Tolerance Testing With a Hairless "Guinea Pig"

SYNOPSIS FOR CORE EXPERIMENT
Students will explore the tolerance of brine shrimp (*Artemia franciscana,* formerly *Artemia salina*) to various concentrations of NaCl. They will determine the effect of increased salinity on the survival of *A. franciscana.*

APPROPRIATE BIOLOGY LEVEL
Introductory, advanced, marine science

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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 89. 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 research lab, students will be able to:

- Define and determine the tolerance limit of *Artemia franciscana* larvae to environmental variables, such as salinity.
- Describe how an environmental variable may be a limiting factor in an ecosystem and act as a selective pressure in the survival of a species.
- Develop the skill of using a serial dilution to prepare various concentrations of solutions.

MATERIALS NEEDED

For the teacher preparation, you will need the following for a class of 24:

- 500 adult brine shrimp or 3 teaspoons of brine shrimp (*Artemia franciscana*) eggs
- 5 clean, plastic 3.8-L (1-gallon) milk jugs
- 200 g NaCl, non-iodized table, canning, or pickling salt
- 20 L distilled water
- 9 culture/dilution containers

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

- 500 adult brine shrimp or 2-day-old culture of brine shrimp with 200 eggs and larvae
- 1 1-L beaker
- 500 mL 1.0% NaCl solution
- 1 clean, white margarine container
- 1 overhead projector or dissecting microscope with video camera
- 5 mL ammonia
- 5 mL liquid soap
- 5 mL spray cleaner
- 5 mL vegetable oil
- 5 mL vinegar
- 5 mL liquid fertilizer
- 5 g road de-icer
- 1 tissue culture plate (*optional*)

LENGTH OF LAB

A suggested time allotment follows:

Day 1 (45 minutes)

- Introduce the lab with a discussion of the law of tolerance, the concept of limiting factors in the ecosystem, and the natural history of *Artemia.* Brainstorm and research ideas for hypotheses.

Day 2 (30 minutes)

- Explain serial dilutions. (15 minutes)
- Have students practice serial dilutions.

Day 3 (45 minutes)

- Observe brine shrimp.
- Develop hypotheses and experimental design.
- Set up the lab.

Day 4 (30 minutes)

- Collect data and analyze.

Day 5 (45 minutes)

- Interpret these data. Generate hypotheses for variations and develop experimental design.

PREPARATION TIME REQUIRED

30 to 45 minutes

- Purchase live brine shrimp or set up a hatchery for brine shrimp. Prepare stock solutions.

15 to 20 minutes

- Gather additional supplies for introduction and student experiments.

TEACHER'S NOTES

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

- 1 10-mL disposable syringe or 10-mL graduated pipette with bulb
- 1 permanent marker
- 10 translucent film cans, 50-mL beakers, test tubes, or 1 divided box with 10 chambers
- 1 teasing needle
- 1 1-mL graduated plastic pipette
- 1 stereo microscope
- 10 mL 8.0% NaCl
- 40 mL distilled H_2O
- 1 dropping pipette
- 1 petri dish with counting grid

SAFETY PROCEDURES

Always wear goggles and lab aprons when working with chemicals.

Label all containers so solutions do not become confused.

Always **add acid to water** when preparing acid solutions.

Use caution when mixing the suggested pollutants during the introduction of the Core Experiment if other "pollutants" are used in addition to those suggested. Be aware of any potential interaction of the chemicals.

Never mix bleach and ammonia or substances containing either substance; toxic fumes will result.

Use only substances that can be poured safely down the sink.

DIRECTIONS FOR SETTING UP THE LAB

Stock Solutions

1.0% NaCl Solution

Dissolve 10 g of salt in 1 L of distilled water. Since a large volume of this brine solution will be needed, you may find it convenient to mix a gallon at a time. Pickling salt is a good choice because it lacks the sodium silico-aluminate added to keep table salt free-flowing. Prepare a gallon by adding 38.8 g NaCl to a well-cleaned milk jug. Fill with distilled water. This is approximately one tablespoon of salt per quart of water (0.25 cup salt per gallon of water); that precision is adequate for the culture stock and as a diluent for the acid.

8.0% NaCl Solution

1. Dissolve 8.0 g NaCl in a small amount of distilled water.
2. Bring to a volume of 100 mL with distilled water.

Brine Shrimp Hatchery (Optional)

1. Tape a piece of acetate parallel to the sides of a sandwich box about 3 cm from one side. Leave a 1-cm gap between the acetate and the bottom of the box. Set the box where it will receive light and be undisturbed. Do not try to move the box after you have added eggs.
2. Fill the box with the 1.0% NaCl stock solution to a depth of at least 3 cm.
3. Sprinkle approximately 3 teaspoons of brine shrimp eggs over the large section of the box. The eggs should be a single layer thick on the surface.

4. Cover the large section, not touching the water, with the opaque lid. The brine shrimp will begin hatching in 1 to 2 days and will swim under the acetate barrier into the small lighted section of the box.
5. Set up additional boxes to produce more larvae. The hatching chamber is shown in Figure 1.

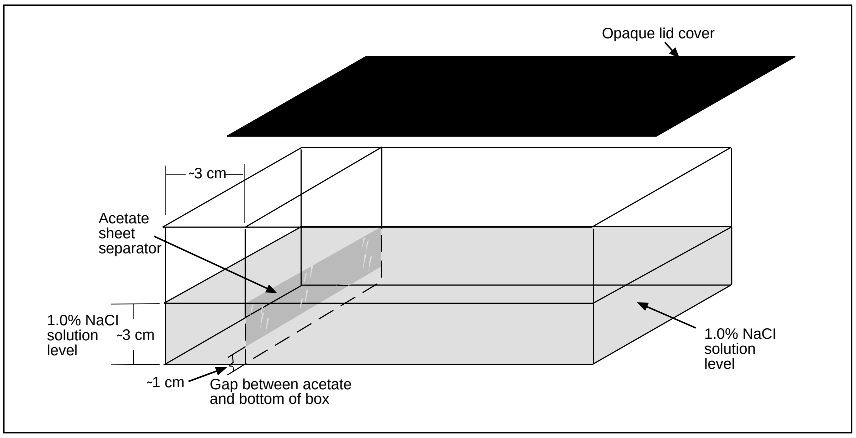


Figure 1. Brine shrimp hatchery.

TEACHER'S NOTES

TEACHER BACKGROUND

Content Information

Brine shrimp (*Artemia*) are members of the class Crustacea and the subclass Branchiopoda (Barnes, 1986). Brine shrimp are not really shrimp, but belong to a separate order (Anostraca) of crustaceans. *Artemia* are known commonly as sea monkeys or fairy shrimp. See Figure 2. While they do require a saltwater environment, they do not live naturally in the ocean. They are found in salt pans and lakes, such as the Great Salt Lake. Fish are rare in these environments, but brine shrimp are able to tolerate these high salt concentration environments. In some western saline bodies of water, brine shrimp occur seasonally in such high numbers they turn the water red or brownish-red. This coloration results from the green algae they eat that appear to turn red in the process of digestion (Schmitt, 1965).

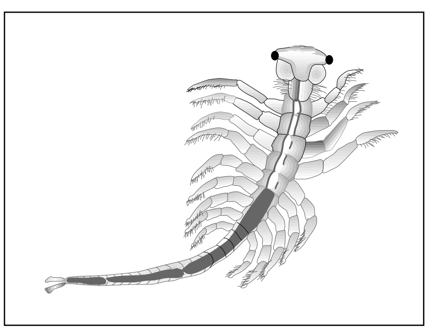


Figure 2. Brine shrimp.

Although brine shrimp can tolerate high salt concentrations, they are very sensitive to changes in their surroundings. Due to this sensitivity, they are useful as an indicator species of the presence of chemicals and temperature fluctuations. Ecologists use indicator species as signals of problematic changes in the ecosystem. Brine shrimp are obtained readily and inexpensively, and the "eggs" hatch quickly. What look like "eggs" are not eggs at all, but cysts containing a dormant gastrula. As unfed larvae, brine shrimp are sensitive to various materials so they can be used as bioindicators of contamination. They even are used in preliminary screens to detect potential tumor suppressing natural products (Meyer et al., 1982).

Pollution is not always obvious. Water that looks clean and clear may be highly contaminated. Humans are not the only creators of pollution on Earth. Volcanoes produce sulfur dioxide; decaying matter and methanogenic bacteria release methane; naturally occurring forest fires emit carbon dioxide; and lightning creates ozone. Even rain could be considered a pollutant to a saline body of water.

Recycling is not a new concept. All matter is recycled. Although the recycle time for some materials, like phosphates, is very long. An example of this recycling process is the nitrogen cycle. Nitrogen is needed by living things for the formation of amino acids and nitrogenous bases (pyrimidines and purines). Nitrogen is the most abundant gas (about 78%) in the air, but most organisms cannot use gaseous nitrogen directly. Nitrogen-fixing bacteria convert about 139 million tonnes (1 tonne = 1 metric ton) of nitrogen each year and aquatic organisms up to 120 million tonnes (Goldsmith & Hildyard, 1988). Nitrogen is converted into the soluble form, nitrate, that can be absorbed and used by plants. The nitrogen in plants can be used by animals that consume them. When the animal dies, bacteria and fungi further use the nitrogen and release it in the form of ammonia. The ammonia may be further used by other kinds of bacteria, ultimately being oxidized to gaseous nitrogen and released into the atmosphere. Human activities have altered the fluxes in this cycle. The application of artificial fertilizers releases at least 55 million tonnes of nitrogen per year. Power plants and automobiles contribute an additional 24 million tonnes in the form of nitrous oxides. Excessive amounts of these forms of nitrogen are partially responsible for environmental degradation by acid rain. Brine shrimp can be used as indicators of these forms of pollution as shown in the Variations of the Core Experiment.

Pedagogical Information

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

Correct Concept	Misconception
• Water that looks clean and safe to drink may contain harmful pollutants.	• Water that looks clean is safe to drink.
• Water pollution is threatening the health of the Earth's waterways and oceans.	• Water pollution is not really a problem because the water and insensitive aquatic organisms can recycle these substances.
• Some substances, even in small quantities, can be hazardous to some organisms, while other substances are only toxic in large quantities and/or to certain organisms.	• If a substance is toxic, even minute amounts of it are toxic.
• Some organisms are adapted to extreme conditions and/or a wide range of environments while others are very sensitive to environmental change.	• What is unhealthy for one organism is unhealthy for all organisms.
• Different organisms have different ranges of tolerance for various chemical and physical factors.	
• Tolerance to one abiotic factor or pollutant does not presuppose tolerance to all factors	• "Good" conditions for one organism will be "good" for all organisms.

TEACHING TIPS

- Purchased brine shrimp are larger and easier to examine. It also reduces the preparation time to prepare and maintain a culture.
- Distinguish between naturally fluctuating abiotic factors and introduced pollutants.
- Always have a supply of brine shrimp that are freshly hatched. If not purchasing brine shrimp, start one culture on Friday before leaving school and another one on Monday afternoon.
- Always keep the brine shrimp culture aerated with a gentle bubble stream or in containers with a large surface area.
- Feed the brine shrimp larvae with baker's yeast and the adults with dry, powdered fish food. *Dunaliella*, a unicellular alga, and whole wheat flour also can be used to rear larvae to the adult stage.
- Brine shrimp can be concentrated by covering the end of a pipette with fragrance-free tissue or fine, clean fabric and suctioning off excess water.
- Students may want to recognize brine shrimp health in more categories than live and dead. They will see some brine shrimp that are alive, but unable to swim in a direct line, merely twitching or swimming in small circles. For the Core Experiment and all variations except Variation 4, the disabled shrimp have been counted with the dead. Variation 4 data illustrate results students might obtain if they recognize the three categories of health.
- Expect responses to a range of concentrations of potential pollutants not to be linear.
- Emphasize the assay potential of this exercise rather than accuracy in creating dilutions.
- The results are quite similar if 8 mL of stock potential pollutant are used with 2 mL of diluent and 1 mL of brine shrimp, or if 8 mL of stock potential pollutant are used with 1 mL of diluent and 1 mL of brine shrimp.
- Keep the dilution math simple. Syringes make the process of making the dilutions very easy. You then can emphasize the concept rather than the manipulation. If

Table 1. Directions for salt concentration preparations.

