

Nature's Early Warning Systems

SYNOPSIS FOR CORE EXPERIMENT

Students will predict and test the effects of the ethanol on the heart rate of *Daphnia*.

APPROPRIATE BIOLOGY LEVEL

Introductory or advanced

TEACHER PARTNERS

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Directions for Teachers

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 345. For a specific variation, check the At-A-Glance Map.

GETTING READY

See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT

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

- Describe possible physiological or behavioral effects of sublethal doses of toxins on *Daphnia*.

MATERIALS NEEDED

You will need the following for a class of 24:

- ♥ 1 *Daphnia* culture
- ♥ 3 1.4 or 3.8-L (0.5 or 1-gallon) jars
- ♥ 1 *Selenastrum capricornutum* culture
- ♥ 3 L spring, aquarium, or reconstituted hard water
- ♥ 4.0 mL ethanol
- ♥ 196.0 mL filtered culture water
- ♥ 1 250-mL graduated cylinder or volumetric flask

You will need the following for each group of three students in a class of 24:

- ♥ 15 *Daphnia*
- ♥ 1 culture dish
- ♥ 2 50-mL jars or beakers
- ♥ 1 wide-bore pipette
- ♥ 2 1-mL plastic, disposable pipettes
- ♥ 1 compound microscope
- ♥ 1 depression slide and cover slip
- ♥ 1 lab journal
- ♥ 1 pencil

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (50 minutes)

- Explain activity. Draw *Daphnia* from microscopic observations. Practice counting heartbeats.

Day 2 (50 minutes)

- Discuss and form hypothesis. Provide an opportunity to read about *Daphnia*.

Day 3 (50 minutes)

- Observe the heart rate under normal conditions and exposure to ethanol. Analyze and interpret results.

PREPARATION TIME REQUIRED

30 to 40 minutes

- Order *Daphnia*, purchase spring water, and gather materials.

20 minutes

- Set up cultures.












5 minutes

- Prepare the solution of ethanol.

TEACHER'S NOTES

- ♥ 1 clock with second hand or a stopwatch
- ♥ 1 calculator
- ♥ several paper towels or filter paper
- ♥ 2 pairs of latex gloves (*optional*)
- ♥ 25.0 mL 2.0% ethanol solution

SAFETY PROCEDURES

-  Wear goggles and a lab apron when working with chemicals.
-  Do not use caffeine or nicotine.
-  Avoid pesticides and insecticides.
-  Use only substances that can be poured safely down the sink to make disposal easier.
-  Work away from any open flames when using ethanol.
-  Prepare the student dilutions in a ventilation hood.
-  Wear latex gloves when working with ethanol. It can be absorbed through the skin.
-  Wash your hands after all work is completed.
-  Do not consume ethanol.
-  Dispose of broken glass in the proper receptacle.
-  Use KittyLitter™ to absorb any large spills. Sweep up the KittyLitter™.

DIRECTIONS FOR SETTING UP THE LAB

- Glassware should be cleaned with hot water and rinsed thoroughly.
- Prepare the 2.0% ethanol solution by combining 4.0 mL ethanol and 196.0 mL filtered culture water in a graduated cylinder or volumetric flask.

TEACHER BACKGROUND

Content Information

Daphnia, commonly called water fleas, are not insects, but small freshwater crustaceans (Class Crustacea, Subclass Brachiopoda, Order Diplostraca, Suborder Cladocera). They are an important source of food for young fish. A transparent exoskeleton covers the entire body. Through it, one can see eggs in a female's brood pouch hatch and develop. While still in the brood pouch their paired lateral eyes combine, forming the large single eye characteristic of adults. The light-colored organ lying above the eye is the brain. Two pairs of antennae protrude from the head. The smaller pair bears sensory organs. The larger, branched pair of antennae is used as oars to move the water flea up and down. It cannot move forward or backward (Kramer, 1987).

Inside the exoskeleton are five pairs of legs with comb-like gills attached to them. When the legs kick forward, they bring a stream of water across the gills and wash bits of food up to the mouth that lies just beneath the beak. The preferred food for *Daphnia* is bacteria, but for most species it is unicellular algae (Weber, 1991). The food travels from the mouth, through the esophagus, to the stomach that connects to the intestine. The dorsal heart is clearly visible even though the blood cells are colorless. In females, a large brood chamber that usually contains eggs ((2 to 40) is posterior to the heart. As the eggs develop they become less distinct. Secretions from the walls of the brood pouch nourish the developing *Daphnia*. See Figure 1.

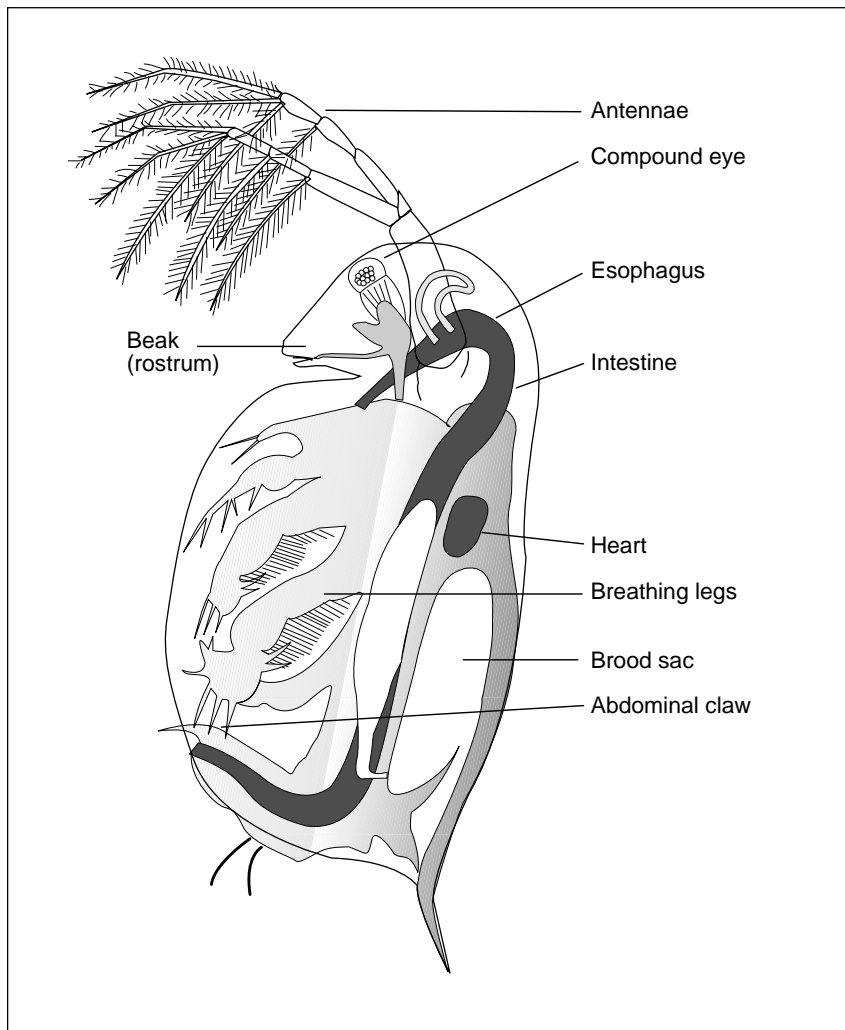
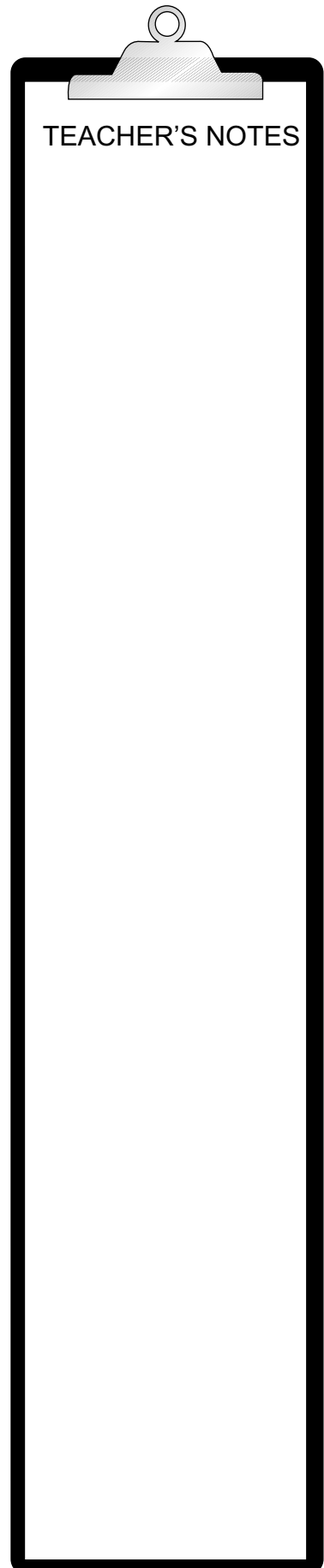


Figure 1. A species of *Daphnia* with several internal structures identified. (Used with permission. Collins, K. & B. (1985). *Daphnia — A Handy Guide*. *The American Biology Teacher*, 47(50), 299.

Only female *Daphnia* can be found in nature, during the spring and summer. These females reproduce parthenogenetically when they are about 10 days old, producing offspring that are identical to themselves. Later in the year, in response to changes in temperature, light and food availability, small males are produced also. Sexual reproduction produces fewer, but larger eggs. These eggs contain more yolk and thicker shells that nourish and protect the offspring until conditions improve. As further protection from a harsh environment, the walls of the female's brood pouch also thicken. When she molts, the walls remain around the eggs.

Daphnia have many uses in the laboratory. Their transparent covering allows their physiology to be studied while they are alive. They also may be used as food for hydra or aquarium fish. They represent an important link in many freshwater ecosystems. The stomach contents of young fish are rarely less than 10% *Daphnia* and may be as much as 95% *Daphnia* (Pennak, 1989). Indicator species serve as an early warning that a community or an ecosystem is being degraded. Since the 1940's, *Daphnia* have been used as an indicator species in lab tests for toxicity thresholds of industrial wastes. They are more sensitive than trout, a fish known for its susceptibility to poisons. Standards for using them in detecting toxic materials in freshwater are established by the Environmental Protection Agency (EPA) (Weber, 1991). Those



TEACHING TIPS

- When transferring *Daphnia* from one container to another, never pour the liquid through the air. Air may become trapped under their carapaces, causing them to float. They are unable to break the surface tension of the water and will be trapped above the water surface and die. Instead, keep the pipette tip submerged.
- If *Daphnia* are to be used more than a couple of days, feed them with a non-filamentous alga such as *Selenastrum capricornutum*. Biological supply companies also sell a concentrated food.
- A 3-L culture of 30 animals should provide about 300 young *Daphnia* per week (Weber, 1991).
- At 28°C, *Daphnia* live an average of 26 days; at 18°C, 42 days; and at 8°C, 108 days (Pennak, 1989).
- Aerate the culture lightly and provide a light cycle of 16 hours light/8 hours dark. Low oxygen concentrations are well tolerated, so you may not need to aerate.
- At weekly intervals, siphon off and discard 90% of the culture medium. Cover the end of the siphon tube with fine mesh, such as organza, to avoid removing *Daphnia*. Gently pour the remaining 10% of the medium with animals into fresh culture medium. The tube may be made from glass tubing, a Pasteur pipette, or plastic transfer pipette.
- Spring water is obtained easily from supermarkets or from biological supply houses. You may have greater survival with aquarium water or with reconstituted hard water (Weber, 1991).
- Use a dishwasher to clean all glassware if one is easily accessible within the school.
- Glass should be cleaned between each use.
- Sublethal solutions of toxins should be measured in terms of the percent of solute in the solvent (weight/volume or volume/volume).
- Encourage your students to think of conditions they might need to control in measuring heart rate. Consider how the temperature and light might change if they leave their slides on the microscope stage with the light.

guidelines even specify the appropriate food to give animals used in toxicity tests. For example, *Daphnia* fed diatoms are more tolerant to pollutants than those fed green algae alone.

