# The Impact of Mollusks on Water Quality

SYNOPSIS

Students will determine how a filter-feeding mollusk, such as an oyster, clam, or mussel, responds to changes in its immediate environment. Students will design experiments to test hypotheses about the effects of environmental variables on filter feeding and identify anatomical structures of a mollusk.

APPROPRIATE BIOLOGY LEVEL

Introductory, advanced, ecology


VARIATIONS 1 TO 9
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CORE EXPERIMENT
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## Directions for Teachers

*Note to Teachers: Information is given for the Core Experiment below. Additional information needed for each variation of the Core Experiment may be found beginning on page 215. 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 the ecological role of filter-feeding mollusks in fresh and/or saltwater ecosystems.
- Identify the major anatomical structures and functions of a filter-feeding mollusk.
- Describe how turbidity and salinity affect the behavior of a filter-feeding mollusk.
- Explain how a population of oysters, clams, or mussels impacts water quality.

### MATERIALS NEEDED

*For the teacher-led demonstration, you will need the following for a class of 24:*

- 1 can of dead oysters or oysters in the shell from the fish market or store
- 1 live bivalve
- 1 algal culture
- carmine powder
- 1 eyedropper
- 1 handheld magnifying glass
- 1 light
- 1 video microscope (*optional*)
- 1 dissecting microscope (*optional*)

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

- 5 live freshwater clams, such as the asiatic clam *Corbicula fluminea* or saltwater hardshell clams *Mercenaria mercenaria*, marine blue mussels *Mytilus edulis* or oysters, such as *Crassostrea virginica*

LENGTH OF LAB

A suggested time allotment is as follows:

*Day 1* (45 minutes)

- Demonstrate filtration and design experiment.

*Day 2* (45 minutes)

- Set up experiment. Place animals into treatment aquaria.

*Day 3* (20 minutes)

- Move animals from treatment tanks to collection containers.

*Day 5* (45 minutes)

- Collect and discuss data.

PREPARATION TIME REQUIRED

Two weeks before lab:

*60 minutes:*

- Set up algal cultures and maintenance aquaria.

*15 minutes:*

- Set up the video camera (optional) and a shallow tray of water for the introductory demonstration.

TEACHER'S NOTES

*(continued from p. 203)*

- 0.5g (3t) kaolin powder
- 3 3.8-L (1-gallon) aquaria or wide-mouthed glass or plastic jars
- 12 L artificial seawater if using marine bivalves or 12 L nonchlorinated water, such as spring water if using freshwater bivalves
- 1 aerator
- 1 thermometer (°C)
- 15 petri dish halves
- 1 Pasteur pipette
- 10 10-mL graduated cylinders

## SAFETY PROCEDURES


Do not collect some species of freshwater mollusks. Some freshwater mussel species are endangered and many are restricted to single drainage systems.


Have students wash their hands before and after the lab.


Caution students not to pick up an aquarium containing water.


Have students carry expensive glass instruments such as hemocytometers in unbreakable containers.


Do not discard mollusks or wash water into local waterways, particularly if the mollusk used is not from the local area.


When adding acid, remember to add acid into water.


Wear safety goggles.


Do not eat any of these organisms.

## DIRECTIONS FOR SETTING UP THE LAB

- Expand the algal cultures by transferring 10 to 20 mL of culture to 1 liter of the appropriate culture medium. For freshwater bivalves, use selected algae of the Division Chlorophyta. *Ankistrodesmus* sp. or mixtures of *Ankistrodesmus* sp., *Chlamydomonas* sp., *Chlorella* sp., and *Scenedesmus* sp. are good food sources. The Bigelow Laboratory for Ocean Sciences does sell starter algal cultures. Contact information is as follows: Bigelow Laboratory for Ocean Sciences, McKown Point, PO Box 475, West Boothbay Harbor, ME 04575-0475; phone: 207.633.9600; fax: 207.633.9641. If you use the bivalve *Corbicula fluminea*, do not use *Selenastrum* sp. They are toxic to the asiatic clam (McMahon, 1991). For marine bivalves, use *Isochrysis* sp., a member of the Division Chrysophyta. Suitable concentrated culture medium for the freshwater species and culture medium for the marine species is available from Aquatic Research Organisms, Inc., PO Box 1271, Hampton, NH 03843-1271; 800.927.1650. The algae grow well at room temperature (22°C) and high light intensity for 12 to 16 hours/day. Use 2 x 4-foot fluorescent light fixtures or a similar setup.
- Set up a maintenance aquarium with aeration, filtration, and the temperature regulated at 18 to 25° C. Aquarium heaters can be used to increase the temperature above ambient. Provide a varied substrate with coarse sand or fine gravel and a few larger rocks. You may find it convenient to set up a smaller aquarium as a feeding station during experimentation. If you will be maintaining the bivalves, you will need to provide algae daily. Add sufficient algae to make the water turn just a very light green/brown. Use care not to overfeed.
- On the first day of this 3-day experiment, set up 3 small aquaria at room temperature with seawater, artificial seawater, or freshwater as appropriate.
- If fresh seawater is unavailable, use commercial instant seawater. Mix with rain, pond or river water, or with dechlorinated tap water to attain a salinity of about 35 parts per thousand (ppt).

• Aerate the water in the aquaria with a small air pump without a filter, or manually aerate the water three or more times a day by dishing out and pouring back water to create bubbles.

TEACHER'S NOTES

## TEACHER BACKGROUND

### Content Information

Clams, mussels, and oysters are essential to the ecology as well as to the economic potential of the waters where they are found. Their population levels and health may indicate the condition of the water and the organisms found in it. They filter phytoplankton and other particulate matter from the water column and transfer it as biodeposits — feces and pseudofeces — to the sediment surface where it is a food resource for other aquatic animals. The removal of this matter allows for better light penetration in the water for benthic plants that are light-dependent.

These mollusks all filter water for feeding and respiration. Otherwise, their life habits are very different. Clams partially bury themselves in sand and clay, living as infaunal burrowers in relatively quiet water. Marine mussels attach to hard objects with byssus threads, living epifaunally in relatively agitated water. Oysters glue their left shell valve to rocks and other shells, living epifaunally cemented in relatively quiet water.

Filter-feeding mollusks are some of the first order consumers that feed on algae. They remove large amounts of these planktonic organisms from the water and thus participate in a natural cleansing process. Normally, they are able to assist in reducing the intensity of algal blooms. It has been estimated that the population of oysters living in the Chesapeake Bay was once so great that they could filter the entire volume of water in a few days, but they have declined so much that this process now takes over a year (Newell, 1988). In other areas, species of mollusks that have been introduced, like the asiatic clam, *Corbicula*, and the zebra mussel, *Dreissena polymorpha*, have proliferated to the point where special removal steps must be taken to prevent them from blocking the water intake pipes of water treatment plants and factories.

Chemical testing and biological assays reveal that human activities have influenced water quality of our rivers, lakes and estuaries significantly. One of the factors contributing to the deterioration of water quality is the disruption of the balance between producers and consumers. This disruption results, for example, in the algal blooms that occur during the summer in many waterways. The prolific growth of the algae is the result of large inputs of nitrates and phosphates. These nutrients come from agricultural runoff, sewage treatment outflows, or even acid rain.

A stable environment is necessary to the survival of all organisms and those that directly or indirectly depend upon them. Some species are unable to adapt even to slight changes in environmental factors, such as salinity, water temperature and movement, food source, oxygen levels. Other organisms are able to adapt and compensate within limits to changes in these factors. For example, organisms in an estuarine environment must be highly tolerant of fluctuating salinity levels.

In 1972, the impact of tropical storm Agnes' continued rainfall was observed not only in the Susquehanna watershed, but many kilometers away throughout the Chesapeake Bay. There were high mortality rates of organisms even in estuaries due to freshwater extending far into the estuaries. In addition, the low density freshwater covering the surface of the estuary prevented the water column from mixing to reoxygenate waters below. Without oxygen in the bottom waters, anaerobic bacteria replaced aerobic bacteria. Instead of oxygen, the anaerobic bacteria produced hydrogen sulfide gas that was toxic to other aerobic organisms. A change in one environmental factor had devastating effects for the entire ecosystem.

TEACHER'S NOTES

In addition to creating nutrient fluxes, rain and irrigation produce periodic changes in turbidity as the runoff carries a heavy sediment load. Where fires rage because smaller natural fires have been reduced or where lands have been cleared for development, the sediment load can be extremely high. Filter-feeding mollusks have a mechanism to clear some of the excess particulates, but when the concentration of suspended particles becomes high, the filtration rate slows and some species starve to death or suffocate.

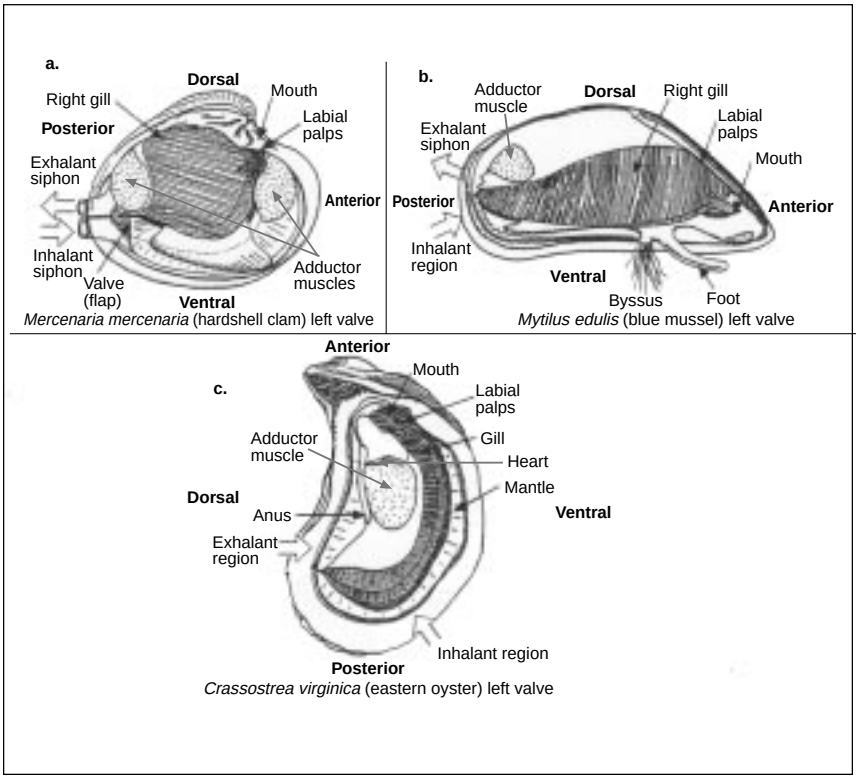



Figure 1. a. *Mercenaria mercenaria* (hardshell clam). b. *Mytilus edulis* (blue mussel). c. *Crassostrea virginica* (eastern oyster).

Bivalves create a current of water through their bodies by the beating of microscopic cilia on the surface of their gills and labial palps. See Figure 1. As water moves over the gills, inorganic and organic particles, which are their source of food, are caught by the gill cilia. They also may filter out the organisms responsible for paralytic shellfish poisoning. Particles larger than 2.0 to 3.0 µm are removed, but zebra mussels and asiatic clams are able to filter even smaller particles (1.0 µm). Non-nutritious particles are discarded as pseudofeces, while food particles are taken into the digestive tract. Pseudofeces are discarded by valve clapping. Indigestible fecal matter is bound together by mucus, released into a cavity near the exhalant siphon, and expelled on exhalant currents.

Many forms of pollutants are detrimental to the health of bivalves. These include organic sewage effluents, chlorine, and industrial effluents. Many times the health of the adult animal is not affected directly but their reproductive capacity is, and an unhealthy population results. Heavy metals may accumulate in their flesh. When the flesh is eaten by a predator such as a bird, a starfish, or even a human, the toxins are transferred to the predator. Many oyster beds have been closed to commercial harvesting due to potential fecal contamination. This has no adverse effects on shellfish, but may be a human health risk.