Variation	Inorganic compound name	Molecular formula	Molecular weight (g/mole)	Amount (g) to make 100 mL of 0.17N (equivalent) solution	Normality (equivalence) of 1.0% solution
1	Sodium hydroxide	$NaOH$	40.01	0.6802	0.25
4	Sodium orthophosphate	$Na_3PO_4 \bullet 12H_2O$	380.16	6.4627	0.17
5	Sodium nitrate	$Na(NO_3)_2 \bullet 6H_2O$	184.37	3.1343	0.05
6	Potassium chloride	KCl	74.55	1.2674	0.13
	Calcium chloride	$CaCl_2$	110.99	1.8868	0.09
	Nickel chloride	$NiCl_2$	129.60	2.2032	0.08
	Lead chloride	$PbCl_2$	278.12	4.7280	0.04
	Ferrous (iron) chloride	$FeCl_2$	126.76	2.1549	0.08
7	Sodium bromide	$NaBr$	102.91	1.7495	0.10
	Sodium iodide	NaI	149.92	2.5486	0.07
	Sodium fluoride	NaF	42.00	0.7140	0.24
8	Sodium hydrogen carbonate	$NaHCO_3$	84.02	1.4283	0.12

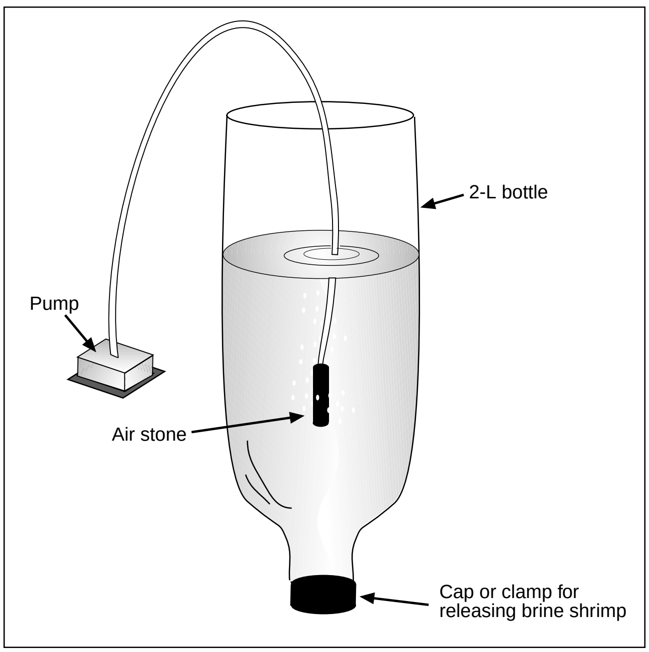


Figure 3. Simple brine shrimp hatchery using a 2-L soda pop bottle.

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

Introduce the class to brine shrimp by placing a small amount of the stock brine shrimp culture into a petri dish on an overhead projector or a dissecting microscope with video camera. Have the class observe the eggs, larvae, and adult brine shrimp. Information about the life history of brine shrimp is important because it will lead students to consider that the impact of the test materials may vary with the maturation of the organism. Discuss the economic importance of brine shrimp and their increasing use in fast, simple, and inexpensive tests to study pollution and potential cytotoxicity.

It is often very difficult to study the effects of a particular abiotic factor in nature. Variables in nature are not controlled easily. Many factors occur simultaneously and some may interact with each other. Once a problem is detected, the specific cause

TEACHING TIPS

you make a dilution series from 1-in-100 to 1-in-1000 to 1-in-10,000 or a series from 1-in-100 (1.0%) to 1-in-200 (0.5%) to 1-in-400 (0.25%), the preparation and the concept will be emphasized in repetition. Recall that pollutants usually are reported in parts per million (ppm) or parts per thousand (ppt), and 1 ppm is 0.0001%.

- Practice making serial dilutions with a dye, such as methylene blue. Use the spectrophotometer to provide quantitative data for solution concentrations.
- Consider the advantage of using Multiwell™ or similar tissue culture plates. They are stable and not easily tipped or spilled. The different dilutions are stored and retrieved easily and some are pre-labeled. Brine shrimp can be observed easily by setting the plate on the microscope stage. Each well in a plate with 24 wells holds just 2.5 mL of test solution plus 0.25 mL of dense brine shrimp culture. Each well in a six-well plate holds 15 mL.
- You may want to reduce the range of dilutions used in a variation. An efficient way to do this is to zero in on the critical range where some, but not all of the organisms, are affected. Test extremes with single samples. Replicate the trials where a difference in mortality is detected.
- Although the hypothesis may be phrased in terms of mortality or survival, the more common way to phrase the results is mortality if you are testing the detrimental effects of a potential pollutant.
- Table 1 contains preparation directions for creating salt concentrations of the same equivalence (normality) (.17N) as 1.0% sodium chloride. See Table 1.

may not be evident. Conducting a laboratory experiment under controlled conditions is one way to isolate the effects of a single variable on a specific organism. The results can be used to make inferences about the impact of this variable in nature.

Discuss the harsh environment where brine shrimp are found and how they deal with the abiotic factors. Does a wide range of tolerance for one factor suggest a similar tolerance for all factors?

Fill a 1-L beaker half full with the saline solution. Tell the students this represents a small ecosystem where brine shrimp might live. If you have a video camera that fits your dissecting microscope, you might do this entire demonstration in the wells of a tissue culture plate. Place a small amount of pollutant, such as ammonia, liquid soap, vegetable oil, vinegar, liquid fertilizer, road de-icer, or spray cleaner, into the jar. Discuss how each of the pollutants may end up in the environment and how they may affect the organisms living there.

Ask students to consider the source and nature of pollutants.
- Who or what is responsible for releasing pollutants into the environment?
- Are any naturally occurring materials pollutants?
- What impact will the addition of these materials have on the living organisms in the environment?
- How much of each material will it take to harm the environment?
- How long will each material stay in the environment?

Ask the students to speculate about the survival of brine shrimp if they are exposed to variations of a key abiotic factor. Pose the following questions:
- Would variations of a key abiotic factor cause the brine shrimp to die?
- Could a small variation of a key abiotic factor be harmless to the brine shrimp?
- How could you design an experiment to test the effects of specific variations in key abiotic factors on living organisms?

HYPOTHESIS GENERATION

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

The hypothesis for the Core Experiment is based on tolerance levels to changes in the environment. The students should consider what would happen if there were an increase or decrease in the salinity of the brine shrimp's environment. There are two possibilities. The addition of NaCl could cause a change in the survival rate or there could be no measurable effect on their survival. An increase in the survival rate would not be noticeable during a short observation period.

Sample Hypotheses

- An increase in the NaCl concentration will cause a decrease in the reproductive rate of the brine shrimp.
- The addition of a small amount of NaCl will kill all the brine shrimp.
- The adult brine shrimp will be able to survive a higher concentration of NaCl than the larvae.

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

TEACHING TIPS

- A normal probability curve is the familiar bell-shaped curve. If you graph the information in that curve as the cumulative probability, the result is an S-shaped curve. The 50% point on this curve is the peak on the original bell-shaped curve and represents 0 standard deviations. One standard deviation above is 84.13% and 15.83% is one standard deviation below. Standard deviations reported as cumulative percentages are probits if 5 is added to their value. The addition of 5 means that negative numbers are not considered.
- For the Core Experiment, if data are collected and solutions recorded as percentages, construct the graph using a log scale for the concentration.
- Brine shrimp are hypo-osmotic, that is, the concentration of water inside their bodies is greater than in the surrounding water. Water and salt are absorbed through their gut and the excess salt is removed by glands in their gills (Engemann & Hegner, 1981).
- The salinity of the Great Salt Lake is variable, but is usually between 17 to 28% (Allen, 1996).
- You can construct an alternate brine shrimp hatchery from a 2-L soda pop bottle as shown in Figure 3. Approximately 1700 mL of water fills this hatchery and the maximum amount of eggs is 4.0 g (1 teaspoon). When you want to collect hatchlings, turn off the air and pull up the air stone. About 10 minutes later, the hatchlings will have settled near the bottom and the eggs and egg shells will have floated to the top. You can draw off 500 mL of culture solution with concentrated brine shrimp by releasing the clamp. Approximately the first 20 mL or so that you draw off will include some abortive larvae and shell contamination. Draw off small samples and check them for undesirable contaminants before collecting some to use. Repeat this process on subsequent days until approximately 100 mL of culture remains in the inverted bottle.

there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each group of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 88.

TEACHER'S NOTES

Question

What is the effect of increased salinity on brine shrimp survival rate?

Hypothesis

Increasing the salinity will cause an increase in the survival rate of brine shrimp larvae.

Rationale

Brine is found in salt evaporation ponds and in the Great Salt Lake where salt concentrations are greater than that of the ocean.

Procedure

1. Prepare a series of NaCl dilutions ranging from distilled water at 0.0% NaCl to 4.0% NaCl with the 8.0% stock solution.
2. Label your dilution experiment containers with the percent NaCl as follows: 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.0625. Use 5.0 mL distilled water for the control at 0.0% NaCl.
3. Dispense 5.0 mL of distilled water into each container except the control.
4. Transfer 5.0 mL of the 8.0% solution to the distilled water in the container marked 4.0% and mix. Transfer 5.0 mL of the resulting 4.0% solution to 5.0 mL of distilled water in the container marked 2.0%. Continue this process until you have reached a dilution of 0.0625%. Discard 5.0 mL of the 0.0625% solution.

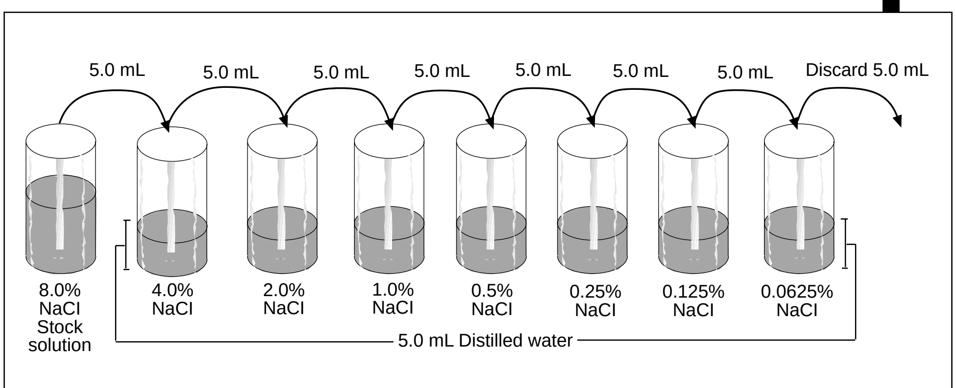
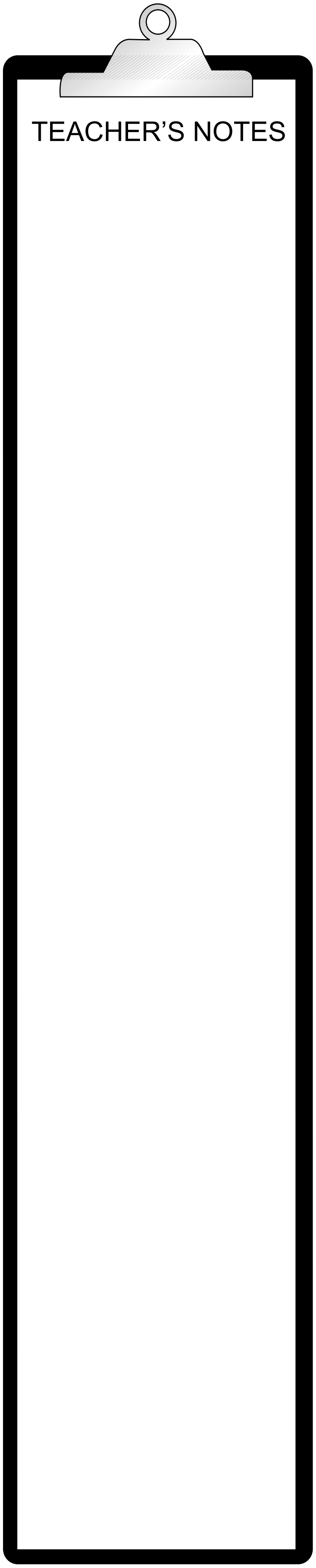


Figure 4. NaCl dilution series.