A chemical may be beneficial, toxic, or lethal to *Daphnia*. A chemical can be beneficial if the exposure is moderate and the chemical is used under the proper circumstances. Under certain conditions, it can be toxic: causing harm, but not killing the *Daphnia*. A chemical is lethal if it kills the *Daphnia*. Factors that affect whether a chemical is beneficial, toxic, or lethal include how it enters the body, the body weight, exposure time, and the amount of chemical. A chemical may be very toxic in a low dosage, but lethal to the same individual at a higher dosage.

Like many planktonic organisms, *Daphnia* migrate up and down. They usually are found actively swimming in the early morning and late afternoon when the sun is low, but have inconspicuously retreated to the bottom of a shallow lake in the middle of the day. Experimentally blinded animals still migrate (Pennak, 1989). Is there a light sensor somewhere else in the body? Is the rhythm endogenous? Can disruptions of this behavior occur if sublethal amounts of toxins are in the environment? At least the last of these questions has been addressed. Goodrich and Lech (1990) developed an assay to assess the disruption of spatial orientation of *Daphnia magna*.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts:

Correct Concept	Misconception
<ul style="list-style-type: none">• Low levels of toxins can affect organisms.	<ul style="list-style-type: none">• It takes high levels of toxins to affect organisms.
<ul style="list-style-type: none">• Clear water may contain toxins.	<ul style="list-style-type: none">• Clear water is free of chemical toxins.
<ul style="list-style-type: none">• Indicator species as small as <i>Daphnia</i> serve as an early warning that a community or an ecosystem is being degraded.	<ul style="list-style-type: none">• Only large, complex organisms are good indicator species for observing the effects of pollution.
	<ul style="list-style-type: none">• Indicator species serve as early warnings that a community or an ecosystem is being depleted.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

The teacher should begin the Core Experiment by giving each laboratory group a flask or culture dish containing *Daphnia*. As the students observe the specimens, the teacher can prompt a discussion with the following questions:

- Are all of the organisms of the same species or different species?
- Compare and contrast the organisms' size, swimming motion, number of appendages, and degree of transparency.
- If they are the same species, what inferences might be made to explain the differences in size?

HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses.

After observing the cultures, have students read “Canaries of the Stream” (Essman & Zarpas, 1990). Follow the reading with a discussion of chemical pollution in our streams and rivers by industry, farms, cities, and individuals. Bring in examples of local industries and other sources. Lawn care products and automobile maintenance wastes are sources of pollution in even the most clean industrial area. Companies whose products involve making or using glues or resins are likely to have the potential to discharge waste alcohol. Ask the following questions:

- What activities did you observe in your *Daphnia*?
- Do you expect the activities to change if the environment of the *Daphnia* changes?
- How could you measure or quantify those activities?
- What environmental changes might occur in a lake or pond as a result of human activity?
- What environmental changes occur in a lake or pond even without human activity?

Sample Hypotheses

- The heart rate of *Daphnia* will decrease when the culture is enriched with lawn fertilizer.
- The exposure of *Daphnia* to rain run-off from the school parking lot will cause a reduction in the population growth.
- Exposure to ethanol will result in a decrease in the percentage of *Daphnia* showing positive phototaxis.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 343.

Question

What is the effect of ethanol on the *Daphnia* heart rate?

Hypothesis

Exposure to ethanol will cause a decrease in the *Daphnia* heart rate.

Rationale

Ethanol depresses the heart rate in humans and may have the same effect on *Daphnia*.

Procedure

1. Obtain 15 *Daphnia* with a wide bore pipette. Place in a 50-mL jar filled with culture water.
2. Set up a second jar or culture dish with culture water to act as a recovery area for treated *Daphnia*.
3. Prepare a wet mount of an adult *Daphnia* using culture water. Use a depression slide to avoid crushing the animal. Pieces of a broken cover glass or a ring of gel may be used to support the cover slip.
4. Observe the *Daphnia* under low power. Identify the following structures: heart, brood sac, swimmers, beak, eye, and antennae. Note the location of the heart and its rate.

TEACHING TIPS

- Many factors may influence the heart rate:
 - The heart rate may increase after feeding (Baylor, 1942 in Maynard, 1960).
 - Light may increase the heart rate for animals kept in dim light (Schulz, 1928 in Maynard, 1960).
 - Circadian rhythms influence the heart rate. *Daphnia's* heart rate is greatest in late afternoon and early evening (Tonolli, 1947 in Maynard, 1960).
 - Some materials in the environment will not affect the heart rate, but if they are in the circulatory system they will affect it (Lévy, 1927 in Maynard, 1960).
 - Increasing temperature, over the normal environmental range, increases the rate of heart contractions about 50 at 5°C to about 200 at 27°C (MacArthur & Baillie, 1929 in Maynard, 1960).
- A constant temperature is necessary to ensure consistent experimental results. For standard toxicity assays, the culture temperature should be 20 ± 2°C (Weber, 1991), but normal growth will occur from 18 to 26°C.
- If you want your students to appreciate the probabilistic nature of science, encourage them to analyze their results statistically. For paired sets of data, a Sign Test or *t*-test of Paired Comparisons is appropriate as is the Wilcoxon's Signed-Rank Test. The latter is explained in the analysis of the Core Experiment. For all of these tests, the tabulated values for comparison should be considered a black box; simply use the values without concern for their derivation.

TEACHER'S NOTES

5. Sketch the *Daphnia* using these observations.
6. After 20 minutes of observation, select a team number assignment from 1 through 10.
7. Student Teams 6 through 10 should go to Step 8. Student Teams 1 through 5 should do the following procedure:
 - a. Remove the cover slip and use a paper towel or filter paper to remove all the culture water carefully from under the cover slip of the depression slide well. Alternatively, use a disposable, plastic 1-mL pipette to remove a majority of the culture water from the depression slide so that area of movement of the *Daphnia* is reduced significantly. This will improve the process of counting heart beats.

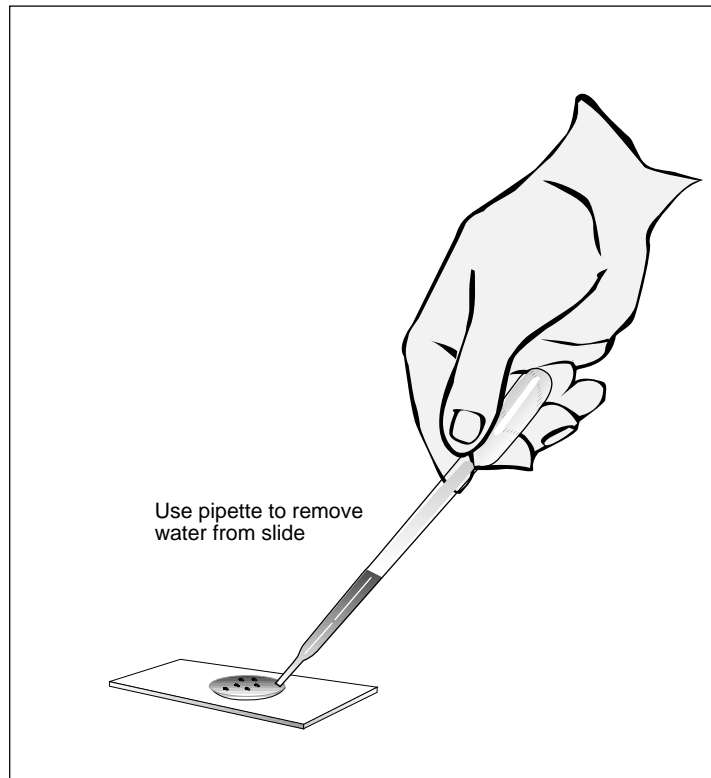


Figure 2. Removing water from *Daphnia* in a depression slide.

- b. Count the number of heartbeats in 15 seconds with a calculator as follows:
 - Step 1:* The student who will be counting the heartbeats should enter $1 + 1$ on the calculator and focus on the *Daphnia's* heart.
 - Step 2:* At the other partner's signal, the student observing the heart rate should count heartbeats by tapping the "=" key on the calculator for each heartbeat observed.
 - Step 3:* After the 15-second signal provided by the partner, the student observing the heartbeats should stop entering heartbeats on the calculator.
 - Step 4:* Calculate the number of heartbeats per minute by subtracting the additional "1" that was entered initially and multiply this value by 4. For example, if the calculator reads 84 beats at the end of the 15-second interval, subtract "1" for a value of 83. Multiply this value by 4 for the number of heartbeats per minute: 332.

(continued on p. 341)

- c. Use a second 1-mL disposable pipette to replace the water with enough 2.0% ethanol solution to submerge the *Daphnia*.
 - d. Count the *Daphnia* heartbeats at 2-minute intervals over a 10-minute period as described in 7b, Steps 1 to 4. Team 1 should observe their *Daphnia* at 2 minutes; Team 2 at 4 minutes; Team 3 at 6 minutes; Team 4 at 8 minutes; and Team 5 at 10 minutes.
 - e. After the count is made, quickly remove the alcohol with the disposable plastic pipette. Exposure time of each specimen should be minimized where possible. Return the specimen to a culture container reserved for specimens that already have been subjected to the alcohol treatment. This will eliminate the chance of using a specimen more than once and reduce the risk of contaminating the culture with residual ethanol.
8. Student Teams 6 through 10 should do the following procedure:
- a. Remove the cover slip and use a paper towel or filter paper to remove all the culture water carefully from under the cover slip of the depression slide well. Alternatively, use a disposable, plastic 1-mL pipette to remove a majority of the culture water from the depression slide so that area of movement of the *Daphnia* is significantly reduced to improve the process of counting heartbeats.
 - b. Replace the water with enough 2.0% ethanol solution to submerge the *Daphnia*.
 - c. Observe the *Daphnia* heartbeat at 2-minute intervals over a 10-minute period as described in Steps 7 a to c. Team 6 should observe their *Daphnia* at 2 minutes; Team 7 at 4 minutes; Team 8 at 6 minutes; Team 9 at 8 minutes; and Team 10 at 10 minutes.
 - d. After the count is made, quickly remove the ethanol from the slide. Replace it with a drop of culture water. Exposure time of each specimen to the ethanol should be minimized.
 - e. After 5 minutes in the culture water solution, count the number of heartbeats in 15 seconds and record the heart rate of the specimen in beats per minute. Return the specimen to a culture container reserved for specimens that have been used already to eliminate the chance of using a specimen more than once and reduce the risk of contaminating the culture with residual ethanol.
 - f. Pool class data for analysis.

TEACHER'S NOTES

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 1. Effect of 2.0% ethanol on the heart rate of *Daphnia*.