Phytoplankton and bacteria are major sources of food for oysters. One particular species of algae, *Isochrysis galbana*, recommended here as a lab food source, is a member of the Chrysophyta phylum. This species has two flagella at its anterior end and is a unicellular, motile sphere approximately 5 µm in diameter. The normal concentration of algae in natural waters may be in the range of several thousand per milliliter. In this lab, concentrations may reach $10^7$ cells per milliliter.

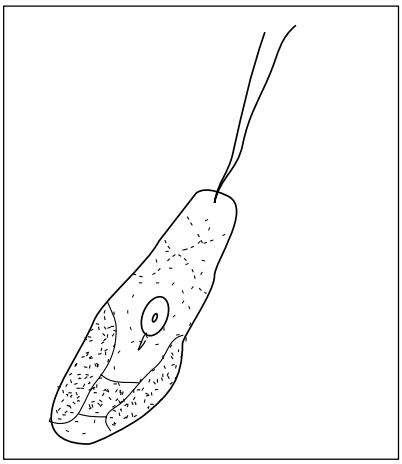


Figure 2. *Isochrysis* sp. (Newell & Newell, 1967).

### Pedagogical Information

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

| Correct Concept | Misconception |
|---|---|
| • Clear water is not a sufficient indicator of water quality. Other indicators such as chemical, bacterial, and pollutant counts provide much more useful information regarding the safety of water. | • Good water quality means that the water is clear without concern for other parameters. |
| • Filter-feeding mollusks play a major role in reducing turbidity in aquatic ecosystems. | • Turbidity in aquatic ecosystems is reduced only by settling of particulate matter. |

## INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

### Introduction

Obtain several live bivalves and demonstrate with carmine powder how water flows through the organisms. This can be done by using an eyedropper to transfer a drop of carmine powder mixed in a few milliliters of water to the water around the mollusk. Ask students what the organisms may add or remove from the water. Refer to Figure 1 to orient students to ventral and dorsal aspects. Bivalves produce two types of biodeposits — feces and pseudofeces — that in oysters are expelled on opposite sides of the organism. In bivalves with siphons, i.e. all clams and mussels, pseudofeces are ejected through the inhalant siphon and feces are expelled through the exhalant siphon. Identify the posterior and anterior ends of the specimen. Siphons or inhalant apertures are at the posterior end and labial palps are at the anterior end.

### TEACHING TIPS

- Keep the list of hypotheses your students generate in class discussion. They may be helpful in the student design phase if a group is having difficulty.
- These data for this variation are presented as a scatter graph. They also could be presented as a bar graph of total deposits.
- A cross-curriculum project could be developed with the home economics or cooking courses.
- Living oysters and mussels are available from biological supply houses or seafood markets.
- If oysters are unavailable, hardshell clams or blue mussels may be substituted. If freshwater mollusks are used, set up freshwater instead of saltwater aquaria.
- If it is not convenient to have both clams and oysters, use one bivalve and study the filtering effect of increased numbers of bivalves. For example, place 3 bivalves in Aquarium 1 and 6 bivalves in Aquarium 2. Sample Data would be as follows:

| Condition | Evidence of filtering | Biodeposit volume (mL) | Biodeposits per bivalve |
|---|---|---|---|
| 3 Bivalves | A little | 0 to 1 | 0.033 |
| 6 Bivalves | A lot | 0 to 2 | 0.033 |
| Unstocked | No | 0 to 0 | 0.0 |

### Interpretation

The greater the number of bivalves, the more material filtered from the water.

- Try to use different individual organisms for each variation.
- Feeding varies by body size. Try to reduce variation between treatments by matching animal sizes in treatment aquaria.
- Remove dead animals promptly because as they decay they foul the water quickly. If an animal's shell is more than 1 mm open, touch the animal lightly. If it does not respond by closing, attempt to pull open the valves. If they open easily, the animal is dead.
- Freshly collected animals can survive for weeks without eating. It is preferable to feed them before you begin the experiments. The best diet is mixed algae.

## TEACHING TIPS

- Inexpensive aquaria may be constructed from plastic, 1-gallon milk containers or 3-L soda bottles by cutting off the tops. Ice cream, cottage cheese, or an assortment of other food storage containers also may make adequate treatment and collection containers. Closely monitor the temperature and water quality in these small containers as well as in the large ones.
- Wide-mouth, glass canning jars, 1-L beakers, or food storage containers make suitable "small collection containers."
- Substitute smaller aquaria for larger ones if your supply of seawater is limited.
- Kaolin is a form of clay used for ceramics and is available at pottery supply stores.
- Carborundum powder or a yeast suspension stained by heating with Congo Red may be substituted for carmine powder. All of these materials are likely to be rejected as pseudofeces after sorting, although some bivalves retain yeast. You also can use pureed frozen spinach with equal parts of water. If it clouds the water too much, rinse it first through a coffee filter, saving the particles on the filter. If you are using a saltwater bivalve, use saltwater to suspend your particles because freshwater floats on saltwater.
- Subtract the amount of material that settles in controls from experimental treatment amounts.
- If the combination of things you use does not show up well with the bright light and white background, try creating a dark field by putting a dark paper on the microscope stage, angling the light in from the side, and shielding the light from your eyes with a paper over the top.
- You do not actually measure filtration rate; you measure biodeposit production. In the oyster, *Crassostrea virginica*, no biodeposits form with 10 mg/L fuller's earth; at higher turbidity biodeposits form, but when turbidity is increased as high as 100 mg/L, water transport is depressed

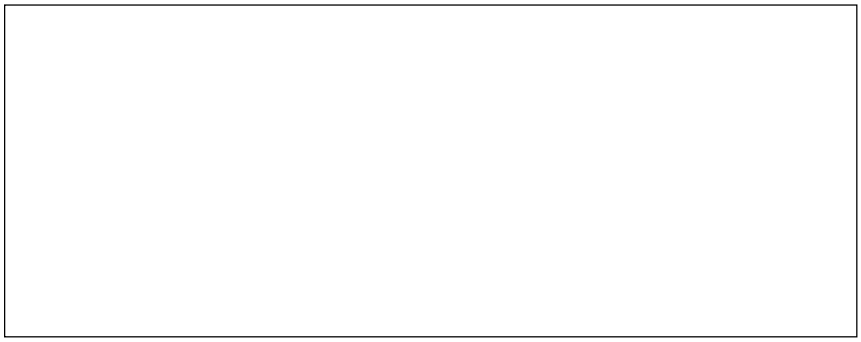


Figure 3. Shells of various bivalves should be labeled so that students can identify their specimens. Shell "1" is *Mercenaria mercenaria*, shell "2" is *Mytilus edulis*, and shell "3" is *Crassostrea virginica*.

Display a can of store-bought oysters to stimulate discussion of their food value. About 90% of food ingested by each successive stage in a food chain is respired, i.e., only 10% is available to be transferred to the next higher level in the food web. Marine producers, at least the phytoplankton, are difficult to harvest and process because of their small size and poor digestibility. As the second link in the food chain, marine bivalves that efficiently harvest from algal primary production provide a palatable, nutrient-rich animal food. Why are oyster bars sometimes declared unsafe? The most common hazard is the accumulation of bacterial pathogens. A natural hazard is the accumulation of dinoflagellate toxins. Industrial pollutants, both organic and inorganic, can accumulate to toxic levels (Boyle, 1981). Move to a discussion of the ecological role of shellfish as well as their economic role.

### HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can transform into hypotheses. Allow the students to reflect on the demonstration, then ask the following questions:

- What do oysters do that can be measured?
- What variables might affect these behaviors?

If necessary, you may suggest examples from the following list. Pair items from the first list with those from the second list and select possible hypotheses. Remind students of their knowledge of the bivalves so that their hypotheses have some rational biological basis.

| Bivalve activities might include: | Variables might include: |
|---|---|
| Respire | Temperature |
| Eat | Light |
| Excrete | Salinity |
| Open and close shells | pH |
| Grow | Dissolved oxygen |
| | Toxic wastes |
| | Food type |
| | Food density |
| | Time of day |
| | Nature of substrate |

## SAMPLE HYPOTHESES

- Oyster feeding rates will remain constant within a fixed range of salinities.
- Feeding rates will be greater in the dark than in the light.
- Heart rates will increase with a decrease in oxygen concentration.

*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 214.*

### Question

What role does filtering play in the life of a mollusk?

### Hypothesis

Both oysters and clams actively remove algae from the water.

### Rationale

Bivalves feed by filtering particulate matter from water. When exposed to increased turbidity, their filtering rate declines although the total amount of particles removed from the water will increase. However, when a mollusk takes in too much inedible particulate matter, it will impact negatively on the life functions of the mollusk. The result will depend on the relative weight and size of the oysters and clams.

### Procedure

*Day 1*

1. Place 3 small marine aquaria with artificial seawater in a dark, quiet place.
2. Aerate the water in the aquaria manually by dishing out and pouring back water to create bubbles, 5 or more times daily. Include when you first arrive in the morning and before you leave school each day.

*Day 2*

1. Place 1 each of 5 small, healthy clams in one half of a petri dish. Place the petri dishes in 1 aquarium. Repeat this procedure with 5 small, healthy oysters in a second aquarium, and with 5 empty clam or oyster shells for the control in a third aquarium. See Figure 4.
2. Acclimate to room temperature for 1 to 2 weeks.

### TEACHING TIPS

by 50%. Some oysters can tolerate up to 700 mg/L silt (Jørgensen, 1966).

- If only small amounts of biodeposits are produced, a measuring device may be made from a tuberculin (1-mL) syringe. Plug the attachment point for a needle with a piece of pipe cleaner or cotton. The syringe can be cleaned and reused. A serological pipette graduated to the tip also makes a good substitute for a small graduated cylinder.

TEACHER'S NOTES

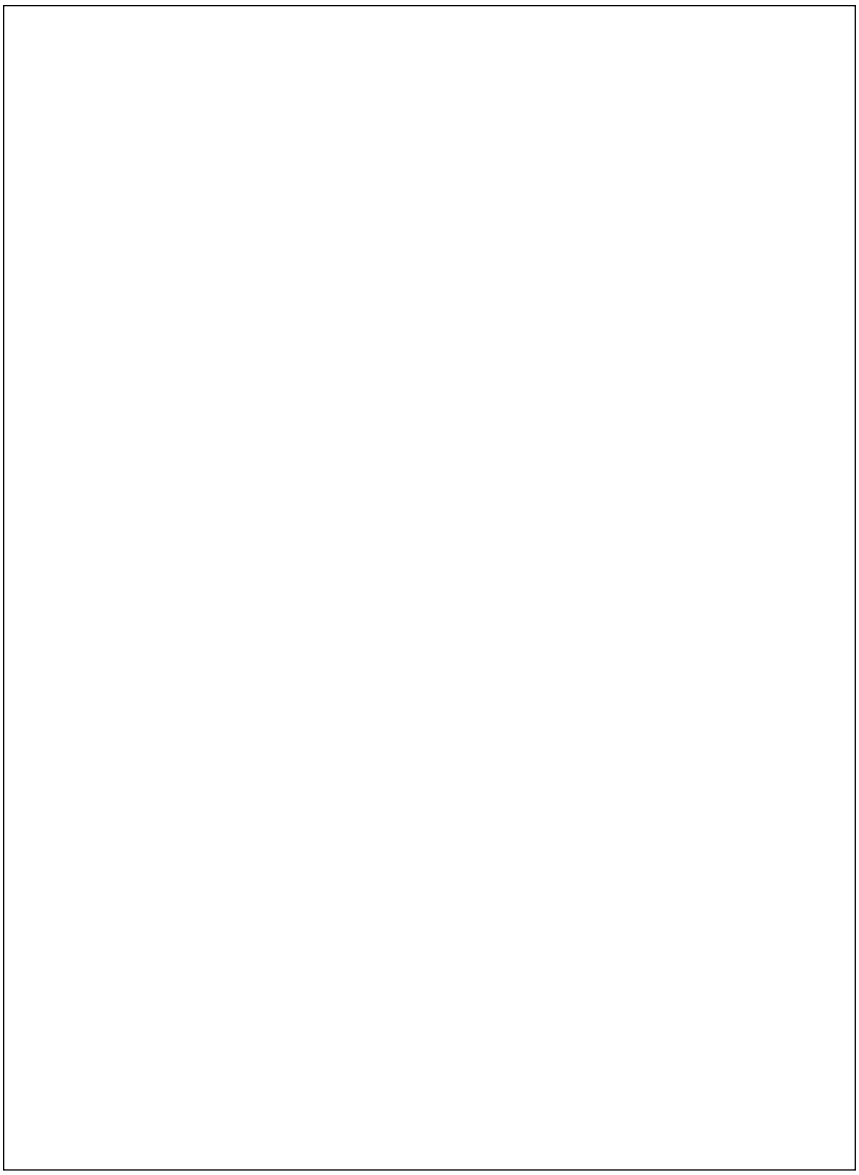


Figure 4. Experimental design setup.