5. Dispense 0.5 mL of brine shrimp culture into each dilution.
6. Incubate the containers in light at room temperature for 24 hours.
7. Determine the mortality of brine shrimp in each NaCl concentration as follows:
   A. Make a copy of the template and tape it to the bottom of a petri dish. See Figures 5a & b. Use the template to keep track of the sample drops of brine shrimp that have been or remain to be counted.



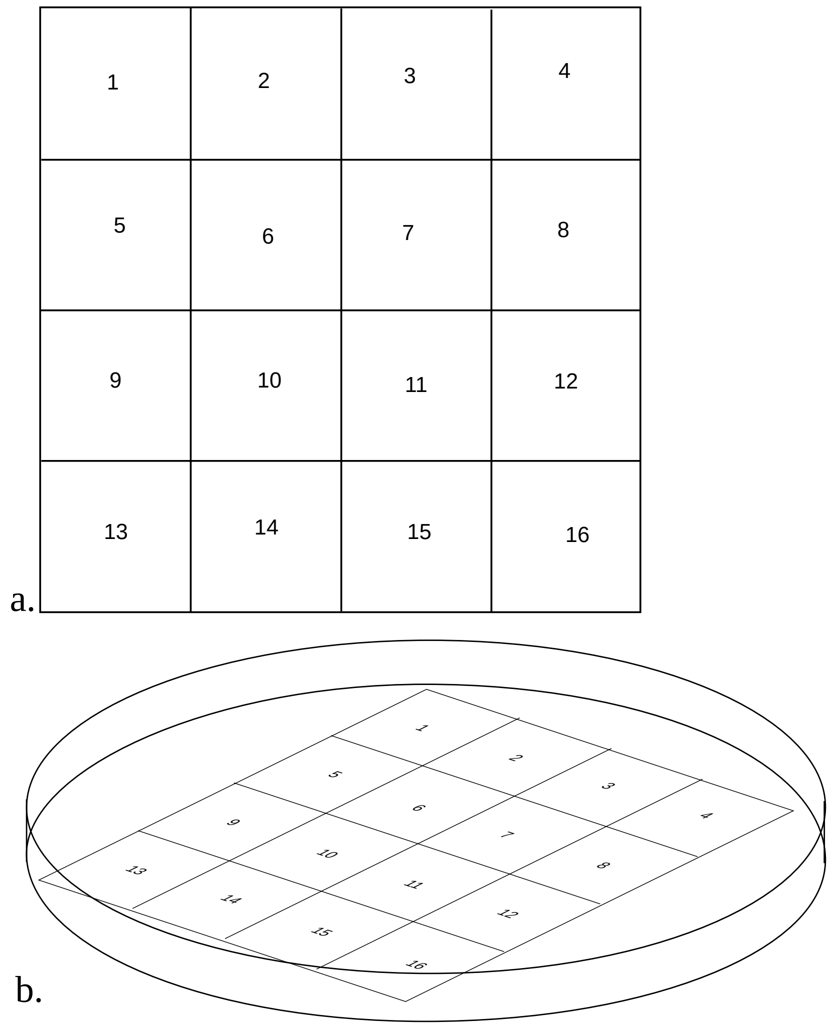


Figure 5 a. Petri dish template. b. Petri dish with template in place.

B. Stir the contents of the experimental container gently with the tip of a 1-mL pipette. Then, remove a small sample with the pipette.

C. Divide this sample into drops, putting one drop in each section of the grid in your petri dish. Vary the size of the drops depending on the activity and concentration of your shrimp. If there are many active shrimp, keep the drops small to count the shrimp as they swim.

D. Count and record the number of live and the number of dead brine shrimp in each sample. Stir the drop with a dissecting needle. Shrimp near the edge of the drop may not be visible. Stirring will bring them into view. Since you are calculating a percent mortality, you can stop counting when you have observed approximately 50 brine shrimp.

E. Calculate the percent mortality by dividing the number of dead brine shrimp by the total number of brine shrimp counted. If brine shrimp have died in the control, subtract the percent mortality of the control from the percent mortality of each treatment.

OR

8. If brine shrimp are purchased from a pet store, the following procedure can replace Steps 5 and 6.

   A. Place brine shrimp population in a wide, open container, such as a culture dish.

   B. Collect 10 brine shrimp and place in a 10-mL graduated cylinder.

C. Withdraw all but 1 mL of the water in the graduated cylinder. The 1 mL of water will contain a concentrated solution of brine shrimp. Dispose of the withdrawn water in a waste container.

D. Add the 1 mL of water containing the 10 brine shrimp to the first treatment container. To insure that all the brine shrimp are removed from the graduated cylinder, add 1 to 2 mL of the acid solution in the treatment container to the graduated cylinder. Swirl and extract the solution back into the original treatment container. **Be sure to start this procedure with the lowest acid concentration.**

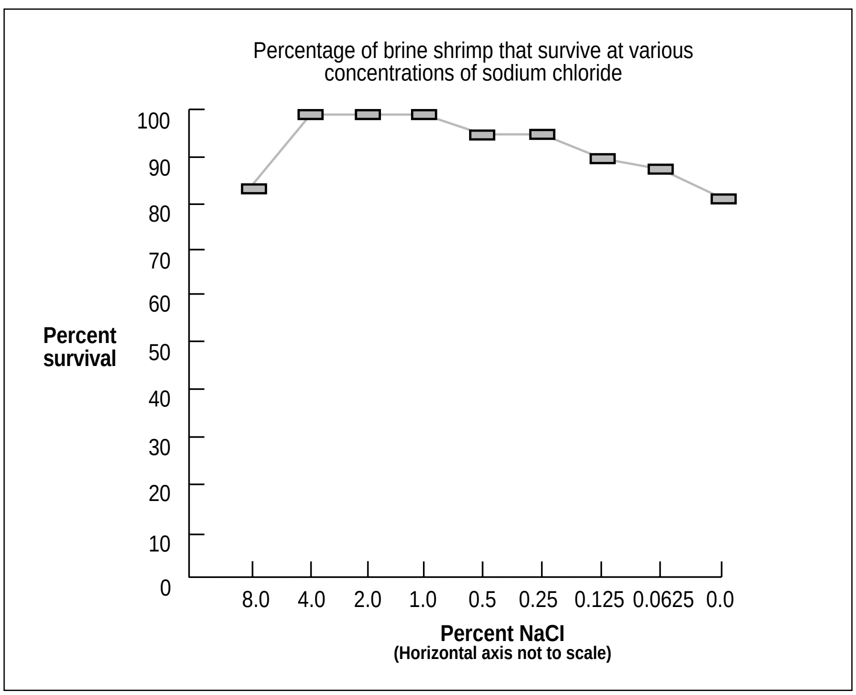
E. Repeat Steps 8 A to D for each treatment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 2. Survival of brine shrimp at various concentrations of sodium chloride.

Percent NaCl	Percent mortality
8.0	17.0
4.0	0.0
2.0	0.0
1.0	0.0
0.5	5.0
0.25	5.0
0.125	10.0
0.0625	12.0
0.0	18.0



Graph A. Salt concentration and brine shrimp survival.

TEACHER'S NOTES

Interpretation

Accept the hypothesis if more brine shrimp survive as the salinity increases. Reject the hypothesis if an increase in the salinity causes a decrease or no change in the survival rate.

Answer to Test Question

In a freshwater environment, the concentration of water outside the organisms would be much higher. Therefore, the osmotic potential would be increased greatly. Water would flow into the organism in larger amounts than normal. Most likely the organism would be incapable of removing this excess water.

TEST QUESTION

Brine shrimp live in a highly saline environment. Based on the results of this experiment, predict what would happen osmotically if brine shrimp were placed into a freshwater environment.

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 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:

- Was the survival of the brine shrimp the same at all NaCl concentrations?
- Was there a specific concentration that was the "best" or was there a range?
- Was there a point when the NaCl became harmful or toxic to the brine shrimp?
- To what other natural conditions or abiotic factors might brine shrimp be exposed? Could you design an experiment to find the optimal point for that organism's range of tolerance?
- To what environmental pollutants might brine shrimp be exposed? Can you test brine shrimp's tolerance to these factors?

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

In this investigation, the participation of students who are visually impaired is limited where observations require the use of a microscope. These students might act as data recorders by recording these data with a braille writer or a tape recorder and providing a typewritten copy for the members of their lab groups.

- Provide labels in braille and in print for the containers.
- Increase the size of the object to be studied so they can use tactile means for observations.
- Consider substituting the larger freshwater crayfish for *Artemia*. The cost of saltwater crayfish would be likely prohibitive. In working with these animals, it is often advantageous to use small rubber bands to immobilize their claws. The investigation would now be to test the tolerance of crayfish for salt (NaCl) in the water. The size of the aquarium housing the crayfish is calculated to determine the appropriate amount of water to be used. The amount of NaCl to make 0.1% solution is measured and packaged. Additional packages of the same amount of salt can be prepared and stored to increase the salinity of the water. The animals should be observed for changes in behavior. Do not try to determine the percent of salt necessary to kill the crayfish. When there has been a drastic change in its actions, move the animal to a container of untreated water.

Gifted

These students may be challenged to determine an LD_{50} (lethal dose to 50% of the population) for a variation by fitting a line to the curve in the linear region or by performing a probit analysis. Although the statistical concepts involved in the probit analysis are complex, performing it with calculators that most students have is not difficult. Table 3 data are unlikely to result from the Core Experiment and are included only to illustrate the technique. Directions for this analysis involve making two transformations of these data: finding the best straight line through the transformed data, and finding out the value of the dose percentage when the mortality value is 50%. The first transformation is to convert dosages or percent acidity to the log of those values. A log transformation converts an exponential function to a linear function. The second transformation is to convert dosages in percent to probits. You make this transformation by using Table 3 from Finney (1971). The best straight line describing the transformed data can be found by using a calculator to find the regression line. Finally, substitute a probit value of 5 (50% mortality) in the equation and determine the dose percentage. Remember to convert by dose percentage that you obtain as a log to its antilog.

TEACHER'S NOTES

Table 3. Transformation of percentages to probits (from Finney, 1971. Reprinted with the permission of Cambridge University Press).