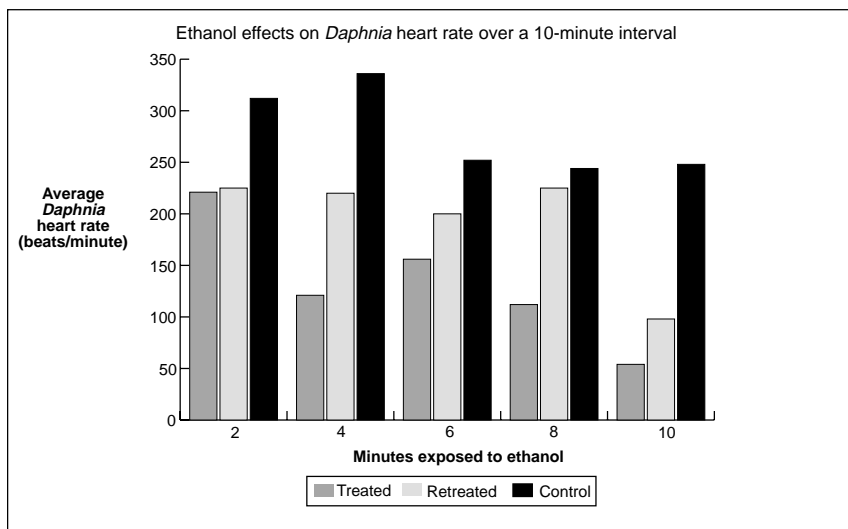
Student team	Untreated (beats/minute)	Ethanol exposed (beats/minute)	Retreated with culture water (beats/minute)
1	296	184	
2	312	221	
3	288	133	
4	336	121	
5	296	65	
6	252	156	224
7	220	155	198
8	244	112	225
9	252	91	150
10	248	54	98

Interpretation

These data support the hypothesis that ethanol will cause a decrease in the heart rate of *Daphnia*. The heart rate of *Daphnia* exposed to ethanol over a 10-minute interval was depressed by 78%. At a concentration of 2.0%, *Daphnia* detect ethanol as a toxin.

Answer to Test Question

Dividing the sample removes the variable of handling introduced by distributing the effect of handling equally between the ethanol-exposed and unexposed specimens, leaving only exposure as the variable to evaluate.



Graph A. *Daphnia* heart rate before and after exposure to 2.0% ethanol, and recovery period.

TEST QUESTION

Why must half the *Daphnia* be evaluated first with ethanol and the other half evaluated first with culture water?

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiments. Ask them what quantifiable experiments could be done based on observations they have made.

Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- Are there physiological or behavioral effects that you observed that might be a result of the low levels of toxin?
- Was time a factor?
- What is the value of indicator species such as *Daphnia*?
- If *Daphnia* is a good indicator organism for low levels of ethanol, to what else might it be sensitive?
- Is there a level at which you can kill the indicator species?
- What might be the long term effects of sublethal doses of toxins?
- How might sublethal doses affect longevity?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

- Provide a complete copy of the laboratory experiment printed in braille. An enlarged diagram of the structures of *Daphnia* (Figure 1) should be included as a raised-line drawing.
- Encourage the visually impaired student to record lab data in braille and make a typewritten copy for the team. A computer enables a visually impaired student to act as a recorder for the team.
- Ask the visually impaired student to provide a typed lab report and to keep a braille copy for personal use.
- Ask the visually impaired student to make graphs of data collected to accompany the team lab report.

Deaf or Hard-of-Hearing

- Allow hearing impaired students to participate fully in the experiment.

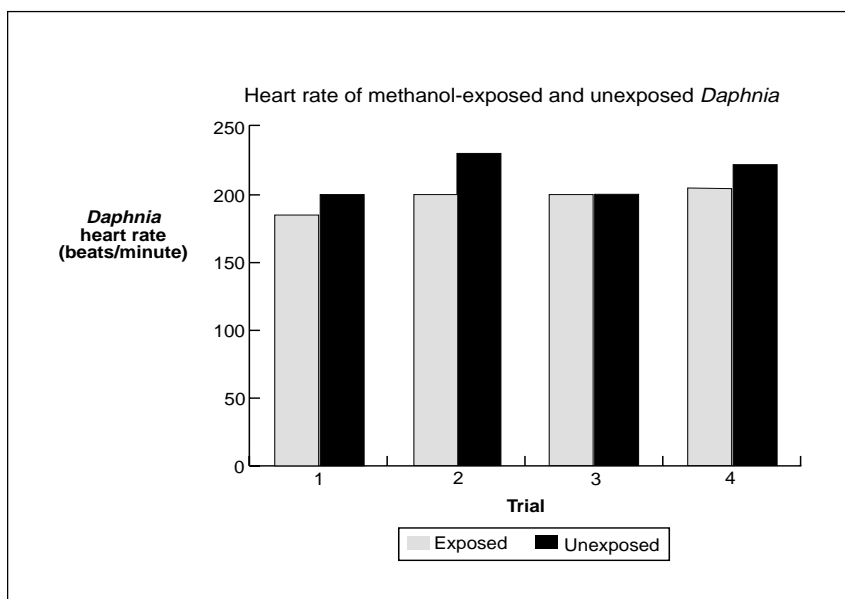
Mobility Impaired

- Provide a lab table with a height that accommodates wheelchair access.
- Permit wheelchair movement if the classroom is large enough to allow the mobility impaired student to obtain supplies as needed.
- Have lab team members prepare slides for the manually impaired student to observe and count the heartbeat of *Daphnia*.
- Provide the manually impaired student with a tape recorder or other means to collect data and to take notes.
- Provide the manually impaired student with a microscope equipped with levers that can focus the fine adjustment.

ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment also may include the following:

1. Ethanol, the alcohol in liquor, is a depressant for humans. If methanol, a poison for humans, is a depressant for offspring, do these data graphed below support your expectation? Explain.



Graph B. Influence of methanol on heart rate of *Daphnia*.

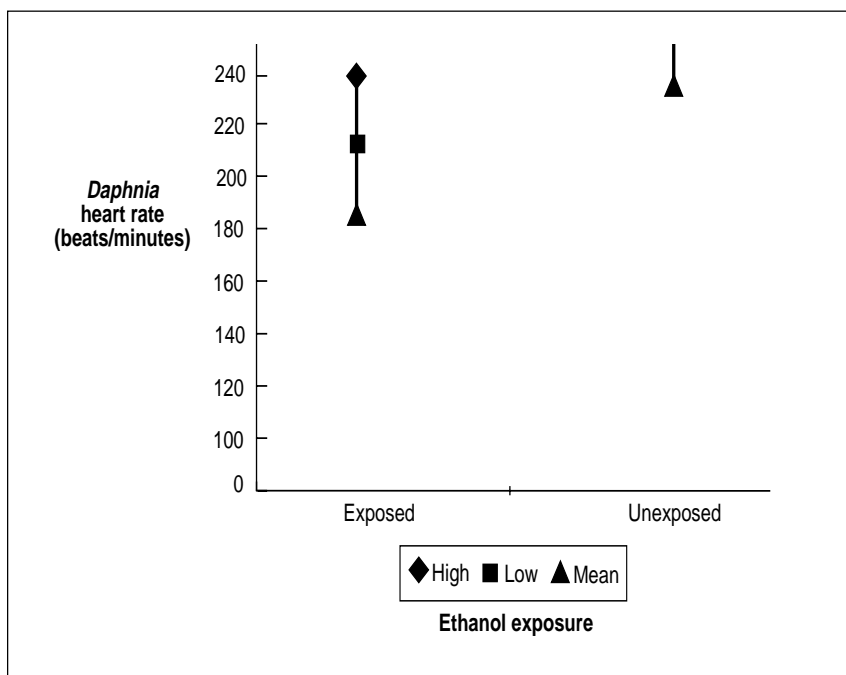
2. Why was it desirable to pool class data to evaluate the effect of ethanol on the heart rate of water fleas?

Answers to Additional Test Questions

1. The expectation was that the methanol would depress or lower the heart rate of water fleas, or maybe even kill them. For three of the four tested animals, the heart rate was lowered and for the fourth there was no change. This supports the hypothesis. The number of water fleas examined is too small to dismiss the possibility that chance alone accounts for these observations.
2. Many factors can influence the responses of an organism. Here, there was no control over or no way to measure the age of the animal, the amount of heat supplied by the microscope light, the oxygen deprivation provided by reduced water, or handling stress. Since these, and possibly other factors, could provide variations in the response, several samples were needed to obtain a measure of the true response.

Answers to Questions and Analysis on Student Page

1. See Graph C for a representative graph.
2. These data support the hypothesis that ethanol would depress the heart rate of *Daphnia*, but there is an overlap in the values. It would be appropriate to analyze these data statistically as well as display them graphically.
3. If half of the animals had depressed heart rates and half had elevated heart rates, reject the hypothesis. Results like these are the same as the results one would expect if a coin were tossed. Half the time one would expect heads and half the time one would expect tails.
4. If the animals showed a depression consistently greater than that shown by another lab, one would question whether the techniques were consistently different. If the exact same procedure was followed and the animals were measured at the same time of day, a consideration may be that the animals differ genetically.
5. An easy way to test whether the animals are different would be to exchange animals and retest. A possible hypothesis is that no difference in the depression of heart rate between the two cultures would be found. Some of each original culture should be retained.



Graph C. Pooled class data on ethanol's effect on *Daphnia* heart rate.

POSSIBLE SOURCES OF MENTORS

- Local college or university toxicology professor.
- Local research or industry that deals with toxicology.

REFERENCES AND SUGGESTED READINGS

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VARIATIONS OF CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

VARIATION 1

The Effect of Different Concentrations of Ethanol on the Heart Rate of *Daphnia*

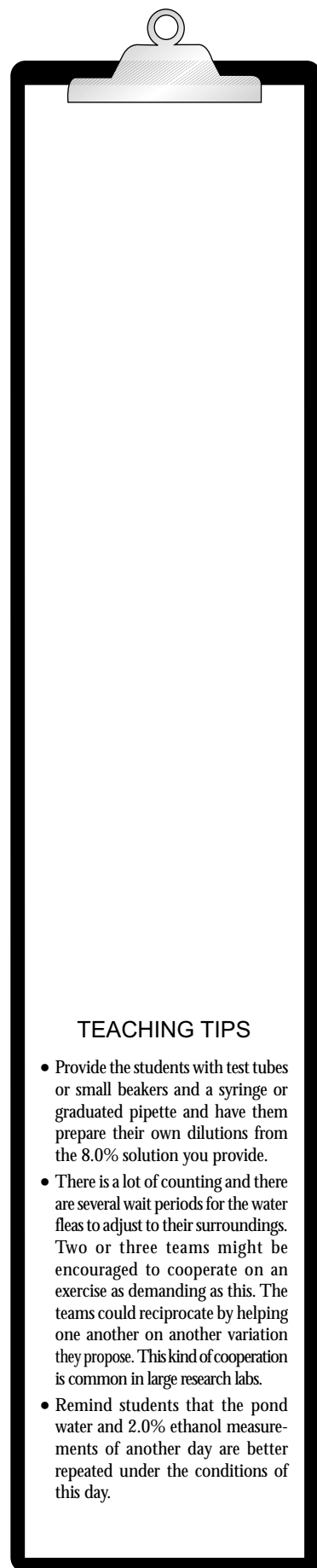
SYNOPSIS

Students will compare the heart rates of *Daphnia* exposed to different concentrations of ethanol.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- ♥ 5 mL 8.0% solution of ethanol
- ♥ 5 mL 4.0% solution of ethanol
- ♥ 5 mL 2.0% solution of ethanol
- ♥ 5 mL 1.0% solution of ethanol
- ♥ 10 to 12 *Daphnia*



TEACHING TIPS

- Provide the students with test tubes or small beakers and a syringe or graduated pipette and have them prepare their own dilutions from the 8.0% solution you provide.
- There is a lot of counting and there are several wait periods for the water fleas to adjust to their surroundings. Two or three teams might be encouraged to cooperate on an exercise as demanding as this. The teams could reciprocate by helping one another on another variation they propose. This kind of cooperation is common in large research labs.
- Remind students that the pond water and 2.0% ethanol measurements of another day are better repeated under the conditions of this day.



TEACHER'S NOTES

SAFETY PROCEDURES



Do not consume ethanol.



Wear goggles and apron when working with ethanol.



Work away from any open flames when using ethanol.



Wear latex gloves when working with ethanol. It can be absorbed through your skin.



Wash your hands after all work is completed.