*One week later*

1. Add a mixture of 0.5 g of kaolin mixed in 0.25 L of seawater to each of the 3 aquaria. Stir the water gently to homogenize.
2. Keep all 3 aquaria in a dark, quiet place and allow the bivalves to filter the water, undisturbed, for 24 hours.

*Next day*

1. After the 24-hour period, examine the water in each of the petri dishes in the 3 aquaria for evidence of filtering to determine the presence of fecal and pseudofecal pellets.
2. Measure the biodeposits produced by each clam and oyster using the following procedure:
   a. With a Pasteur pipette, remove the biodeposits produced by each organism from the petri dish.
   b. Transfer the biodeposits produced to separate 10-mL graduated cylinders.
   c. Allow the biodeposits to settle and read the number of milliliters on the cylin-

der. This number gives a crude measure of biodeposits produced that is an indirect measure of filtration and feeding rate.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data

| Condition | Evidence of filtering | Biodeposit volume |
|---|---|---|
| Clams | Only a little | > 0.1-mL |
| Oysters | Yes | 0.2-mL |
| Unstocked | No | 0.0-mL |

### Interpretation

Oysters are more productive at filtering particulate matter from the water.

## TEST QUESTION

Why would you expect extremely high concentrations of suspended particles to reduce filtration rates? Do your data support a reduction in rates at high concentrations?

### Answer to Test Question

Filter-feeding bivalves depend on ciliary filtering and sorting. If the filtering mechanism is overwhelmed by sediment, the organism will be unable to obtain adequate oxygen or adequate nutrients. Shutting down the filtration system while suspended particle density is high could be more efficient than continuing to clean debris from the gills. Many bivalves are unable to survive in waters with high silt. These data do not support the hypothesis that there is a reduction in filtration rate at high concentrations of suspended particles because only one particle concentration was tested. At least two concentrations are needed to test this hypothesis.

## STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for additional experiments. They should think of questions that occurred to them as they conducted their first experiment. 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:

- How will factors such as temperature, pH, or salinity affect biodeposition rates?
- Do the rates of biodeposit production differ among oysters, clams, and mussels?

## 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

- Have the student who is visually impaired do library research on a scientist, such as Dr. Geerat Vermeij. Dr. Vermeij has been blind since the age of 3 and is well known for his ability to identify mollusks simply by the use of touch (Vermeij, 1996b). Sighted students may wish to identify different species of mollusks provided by the instructor by using just the sense of touch.
- Prepare raised-line drawings or braille paintings of the cross section of the mussel, clam, and oyster shown in Figure 1 of the Core Experiment. Enlarge each mollusk to the size of a sheet of braille paper. See Figure 5.

TEACHER'S NOTES

Figure 5. Making a raised-line drawing with a stylus on braille graph paper.

- Transfer a drop of carmine powder in solution to the water around the mollusk. Visually impaired students may be able to detect the path of the carmine in the gills with a light sensor.
- Provide students who are blind with a variety of mollusks to understand the differences in the animals used in this activity.
- Have a sighted lab partner remove the biodeposits produced by each mollusk with a pipette and place them in a 10-mL plastic graduated cylinder with raised lines for volume amounts. The student who is visually impaired can use a thumbnail or plastic card to determine the number of spaces indicating volume. With a light sensor, the student can determine the amount of pseudofeces produced by locating the line that separates the pseudofeces from the liquid. See Figure 6.

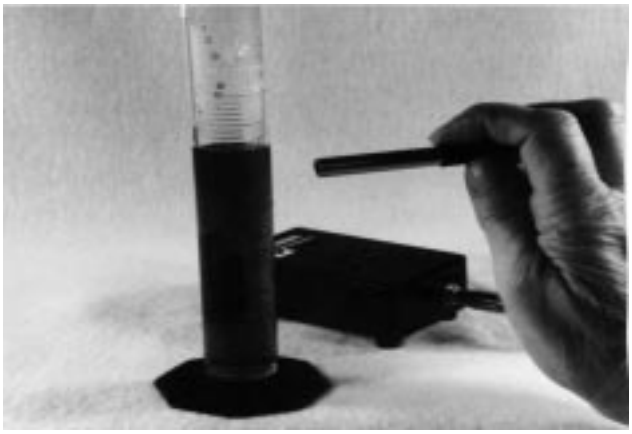


Figure 6. Using the light probe to determine liquid level in a plastic graduated cylinder with raised lines for volume amounts. Accuracy from ± 1 or 2 mL varies with the experience of the operator and the type of probe used.

### Deaf or Hard-of-Hearing

There are no specific concerns related to this investigation for the hearing impaired. Evaluate the communication skills within the groups containing members who are deaf.

TEACHER'S NOTES

### Gifted

Have the students who are gifted do independent research on the local mollusks, gathering information for a class report on the population, habitat characteristics, life cycle, and ecology of the organisms. They could contact mentors from local universities, museums, or shell-collecting clubs and work on ways to use computerized data collection methods both in the lab and in the field.

### Mobility Impaired

- Provide a comfortable position for microscope work for students in wheelchairs. If counters in the laboratory are too high, consider transferring the student to a platform chair that is secured to a 20 x 24-inch wooden platform with sturdy caster wheels. See Figure 7.

Figure 7. Wheelchair platform for microscope work.

- Offer students with only one arm access to clipboards or other available clamping devices that hold objects or writing materials.
- Offer students who are palsied access to a microscope that has been secured to the counter. A frame that accommodates a microscope can be fastened to the lab table.
- Provide students who are palsied with a guard on the oculars to steady their heads

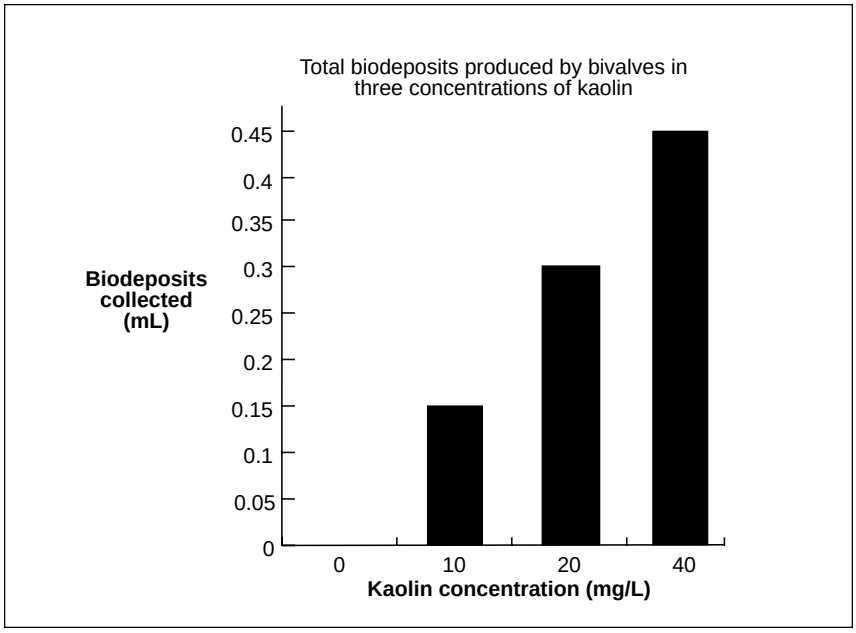
while using a microscope. The small, inexpensive rubber shields such as those used on cameras to keep out stray light work well.

- Provide a bar on the microscope's fine and course adjustments that will allow them to be turned easily.

## ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment may include the following:

1. Identify the independent and dependent variables in this experiment.
2. Identify the limitations in this experiment in terms of number of trials and explain how the experiment could be improved.
3. Design an experiment to determine the effect of temperature on the ability of oysters to remove particles from the water.




Graph A. The production of biodeposits by bivalves exposed to different concentrations of kaolin.

### Answers to Additional Test Questions

1. The dependent variable is total biodeposits; the independent variable is the number of bivalves. The number of bivalves is the independent variable because it can be controlled by the investigator, and the amount of biodeposits produced is dependent on the total number of animals used in each experiment.
2. All animals exhibit individual variation. In order to smooth out this variability, at least 3 aquaria per treatment should be used, i.e., increase replication.
3. Use one turbidity level, a moderate value, and expose 3 to 5 bivalves to varying temperatures from 5 to 30°C. Measure the change in feeding rate with each temperature.

### Answers to Questions and Analysis on Student Page

1. The answers will vary depending on the students' data. The graph title and axes should be similar to those in Graph A.
2. These data support the hypothesis that more biodeposits will be produced when the water is turbid.
3. Doubling the amount of suspended material, kaolin, resulted in a doubling in the amount of biodeposits produced. Compare 10 and 20 mg/L.
4. Results will vary. Students may comment on small sample sizes, inadvertent temperature differences, or student techniques.
5. The amount of biodeposits produced under the identical conditions may vary depending on the physiology of the organisms.
6. The overall health of the animals may influence the rate of filtration, and consequently the amount of biodeposits produced.

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### POSSIBLE SOURCES OF MENTORS

National Marine Fisheries Service, Milford, CT 06460

Access shellfish groups on the Internet and ask for a response from a nearby scientist.

TEACHER'S NOTES

# VARIATIONS ON THE 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.*

TEACHER'S NOTES

# VARIATION 1
# The Effect of Turbidity on Bivalve Filtering Rates

*Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 1.*

## SYNOPSIS

Students will compare the effects of increased turbidity on bivalve filtering rates.

## ADDITIONAL MATERIALS NEEDED

*You will need the following for a class of 24:*

- 12 live bivalves, such as eastern oysters (*Crassostrea virginica*), mussels (*Mytilus edulis*), or asiatic clams (*Corbicula fluminea)*
- 1 permanent marker
- 1 scrub brush
- 5 treatment aquaria
- 20 L natural or artificial seawater or 20 L freshwater
- 12 small collection containers
- 2 g kaolin
- 1 thermometer ($^{o}$C)
- 5 air lines
- 5 air stones
- 1 aquarium pump
- 1 Pasteur pipette with bulb or transfer pipette
- 4 10-mL graduated cylinders

## HYPOTHESIS GENERATION

### Question

How does the influence of particle concentration on feeding rate differ between different species of bivalves?

### Sample Hypothesis

Bivalve filtering rates will increase as the turbidity increases within a fixed range.

### Rationale

There is an optimum filtering rate with increasing turbidity. At some level "x," the bivalve will not be able to filter at a capacity that will maintain a healthy state, and the organism may suffocate.