Percent	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.42	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.82	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Example:

Percent HCl dose	Log dose	Percent mortality	Mortality point
0.00001	-5.00	17	4.05
0.00005	-4.30	45	4.87
0.0001	-4.00	59	5.23
0.0005	-3.30	66	5.41
0.001	-3.00	90	6.28
0.005	-2.30	98	7.05
0.01	-2.00	100	—-
0.05	-1.30	93	6.48
0.10	-1.00	100	—-

TEACHER'S NOTES

Answers to Additional Test Questions

1. B
2. A scientist could raise brine shrimp in a solution with a slightly higher concentration of acid. After several days, the living brine shrimp could be removed and allowed to reproduce. Although all of the surviving brine shrimp are able to cope with a higher acid concentration, their mechanisms for survival may not be the same. The scientist could repeat this procedure, increasing the acidity slightly, for several generations; and each generation should be better able to live in an environment with a higher acid concentration. This demonstrates natural selection; individuals with beneficial characteristics will survive to pass these genetic traits on to their offspring.

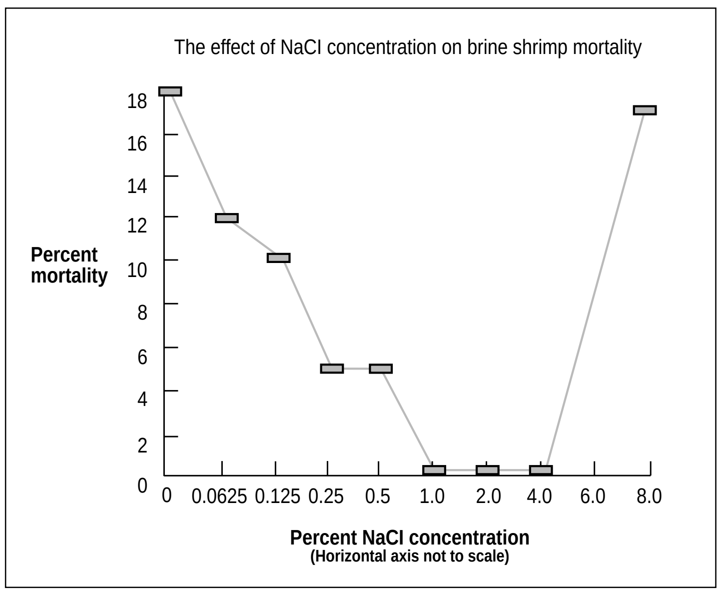
Mobility Impaired

- Review the selection of laboratory partners. A considerable amount of the time for this investigation is spent using a microscope. The instructor should check the access and comfort level of the student while using a microscope.
- Discuss the ability of the student who is mobility impaired to obtain supplies.
- Provide a microscope that can be operated by levers for students who are manually impaired. Sighted lab partners may assist with adjusting the field of view.
- Provide a tape recorder for taking notes for manually impaired students.
- Arrange for a lab partner to make the graphs of these data summarized by the student who is manually impaired.
- See “Allelopathy” for further considerations.

ADDITIONAL TEST QUESTIONS

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

1. According to the following graph, at what salt concentration did the brine shrimp have the highest survival rate?
   A. 8.0%
   B. 4.0%
   C. 0.5%
   D. 0.125%
   E. 0.0625%



Graph B. The effect of salt concentration on brine shrimp mortality.

2. Natural selection occurs when environmental conditions change and some individuals have a difference that allows them to survive and reproduce. When they reproduce, the offspring inherit the difference that allowed their parents to survive. Using brine shrimp, how could a scientist illustrate the process of natural selection?

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VARIATIONS OF 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.

VARIATION 1
The Effect of Increased Alkalinity on Brine Shrimp

SYNOPSIS

Students will determine if an increase in the concentration of the base NaOH will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

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

- 10 mL 1.0% NaOH solution

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Follow the same procedure as in the Core Experiment substituting sodium hydroxide for the NaCl.
- Dissolve 1.0 g of NaOH in 100 mL of 1.0% NaCl solution.

Answers to Questions and Analysis on Student Page

1. These laboratory data correlate with the hypothesis that if salt concentration increases above 4.0%, then the survival rate decreases.
2. Answers will vary. Examples of possibilities are miscounting, poor laboratory skills, old brine shrimp eggs, unknown impurities in the water such as fluorine.
3. Practice laboratory procedures such as using a pipette, syringe, or graduated cylinder before conducting the actual experiment. Repeat your experiment looking for discrepancies. Use large sample sizes and average your results. Check all of your math work for errors.
4. The graph shows that brine shrimp have an optimum tolerance level for salt. While a few will survive in a low concentration of salt, those shrimp in less concentrated solutions had the lowest survival rate.
5. Answers will vary. Examples could be improved laboratory skills and math skills, a greater understanding of the scientific inquiry process, and appreciation of the delicate balance that exists in nature.
6. Answers will vary depending on the topic you are studying. You could relate this laboratory to studies of ecology, marine biology, osmo-regulation, tolerance, and cell biology.
7. The laboratory results indicated that brine shrimp have an optimum tolerance level for salt. Also, while brine shrimp survival is reduced at certain concentrations, we do not know if similar results would occur in other organisms. These pose questions that could serve as topics for further investigation.
8. This laboratory uses an understanding of many areas of science. In order to appreciate fully and understand the laboratory, one needs a knowledge of chemistry, ions, chemical formulas, membrane transport, ecology, and marine systems.

TEACHER'S NOTES

HYPOTHESIS GENERATION

Question

How will increasing the pH of the water environment, i.e., making it more basic, affect brine shrimp?

Sample Hypothesis

Increasing the concentration of the base NaOH will cause an increase in the mortality rate of brine shrimp larvae.

Rationale

Animal tissues dissolve in bases. Increased concentration of a base should result in increased physiological stress and increased mortality.

Sample Experimental Procedure

1. Label the dilution experimental containers to represent the desired final concentration of NaOH. Include 1 container with 1.0% NaCl, but no NaOH.
2. Prepare several concentrations of NaOH as follows:
   A. Dispense 9 mL of 1.0% NaCl into each of the labeled containers.
   B. Add 1 mL of 1.0% NaOH to the dilution container labeled 0.1%. Mix thoroughly.
   C. Use the same pipette to transfer 1 mL of the 0.1% solution to the dilution container labeled 0.01%.
   D. Use the same pipette to repeat the process of Steps A to C to create your additional dilutions. See Figure 6.

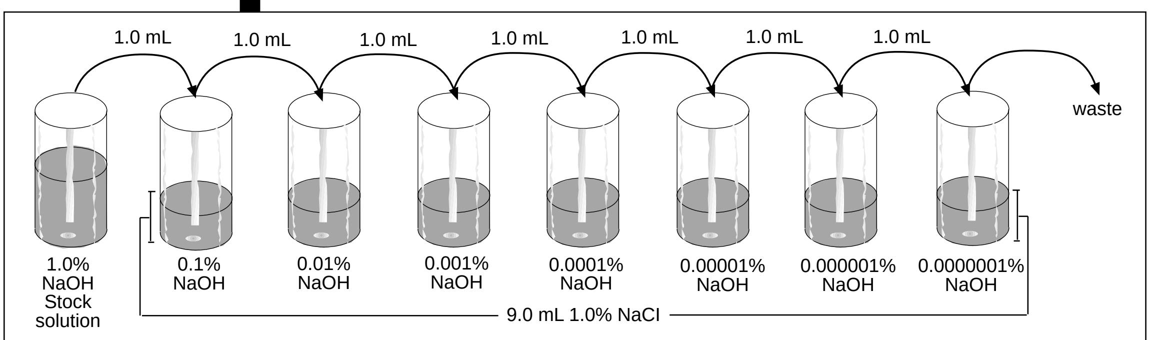


Figure 6. Serial dilution of NaOH.

   E. Remove and discard 1 mL from the weakest solution.
3. Dispense 1 mL of brine shrimp with a plastic, disposable pipette into each container.
4. Incubate the containers in light at room temperature for 24 hours.
5. Follow the procedure of the Core Experiment to determine the mortality of brine shrimp in each basic concentration.

TEACHING TIPS

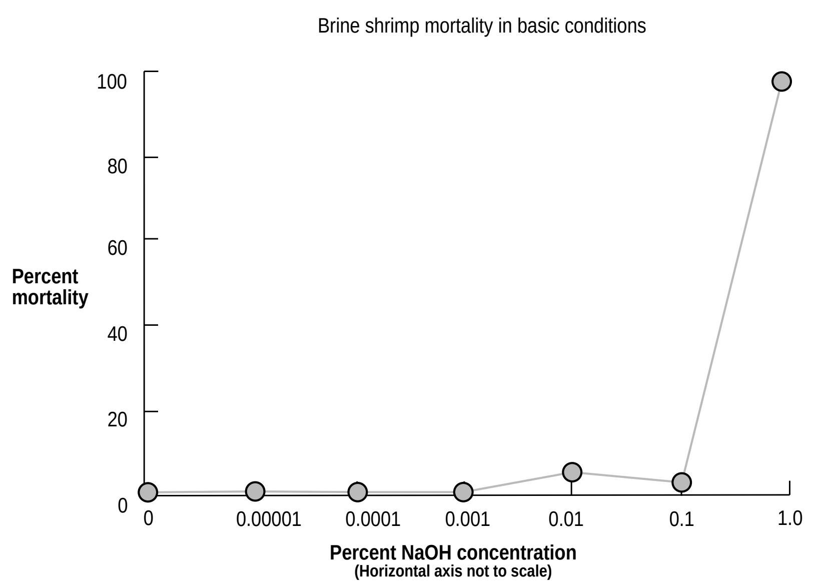
Seawater and the Great Salt Lake are alkaline. Allen (1996) found increased survival of brine shrimp if he used sodium carbonate to increase the pH value to 8.5.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 4. Brine shrimp mortality in basic conditions.

Percent NaOH	Number of live brine shrimp	Number of dead brine shrimp	Percent mortality
0.0	50	0	0
0.00001	50	0	0
0.0001	50	0	0
0.001	50	0	0
0.01	48	2	4
0.1	49	1	2
1.0	0	50	100



Graph C. NaOH effect on brine shrimp mortality.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Design investigations similar to those using brine shrimp.
- Have students who are blind observe crayfish manually and by listening to the sounds as the animals move through the water.
- Make a raised-line drawing or a braille painting of the brine shrimp and the crayfish if models are not available in the following ways:

Raised-line sketch. Sketch a cross section of the brine shrimp and of the crayfish on braille paper. Place the sketch on a rubber mat or on a wooden board covered with several layers of heavy cloth. Trace the sketch with a variety of tools that give various sizes and depths of lines. Use a stapling tool to give variety to the surface texture or a sewing tracing wheel to give the feel of a dotted line.

Braille painting. Draw the cross section of the animal desired on canvas board or heavy cardboard. Use Elmer's Glue® to make a narrow raised line covering the

TEACHER'S NOTES

Interpretation

Accept the hypothesis if fewer brine shrimp survived as the concentration of NaOH increased. Reject the hypothesis if there was no change in the survival rate as the concentration of the NaOH increased. Also, reject the hypothesis if the survival rate peaks at a certain pH then decreases as the pH changes (either higher or lower) from that level. This suggests there is an optimal pH value.

TEACHER'S NOTES

pencil marks. Obtain variety by placing string, wire, rope, and other items in the glue. Sprinkle rice or sand in an area of glue to designate other tissue. There are no rules for making these 'paintings.' Just be creative. Some students gladly do these. Others do them to help a friend or to try something different. They are done in a thin cardboard box with a lid and can be stored for years.

VARIATION 2
The Effect of Thermal Pollution on *Artemia*

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 determine the optimal temperature for survival of *Artemia* larvae.

ADDITIONAL MATERIALS NEEDED

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

- 1 thermometer (°C)
- 6 beakers
- 6 Styrofoam™ cup insulators or wide mouth thermoses
- 1 ice bath
- 1 hot water bath

HYPOTHESIS GENERATION

Question

What is the effect of temperature on brine shrimp survival?