Broken glassware presents a hazard. Dispose of broken glassware in appropriate containers.



Put KittyLitter™ on any large spills. Sweep up KittyLitter™ after it has absorbed the spilled material.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 8.0% ethanol solution by diluting 8.0 mL of ethanol with 92.0 mL of culture water.
- A 4.0% solution is made by diluting 50.0 mL of the 8.0% solution with 50.0 mL of culture water.
- A 2.0% solution is made by diluting 50.0 mL of the 4.0% solution with 50.0 mL of culture water.
- A 1.0% solution is made by diluting 50.0 mL of the 2.0% solution with 50.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of ethanol on *Daphnia* heart rate?

Sample Hypothesis

The higher the concentration of ethanol, the greater the decrease in *Daphnia* heart rate.

Rationale

If some is harmful, more might be even more harmful. The model for humans is that ethanol in large quantities is lethal.

Sample Experimental Procedure

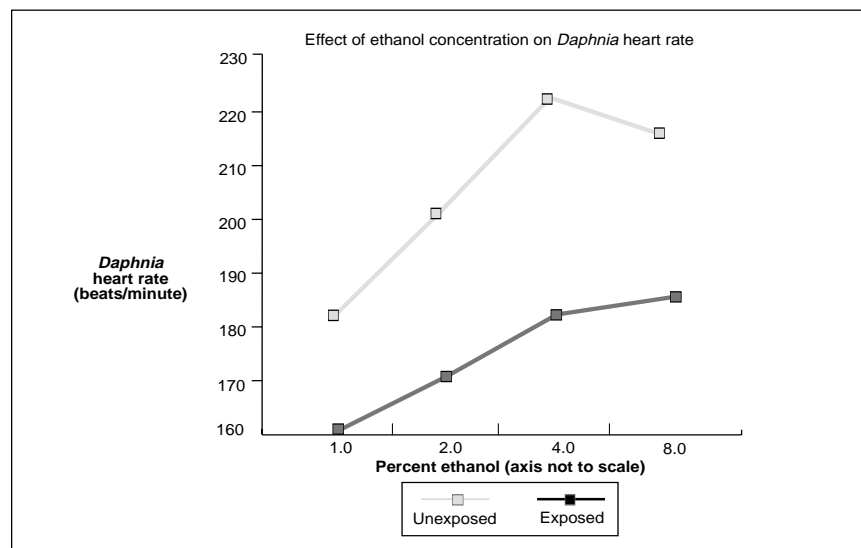
1. Repeat the Core Experiment using these 4 concentrations of ethanol and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. Effect of ethanol concentration on *Daphnia* heart rate. Values reported are the average rates for 10 animals.

Percent ethanol	Exposed	Unexposed
1.0	160	184
2.0	170	200
4.0	184	224
8.0	186	216



Graph D. Effect of varying ethanol concentrations on *Daphnia* heartbeat rate per minute.

TEST QUESTION

How could you determine if the results of your experiment represent differences in diffusion rates resulting from using solutions of different concentrations?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide syringes or plastic graduated cylinders in a variety of sizes to allow the visually impaired student to prepare the ethanol solutions indicated in the lab.

Interpretation

These data show a slight increase of 13% at 1.0% ethanol to 18% at 4.0% ethanol. These three concentrations of 1.0%, 2.0%, and 4.0% support the hypothesis that increasing concentrations of ethanol will result in increasing depressions of heart rate. The depression of heart rate was less with exposure to 8.0% ethanol than it was with exposure to 4.0% ethanol. These trials were done sequentially and early in the day with the 8.0% observations made first. It may be that some daily rhythm in the water fleas has confounded the results. It is recommended that this exercise be repeated randomizing the treatments.

Answer to Test Question

One would expect that the greater the difference in the concentration of solutions of the internal and external environments of the water flea, the greater the diffusion will be and the more quickly the effect will be detected. With the exception of the most concentrated solution, that is what was found. While the results are consistent with the effects of diffusion, they do not prove that the observed differences are caused by diffusion. It could be that water fleas are able to detoxify ethanol. One could test this by leaving them in a dilute ethanol solution for a longer time. If they are able to detoxify the ethanol, the extended time will not result in their death.

TEACHING TIPS

- Although automatic dishwashing detergents contain phosphates, they also contain many other potentially toxic materials, such as chlorine bleach. They should not be used.
- Trisodium phosphate (TSP) is available at hardware stores and may be used. It is a recommended cleaner for surfaces to be painted. You may substitute sodium orthophosphate from the chemical storeroom, but students should know that the phosphate should be something the water fleas likely would encounter in the environment.

VARIATION 2

The Effect of Phosphate on the Heart Rate of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS

Students will compare the heart rate of *Daphnia* before and after exposure to phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 2.0% trisodium phosphate (TSP)

SAFETY PROCEDURES



Do not ingest TSP. It may cause burns to eyes and skin.



Wear goggles, gloves, and a long-sleeved garment.



Do not allow the solution to be absorbed through gloves or clothing.



Keep TSP away from eyes.



Wash all contaminated clothing thoroughly before use.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

To prepare a 2.0% phosphate solution, dissolve 2 grams of TSP in a small quantity of culture water and use culture water to dilute the resulting solution to 100 mL.

HYPOTHESIS GENERATION

Question

What is the effect of phosphate on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* will be decreased if they are exposed to phosphate.

Rationale

Phosphates have been reduced in cleaning products because phosphate enrichment of water causes algal blooms and subsequent loss of aquatic animals. Phosphates may cause problems for some aquatic animals directly.

Sample Experimental Procedure

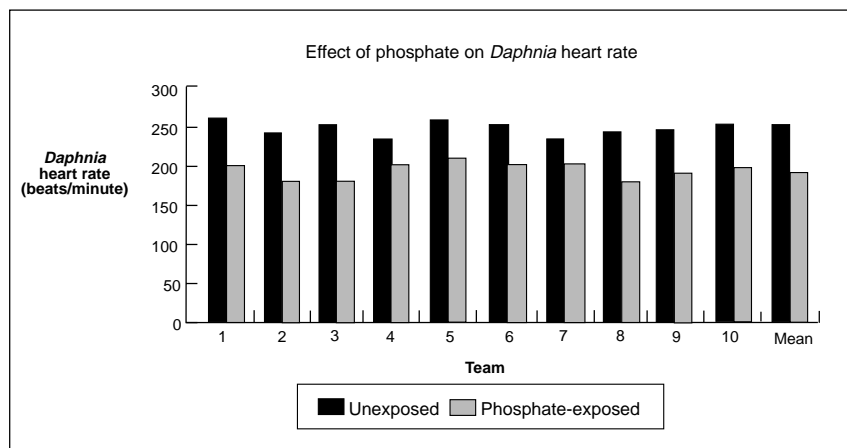
1. Repeat the Core Experiment using a 2.0% solution of phosphate instead of 2.0% ethanol.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 3. Results of phosphate exposure on *Daphnia* heart rate.

Student team	Unexposed <i>Daphnia</i> (beats/minute)	Phosphate-exposed <i>Daphnia</i> (beats/minute)
1	262	200
2	248	180
3	256	180
4	240	200
5	260	212
6	252	200
7	240	200
8	244	180
9	248	192
10	252	196
Mean	250	194



Graph E. Effect of phosphate on *Daphnia* heart rate.

TEST QUESTION

The graph on page 350 displays data collected by another group of students. Interpret their results.

Interpretation

The mean number of beats per minute was 22% lower in the phosphate-exposed animals compared with the unexposed animals. These data support the hypothesis that phosphate pollution will depress the heart rate of water fleas.

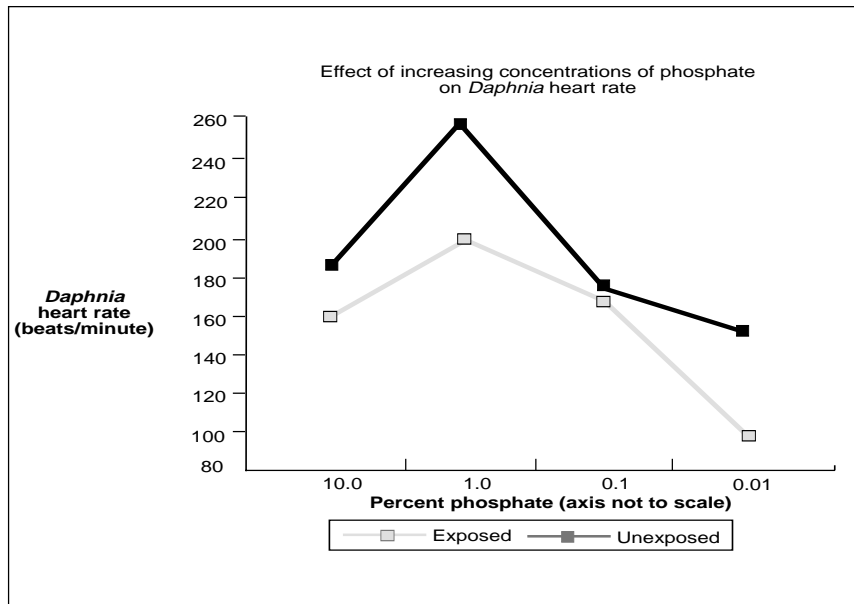
Answer to Test Question

These students found that the heart rates of water fleas increased when they were exposed to phosphate. This could represent a dying response or an initial response that will be reduced later. One needs to know whether these students used the same procedure as others who did this variation. If they did, one would suspect that they did not use the same kind of water fleas or that they used a different source for their phosphate amendment.

TEACHER'S NOTES

TEACHING TIP

Provide students with syringes or pipettes, several test tubes or small beakers, and an 8.0% phosphate solution. Allow them to prepare their own dilutions.



Graph F. Influence of phosphate on *Daphnia* heart rate.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 2.

VARIATION 3

The Effect of Different Concentrations of Phosphate on the Heart Rate of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

SYNOPSIS

Students will compare the heart rates of *Daphnia* exposed to different concentrations of phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 8.0% solution of phosphate
- ♥ 4.0% solution of phosphate
- ♥ 2.0% solution of phosphate
- ♥ 1.0% solution of phosphate
- ♥ 10 *Daphnia*

SAFETY PROCEDURES

See Variation 2.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 8.0% phosphate solution by dissolving 8.0 g of phosphate (trisodium phosphate, TSP) in a small amount of culture water and dilute the resulting solution to 100.0 mL.
- A 4.0% solution is made by diluting 50.0 mL of the 8.0% solution with 50.0 mL of culture water.
- A 2.0% solution is made by diluting 50.0 mL of the 4.0% solution with 50.0 mL of culture water.
- A 1.0% solution is made by diluting 50.0 mL of the 2.0% solution with 50.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of phosphate on *Daphnia* heart rate?

Sample Hypothesis

The higher the concentration of phosphate, the greater the decrease in *Daphnia* heart rate.

Rationale

If some is harmful, more should be even more harmful. A decrease in the heart rate may signal impending death.

Sample Experimental Procedure

1. Repeat the Core Experiment using these 4 concentrations of phosphate and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. Effect of increasing phosphate concentrations on *Daphnia* heart rate. Values reported are the average rates of 10 animals.