### Sample Experimental Procedure

1. On the first day of the experiment, set up 4 treatment aquaria with water like that in the maintenance aquarium described in the Core Experiment.
2. Aerate and heat the water to the same temperature as the maintenance aquarium. Set the treatment aquaria in a place to maintain temperatures.
3. Add kaolin to the containers to produce the concentrations shown in Table 1. Make sure students know that kaolin is not a food source for the mollusks. Kaolin must be added to a few milliliters of tap water to produce a slurry before dissolving it in a larger volume of water. Remember to use dechlorinated water as the base for freshwater organisms and seawater as the base for marine organisms. If supplies of lab materials are limited, each group of students can set up the experiment with a different container. Class data may be pooled.

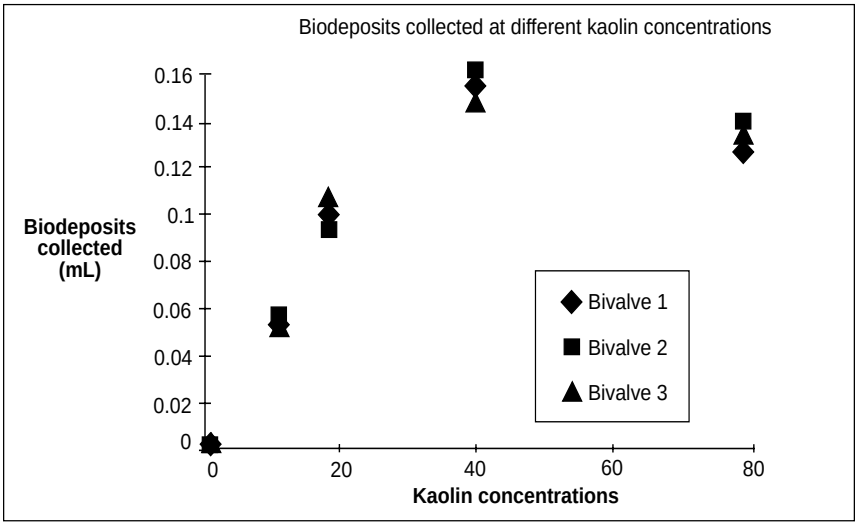
Table 1. Setup for Variation 1.

| Treatment aquarium | Number of bivalves | Kaolin concentration (mg/L) |
|---|---|---|
| 1 (Control) | 3 | 0 |
| 2 | 3 | 10 |
| 3 | 3 | 20 |
| 4 | 3 | 40 |
| 5 | 3 | 80 |

4. Day 2, obtain 12 healthy bivalves. Wash each organism with a scrub brush under running tap water, pat dry, and number with a waterproof marker.
5. Place 3 bivalves in each treatment aquarium.
6. Check all organisms to make sure they are producing biodeposits. If some are not functioning, replace them with others.
7. Leave the organisms in the treatment aquaria for 24 hours.
8. After 24 hours, transfer the organisms to individual small collection containers with the same conditions as the maintenance aquarium. Keep records of which organisms were in each treatment aquarium.
9. After 48 hours in the small collection containers, measure the biodeposits produced by each organism.
   a. With a Pasteur pipette, remove the biodeposits produced by each organism from the small collection container.
   b. Transfer the biodeposits produced by each organism to separate 10-mL graduated cylinders or 15-mL graduated centrifuge tubes.
   c. Allow the biodeposits to settle and read the number of milliliters on the measuring container. This number gives a crude measure of biodeposits produced, which is an indirect measure of the filtration or feeding rate.
10. Return the organisms to the maintenance aquarium. Pool the class data.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data




Graph B. The volume of biodeposits produced in 48 hours by freshwater bivalves incubated for 24 hours in turbid water. These data are hypothetical, but fit the pattern expected with increasing turbidity and are of the magnitude reported in some student experiments.

TEACHER'S NOTES

### Interpretation

More deposits were produced when the amount of suspended material was increased. The relationship was not linear. At the highest kaolin concentration less material was deposited than expected. The bivalves may reduce their filtration rate when the turbidity is very high.

### Answer to Test Question

Filter-feeding bivalves depend on ciliary filtering and sorting. If the filtering mechanism is overwhelmed by sediments, the organism would be unable to obtain adequate oxygen or adequate nutrients. Shutting down the filtration system while suspended particle density is high could be more efficient than continuing to clean debris from the gills. Many bivalves are unable to survive in waters with high silt. Our data support a reduction in filtration rate at high concentrations of suspended particles.

### TEACHING TIPS

- Begin the experiment immediately after putting the organisms in the new temperature. An immediate temperature change is expected to cause an abrupt metabolic change in ectothermic animals. Bivalves show seasonal variation related to both temperature and reproductive cycles. If the organisms are allowed to acclimate to the new temperatures, they may show no change in metabolism, show the expected immediate change in metabolism, or show a reverse metabolic response (McMahon, 1991). Bivalves are very diverse in their temperature responses.
- Metabolic activity can be suppressed by decreased oxygen availability, so be certain to maintain aeration in all aquaria.
- Although different species have specific upper and lower temperature limits for survival and reproduction, temperatures of 2 to 25°C are probably safe for most bivalves.
- Sudden large temperature changes may be lethal. Ten degrees above and below the maintenance aquarium are likely to give good results. This may mean using fewer treatment aquaria than you used for the Core Experiment.

### TEST QUESTION

Why would you expect extremely high concentrations of suspended particles to reduce filtration rates? Do your data support a reduction in rates at high concentrations?

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Use a Talking Thermometer to check the temperature of the water in each aquarium.
- Provide a balance with a taped midpoint for the student who is blind to locate the pointer as it comes to rest. Blind students can measure the amounts of kaolin. The weights on the beams slide into notches and are counted by touch to determine the value. For the balance slide with no grooves, the student's braille ruler can be calibrated with the scale.
- Have the student who is visually impaired prepare graphs of data on braille graph paper. The bars may be filled in with a crayon or tool that gives a raised line.

## VARIATION 2

## The Effect of Temperature on Bivalve Production of Biodeposits

*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 measure and compare the production of biodeposits from bivalves held at different temperatures.

### ADDITIONAL MATERIALS NEEDED

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

- 1 thermometer (°C)
- 2 aquarium heaters

### HYPOTHESIS GENERATION

#### Question

How will temperature affect the production of biodeposits?

#### Sample Hypothesis

The production of biodeposits by bivalves will increase with increasing temperature.

#### Rationale

An increase in temperature typically results in an increase in metabolic rate. Increasing the metabolic rate should require additional food intake which could be accomplished by faster filtration rates. Faster filtration would result in the production of more biodeposits.

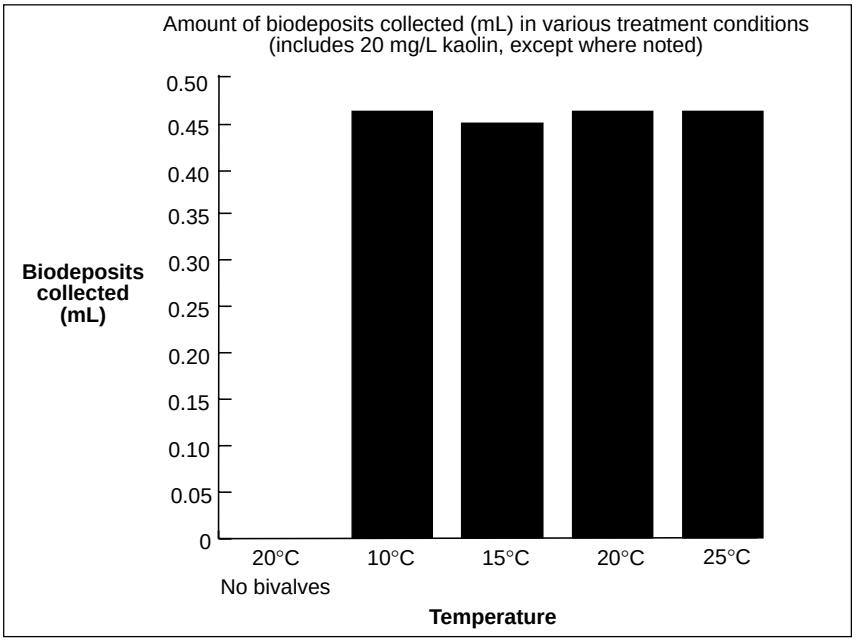
#### Sample Experimental Procedure

1. Use the kaolin concentration for which you observed the highest rate of biodeposit production in the linear part of the curve from the Variation 1 experiment. In the hypothetical data for the Variation 1, it would be 20 mg/L.

2. Maintain the temperature of 1 aquarium and the control aquarium, i.e., shells only to account for the passive settling of the kaolin, at the same temperature used in the maintenance aquarium.
3. Maintain constant temperatures 5 to 10°C different than the maintenance aquarium in each of the other 2 aquaria.
4. Repeat the incubation and data collection procedure of Variation 1.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data




Graph C. Relationship between biodeposit production and temperature. Note that at 20°C, there is no kaolin.

## TEST QUESTION

A student hypothesizes that bivalve filtration rates will increase with increasing temperature. Do the following data support that hypothesis? What biological explanation might account for these data?

Table 2. Biodeposit production at two temperatures.

| Replicate | Biodeposits (mL) collected at | |
|---|---|---|
| | 10°C | 20°C |
| 1 | 1.5 | 2.2 |
| 2 | 1.7 | 2.0 |
| 3 | 1.4 | 1.9 |
| Mean | 1.53 | 2.03 |

### TEACHING TIPS

- Some freshwater clams of the genera *Sphaerium*, *Musculium*, and *Corbicula* do not show significant correlation between filtering rate and temperature (McMahon, 1991).
- To maintain a temperature of 5°C, put the aquarium in a cooler on ice.
- In the oyster, *Crassostrea virginica*, optimum temperature for water transport is around 20°C.
- The control in this experiment should be dead bivalves, i.e., shells only, to account for the passive settling of the kaolin.

### Interpretation

Accept the hypothesis if more biodeposits are collected at high temperatures than at low temperatures. This would suggest that metabolic activities associated with filtration are sensitive to environmental temperature as you would expect in a ectotherm. Reject the hypothesis if there are fewer or the same amount of biodeposits collected at higher temperatures than at lower temperatures. This would suggest that some other factor controls the rate of filtration.

### Answer to Test Question

These data support the hypothesis that the filtration rate will be greater at a higher temperature because 0.5 mL more biodeposits were produced at the higher temperature. Metabolic rate usually increases with increasing temperature. The increase could be related partly to the increased rate of enzymatic functions at higher temperatures. This rate is less than an expected doubling, but still supports the hypothesis.

### TEACHING TIPS

- For the oyster, *Crassostrea virginica*, salinities from 25 to 39 ppt resulted in normal pumping activity, 20 ppt in slower pumping, and below 13 ppt no pumping (Jørgensen, 1966).
- Use artificial seawater salts rather than sodium chloride, because ion imbalances with sodium chloride may result in the death of animals (Dietz et al., 1996).
- Provide background on estuarine communities if students have not observed this community previously.
- Salts to make seawater of different concentrations, such as Instant Ocean™, are available from most pet stores.
- Note that only marine bivalves can be used in this variation.
- The control in this experiment should be just shells to account for the passive settling of the kaolin.

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See the Core Experiment and Variation 1.

## VARIATION 3

## The Effect of Water Salinity on Oyster Biodeposit Production

*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 measure and compare the biodeposit production of oysters or other marine mollusks held at different levels of water salinity.

### ADDITIONAL MATERIALS NEEDED

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

- Instant Ocean™
- 1 balance
- 1 weigh boat
- 1 scoop
- 1 1-L graduated cylinder

### HYPOTHESIS GENERATION

#### Question

Are oysters adaptable to a range of salinities?

