that the effect you see is due to the drugs. Perhaps the change in heartbeat rate is caused by the heat of the microscope light. The removal or addition of the various test solutions may have an impact on your test subject. This is why we will perform a control experiment, otherwise your data is meaningless.

THE CONTROL EXPERIMENT:

The control procedure must be performed exactly as the experimental procedure. The only difference is the variable is omitted. In this instance that means that the alcohol and caffeine are not added. Again place a *Daphnia* on a slide. Using the same time intervals and following the same procedure, substitute 1 drop of water for another. Record the heart rates on the worksheet.

COLLECTING DATA:

You will occasionally perform an experiment that does not work. This does not necessarily mean that you have disproved the hypothesis. It does mean that the experiment must be repeated so that variations in technique or in an individual organism's response can be put into perspective. For example, having used a different *Daphnia* for the control procedure, how can you be sure that its responses compare adequately to the *Daphnia* that you used for the alcohol and caffeine procedures? The answer is to repeat the experiment many times. Instead of you repeating the experiment several times in order to get some good data, collect your class data. Then individual results can be compared to a larger sample size. Use the Group Data Sheet included in this lab to record your results.

ANALYSIS OF THE DATA:

Results from experiments must be presented in a clear, scientific way. The first lab of the year is the time to learn this procedure. If tables and graphs are well constructed, they can provide a concise summary and allow the reader to see at a glance the pertinent results of the experiment. Remember, a picture is worth a thousand words.

INTERPRETATIONS AND CONCLUSIONS:

One of the most important features of scientific inquiry is the exchange and sharing of information. Scientists publish experimental results to make them known to others and include their interpretations of what those results mean. When writing a discussion of an experiment be careful not to let preconceived notions or personal convictions interfere with an objective analysis.

FURTHER EXPERIMENTS:

The scientific method is a continuing cycle of questions and answers. A good experiment not only answers the question that was originally posed, but also gives rise to further questions that may lend themselves to additional experiments. This is how scientists begin to understand a particular system.

CLEANING UP:

This is the final step in every laboratory. Return all materials and solutions then clean up your work area as directed by your instructor. Always wipe your area down with a paper towel. Remember that there will be other classes using the same lab space to perform their experiments. Please remember to leave your area clean. Some lab solutions can stain clothes, damage books and perhaps bodily harm so please be considerate.

Laboratory Questions

Student___________________ **Section________________**

Date_____________________

1) What did you observe under the dissecting microscope. What did you find interesting about that object.

2) Approximately what was the magnification of your sample under the scope?

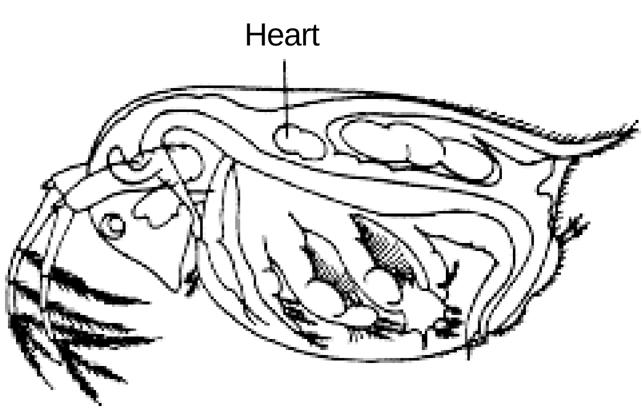
3) Present a hypothesis for this experiment.

4) Can you prove a hypothesis?

5) How do you store a microscope properly?

6) What is the purpose of a control experiment?

7) Label the picture of the *Daphnia* below.



8) What is the importance of having a large test sample? In other words why is it better to test more than one sample?

9) List a few questions that this experiment raised in your mind and procedures that could be used to test these ideas.

10) Report any unique or strange observations that may have occurred during your analysis of the *Daphnia.*

11) What did you gain as a result of performing this lab? If you could change something from this lab exercise what would it be?