Percent phosphate	Exposed	Unexposed
1.0	160	184
2.0	227	250
4.0	170	174
8.0	92	153



TEACHER'S NOTES

Interpretation

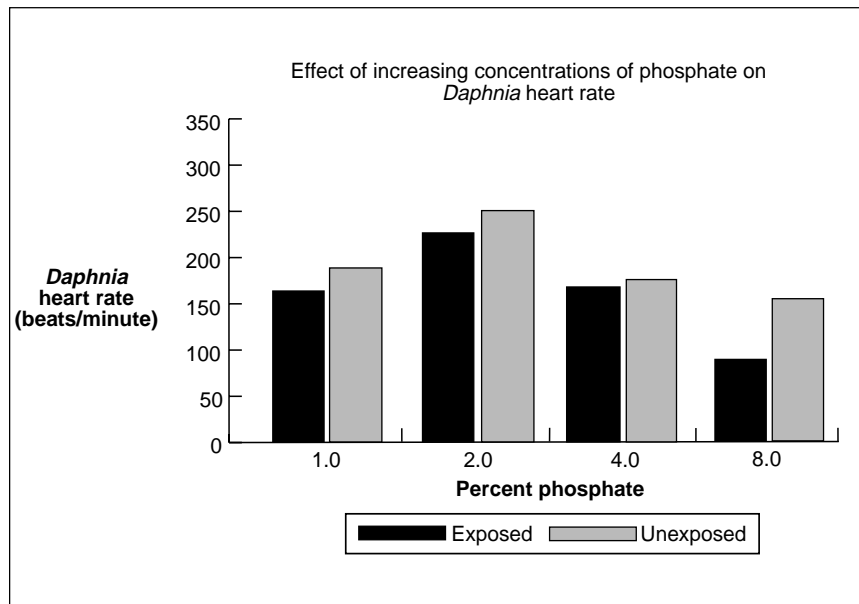
These data show irregular changes in heart rate. The greatest depression (40%) occurs with the highest phosphate concentration, but the next greatest depression (13%) occurs at the lowest concentration. If these were the only 2 concentrations tested, one would conclude that these data support the hypothesis. Here, however, less depression in the heart rate was found at 2.0% phosphate (9%) and 4.0% phosphate (2%) than at the extremes tested. Perhaps the other team that collected data did not follow the same procedure exactly. The 5-minute adjustment period could be crucial for obtaining repeatable results.

Answer to Test Question

The initial increase in water flea numbers could be caused by an increase in unicellular algae on which they feed. The subsequent decrease could be caused by an increase in the size and appetite of the fish predators of the water fleas. It also could be caused by a decrease in the oxygen concentration of the water as the algae die and bacterial decay of their carcasses reduces the oxygen in the water.

TEACHING TIPS

- Lowering an alkaline pH causes more oxygen to be released from some crustaceans' hemoglobin. The oxygen is released until a critical value is reached where the effect is reversed (Wolvekamp & Waterman, 1960).
- Lowering the pH mimics the metabolic effects of increasing the carbon dioxide concentration. For some crustaceans, the result is an initial increase in respiration rate followed by a decrease in respiration rate. For others, an initial decrease is followed by an initial increase (Wolvekamp & Waterman, 1960).



Graph G. Effect of increasing concentrations of phosphate on *Daphnia* heart rate.

TEST QUESTION

Over a period of 2 to 3 weeks, an increase in phosphate concentration in a pond or lake initially can cause an increase in water flea population, then a decrease. These observations are independent of any changes in the heart rates, but involve the changes in the lake community. How could you account for the change in water flea populations?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 3.

VARIATION 4

The Effect of Vinegar on the Heart Rate of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS

Students will compare the heart rates of *Daphnia* before and after exposure to vinegar.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 5.0 mL 2.0% distilled vinegar in culture water
- ♥ 10 to 12 *Daphnia*

SAFETY PROCEDURES



Practice safe handling of acids. Add acid to water rather than water to acid.



Wear goggles and lab apron when working with acids.



Clean all spills immediately.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Prepare a 10.0% vinegar solution by adding 10.0 mL of distilled vinegar (5.0% acetic acid) to 90.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of vinegar on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* will decrease if they are exposed to vinegar.

Rationale

Acid rain has killed the animals in many lakes. Vinegar is an acid that may have the same effect as the acids of acid rain.

Sample Experimental Procedure

1. Repeat the Core Experiment with the 10.0% vinegar solution and unadulterated culture water.
2. Expose the *Daphnia* to various concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Effect of vinegar on *Daphnia* heart rates.

Student team	Unexposed (beats/minute)	Vinegar-exposed (beats/minute)
1	262	300
2	248	280
3	256	260
4	240	290
5	290	300
6	262	290
7	280	280
8	278	280
Mean	265	285

TEACHER'S NOTES

Interpretation

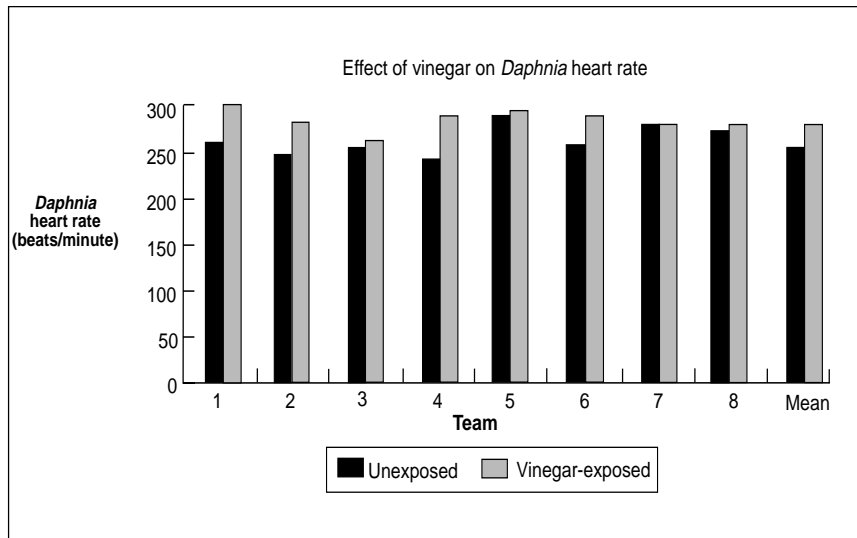
These data show increased heart rate with exposure to vinegar. These data do not support the hypothesis that heart rate would be depressed. An increase in heart rate still indicates that the water fleas are stressed by the treatment. In humans, an increase in heart rate occurs when there is an increased demand for oxygen, such as with exercise. Somehow, the vinegar may be reducing the available oxygen causing the water fleas' heart rate to increase.

Answer to Test Question

The water fleas were very sensitive to acid. Copepods may be even more sensitive. They probably have hemoglobin similar to water fleas and may have problems getting enough oxygen when the water is acidic. In addition, they may have problems with the calcium in their exoskeletons dissolving in acid.

TEACHING TIPS

- Provide students with syringes or pipettes, several test tubes or small beakers, and undiluted vinegar. Allow them to prepare their own dilutions.
- See Teaching Tips, Variation 4.



Graph H. The relationship of vinegar on *Daphnia* heart rate.

TEST QUESTION

Crustaceans belonging to the subclass containing water fleas have soft, pliable skeletons of chitin. Other planktonic crustaceans, like copepods and ostrapods, have calcareous plates reinforcing their chitin exoskeletons. Do you expect that water fleas or copepods will be more sensitive to acidification?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use the chemicals in Variation 4.

VARIATION 5

The Effect of Different Concentrations of Vinegar on the Heart Rate of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS

Students will compare the effects of different vinegar concentrations on *Daphnia* heart rate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 5.0 mL 10.0% distilled vinegar in culture water
- ♥ 5.0 mL 1.0% distilled vinegar in culture water
- ♥ 5.0 mL 0.1% distilled vinegar in culture water
- ♥ 5.0 mL 0.01% distilled vinegar in culture water

SAFETY PROCEDURES

See Safety Procedures, Variation 4.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare the 10.0% vinegar solution by adding 10.0 mL of distilled vinegar (5.0%) to 90.0 mL of culture water.
- A 1.0% solution is made by adding 1.0 mL of the 10.0% solution to 9.0 mL of culture water.
- A 0.1% solution is made by adding 1.0 mL of the 1.0% solution to 9.0 mL of culture water.
- A 0.01% solution is made by adding 1.0 mL of the 0.1% solution to 9.0 mL of culture water.

HYPOTHESIS GENERATION

Question

What is the effect of varying concentrations of vinegar on *Daphnia* heart rate?

Sample Hypothesis

The heart rate of *Daphnia* decreases more with exposure to increasing concentrations of vinegar.

Rationale

Acid rain has killed the animals in many lakes. Vinegar is an acid that may have the same effect as the acids of acid rain. In the Core Experiment, the ethanol-stressed, sometimes dying, water fleas had reduced heart rates. Increasing the concentration of a toxin should increase the stress it creates in an animal.

Sample Experimental Procedure

1. Repeat the Core Experiment using these 4 concentrations of vinegar and unadulterated culture water.
2. Expose the *Daphnia* to the concentrations for 5-minute intervals. At the end of 5 minutes, take the heart rate as described in the Core Experiment.
3. Analyze patterns of change in heart rate.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Effect of increasing concentrations of vinegar on the *Daphnia* heart rate. Values reported are the average of rates of 10 animals.

Percent vinegar	Exposed	Unexposed
10.0	340	184
1.0	288	250
0.1	270	174
0.01	240	153

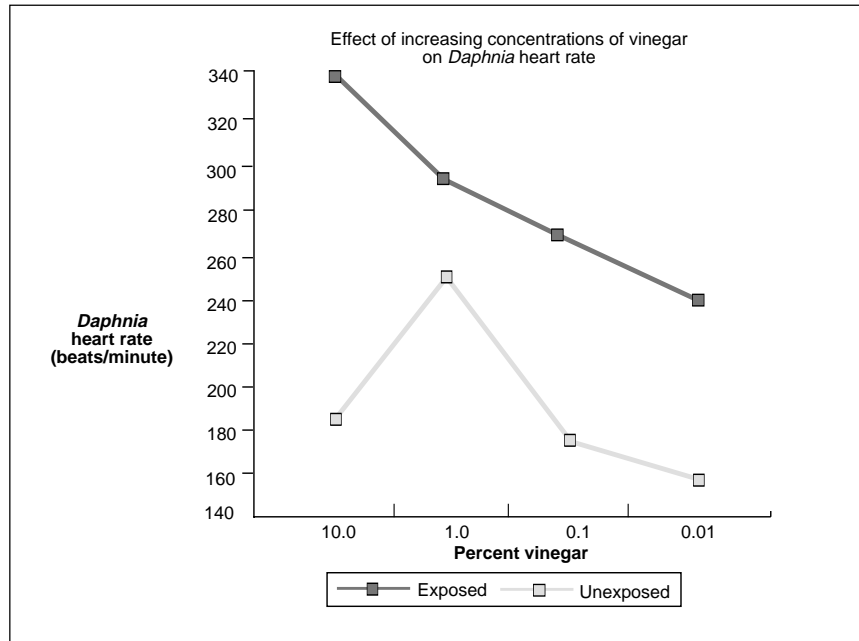
TEACHER'S NOTES

Interpretation

These data show increasing heart rates with increasing vinegar concentration. These data support the hypothesis that heart rates would be increased and indicate that the water fleas are stressed by the treatment.

Answer to Test Question

Anything that increases the carbon dioxide concentration of water could increase the hydrogen ion concentration. This would include decay of an algal bloom. Sulfur and nitrogen oxides are produced by the burning of fossil fuels and eruption of volcanoes that react with water in the air to form acids. Industrial spills also could introduce acids to waterways.



Graph I. The effect of increasing vinegar concentrations on *Daphnia* heart rate.

TEST QUESTION

Adding vinegar to water increases the hydrogen ion concentration, thus lowering the pH. What activities by man or nature could cause an increase in the hydrogen ion concentration of lakes?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1. Use chemicals specified for Variation 5.