#### Sample Hypothesis

Within a range of salinities, oyster feeding behavior will be constant.

#### Rationale

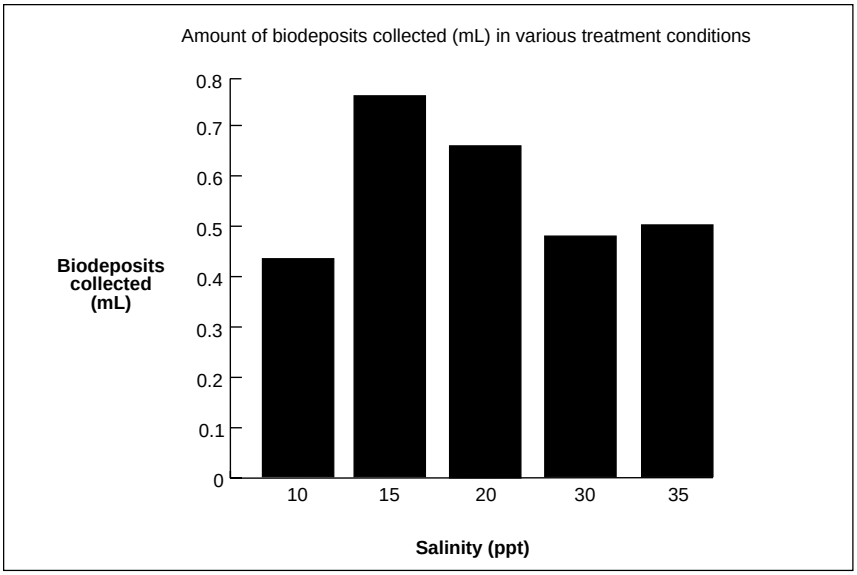
Living in an estuarine environment requires the ability to adapt to ever-changing conditions of salinity.

#### Sample Experimental Procedure

1. Use the kaolin concentration for which you observed the highest rate of biodeposit production in the linear part of the curve from the Variation 1 experiment. In the hypothetical data for Variation 1, this is 20 mg/L.
2. Maintain the salinity of 1 aquarium and the control aquarium with only bivalve shells at the same salinity used in the Core Experiment. In each of the other 3 aquaria, use 10 to 35 ppt.
3. Allow the bivalves to acclimate to their saline environments for 24 hours before adding the kaolin. Incubate and collect data as in Variation 1.

## DATA ANALYSIS AND INTERPRETATION

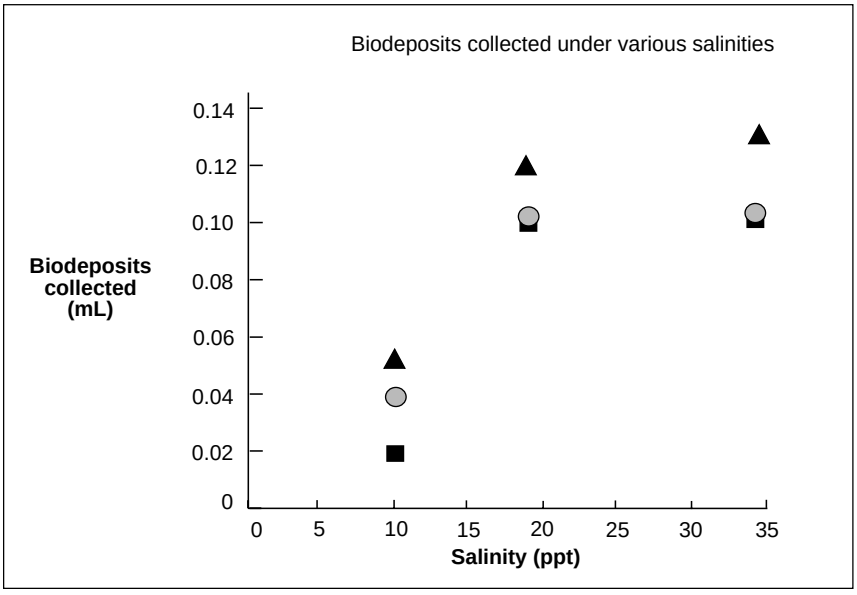
### Sample Data




Graph D. Relationship between salinity and biodeposit production. Note that at 30 ppt, there are no bivalves and no kaolin. The control in this experiment is just shells to account for the passive settling of the kaolin.

## TEST QUESTION

These data in Graph E represent the results of a student experiment with oysters in 3 aquaria of different salinities with a constant amount of kaolin. The student had hypothesized that there would be no difference in the production of biodeposits with different salinities. Do these data support the hypothesis? How would you answer this question if the student had only collected data at 25 and 35 ppt?




Graph E. Biodeposit production at different salt concentrations.

### Interpretation

The hypothesis should be accepted if the amount of biodeposits is constant over the range of tested salt concentrations. This would mean that the bivalves filter equally under all tested salinities. The hypothesis should be rejected if the amount of biodeposits varies with salinity.

### Answer to Test Question

These data show increased production of biodeposits at the two highest salinities. Reject the hypothesis because it predicts equal amounts of biodeposits at all salinities. If this student had only measured biodeposits at the two highest salinities, one would have accepted the hypothesis for that range of salinities because the amounts collected were the same.

## TEACHING TIPS

- Use a video microscope to introduce the Neubauer hemocytometer shown in Figure 8. Explain the method of counting the 4 corner 1-mm squares and the center 1-mm square, dividing that number by 5 to estimate the number (n) of cells per 1-mm square. Because the depth of the counting chamber is 0.1 mm, the number of cells per 1 $mm^3$ equals n x 10. In order to express this as cells per mL, multiply by 1000. Put the algal culture on the slide and have students determine the density of the culture.
- Some observations of *Crassostrea* show very little retention of low concentrations of algal cells unless some other particulate matter is present in the water, such as kaolin to increase particle concentration (Jørgensen, 1966).
- You should be able to detect differences in algal concentration at levels too low to show pseudo-feces formation.
- Dark can be created by covering the aquaria or putting them in a cabinet. Be certain, however, to keep all water aerated.
- If you do not have a hemocytometer, you can count cells per microscope field to obtain comparative data. Be certain everyone uses the same magnification.

## SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

### Blind or Visually Impaired

- See Variation 1.

# VARIATION 4
## The Effects of Light on Feeding Activity

*Note to Teachers: In addition to the information found in Variation 1, the following material has been provided for Variation 4.*

### SYNOPSIS

Students will compare the disappearance of algae in the aquarium water in the light and the dark.

### ADDITIONAL MATERIALS NEEDED

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

- 1600 mL culture containing a thriving algal culture (*Isochrysis*, sp. for marine mollusks, *Ankistrodesmus* or mixed culture for freshwater mollusks)
- 8 aquaria
- 1 hemocytometer slide (see Figure 8) and cover slip
- 1 Pasteur pipette with bulb
- 1 compound microscope

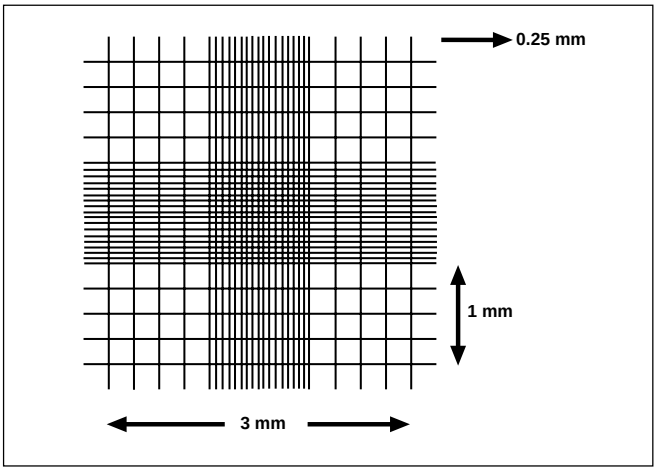



Figure 8. Hemocytometer.

### SAFETY PROCEDURE

Carry expensive, breakable instruments like hemocytometers in unbreakable containers.

### HYPOTHESIS GENERATION

#### Question

What effect does light have on the filtering rates of bivalves?

#### Sample Hypothesis

The rate of clearing of algae from the water by bivalves will be greater in the dark than in the light.

### Rationale

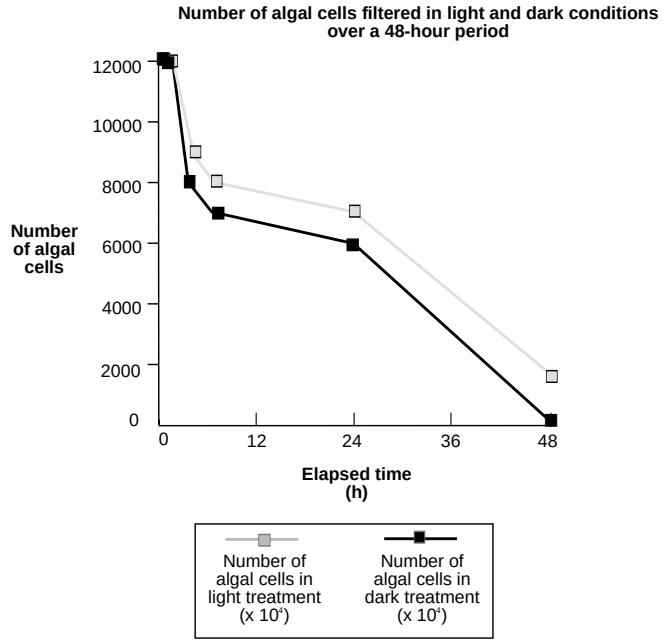
Phytoplankton, while abundant at the surface during the day, often sink to greater depths at night. Feeding at night may be more efficient than feeding during the day.

### Sample Experimental Procedure

1. Set up 8 treatment aquaria with 1 L of water each. The water should be at the temperature of the maintenance aquarium.
2. Place 3 bivalves in each of the 6 aquaria.
3. One hour later, add 200 mL of algal culture to each treatment aquarium and take a sample from one to estimate the initial cell concentration.
4. Place 3 aquaria with bivalves and 1 control aquarium without bivalves in the light. Place the other 3 treatment and 1 control aquaria in the dark.
5. After 30 minutes, sample approximately 1 mL from each aquarium and estimate the cell density with a hemocytometer.
6. Repeat Step 5 at 2, 4, 6, 24, and 48 hours.
7. Correct the density values for any algal growth or disappearance unrelated to the presence of bivalves with data from the light and dark control aquaria maintained without bivalves.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data




Graph F. The number of algal cells present after incubating bivalves in aquaria in light and dark. These values are corrected for the controls.

## TEST QUESTION

Why was it necessary to keep an aquarium in the light and one in the dark with algae, but without bivalves.

### Interpretation

If the line for the number of algal cells in the dark treatment has a steeper negative slope than the line representing the number of algal cells in the light treatment as determined by a linear regression statistical test, these data support the hypothesis.

### Answer to Test Question

In the light, it is likely that the algal population would grow. It was necessary to correct the counts for the possible increase in the number of algae in the light.

## TEACHING TIPS

- Acid rain or other sources of low pH, such as mine effluents, can adversely affect freshwater bivalve populations by eroding shells (McMahon, 1991).
- Low pH decreases the availability of calcium.
- Use a pH meter or pH test papers to determine the acidity of the treatment aquaria.
- For *Crassostrea virginica*, lowering the pH slightly from the normal 7.75 produced a transient increase in pumping rate and later a decrease below the normal rate. At pH 6.5, activity always was reduced. At pH 4.25, it was reduced to 10% of the rate measured at a normal pH (Loosanoff & Tommers, 1947 in Jørgensen, 1966).
- Most freshwater bivalves prefer water with pH greater than 7. Some species are relatively insensitive to pH and can be found at a pH of 4.7 to 8.3 (McMahon, 1991).