Sample Hypothesis

Survival will increase with increasing temperature up to a moderate temperature and then decline as the temperature is further increased.

Rationale

Survival depends on several enzyme systems working. In general, enzyme activity is slowed by cold and stopped by high temperatures. Optimal enzyme activity occurs at a moderate temperature or at a temperature close to the normal temperature of the organism.

Sample Experimental Procedure

1. Place 5 mL of a 1.0% NaCl solution into each of your sample vials.
2. Adjust the water in thermostatically controlled water baths or in vacuum bottles to the desired temperatures.
3. Balance or float one of your sample vials in each water bath for about 2 minutes to allow the salt solution to come to the desired temperature.
4. Add 0.5 mL of a stock brine shrimp solution to each jar. Monitor and maintain the temperature for at least 10 minutes by adding warm or cold water to the water bath.
5. Allow the water sample vials to return slowly to room temperature and maintain them at this temperature for 24 hours.
6. Follow the procedure of the Core Experiment to determine percent mortality.

TEACHING TIPS

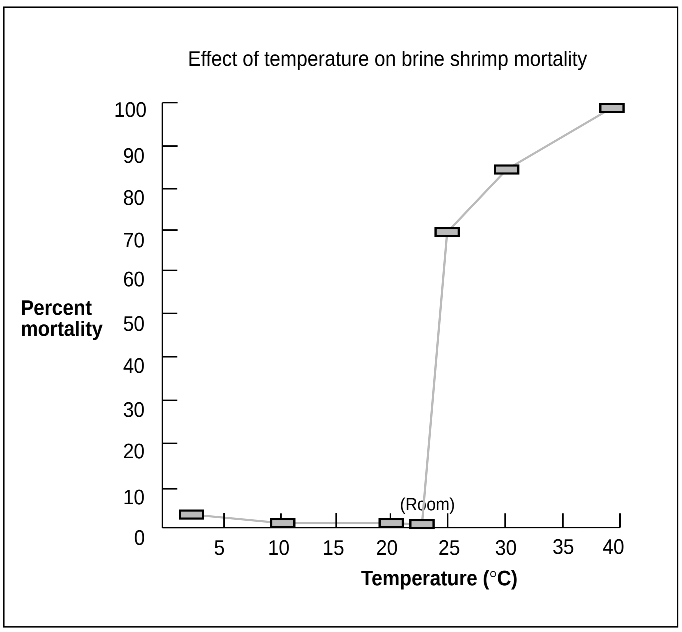
- Use temperatures of 2, 10, 20, 25, 30, 40°C.
- You can create Styrofoam™ collars from food trays to float sample vials.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Percent mortality of brine shrimp surviving after exposure to various temperatures for 10 minutes.

Incubation temperature (°C)	Percent mortality of brine shrimp
2	4
10	2
20	0
22 (Room)	0
25	70
30	86
40	100



Graph D. Relationship of temperature to brine shrimp mortality.

TEST QUESTION

Graph E displays slightly different data from another group of students who conducted the same experiment as the students in Graph D. According to this set of data, what is the optimal temperature or range of temperatures for brine shrimp survival? How can you account for the difference in survival at different temperatures?

Interpretation

The hypothesis should be accepted if the greatest number of living brine shrimp are counted at an intermediate temperature. Reject the hypothesis if the highest survival occurs at extreme temperatures.

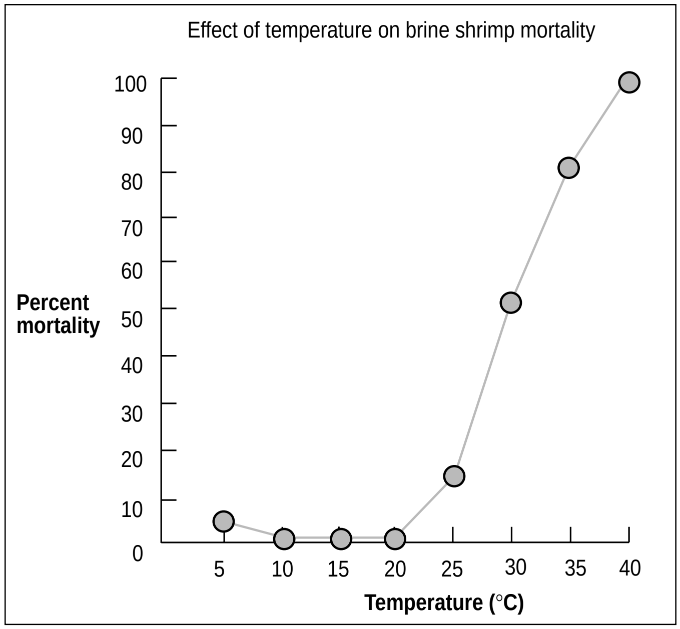
Answer to Test Question

Brine shrimp survival is optimum where mortality is minimal. This occurs over the tested temperature range of 10 to 20°C. Temperatures lower than 10°C may be too cool to provide the necessary heat for molecular interactions, while temperatures greater than 20°C may alter the shape of the enzymes necessary for life-sustaining metabolic reactions.

TEACHER'S NOTES

TEACHING TIPS

- Brine shrimp may show diurnal rhythms independent of the light or dark period you provide. Many planktonic organisms migrate diurnally, following their food source. The rhythm continues in continuous dark.
- If brine shrimp depth preference is related to their choice of a particular light intensity, you should get different results in a tube continuously lighted and one wrapped in dark paper or foil and lighted only from the top. However, light intensity provided by classroom fluorescent lights may not be adequate to show a difference.



Graph E. Brine shrimp mortality.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 3
Determination of Preferred Depth of Migration of *Artemia*

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 determine if the larvae of *Artemia* show a preference for a specific depth.

ADDITIONAL MATERIALS NEEDED

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

- 1 2-m piece of Tygon™ tubing
- 42 plastic pinch or screw clamps
- 1 meter stick
- 1 pair of scissors
- 40 67-mL (2-oz) jars, small beakers, or vials

HYPOTHESIS GENERATION

Question

Will *Artemia* show a preference for shallow depths?

Sample Hypothesis

The larvae of *Artemia* will show a preference for shallow depths.

Rationale

Brine shrimp larvae feed on algae that require sunlight to photosynthesize. Consequently the brine shrimp will prefer shallow depths.

SAMPLE EXPERIMENTAL PROCEDURE

1. Close off the bottom of a 2-m piece of Tygon™ with a clamp or a rubber stopper.
2. Use a meter stick to position additional clamps onto the tubing at 5-cm intervals with enough pressure so that they will not slide, but not tight enough to seal the tubing. See Figure 7.

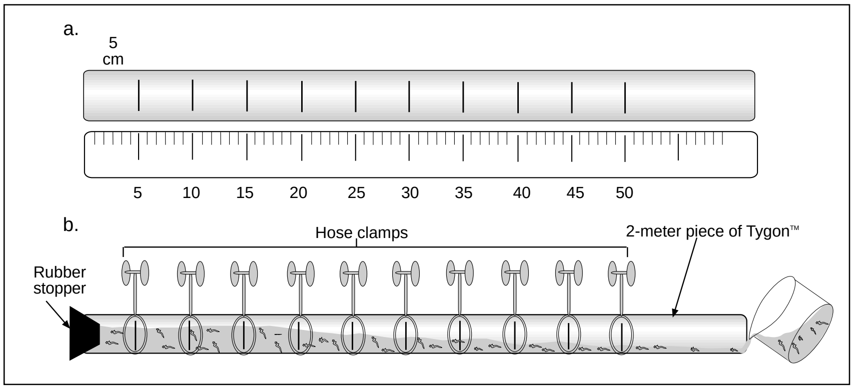


Figure 7. Position clamps every 5 centimeters.

3. While holding the tubing parallel to the floor, fill it with brine shrimp solution until it almost reaches the top of the tube.
4. Hang the tube upright.
5. Allow brine shrimp to migrate for several hours.
6. Without disturbing the location of brine shrimp, seal off the clamps one at a time beginning at the bottom.
7. Position a labeled collection container under the tube and remove the stopper or closing clamp. See Figure 8.

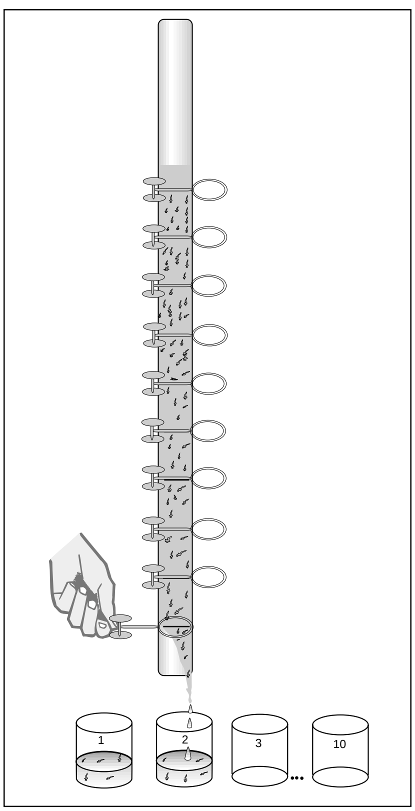
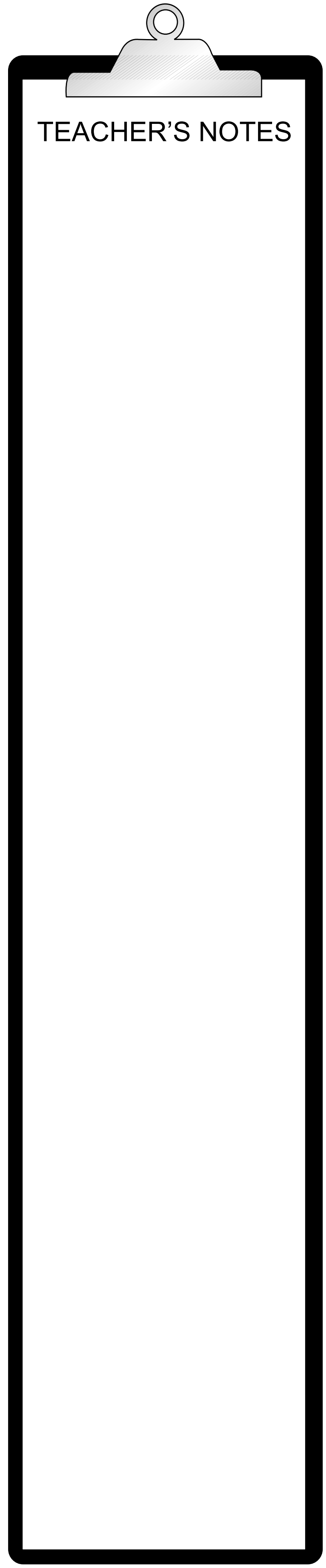


Figure 8. Tygon™ in vertical position with collection chamber.