VARIATION 6

The Effect of Vinegar on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed and not exposed to vinegar.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 20 *Daphnia*
- ♥ 2 large paper “backgrounds” divided evenly into dark and light sides
- ♥ 1 10-mL graduated cylinder or pipette with bulb
- ♥ 10.0 mL 2.0% solution of vinegar
- ♥ 10.0 mL culture water
- ♥ 2 petri dishes
- ♥ 2 pieces of filter paper
- ♥ 1 5-mL plastic, disposable pipette
- ♥ 2 15-cm squares of dark opaque plastic (garbage bag)

HYPOTHESIS GENERATION

Question

What is the effect of vinegar on the phototactic response of *Daphnia*?

Sample Hypothesis

The phototactic response of *Daphnia* will be affected if they are exposed to a weak solution of vinegar.

Rationale

If exposure to acid results in oxygen starvation, nervous system damage could result in long term disorientation.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto a piece of filter paper.
2. Transfer them quickly to a petri dish containing 10.0 mL of 2.0% vinegar.
3. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
4. Prepare 2 large paper “backgrounds” divided evenly into dark and light sides.
5. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
6. Drape the dark, opaque plastic over the dark side of the dish. See Figure 3.

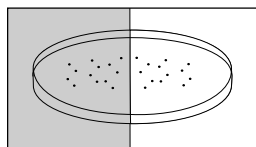


Figure 3. Experimental setup for phototactic behavior observation.

7. Every minute for 15 minutes, count the number of *Daphnia* in the light in each dish.
8. At the end of the 15 minutes, transfer them to the culture container reserved for specimens that have been stressed previously.
9. Calculate the total animal-minutes spent in the light in each container.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Percent of *Daphnia* in the light during each minute of a 15-minute observation.

Time (minutes)	Culture water	2.0% Vinegar
1	18	12
2	20	12
3	20	14
4	20	10
5	14	12
6	10	14
7	14	16
8	10	12
9	14	14
10	10	16
11	12	12
12	8	18
13	4	12
14	9	8
15	8	18

TEACHER'S NOTES

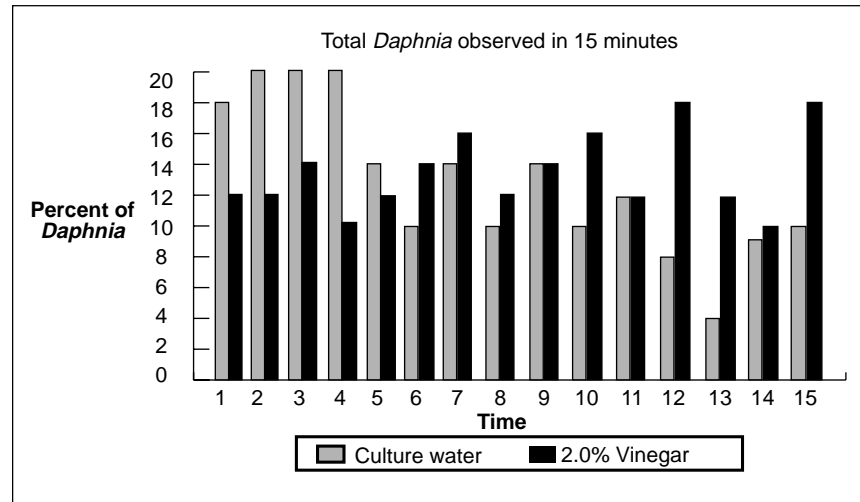
Interpretation

Nearly the same amount of time was spent in the light in both the treatment (acid) and in the control (culture water). If all the water fleas had spent the entire time in the light, there would have been 300 animal-minutes recorded for each petri dish. Nearly two-thirds of the possible animal-minutes were in the light (191/300 in the culture water and 200/300 in vinegar). No disorientation to the light is apparent when the water fleas are in 2.0% vinegar. Both vinegar and culture water *Daphnia* prefer the light.

Answer to Test Question

Daphnia are filter feeders. Their primary food source is probably single-celled algae. The algae require light to photosynthesize. If the water fleas are attracted to light, they will be attracted to the area of the lake where their food source is most abundant.

Total the number of animals observed in the light throughout the 15 minutes. An appropriate analysis here would be Chi-Square Goodness-of-Fit with the hypothesis that there will be no difference in light preference; that is, that the total number of animal-minutes in the light will be the same in the culture water and in the vinegar.



Graph J. Percent *Daphnia* observed in light over a 15-minute period.

TEST QUESTION

In this experiment *Daphnia* were found to prefer light to dark. Why is it advantageous for them to have the ability to orient to the light?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL Blind or Visually Impaired

- Have the visually impaired student collect data and observations of phototactic responses from the team and present in bar and line graphs as needed.

VARIATION 7

The Effect of Temperature on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS

Students will determine if temperature influences the phototactic behavior of *Daphnia*.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three to four students in a class of 24:

- ♥ ice
- ♥ 1 refrigerator
- ♥ 1 large paper “background” divided evenly into dark and light sides
- ♥ 4 15-cm squares of dark opaque plastic (garbage bag)

(continued on p. 359)

- ♥ 10-mL graduated cylinder or pipette with bulb
- ♥ 1 thermometer (°C)
- ♥ 4 petri dishes
- ♥ 40 *Daphnia*
- ♥ 4 culture dishes larger than the petri dishes to serve as water baths
- ♥ 2 water baths
- ♥ 1 ice bath (*optional*)
- ♥ 1 bright light source
- ♥ 2 pieces of filter paper

SAFETY PROCEDURES

Use caution when preparing and using the hot water bath.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Preheat 100.0 mL of culture water to 25°C and another 100.0 mL to 30°C. Maintain the temperatures with water baths.
- Chill 100.0 mL of culture water in the refrigerator or an ice bath to 5°C and another 100.0 mL to 15°C.

HYPOTHESIS GENERATION

Question

What is the effect of temperature on the phototactic behavior of *Daphnia*?

Sample Hypothesis

Water temperature will affect the phototactic behavior of *Daphnia*.

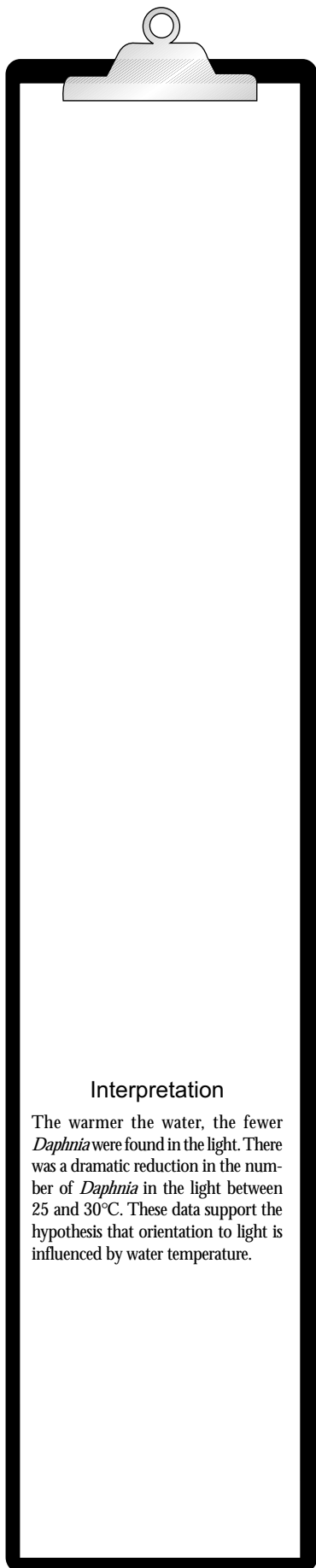
Rationale

During a bright sunny day, *Daphnia's* algal food source should be at the surface of the water that is slightly warmer than the water below it. Optimal temperature for enzyme activity is fairly narrow. *Daphnia* should be able to migrate to their optimal temperature independent of the light intensity.

Sample Experimental Procedure

1. Set up 4 petri dishes containing 100.0 mL water each at the following temperatures: 5°C, 15°C, 25°C, and 30°C.
2. Maintain the desired temperature in each petri dish by setting it in a larger dish with water of the same temperature.
3. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of water at the desired temperature.
4. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of water at another desired temperature. Continue this process until dishes of all desired temperatures are prepared.
5. Prepare 1 large plastic or laminated paper “background” divided evenly into dark and light sides to hold the 4 petri dishes.
6. See Sample Experimental Procedure Variation 6. Repeat Steps 6 and 7.
7. Return *Daphnia* to container of previously stressed organisms.
8. Calculate the total animal-minutes spent in the light in each container.

TEACHER'S NOTES



DATA ANALYSIS AND INTERPRETATION

Sample Data

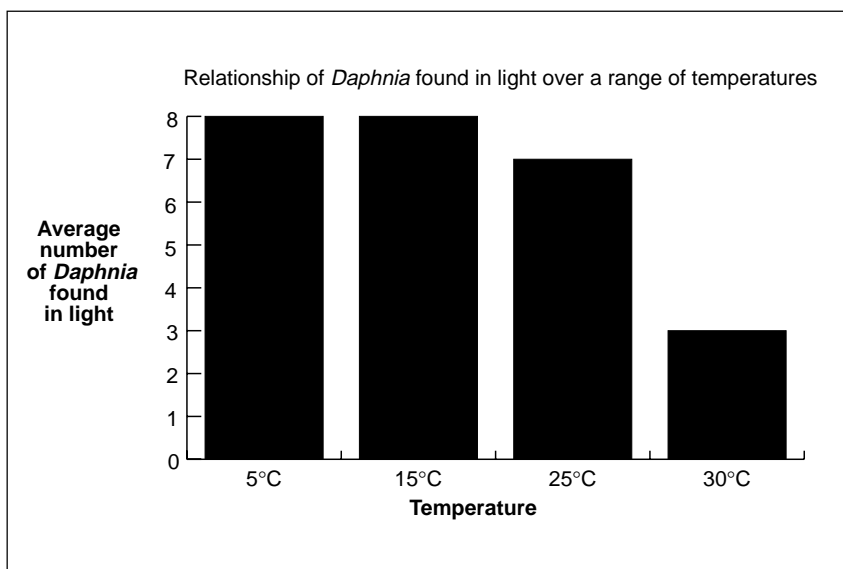
Table 8. Number of *Daphnia* in light at several temperatures. Ten *Daphnia* were placed in each container for 15 minutes.

Time	5°C	15°C	25°C	30°C
1	8	8	7	5
2	9	8	7	4
3	7	8	7	4
4	8	7	8	6
5	9	7	6	3
6	9	8	6	3
7	8	9	8	5
8	9	8	5	2
9	10	7	7	2
10	7	8	6	1
11	7	8	8	1
12	10	7	5	2
13	9	9	5	1
14	8	8	7	1
15	7	8	8	1

An appropriate analysis would be Chi-Square Goodness-of-Fit using the hypothesis that there will be no difference in the number of *Daphnia* in the light in water at different temperatures.

Interpretation

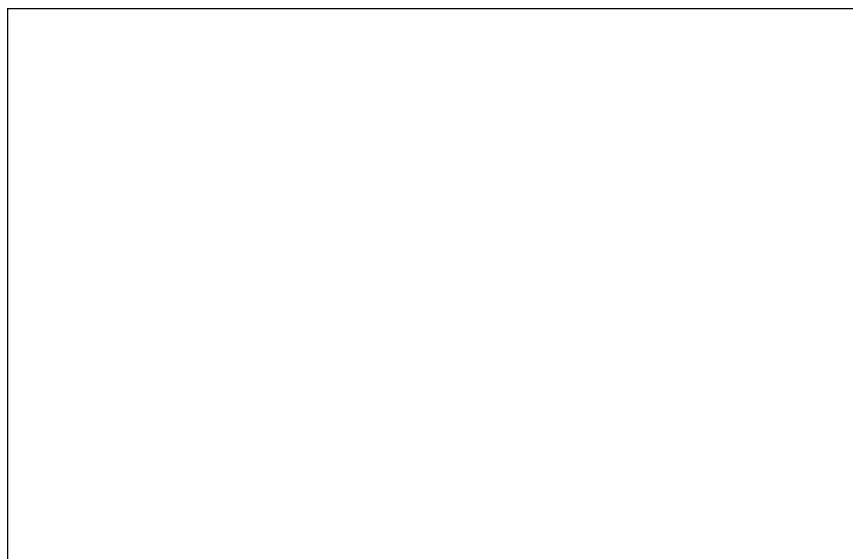
The warmer the water, the fewer *Daphnia* were found in the light. There was a dramatic reduction in the number of *Daphnia* in the light between 25 and 30°C. These data support the hypothesis that orientation to light is influenced by water temperature.