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

• Use a light sensor to find changes in cultures due to algal growth. For more accurate measurement of single-cell green algae growth, use a spectrophotometer.

# VARIATION 5
## The Effect of pH on Food Uptake

*Note to Teachers: In addition to the information found in Variation 1, the following material has been provided for Variation 5.*

### SYNOPSIS

Students will compare biodeposit production of oysters exposed to vinegar with those not exposed to vinegar.

### ADDITIONAL MATERIALS NEEDED

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

- 10 mL undiluted 5.0% distilled vinegar
- pH test papers or pH meter (optional)

### HYPOTHESIS GENERATION

#### Question

Is biodeposit production affected by acidic conditions?

#### Sample Hypothesis

The production of biodeposits will decrease if bivalves are exposed to acidic conditions.

#### Rationale

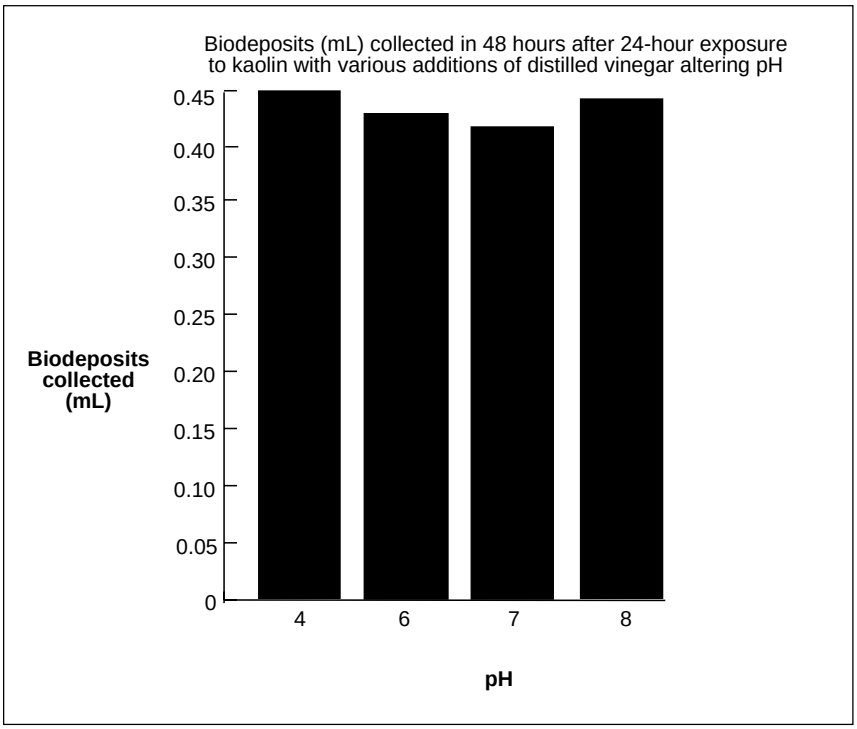
Bivalves respond to adverse environmental conditions, such as poor water quality, by closing the valves of their shells. When bivalves sense a reduction in pH, they respond by closing their valves and consequently their feeding activity also declines.

#### Sample Experimental Procedure

1. Do not add vinegar to 1 aquarium; it will be the control.
2. Amend each of 3 other treatment aquaria with different concentrations of vinegar to obtain pH values from 4 to 7.
3. Incubate and collect data as in Variation 1.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data




Graph G. Amount of biodeposits (mL) collected in 48 hours after 24-hour exposure to kaolin with various additions of distilled vinegar.

### Interpretation

If there is no difference in the total deposits collected in each treatment, reject the hypothesis. Here, there is a nearly complete overlap in individual values between the treatments, and there is no pattern in the production of biodeposits. These bivalves are not sensitive to a pH change in the aquarium water.

## TEST QUESTION

Based on the results of your experiment, do you expect to find a reduction in the number of bivalves in an estuary receiving acidic industrial pollution compared with an estuary not receiving this form of pollution? Why or why not?

### Answer to Test Question

One would not expect to find differences in the number of bivalves between undisturbed estuaries and those polluted with acid because no effect of the acid amendments was detected in the bivalves. Students may wish to re-evaluate their answer after learning what the pH created by the acid pollution was and what it was in the experiment.

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Provide electrical pH meters with module that speaks accurately and directly to the student. With current models, the larger the dial on the pH meter, the more accurately a light sensor can detect the location of the needle on the pH meter.

# VARIATION 6
## The Effects of Temperature on the Ciliary Action of Bivalves

*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 measure the ciliary action of bivalves exposed to different temperatures.

TEACHING TIP

Only a small amount of carmine powder is needed. Dip the small end of the toothpick in the powder and tap off the excess.

## ADDITIONAL MATERIALS NEEDED

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

- 1 dissecting microscope
- 1 screwdriver
- 1 pair heavy gloves
- 1 metal kitchen knife or scalpel
- carmine powder
- 1 stopwatch
- 1 metric ruler
- 1 flat toothpick
- 1 dissecting tray

### SAFETY PROCEDURES

It is highly recommended that only the instructor open the mollusk shells and use the following precautions:

Wear a heavy glove and use a screwdriver in a direction away from the body to pry open the hinge of the oyster. Other species of bivalves are easier to open.


Exercise extreme caution when using the knife or scalpel. Cut in a direction away from the body. An accident is less likely to happen if the scalpel is very sharp and can cut the mollusk muscle easily. A dull scalpel may require force to open the shell.

## HYPOTHESIS GENERATION

### Question

What effect will temperature have upon the ciliary action of bivalves?

### Sample Hypothesis

The higher the temperature is, the faster the ciliary action of bivalves.

### Rationale

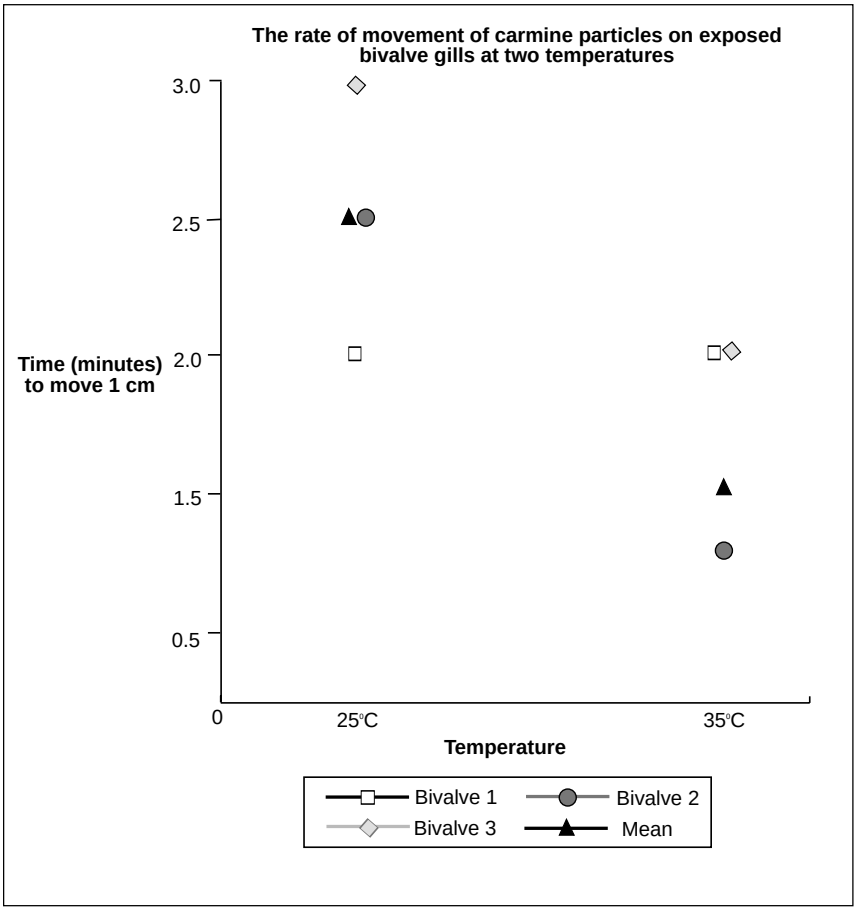
Metabolic rates are usually higher at higher temperatures in cold-blooded or ectothermic animals, such as mollusks. It seems likely that oysters will process particles faster. Faster processing would make more food available to support their higher metabolic rate.

### Sample Experimental Procedure

1. Set up live bivalves in aquaria held at different temperatures. Allow them to acclimate 10 minutes before cutting open the valves.
2. Measure the effects of temperature on bivalve ciliary action in this way:
   a. Carefully separate the 2 halves of the shell that your instructor has opened previously.
   b. Immerse the bivalve in water at the same temperature as the aquarium from which it was taken.
   c. Use a dissecting microscope to focus on the gills.
   d. Apply the carmine powder by touching the water above the gill surface with the toothpick. See Figure 1. Watch the movement of the darkly colored particles along the surface of the gills. You will notice several particles making a streak. Time one end of the streak as it moves across the gills.
   e. Measure the distance the particle moved by putting a metric ruler on the microscope stage.

## DATA ANALYSIS AND INTERPRETATION

Sample Data




Graph H. Relationship of temperature to ciliary action in bivalves showing faster rates of particle movement at higher temperature.

### Interpretation

If these data overlap, reject the hypothesis. If it took longer to move the same distance at the cooler temperature, accept the hypothesis. If it took longer to move that same distance at the warmer temperature, reject the hypothesis.

## TEST QUESTION

If you had maintained the bivalves at a temperature of 10°C for a period of several weeks, how would you expect the results to differ? Why?

### Answer to Test Question

If the bivalves initially were acclimated to the cooler temperature, one would expect them to move the carmine powder faster at that temperature than was measured in Graph H. When bivalves are held at a certain temperature for a period of weeks, they undergo biochemical adaptations that enable them to function optimally at that particular temperature. Also, this stress will be a general slowing of metabolic processes until the acclimation is complete.

## SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

### Blind or Visually Impaired

- Have students who are visually impaired act as reporters and take temperatures with a Talking Thermometer.
- Have students who are visually impaired devise a classification key of mollusks if a collection of shells is available. There are directions in most biology texts for making a key.

## TEACHING TIPS

- For the oyster, *Crassostrea virginica*, salinities from 25 to 39 ppt resulted in normal pumping activity; 20 ppt resulted in slower pumping; and below 13 ppt resulted in no pumping (Jørgensen, 1966).
- Use artificial seawater salts rather than sodium chloride, because ion imbalances with sodium chloride may result in the death of animals (Dietz et al., 1996).
- Provide background on estuarine communities if students have not observed this community previously.

# VARIATION 7
# The Effects of Salinity Level on the Ciliary Action of Bivalves

*Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 7.*

## SYNOPSIS

Students will measure the ciliary action of bivalves exposed to different levels of water salinity.

## HYPOTHESIS GENERATION

### Question

Will salinity affect ciliary action in bivalves?

### Sample Hypothesis

The ciliary action of bivalves will not vary within a range of salinities.