DATA COLLECTION SHEET

Experimental Results

Drop	Drug %	Beats in 15 sec	Beats in 1min

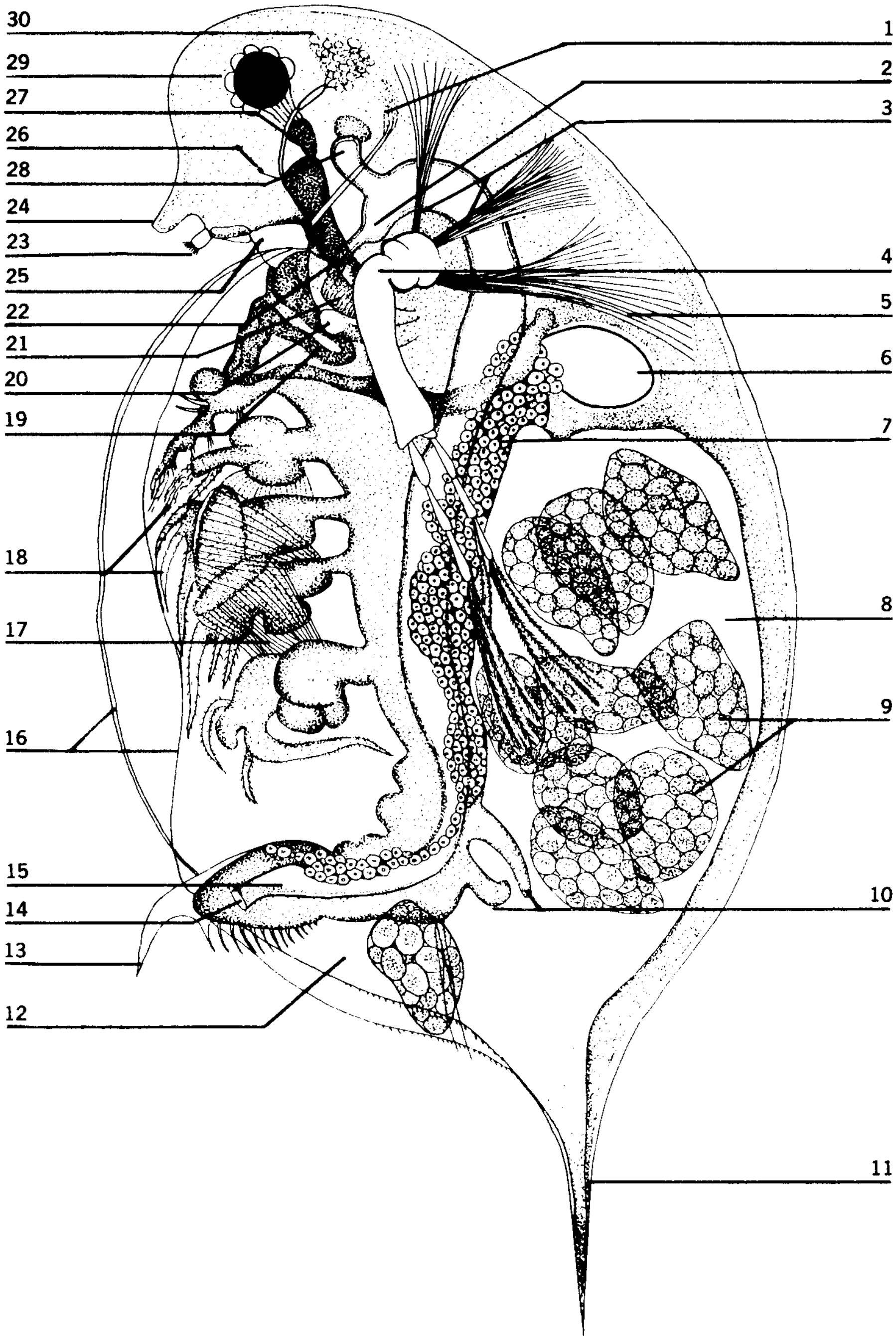
Control Results

Drop	Drug %	Beats in 15 sec	Beats in 1min

Group Data Sheet

	Experiment (beats/min)											
% Drug	**Group Number**											
	1	2	3	4	5	6	7	8	9	10	11	12
Blank	**Blank**											
water												
Alcohol	**Alcohol**											
2.0% alcohol												
4.0% alcohol												
6.0% alcohol												
8.0% alcohol												
Caffeine	**Caffeine**											
0.5% caffeine												
1.0% caffeine												
2.0% caffeine												
Water	**Control Water (beats/min)**											
drop 1												
drop 2												
drop 3												
drop 4												
drop 5												
drop 6												
drop 7												
drop 8												

Daphnia



Daphnia, Diagrammatic Anatomy of Female

1. Frontal organ
2. Esophagus
3. Antennal adductor muscles
4. Antenna
5. Antennal levator muscle
6. Heart
7. Ovary
8. Brood chamber
9. Thin-shelled "summer eggs"
10. Abdominal process
11. Apical spine
12. Post abdomen
13. Post abdominal claw (paired)
14. Anus
15. Rectum
16. Carapace
17. Filter setae
18. 2nd and 3rd thoracic appendages
19. Shell gland
20. Maxillae (vestigal)
21. Mandible
22. Mouth
23. Antennule
24. Rostrum
25. Labrum
26. Ocellus or nauplius eye
27. Optic ganglion
28. Hepatic cecae
29. Compound eye with optic nerve
30. Nuchal or neck organ

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