Graph K. Effect of varying temperatures on *Daphnia's* presence in the light.

TEST QUESTION

Compare the student data graphed below with the results of your experiment.



Graph L. Influence of temperature on the phototactic response of *Daphnia*.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Provide blind students with Talking Thermometers so they can record temperatures of various cultures.

VARIATION 8

The Effect of Seltzer Water on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed to seltzer water with those of *Daphnia* not exposed to seltzer water.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 1 large paper “background” divided evenly into dark and light sides.
- ♥ 10.0 mL seltzer water
- ♥ 10.0 mL culture water
- ♥ 1 1-mL disposable pipette
- ♥ 20 *Daphnia*
- ♥ 2 pieces of filter paper
- ♥ 2 petri dishes
- ♥ 2 15-cm squares of dark opaque plastic (garbage bag)
- ♥ 1 10-mL graduated cylinder

Answer to Test Question

These students should find a similar trend in their water fleas' response to light at different temperatures.



TEACHER'S NOTES

HYPOTHESIS GENERATION

Question

What is the effect of seltzer water on the phototactic behavior of *Daphnia*?

Sample Hypothesis

The phototactic responses of *Daphnia* will be affected if they are exposed to seltzer water.

Rationale

Students should provide their own rationale for the hypothesis.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of 10% seltzer water.
2. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
3. Prepare 1 large paper “background” divided evenly into dark and light sides.
4. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
5. See Sample Experimental Procedure, Variation 6. Repeat Steps 6 to 9 with the exception of Step 8 where the draining process will remove seltzer water, not acid as described in Variation 6.

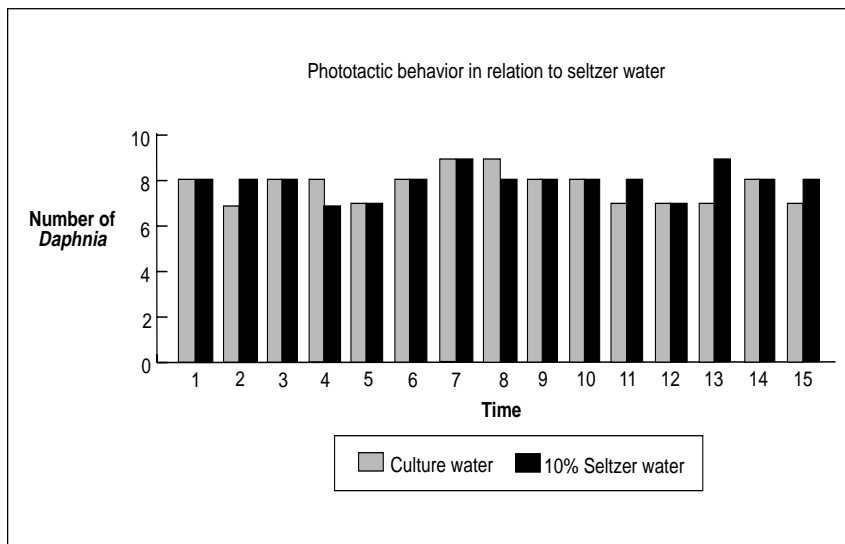
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. Number of *Daphnia* in light when incubated in 10% seltzer water and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	10% Seltzer water	Culture water
1	8	8
2	7	8
3	8	8
4	8	7
5	7	7
6	8	8
7	9	9
8	9	8
9	8	7
10	8	8
11	7	8
12	7	7
13	7	9
14	8	8
15	7	8

Total the number of animals observed in the light throughout the 15 minutes. An appropriate analysis here would be Chi-Square Goodness-of-Fit with the hypothesis that there will be no difference in light preference, that is, that the total number of animal-minutes in the light will be the same in the culture water and in the seltzer water.



Graph M. Effect of seltzer water on *Daphnia*.

TEST QUESTION

Although the phototactic response of *Daphnia* was not altered by exposure to seltzer water, the activity level was altered. Would you consider seltzer water a toxin? Explain.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

VARIATION 9

The Effect of the Time of Day on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS

Students will determine if the diurnal cycle of *Daphnia* influences their phototactic response.

HYPOTHESIS GENERATION

Question

What effect does time of day have on the phototactic response of *Daphnia*?

Interpretation

A decrease in activity of the *Daphnia* in the treatment was noticed, but there was no difference in the number of animal-minutes in the light in the treatment and in the culture water.

Answer to Test Question

Seltzer water could be considered a toxin for the *Daphnia* because they did not behave normally when exposed to it. The failure to measure a difference may mean that the correct response to detect the effect of seltzer water was not chosen.

TEACHING TIP

Students in two different biology classes could perform this variation and compare data. One class could be scheduled for the early morning hours, and the other for the late afternoon. The early class should plan to come in about 7 a.m.



TEACHER'S NOTES

Sample Hypothesis

Daphnia will show a stronger phototactic behavior in the early morning than in the late afternoon.

Rationale

During the early morning hours, *Daphnia* should be following their food source to the surface. In the heat of the afternoon or in the waning hours of light, they may retreat from the lighted surface, again following their food source.

Sample Experimental Procedure

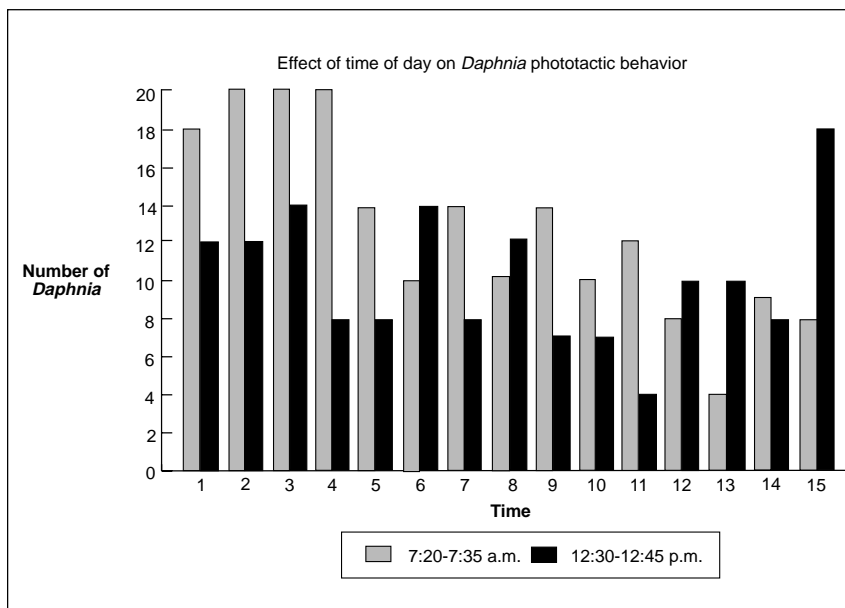
1. Drain the water from 20 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of culture water.
2. Prepare 1 large paper "background" divided evenly into dark and light sides.
3. Set the dish on the divided background so that half the dish is over light and half is over dark.
4. Count the number of water fleas in the light in each dish for 15 minutes at 2 different times of the day, such as in the morning and at midday.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Number of *Daphnia* in the light during each minute of a 15-minute observation at different times of day. There were 20 *Daphnia* in each observation dish.

Time (minutes)	7:20 to 7:35 a.m.	12:30 to 12:45 p.m.
1	18	12
2	20	12
3	20	14
4	20	8
5	14	8
6	10	14
7	14	8
8	10	12
9	14	7
10	10	7
11	12	4
12	8	10
13	4	10
14	9	8
15	8	18



Graph N. Effect of time of day on *Daphnia's* phototactic behavior.

TEST QUESTION

Do *Daphnia* show a positive or negative phototactic response in the early morning? What evidence supports your answer?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 6.

VARIATION 10

The Effect of Phosphate-Containing Detergent on the Phototactic Behavior of *Daphnia*

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS

Students will compare the phototactic responses of *Daphnia* exposed to phosphate with those of *Daphnia* not exposed to phosphate.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 1 large paper “background” divided evenly into dark and light sides
- ♥ 2.0% trisodium phosphate (TSP)

SAFETY PROCEDURES

See Safety Procedures, Variation 2.

Interpretation

In the early morning, 191 animal-minutes were spent in the light. In the early afternoon, only 152 animal-minutes were spent in the light. These data support the hypothesis. During the early morning hours, *Daphnia* should be following their food source to the surface. In the heat of the afternoon or in the waning hours of light, they may retreat from the lighted surface, again following their food source.

Answer to Test Question

Daphnia show a positive phototactic response in the early morning. If there were no phototactic response, one would have expected to find only 150 animal-minutes in the light. If the response were negative, one would have expected to find still fewer. The observation of 191 animal-minutes in the light supports a positive phototactic response.

TEACHING TIPS

See Teaching Tips, Variation 2.



TEACHER'S NOTES

HYPOTHESIS GENERATION

Question

What is the effect of phosphate-containing detergent on the phototactic response of *Daphnia*?

Sample Hypothesis

The phototactic responses of *Daphnia* will be affected if they are exposed to phosphate-containing detergent.

Rationale

Phosphate is a recognized pollutant. It should be assumed to be harmful to the normal functioning of an organism.

Sample Experimental Procedure

1. Drain the water from 10 *Daphnia* by gently pipetting them onto filter paper. Quickly transfer them to a petri dish with 10.0 mL of 2.0% TSP.
2. Repeat the same draining process for another 10 *Daphnia*, but transfer these water fleas to a petri dish with 10.0 mL of culture water.
3. Prepare 1 large paper “background” divided evenly into dark and light sides.
4. Set both dishes on the divided background so that half the dish is over light and half the dish is over dark.
5. See Sample Experimental Procedure, Variation 6. Repeat Steps 6 to 9 with the exception of Step 8 where the draining process will remove TSP, not acid as described in Variation 6.

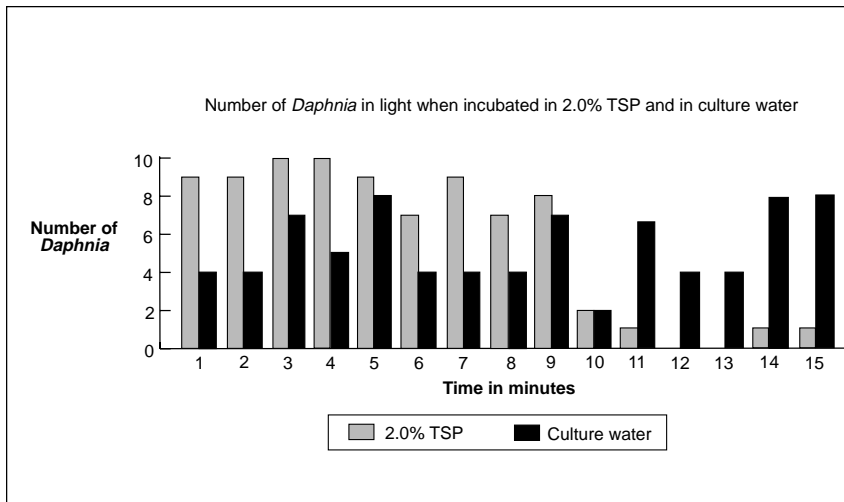
DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 11. Number of *Daphnia* in light when incubated in 2.0% TSP and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	2.0% TSP	Culture water
1	9	4
2	9	4
3	10	7
4	10	5
5	9	8
6	7	4
7	9	4
8	7	4
9	8	7
10	2	2
11	1	7
12	0	4
13	0	4
14	1	4
15	1	4

An appropriate analysis would be Chi-Square Goodness-of-Fit using the hypothesis that there will be no difference in the number of *Daphnia* in the light in different solutions.