### Rationale

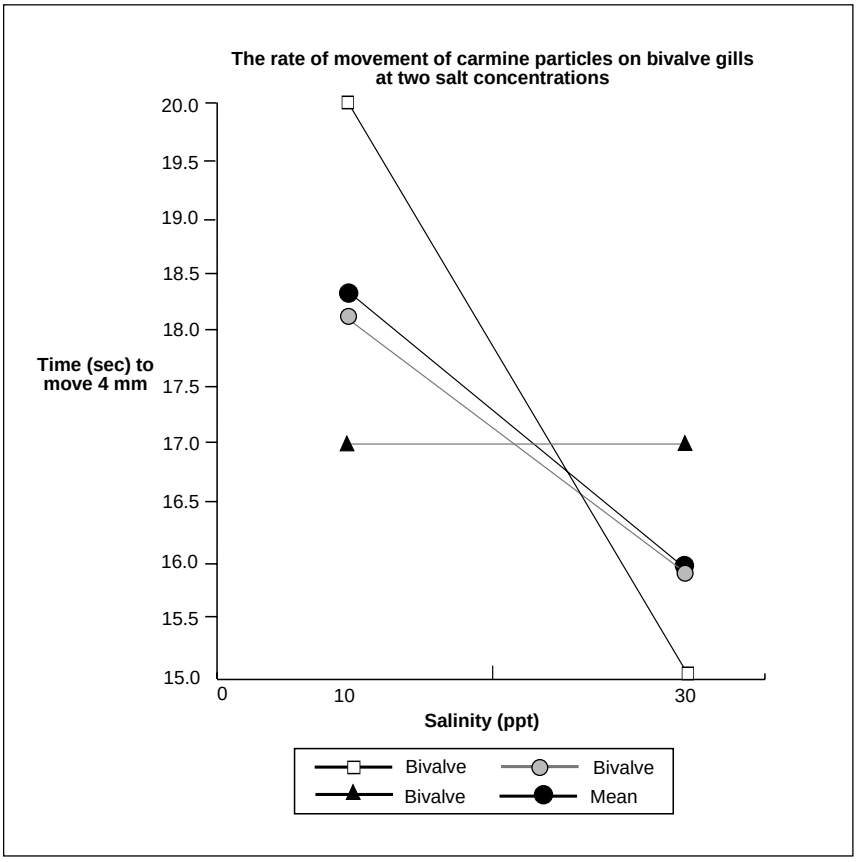
Living in an estuarine environment requires the ability to adapt to changing conditions of salinity.

### Sample Experimental Procedure

1. Set up bivalves in aquaria held at 2 different salinities with one at the same salinity as the maintenance aquarium.
2. Proceed with Step 2 in Variation 6 measuring the effects of salinity and not temperature on ciliary action.

## DATA ANALYSIS AND INTERPRETATION

Sample Data



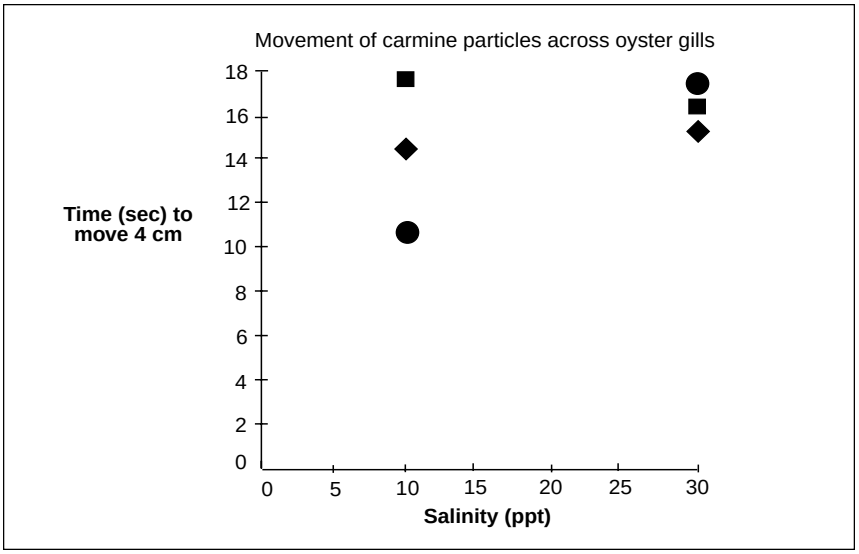

Graph I. Relationship of ciliary action and salinity concentration showing faster particle transport at higher salinities.

### Interpretation

If these data overlap, accept the hypothesis. If it took longer to move the same distance at the lower salinity, reject the hypothesis. If it took longer to move that same distance at the higher salinity, reject the hypothesis.

## TEST QUESTION

If you obtained results like those shown in Graph J, how would you interpret them?




Graph J. The movement of carmine particles over bivalve gills at 2 salt concentrations.

### Answer to Test Question

There is more variability in the rate of particle movement at the lower salinity. There is no difference in the rate of movement at the high and low salinities because these data overlap completely. These oysters are not sensitive to the differences in salinity that were tested.

TEACHING TIP

Copper sulfate concentrations greater than 0.1 g/L are likely to be lethal after several days.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

• See Variation 6.

# VARIATION 8

## The Effect of Heavy Metal on Bivalve Biodeposit Production

*Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 8.*

### SYNOPSIS

Students will measure and compare the biodeposits produced by bivalves exposed to a heavy metal with those not exposed.

### ADDITIONAL MATERIALS NEEDED

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

- $CuSO_4 \bullet 5H_2O$

### DIRECTIONS FOR SETTING UP THE LAB

Add all amendments to the treatment aquaria before adding the bivalves.

### SAFETY PROCEDURES

- Cupric sulfate is a skin and respiratory irritant, and is toxic by ingestion and inhalation. It is strongly recommended that $CuSO_4$ solutions be purchased and that $CuSO_4$ powder not be used. If the powdered form is used to make solutions, the teacher should be the only individual who works with $CuSO_4$. Wear a mask, gloves, and lab apron.
- Students should avoid contact with $CuSO_4$ solutions.
- Wash hands after using the solutions.
- Do not ingest the solutions.
- Wear gloves and a lab apron.
- Check your local safety guidelines for additional information about the safe use of $CuSO_4$, including storage and disposal.

### HYPOTHESIS GENERATION

#### Question

Do heavy metals affect the filtering rate of bivalves?

#### Sample Hypothesis

The filtering rate of oysters exposed to a heavy metal will decrease as the concentration of the heavy metal increases.

#### Rationale

Heavy metals in large concentrations are toxic to bivalves.

### Sample Experimental Procedure

1. Use the kaolin concentration when the highest rate of biodeposit production in the linear part of the curve in all treatment aquaria was observed. Hypothetical data for the Core Experiment would be 20 mg/L.
2. Set up 4 treatment aquaria as follows:
   - Aquarium 1 Control with no cupric sulfate
   - Aquarium 2 0.001 g/L
   - Aquarium 3 0.01 g/L
   - Aquarium 4 0.1 g/L
3. Incubate and collect data as in the Core Experiment.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data

Table 5. Amount of biodeposits produced by asiatic clams exposed to various amounts of cupric sulfate.

| | Amount of Copper Amendment (g/L) | | | |
|---|---|---|---|---|
| | 0 | 0.001 | 0.01 | 0.1 |
| Organism 1 | 0.17 | 0.02 | died | 0 |
| Organism 2 | 0.15 | Died | 0 | died |
| Organism 3 | 0.16 | Died | 0 | 0 |
| Mean | 0.16 | <0.01 | 0 | 0 |

Create a scatter diagram of these data. Indicate the deaths on the graph.

### Interpretation

If fewer biodeposits are collected with high copper accept the hypothesis. If the amount does not change or increases, reject the hypothesis. Copper is very toxic. Organisms may die even in the least concentrated copper amendments. Clearly, filtration rate is less with high copper concentration. The sample size is so small that no significance can be attached to the death of organisms in the least concentrated amendment compared to the higher amendments.

## TEST QUESTION

If copper or other heavy metals are so toxic that they cause bivalve death, how can they be used as indicators of contamination?

### Answer to Test Question

Even when bivalves die, they can be used as indicators of past contamination because their shells remain as an historical record of the population. Copper can accumulate in the shell of the animals as it lays down new shell throughout the growing season.

# VARIATION 9

## The Effect of Temperature on Bivalve Heart Rate

*Note to Teachers: In addition to the information found in Variation 6, the following material has been provided for Variation 9.*

## SYNOPSIS

Students will measure the heart rate of bivalves exposed to different temperatures.

## TEACHING TIPS

- There are no respiratory pigments in the hemolymph of most marine and freshwater bivalves. Their oxygen is dissolved directly in the hemolymph fluid, so their oxygen carrying capacity is the same as the water (McMahon, 1991).
- See Variation 6 for instructions on how to open bivalves.
- The pericardial cavity is equivalent to the coelom. In oysters, the heart is located immediately anterior to the adductor muscle. See Figure 1. The intestine travels the length of the cavity. The ventricle of the heart surrounds the alimentary tube in this cavity in most bivalves, but not oysters. If you dissect away the heart, you will reveal the digestive cavity. Refer to Figure 9 to locate the heart.
- All reports of molluscan heart rates show the beat is slowed by cooling and increased by heating within physiological ranges (Hill & Welsh, 1966).
- If the heart is large enough, you can see the beating without a microscope.
- The vast majority of bivalves do not have respiratory pigments. Their blood, a colorless fluid, is called hemolymph.

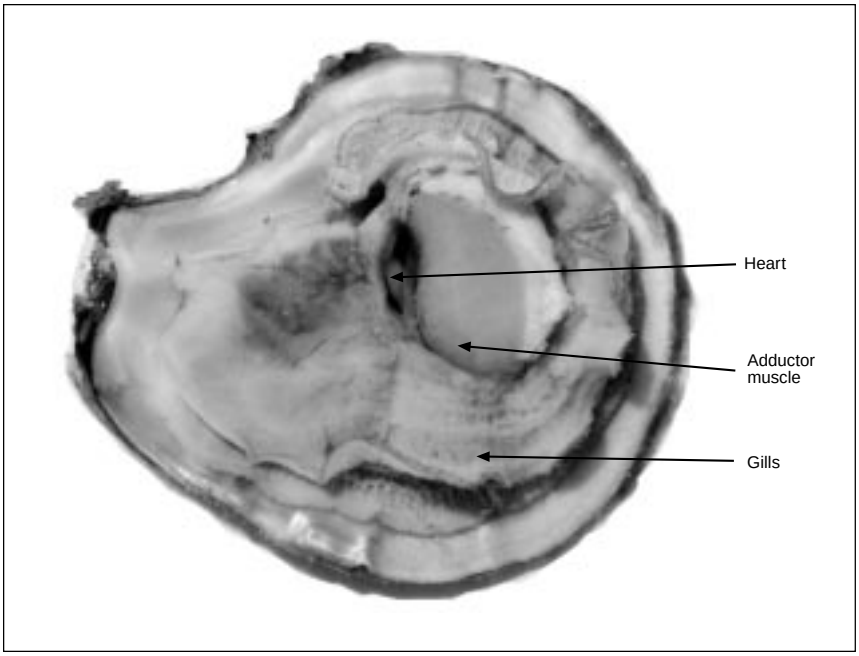



Figure 9. Cutting into the pericardial cavity just anterior to the posterior adductor muscle reveals the heart.

### SAFETY PROCEDURES

See Variation 6 for instructions on how to open bivalves.

### HYPOTHESIS GENERATION

#### Question

How does temperature affect the heart rate of bivalves?

#### Sample Hypothesis

The higher the temperature is, the faster the heart rate of bivalves.