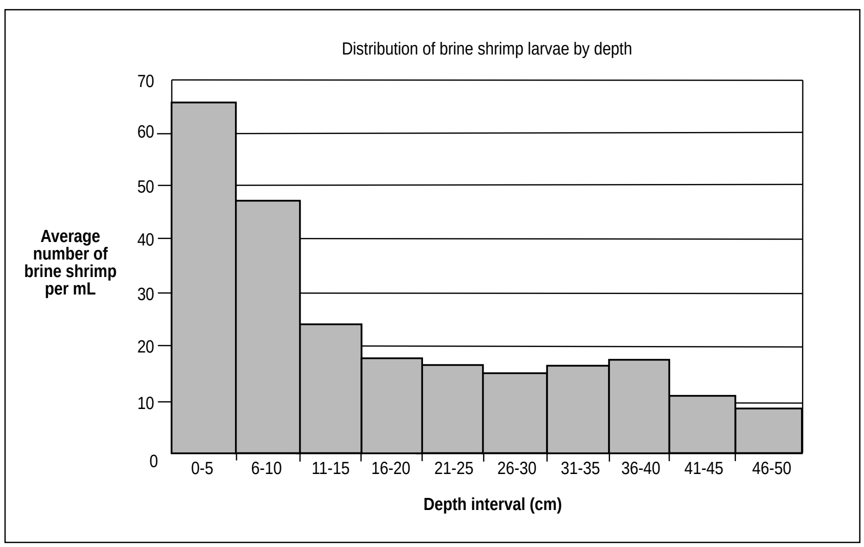
8. Repeat the process for each section until the tube is emptied.
9. Count the number of living brine shrimp in a measured volume from each section. Use the drop counting technique of the Core Experiment, but vary the volume that you count. Where 1 mL contains several hundred brine shrimp, a 0.25 mL sample will be enough, but where the density is very low, you may need to count more milliliters to find 50 organisms.
10. Adjust all counts to per milliliter. For example, if you have counted only 0.25 mL, multiply your value by 4. If you have counted 2 mL, divide your value by 2.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Distribution of brine shrimp larvae by depth in a narrow column of salt water.

Depth interval (cm)	Average number of live brine shrimp (per mL)
0 to 5	68
6 to 10	48
11 to 15	24
16 to 20	16
21 to 25	14
26 to 30	13
31 to 35	14
36 to 40	16
41 to 45	11
46 to 50	9

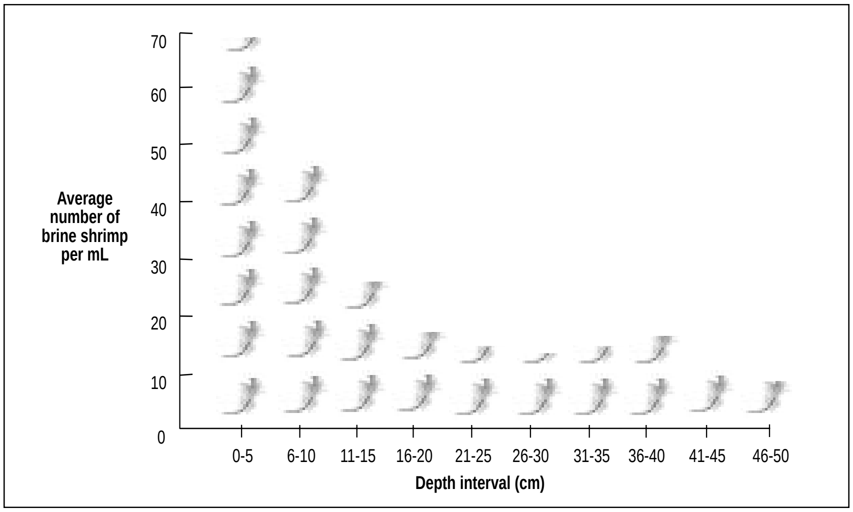


Graph F. Column graph showing the distribution of brine shrimp larvae by depth.

Alternatively, you can display these data in a pictograph such as the one shown in Graph G.

Interpretation

Accept the hypothesis if more brine shrimp were counted in the sections of tube nearest to the surface of the water. Reject the hypothesis if there was an even distribution of the brine shrimp throughout the length of the tubing or if more individuals were counted at lower depths.



Graph G. A pictograph showing the distribution of brine shrimp larvae by depth.

TEST QUESTION

Life exists at various depths in the aquatic environment. In this experiment, where were the majority of brine shrimp found? Explain why they tend to stay at this depth.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

The topic of 'determining the depth of migration of brine shrimp' is not practical to do with a crayfish. The blind student might investigate the activity of crayfish in the dark and various amounts of light.

VARIATION 4

The Effect of Increased Sodium Phosphate Concentration on Brine Shrimp

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 determine if an increase in sodium phosphate concentration will affect the survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

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

- 10 mL 8.0% sodium orthophosphate ($Na_3PO_4 \bullet 12H_2O$)

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Follow the same procedure as in the Core Experiment substituting a series of solutions from 0.0% to 8.0% of sodium phosphate. To prepare 8.0% sodium phosphate, dissolve 8.0 g of sodium phosphate in a small amount of distilled water, then dilute it to 100 mL with distilled water. Sodium phosphate makes available sodium, so do not use 1.0% NaCl as the diluent.

Answer to Test Question

They are filter feeders who primarily eat autotrophic phytoplankton that should be located near the surface where there is enough light for them to photosynthesize.

HYPOTHESIS GENERATION

Question

Will increasing phosphate concentration affect the survival rate of brine shrimp?

Sample Hypothesis

Increasing the phosphate concentration will cause a decrease in the survival of brine shrimp larvae.

Rationale

Phosphates have been eliminated from most cleaning products because of their damage to the environment so phosphate may harm organisms directly.

Sample Experimental Procedure

1. Label the vials with the following Na_3PO_4 dilutions: 8.0%, 4.0%, 2.0%, 1.0%, 0.5% and 0.25%.
2. Dispense 5 mL of distilled water into all the treatment vials except the one reserved for 8.0% sodium phosphate.
3. Transfer 5 mL of the 8.0% solution into the 4.0% vial. Mix well and remove 5 mL.
4. Transfer the 5 mL you removed from the 4.0% vial to the vial labeled 2.0%. Mix this well and remove 5 mL.
5. Continue the transfer-mix process until you have made all the desired dilutions.
6. Remove 5 mL from the last dilution so that the volume is the same as in your other vials.
7. Add 0.5 mL of culture to each dilution.
8. After 24 hours, count and classify the organisms as lively, barely alive, or dead using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

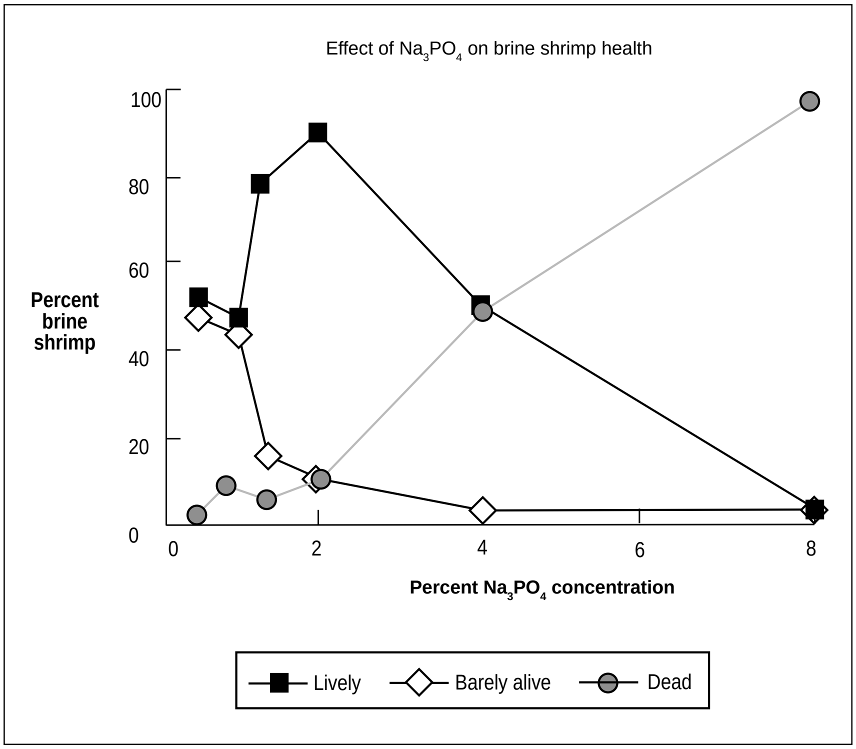
Sample Data

Table 7. Effect of sodium phosphate on apparent brine shrimp health.

Phosphate concentration (%)	Number lively	Number barely alive	Number dead	Percent lively	Percent barely alive	Percent dead
8.0	0	0	50	0	0	100
4.0	0	34	33	0	51	49
2.0	3	45	3	6	88	6
1.0	4	61	3	16	80	4
0.5	34	35	5	46	47	7
0.25	42	43	0	49	51	0

TEACHING TIP

The hypothesis is written for testing various concentrations of sodium phosphate. If your students want to compare the effect of phosphate separate from the effect of sodium, they should calculate the solutions in equivalents. An 8.0% sodium orthophosphate solution is 0.17N and offers three times the amount of sodium as a 0.17N sodium chloride solution. They will want to have a separate sodium chloride control for each sodium orthophosphate dilution. For the 8.0% sodium orthophosphate solution, the control would be a 2.98% sodium chloride solution. When doing their calculations, they will subtract the mortality in the matching control from the mortality in the treatment.



Graph H. Graphical display of apparent health of brine shrimp after 24 hours exposure to various concentrations of sodium orthophosphate.

TEST QUESTION

In a natural system, brine shrimp eat photosynthetic algae that they filter from the water. How might the concentration effects of phosphate be different if algae were present in your experimental system? Consider both the short term 1 to 5 days and long term 1 to 2 months effects.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 5
The Effect of Nitrate Concentration on *Artemia*

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 determine if an increase in nitrate concentration will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

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

10 mL 10.0% $NaNO_3$

Interpretation

Accept the hypothesis if a decrease in the survival rate is observed whn there is an increase in the phosphate concentration. Reject the hypothesis if there is no change in the survival rate as the concentration of phosphate increases or if the survival mrate decreases as the phosphate concentration decreases.

Answer to Test Question

If algae were present and the systems were in the light, one would expect the algae to take up phosphate from the solution. That would reduce quickly the concentration making a given addition of phosphate less toxic to the brine shrimp. One would expect the low concentrations of phosphate then to enhance the growth of algae. More algae would mean more food for the brine shrimp, resulting in greater survival and reproduction. Over a still greater time with high phosphate concentrations, algae should grow so well that as they die they create a large source of food for bacteria. The bacterial growth then will reduce the availability of oxygen and result in the asphyxiation of the brine shrimp.

TEACHING TIP

The hypothesis is written for testing various concentrations of sodium phosphate. If your students want to compare the effect of phosphate separate from the effect of sodium, they should calculate the solutions in equivalents. An 8.0% sodium orthophosphate solution is 0.17N and offers three times the amount of sodium as a 0.17N sodium chloride solution. They will want to have a separate sodium chloride control for each sodium orthophosphate dilution. For the 8.0% sodium orthophosphate solution, the control would be a 2.98% sodium chloride solution. When doing their calculations, they will subtract the mortality in the matching control from the mortality in the treatment.

Interpretation

The hypothesis should be accepted if a decrease in the survival rate is observed when the concentration of nitrate increases. The hypothesis should be rejected if there is no change in the survival rate as the concentration of nitrate increases or if the survival rate decreases as the nitrate concentration decreases.

DIRECTIONS FOR SETTING UP THE EXPERIMENT

Follow the same procedure as in the Core Experiment substituting a series of sodium nitrate solutions from 0.0% to 8.0%. To prepare an 8.0% sodium nitrate solution, dissolve 8.0 g of sodium nitrate in a small amount of distilled water and dilute to 100 mL with distilled water. Sodium nitrate makes sodium ions available, so do not use 1.0% NaCl as the diluent.

HYPOTHESIS GENERATION

Question

What effect will the increase in nitrate concentrations have on brine shrimp?

Sample Hypothesis

An increase in the concentration of nitrate will cause a decrease in the survival of brine shrimp larvae.

Rationale

Nitrate pollution is viewed as a serious problem resulting from wide use of fertilizers in agriculture and horticulture.