Graph O. The effect of phosphate on the phototactic behavior of *Daphnia*.

TEST QUESTION

Create graphs to display the following experimental results obtained by two different researchers.

Table 12a. Number of *Daphnia* in light when incubated in 2.0% TSP and in culture water. Ten *Daphnia* were in each container for 15 minutes.

Time	2.0% TSP	Culture water
1	9	8
2	9	7
3	10	8
4	10	9
5	9	7
6	7	7
7	9	7
8	7	7
9	8	8
10	2	8
11	1	7
12	0	6
13	0	8
14	1	8
15	1	8

Interpretation

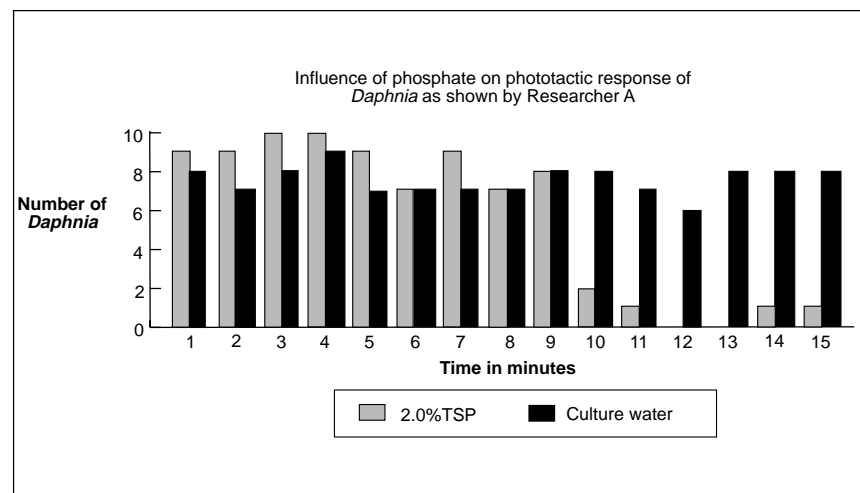
More animal-minutes in the light were observed in the phosphate treatment (83) than in the culture water (72). These data support the hypothesis that phosphate pollution alters the phototactic response of *Daphnia*.

**Answer to Test Question
Graphs P and Q.** Influence of phosphate on phototactic response of *Daphnia* as found by two different researchers.

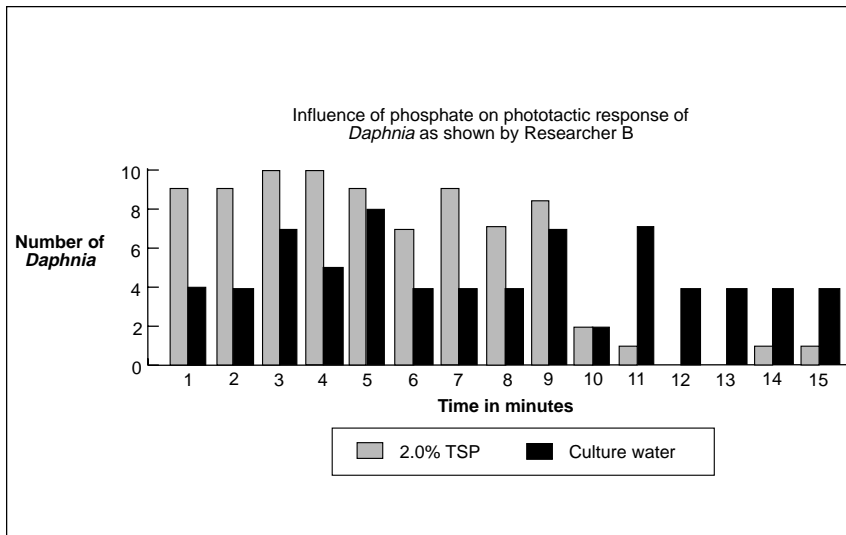
TEACHER'S NOTES

Table 12b.

Time	2.0% TSP	Culture water
1	9	4
2	9	4
3	10	7
4	10	5
5	9	8
6	7	4
7	9	4
8	7	4
9	8	7
10	2	2
11	1	7
12	0	4
13	0	4
14	1	4
15	1	4



Graph P. Researcher A's graphed results.



Graph Q. Researcher B's graphed results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL
Blind or Visually Impaired

- See Variation 6.

Interpretation

These results are the opposite. Group P shows a stronger, positive phototactic response in TSP than in culture water. Graph Q shows the stronger response in culture water. It is possible that Researcher A did the experiment at a different time of day or at a different temperature. The influence of these variables on the response was not tested.

NATURE'S EARLY WARNING SYSTEMS

Directions for Students

INTRODUCTION

The sun is barely up, and near the shore of a shallow lake there seem to be hundreds of identical little water fleas about 5 centimeters long. By early afternoon this bright sunny day, the fleas have nearly all disappeared. Where did they go? Are they really fleas? Could they bite you? Are they really all identical, or do you just need to look closer to recognize differences between them? Are they in all shallow lakes and ponds?

Some of these questions are answered easily. For instance, if the small fish have not eaten them and if the lake has not changed during the day, they are probably resting on the mud at the bottom in the early afternoon. They will be back to the surface later in the afternoon, as they follow a rhythm set by light reception and their biological clocks. They are not really fleas, even though they make a jerky, flea-like movement, and they will not bite you. A good meal for these crustaceans (*Daphnia*) is single-celled green algae, a few diatoms, and some bacteria. At some times of the year, they really are all alike. Under some conditions, *Daphnia* produce nearly identical offspring—at least, they're all daughters!

The last two questions are harder to answer. What accounts for the absence of an animal could be just that it was never there, or that conditions were not right for its survival when it arrived, or that conditions have changed since it arrived. According to wildlife officials, *Daphnia* are transported easily by birds. We also know that *Daphnia* are extremely sensitive to some toxins, such as low concentrations of industrial wastes or run-off from lawn care chemicals. They are more sensitive to most materials that will harm them than are the fish who feast on them. Their sensitivity, small size, and ease of culture make them excellent subjects for assaying the toxicity of waters before other organisms show any signs of damage. You will explore the effect of a toxic material on the physiology of *Daphnia* at levels too low to be lethal. See Figure 1.

OBJECTIVES

At the end of this lab, you should be able to:

- Describe possible physiological or behavioral effects of sub-lethal doses of ethanol on *Daphnia*.

SAFETY PROCEDURES



Wear goggles and a lab apron when working with chemicals.



Work away from any open flames when using ethanol, especially when wearing gloves.



Wear latex gloves when working with ethanol. It can be absorbed through your skin.



Wash your hands after all work is completed.



Do not consume ethanol.



Dispose of broken glassware in appropriate containers.



Use KittyLitter™ to absorb any large spills. Sweep up the KittyLitter™ after it has absorbed the spilled material.

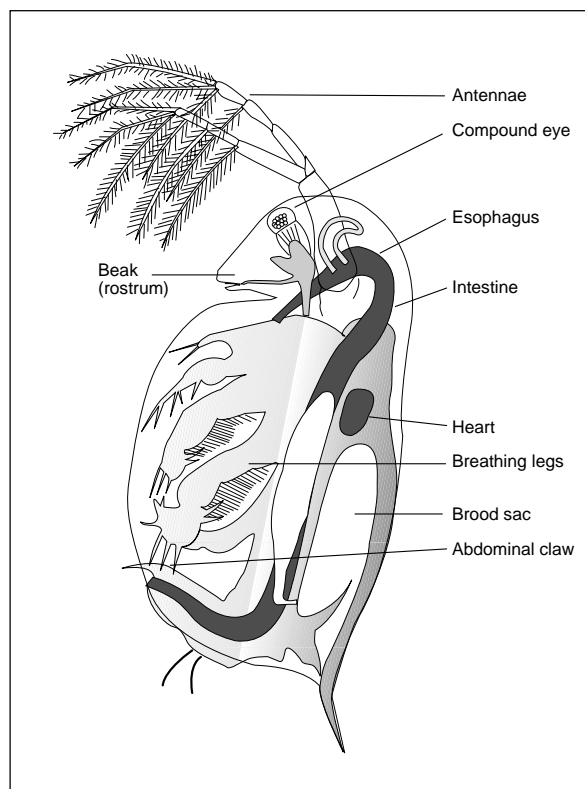


Figure 1. A species of *Daphnia* with several internal structures identified. (Used with permission. Collins, K. & B. (1985). *Daphnia — A Handy Guide*. *The American Biology Teacher*, 47(50), 299.



MATERIALS NEEDED

You will need the following for each group of three students in a class of 24:

- ♥ 10 *Daphnia*
- ♥ 1 culture dish
- ♥ 1 50-mL jar or beaker
- ♥ 1 wide-bore pipette
- ♥ 2 1-mL plastic, disposable pipettes
- ♥ 1 compound microscope
- ♥ 1 depression slide and cover slip
- ♥ 1 lab journal
- ♥ 1 pencil
- ♥ 1 clock with second hand or a stopwatch
- ♥ 1 calculator
- ♥ several paper towels or filter paper
- ♥ 2 pairs of latex gloves (*optional*)
- ♥ 25.0 mL of 2.0% ethanol solution

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of chemical toxicity and *Daphnia*. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

HYPOTHESIS GENERATION

From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis. Answer the following questions:

1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. What is the variable in your experiment?
4. What other variables will you need to control?
5. Why is controlling variables important?

PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

You must have your teacher approve this protocol before you begin this experiment.

QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Combine your results with those of all your classmates. Construct a graph showing the heart rates of *Daphnia* exposed to ethanol and not exposed to ethanol.
2. Do these data support or refute your hypothesis? Explain.
3. If half of the ethanol-treated animals had depressed heart rates and the other half did not, would you accept or reject your hypothesis? Explain.
4. Suppose all of the animals showed a 20% depression in heart rates when exposed to ethanol, while those tested from another lake by students across town showed a 10% depression. How could you explain the difference?
5. Propose a test of your explanation to the question above.

DESIGN AND VARIATIONS OF THE CORE EXPERIMENT

After collecting and analyzing these data from the Core Experiment and sharing the results and conclusions with the class, brainstorm ideas for experiments you could do next. Think of questions that occurred to you as you conducted the Core Experiment on *Daphnia* and their role as environmental indicators. What quantifiable experiments could be done based on the observations you made? Questions other students have studied include the following:

- What is the effect of ethanol on *Daphnia* heart rates?
- What is the effect of varying concentrations of ethanol on *Daphnia* heart rates?
- What is the effect of phosphate on *Daphnia* heart rates?
- What is the effect of varying concentrations of phosphate on *Daphnia* heart rates?
- What is the effect of vinegar on *Daphnia* heart rates?
- What is the effect of varying concentrations of vinegar on *Daphnia* heart rates?
- What is the effect of vinegar on the phototactic response of *Daphnia*?
- What is the effect of temperature on the phototactic behavior of *Daphnia*?
- What is the effect of seltzer water on the phototactic behavior of *Daphnia*?
- What is the effect of phosphate-containing detergent on the phototactic response of *Daphnia*?