#### Rationale

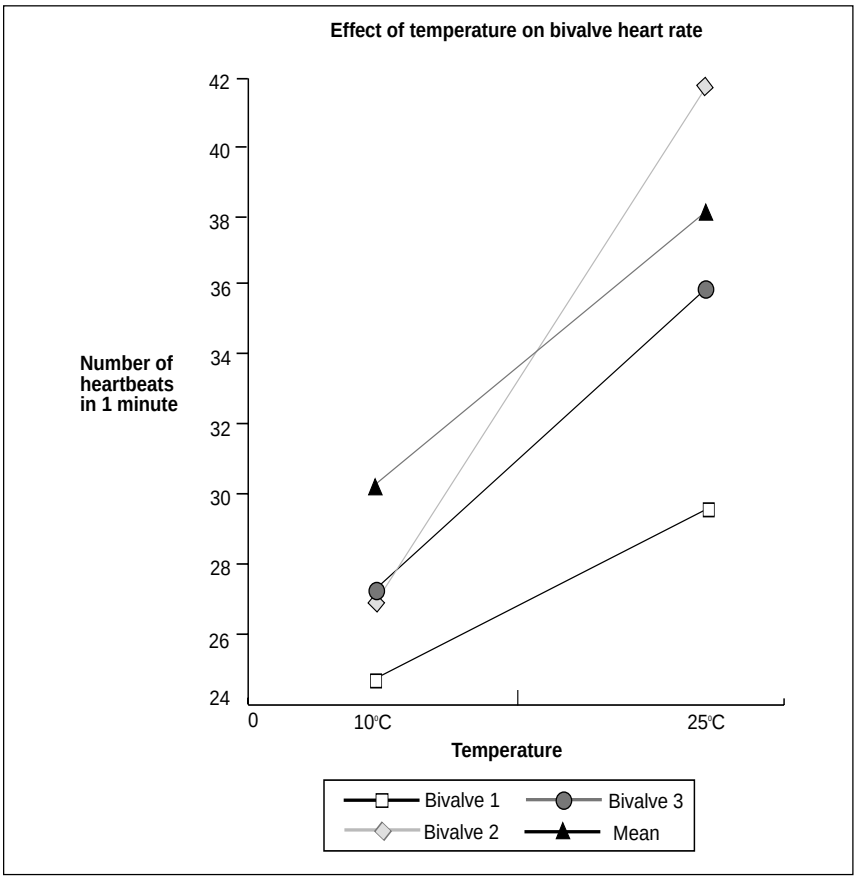
Higher temperature usually means greater metabolic rate and this should require more rapid circulation.

#### Sample Experimental Procedure

1. Set up bivalves in aquaria held at 2 different temperatures as in Variation 6. One aquarium should be the same temperature as the maintenance aquarium.
2. Measure the effects of temperature on bivalve heart rate in this way:
   a. Carefully separate the 2 halves of the shell that your instructor has opened previously. See Variation 6 for instructions on how to open bivalves.
   b. Immerse the bivalve in water at the same temperature as the aquarium from which it was taken.
   c. Cut the pericardium to see the heart beating.
   d. Using a dissecting microscope, locate the heart. If you have a medium-to-large size bivalve, you should be able to count the heart pulses without the microscope.
   e. Time and record the heart rate in 1 minute for each bivalve. Repeat the count 2 more times for each bivalve, and average the 3 numbers.

## DATA ANALYSIS AND INTERPRETATION

### Sample Data




Graph K. Effect of temperature on bivalve heart rate.

### TEST QUESTION

If you had maintained the bivalves at a temperature of 10°C rather than at 25°C, how would you expect the results to differ? Why do you expect this difference?

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Provide students who are visually impaired with large oysters so they will be able to count the heart beat without the use of a microscope. A raised-line drawing of the cross section of the heart plus graphs of heartbeats should be included with this investigation.
- Record temperatures with a Talking Thermometer.

#### Interpretation

If these data overlap, reject the hypothesis. If there were fewer heartbeats at the lower temperature, accept the hypothesis. These data were quite variable at the higher temperature, but there was very little overlap. If there were more heartbeats at the higher temperature, the hypothesis can be accepted.

#### Answer to Test Question

If the bivalves were maintained at the cooler temperature, one would have expected them to have a lower heartbeat at the cooler temperature. Until they fully acclimate, all metabolic processes will be slow.

# The Impact of Mollusks on Water Quality

## Directions for Students

### INTRODUCTION

FLAMING RIVER! The headlines of mid-1969 proclaimed. Indeed, pollution had created a combustible mess of the Cuyahoga River, a tributary to Lake Erie. All it took to ignite the river was a spark from a steel-mill rail car to create the absurd situation that alerted many complacent Americans to the damage we had done to our waterways. The first Earth Day was celebrated the following year, and we were made aware of extreme pollution in many waterways. Lake Erie looked like pea soup (Luoma, 1996). The Chesapeake Bay where oysters were once so abundant that they filtered the entire volume of the bay in just a few days, now had so few oysters that it required a year to filter the same volume of water (Newell, 1988). Filter-feeding mollusks are among the losses attributed to pollution. These creatures and many other endangered aquatic organisms are less charismatic than the familiar endangered wolves or otters. In Illinois alone, 67% of all state-listed endangered animals and 22% of all threatened and endangered species are aquatic (Cummings & Mayer, 1996).

Clams, oysters, and mussels can be forms of natural pollution, too. See Figure 1 for representative mollusks.

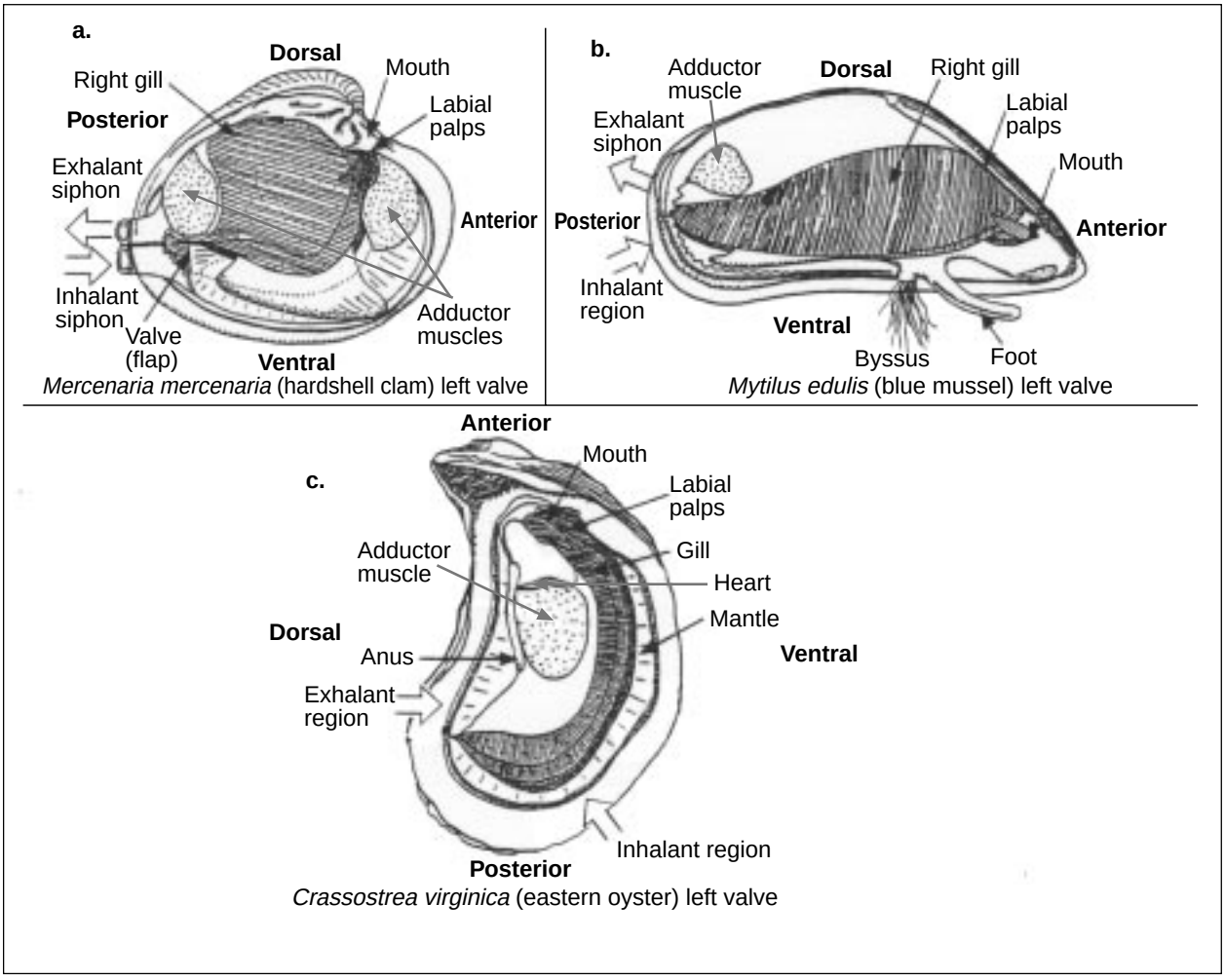



Figure 1. a. *Mercenaria mercenaria* (hardshell clam). b. *Mytilus edulis* (blue mussel). c. *Crassostrea virginica* (eastern oyster).

For example in 1988, zebra mussels arrived in the Great Lakes probably in the ballast water of a ship from Europe. Since they reproduce so quickly and have few natural predators, they quickly clogged water-intake pipes, coated boat hulls, and contributed to extensive mortality or complete elimination of some native bivalves. By the end of 1995, 20 of the 38 states east of the Rocky Mountains had been invaded by zebra mussels (Ram & McMahon, 1996). The news about zebra mussels is not all bad. They have done such a terrific job of filtering that light now penetrates deep into Lake Erie and eight native species of plants have returned after an absence of 30 to 50 years (Anonymous, 1996).

When planktonic algae containing a powerful nerve-toxin are abundant, shellfish concentrate the poisons. They also accumulate other toxins including heavy metals, like copper and mercury, and bacterial pathogens from human sewage (Boyle, 1981).

In this exercise you will investigate some of the behavior of bivalves that makes them good news and bad news for the environment.

## OBJECTIVES

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

- Describe the ecological role of filter-feeding mollusks in fresh and/or saltwater ecosystems.
- Identify the major anatomical structures and functions of a filter-feeding mollusk.
- Describe how turbidity and salinity affect the behavior of a filter-feeding mollusk.
- Explain how a population of oysters, clams, or mussels impacts water quality.
- Maintain strict procedures needed to support the organisms' health and basic functions.

### SAFETY NOTES


Wash your hands before and after the lab.


Do not pick up an aquarium containing water.


Carry expensive glass instruments like hemocytometers in unbreakable containers.


Do not discard mollusks or wash water into local waterways.


Do not eat any of these organisms.

Do not open any shellfish using a sharp instrument, such as a scalpel. Your instructor will do this.


Wear safety goggles and lab apron.

## MATERIALS NEEDED

*For each team of four students, you will need the following:*

- 5 live freshwater clams, such as *Corbicula fluminea* or saltwater hardshell clams *Mercenaria mercenaria*, marine blue mussels *Mytilus edulis*; 5 live oysters, such as *Crassostrea virginica*
- 0.5g (3t) kaolin powder
- 3 3.8-L (1-gallon) aquaria or wide-mouthed glass or plastic jars
- 12 L artificial seawater
- 1 aerator
- 1 thermometer (°C)
- 15 petri dish halves
- 1 Pasteur pipette
- 10 10-mL graduated cylinders

## STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature or web search on the topic of filter-feeding bivalves and pollution. 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. Why is it important to control variables?
4. What is the variable in your experiment?
5. What will you measure?
6. How will you control other potential variables?

## 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. Construct a bar graph showing the total amount of biodeposits collected for bivalves in each treatment.
2. Do your data support or refute your hypothesis?
3. Using your specific data, explain your answer to Question 2.
4. Compare your data with that of other groups. Are these data the same or different? Why or why not?
5. Will the amount of biodeposits always be the same under the conditions you used?
6. What could cause differences? Be specific.

## DESIGN OF VARIATIONS OF CORE EXPERIMENT

- How do filtering rates compare for different species of bivalves?
- How will temperature affect the production of biodeposits?
- Are oysters adaptable to a range of salinities?
- What effect does light have on the filtering rates of bivalves?
- Is biodeposit production affected by acidic conditions?
- What effect will temperature have upon the ciliary action of bivalves?
- Will salinity affect ciliary action in bivalves?
- Do heavy metals affect the filtering rate of bivalves?
- How does temperature affect the heart rate of bivalves?