Sample Experimental Procedure

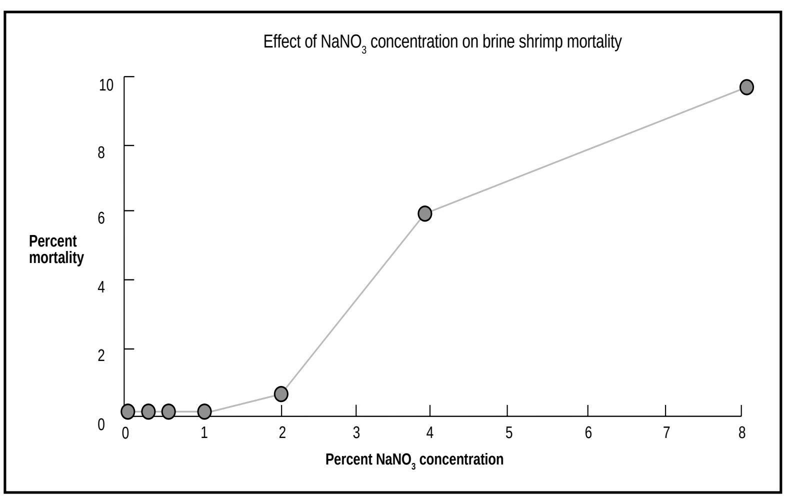
1. Repeat Steps 1 to 7 from Variation 4, substituting sodium nitrate for sodium phosphate.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Mortality of brine shrimp in various sodium nitrate solutions.

Percent $NaNO_3$ concentration	Percent mortality
8.0	100
4.0	58
2.0	5
1.0	0
0.5	0
0.25	0
0.125	0
0.0625	0



Graph I. Effect of nitrate concentrations on brine shrimp mortality.

TEST QUESTION

Most commercial fertilizers contain nitrates. As rain falls on an agricultural field, the runoff may dissolve the nitrates in the fertilizers and carry them into the groundwater that may eventually reach rivers and oceans. What might this do to organisms living in the water?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 6
The Effect of Substituting a Different Metal Ion for the Sodium Ion in the Culture Solution on the Survival of the Larvae of Brine Shrimp

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 determine if replacing the sodium ion of NaCl will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

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

- 1.0% solution of any of these possible salts: KCl, $CaCl_2$, $NiCl_2$, $FeCl_3 \bullet H_2O$, $CuCl_2$

HYPOTHESIS GENERATION

Question

What effect do other metal ions have on brine shrimp larvae survival?

Sample Hypothesis

A substitution for the sodium ion in the culture solution will cause a decrease in the survival rate of the brine shrimp larvae.

Rationale

Brine shrimp would be unlikely to have protection from high levels of other metal ions because they would not have experienced them during their evolution.

Sample Experimental Procedure

1. Repeat Steps 1 to 7 from Variation 4, substituting the appropriate salt concentrations from 0.0625 to 1.0% only for each salt.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

Answer to Test Question

In the same way that nitrates fertilize crops, they act as fertilizers to algae and plants in the water. They stimulate their growth and the population increases. As the plants increase in size and number, they use oxygen. The plants compete with the animal life for oxygen. Ecosystems can be threatened seriously by increased levels of nitrates.

TEACHING TIPS

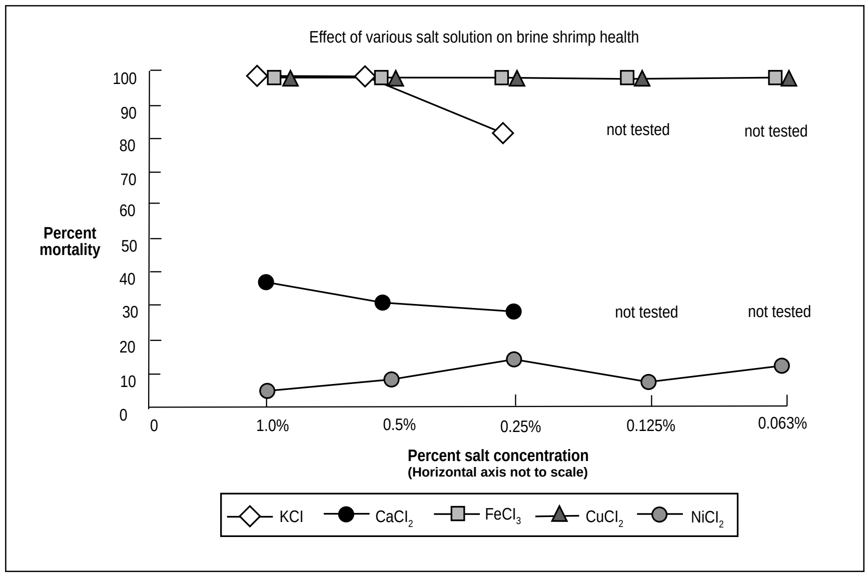
- Concern over the damaging effects of sodium chloride used to de-ice roads in winter has led to the replacement of sodium with other metal ions. Students might be interested in discovering what is used in your area or in an area where they have family or where they vacation. Substituting this material for the shelf chemicals would be a relevant variation.
- The appropriate controls are sodium chloride concentrations equivalent to the chloride ion concentrations provided by the test salt solutions. Over most of the range of concentrations students will not find depressed mortality due to chloride ions. Coordinate with a group planning to study the effect of sodium chloride concentrations as in the Core Experiment, or through a coordinated dilution scheme.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 9. Average brine shrimp mortality (%) in various salt solutions of several concentrations corrected for mortality in sodium chloride.

Salt solution	1.0%	0.5%	0.25%	0.125%	0.0625%
KCl	100	100	86	Not tested	Not tested
$CaCl_2$	37	30	26	Not tested	Not tested
$FeCl_3$	100	100	100	100	100
$CuCl_2$	100	100	100	100	100
$NiCl_2$	4	5	8	4	7



Graph J. Effect of metal ion substitution on brine shrimp mortality.

TEST QUESTION

Normally brine shrimp live in a NaCl solution. What role does the sodium ion play in the survival of organisms? What would happen if you substituted calcium chloride for sodium chloride?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

Interpretation

The hypothesis needs to be tested and either accepted or rejected separately for each ion substitution made. Accept the hypothesis if the substitution of an ion causes a decrease in the survival rate. Reject the hypothesis if the ion substitution causes an increase or no change in the survival rate.

Answer to Test Question

If the sole purpose of sodium ions is to maintain the osmotic potential, changing the metal ion would have little effect. The results of the experiment suggest that sodium has additional functions in the survival of brine shrimp. It is possible that brine shrimp rely on a sodium-potassium pump mechanism for osmotic regulation.

VARIATION 7
The Effect of Substituting a Different Halogen Anion for the Chloride Ion on the Survival of Brine Shrimp Larvae

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 replacing the ion in NaCl will affect survival of brine shrimp larvae.

ADDITIONAL MATERIALS NEEDED

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

- 1.0% solution of any of these possible salts: NaI, NaBr, NaF

HYPOTHESIS GENERATION

Question

What effect will the substitution of a halogen ion have on the survival rate of brine shrimp?

Sample Hypothesis

A substitution for the chloride ion in the culture solution will cause a decrease in the survival rate of the brine shrimp larvae.

Rationale

The concentration of bromine in the ocean is 65 ppm with fluorine at 1.4 ppm and iodine at 0.05 ppm. All are very small when compared to chlorine at 18,980 ppm (Hodgman, Weast, & Selby, 1961). You would not expect brine shrimp that have had little contact with high concentrations of halogens, other than chlorine, throughout their evolution to have established high tolerances for them.

Sample Experimental Procedure

1. Repeat Steps 1 to 7 from Variation 4, substituting the appropriate concentrations of the halogen from 0.032 to 1.0%.
2. After 24 hours, count and classify the organisms using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 10. Average brine shrimp mortality (%) in various salt solutions of several concentrations.

Salt solution	1.0%	0.5%	0.25%	0.125%	0.0625%	0.032%
NaF	100	100	100	100	100	Not tested
NaBr	70	40	10	8	8	30
NaI	56	55	60	55	61	77

TEACHER'S NOTES

TEACHING TIPS

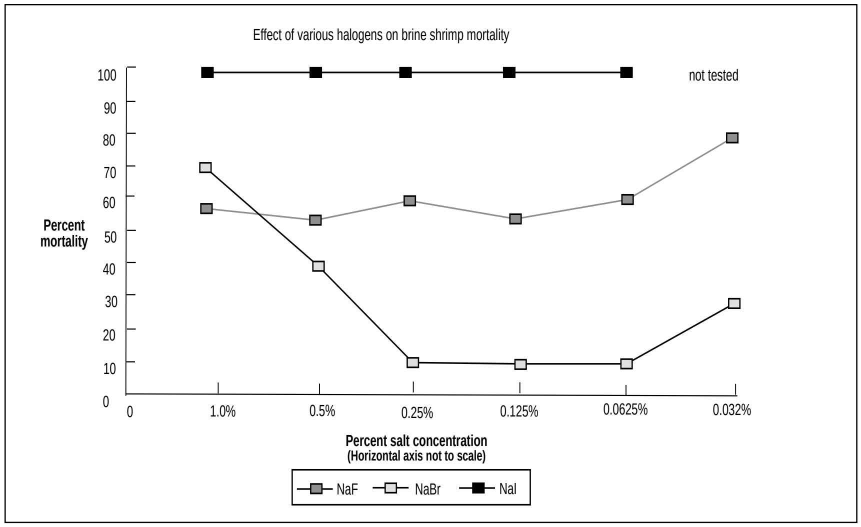
- The appropriate control is a sodium chloride concentration equivalent to the sodium ion concentration provided by the test salt solution. Over the range of salt concentrations tested here, most students would have found 100% survival of brine shrimp larvae. Coordinate with a group planning to study the effect of sodium chloride concentrations as in the Core Experiment, or through a coordinated dilution scheme.
- If your students are capable of constructing an experiment as complex as Variation 7, consider encouraging someone to test baking soda here, although the bicarbonate anion (HCO_3^{-1}) is a complex ion rather than a halogen anion.

Interpretation

The hypothesis needs to be tested and either accepted or rejected separately for each ion substitution made. Accept the hypothesis if the substitution of an ion causes a decrease in the survival rate. Reject the hypothesis if the ion substitution causes an increase or no change in the survival rate.

Answer to Test Question

If one used iodized table salt to prepare the culture salt solution for brine shrimp, one would expect to have a very poor harvest. If sodium iodide was used instead of sodium chloride, the harvest would be even worse because iodide ions would be more concentrated.



Graph K. The relationship of salt concentration to brine shrimp survival.

TEST QUESTION

Normally the brine shrimp live in a NaCl solution. What would happen if you substituted sodium iodide for sodium chloride or if you prepared brine shrimp culture solutions using iodized table salt?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 8
The Effect of Combining a Harmful Cation with an Apparently Safe Anion on Brine Shrimp Survival

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 determine how the exposure of *Artemia* to the combination of a 0.125% $FeCl_3 \bullet 6H_2O$ solution and a 0.125% $NaHCO_3$ solution will affect survival of the larvae.

ADDITIONAL MATERIALS NEEDED

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

- 10 mL of 1.0% $FeCl_3 \bullet 6H_2O$
- 10 mL of 1.0% $NaHCO_3$

DIRECTIONS FOR SETTING UP THE EXPERIMENT

- Prepare a 1.0% solution of $FeCl_3$ by dissolving 1.0 g of $FeCl_3$ in a small amount of distilled water and diluting the resulting solution to 100 mL with distilled water.
- Prepare a 1.0% solution of sodium hydrogen carbonate (baking soda) by dissolving 1.0 g of $NaHCO_3$ in a small amount of distilled water and diluting the resulting solution to 100 mL with distilled water.

HYPOTHESIS GENERATION

Question

What effect will combining a harmful cation with a "safe" anion have on brine shrimp survival?

Sample Hypothesis

The addition of $NaHCO_3$ to a $FeCl_3$ solution will cause an increase in the survival of brine shrimp larvae.

Rationale

Students should provide their own rationale.

Sample Experimental Procedure

1. Label your vials for a dilution series producing the desired dilutions. If you want to test the effect of 0.125% $NaHCO_3$ and 0.125% $FeCl_3$ you will need that concentration of each solution and a 0.25% solution of each compound. If you combine 2.5 mL of 0.25% of $NaHCO_3$ with 2.5 mL of 0.25% $FeCl_3$, the resulting solution will be 0.125% in each compound.
2. Label experimental containers: Na_3Cl control, $NaHCO_3$, $FeCl_3$, and $NaHCO_3$ + $FeCl_3$.
3. Dispense 10 mL of distilled water into all the dilution containers.
4. Into the 0.5% vial, transfer 10 mL of the 1.0% solution. Mix this well and remove 10 mL.
5. Transfer the 10 mL you removed from the 0.5% vial to the vial labeled 0.25%. Mix this well and remove 10 mL.
6. Continue the transfer-mix process once more to create 10 mL of 0.125%.
7. Transfer 5 mL of the final dilution to the vials labeled for a single compound. Transfer 2.5 mL of the next to the last dilution of each solution to the vial labeled for both solutions.
8. Put 5 mL of 1.0% NaCl in the NaCl control container.
9. Add 0.5 mL of culture to each experimental container.
10. After 24 hours, count and classify the brine shrimp larvae using a slight modification of the drop counting technique of the Core Experiment. Here count exactly 50 brine shrimp from each solution and record the percent of survivors.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 11. Survivorship of brine shrimp larvae in various solutions of $FeCl_3$ and $NaHCO_3$. The solution that has no iron or bicarbonate is 1.0% NaCl.

Solution	0.0% $FeCl_3$	0.125% $FeCl_3$
0.0% $NaHCO_3$	50	0
0.125% $NaHCO_3$	47	44

Table 12. Survivorship percentage of brine shrimp larvae in various solutions of $FeCl_3$ and $NaHCO_3$.

Solution	0.0% $FeCl_3$	0.125% $FeCl_3$
0.0% $NaHCO_3$	100	0
0.125% $NaHCO_3$	94	88

Display the percent survivors for each treatment plotted against the treatment on the x-axis.

TEACHING TIPS

- Have students mix a drop of the 1.0% solutions together to observe what happens. The precipitate that forms will resemble tomato juice. They will see that it would be difficult to count larvae in such a solution. Encourage them to consider more dilute solutions.
- The reaction that occurs when the solutions are mixed reduces the concentration of iron ions:

  $2FeCl_3 + 2NaHCO_3 \rightarrow$
  $Fe_2(CO_3)_3\downarrow +$
  $2NaCl + CO_2\uparrow + H_2O$

  To clean up, students can precipitate a pollutant and remove the precipitate.
- Students may want to consider whether these solutions should be made in 1.0% NaCl and all dilutions made using 1.0% NaCl. The directions are written with only distilled water, but you easily can substitute 1.0% NaCl for distilled water.

Interpretation

Accept the hypothesis only if both the $FeCl_3$ solution causes a decrease in the survival rate and the addition of $NaHCO_3$ to the $FeCl_3$ solution reduces its negative effects. Reject the hypothesis if the $FeCl_3$ did not cause a decrease in the survival rate. Also reject the hypothesis if the $FeCl_3$ caused a decrease in the survival rate but the $NaHCO_3$ did not reduce the negative effects of the $FeCl_3$.

Answer to Test Question

There will be many answers to this question and you should be more concerned with the thought process rather than the “correct” answer. The most likely explanation is the sodium bicarbonate chemically ties up the Fe ions, thereby removing them from the solution.

TEACHING TIPS

- Film cans work especially well for this variation because the surface area is larger than in a test tube, but not so large as to require a lot of oil to cover the surface.
- Have the students draw several circles the same size as the film can and fill in 20%, 40%, 60%, and 80% of the surface so that they have a model for what they expect when they adequately have covered the surface for each treatment.
- Alternatively, have students count the number of drops to determine these percentages. Table 13 shows a representative set of number of drops in relation to the percent surface area covered. See Figure 9 for a graphic representation of this information.

TEST QUESTION

Iron can be leached from the soil by acid rain thereby releasing Fe ions into the water. Iron ions greatly reduce survivorship of brine shrimp larvae. Adding sodium bicarbonate reduces the effects of the iron. How would you explain this?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- See Variation 1.

VARIATION 9

The Effect of a Simulated Oil Spill on Brine Shrimp Larvae

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 compare mortality of brine shrimp when exposed to increasing amounts of motor oil.

ADDITIONAL MATERIALS NEEDED

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

- 1 mL new motor oil
- 1 fine-tipped dropping pipette

Table 13. Calculation of percent surface area covered in drops.

Diameter (cm) covered	Percent covered	Number of oil drops
0.8 cm	20%	2 drops
1.6 cm	40%	10 drops
2.4 cm	60%	41 drops
3.2 cm	80%	52 drops
4.0 cm	100%	55 drops

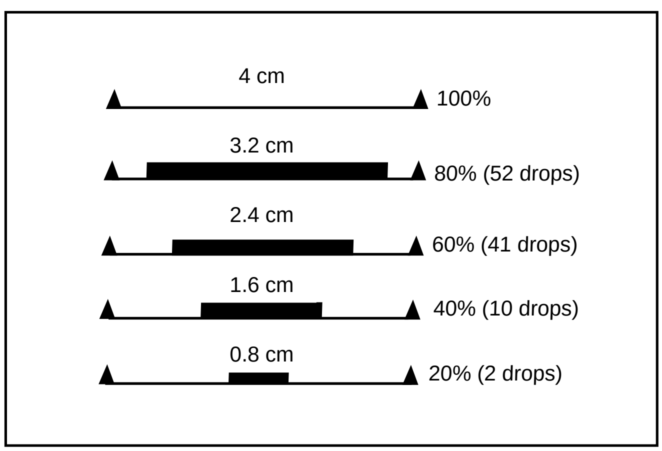


Figure 9. Side view of area covered with number of oil drops indicated in Table 13. Scaled to the 4-cm diameter of a film canister.

HYPOTHESIS GENERATION

Question

What effect will increasing the concentration of oil have on brine shrimp survival?

Sample Hypothesis

Increasing the amount of oil on the surface of water will increase the mortality of brine shrimp larvae.

Rationale

The oil will form a surface over the water preventing the access to aeration that the brine shrimp need to survive.

Sample Experimental Procedure

1. Add 6 mL of brine shrimp larvae in 1.0% NaCl culture to 54 mL of 1.0% NaCl and mix them well.
2. Aliquot 10 mL of the diluted culture to each of 6 film cans.
3. Leave 1 film can without an oil addition as the control.
4. To each of the film cans add oil by the drop. Vary the amount of surface covered so that you create 20%, 40%, 60%, 80%, and 100% surface cover.
5. Incubate the brine shrimp in the light for 24 hours.
6. Mix the contents of each container well and count and classify the brine shrimp larvae using the drop counting technique of the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 14. Brine shrimp mortality and survivorship in salt water contaminated with new motor oil.

Percent of surface covered with oil	Number of live brine shrimp	Number of dead brine shrimp
0 (Control)	48	2
20	46	4
40	45	5
60	30	29
80	15	39
100	0	50

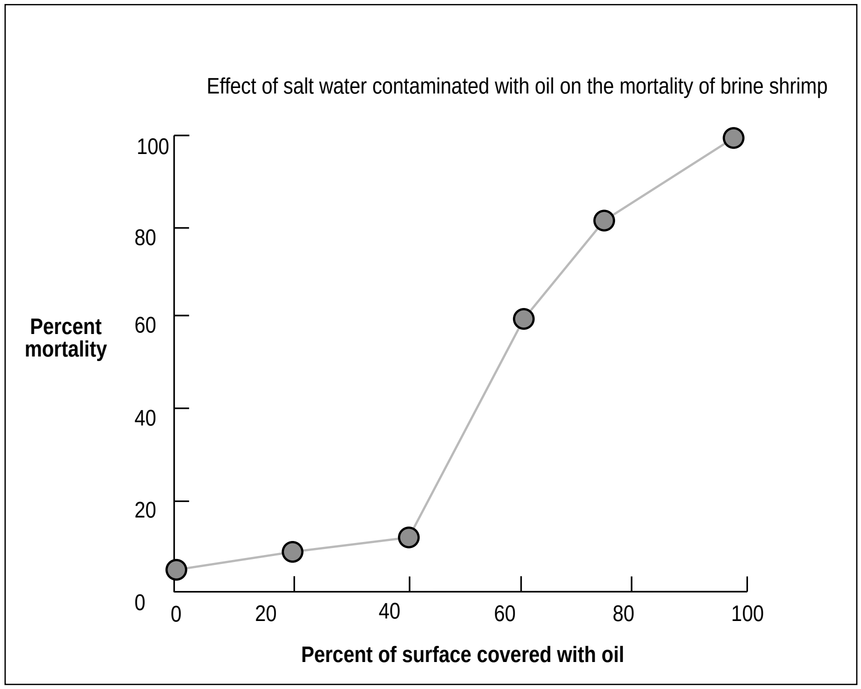
TEACHER'S NOTES

Interpretation

Accept the hypothesis if you calculate a higher percent mortality for higher oil coverage. Reject the hypothesis if you calculate no difference in mortality between treatments or if you calculate a higher percent mortality for greater oil coverage.

Answer to Test Question

Increasing mortality with increasing oil contamination was observed. In some cases, the animals seemed to become coated with the oil so that they could not move well. In other cases, it is suspected that they got oil in their gills so that extracting oxygen was difficult. In the most extreme case of 100% oil coverage, they could have been deprived of oxygen because there was no opportunity for gas exchange between the water and the atmosphere. If the oil were unrefined or used, one would expect that the mortality would have been even higher because toxic materials in the oil would also affect brine shrimp survival.



Graph L. Relationship of percent surface area covered with oil to brine shrimp survival.

TEST QUESTION

Oil pollution of water is a serious problem, whether from maritime accidents or the casual improper disposal of used motor oil. If your brine shrimp mortality increased with increasing oil pollution, what might the cause have been? Would you expect different results if the oil had been unrefined or used?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

- Have students who are visually impaired obtain data for calculations from sighted students.

VARIATION 10

The Effect of Acid Concentrations on Brine Shrimp Survival

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 examine the effects of increased acid concentration on brine shrimp survival.

ADDITIONAL MATERIALS NEEDED

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

- 5 mL 1.0% HCl prepared with saline solution
- 100 mL 1.0% NaCl solution

HYPOTHESIS GENERATION

Question

What effect will an increase in acid concentration have on brine shrimp survival?

Sample Hypothesis

An increase in the acid concentration of the stock water will cause a decrease in the number of living brine shrimp in the sample after a 24-hour period.

Rationale

Every organism has an optimum pH for survival. Even a slight decrease in pH may have an adverse effect on an organism's survival.

Sample Experimental Procedure

1. Label your dilution treatment containers to represent the desired final concentrations of acid; include one container with 1.0% sodium chloride, but no acid.
2. Prepare your dilutions as follows (See Figure 10):
   A. Dispense 9 mL of 1.0% sodium chloride into each of your labeled containers.
   B. Add 1 mL of 1.0% acid to the dilution container labeled 0.1%. Mix thoroughly.
   C. Using the same pipette, transfer 1 mL of the 0.1% solution to the dilution container labeled 0.01%.
   D. Use the same pipette to repeat the process of Steps A to C to create your additional dilutions. From 0.01% create 0.001%, and so on.
   E. Remove and discard 1 mL from the weakest solution.

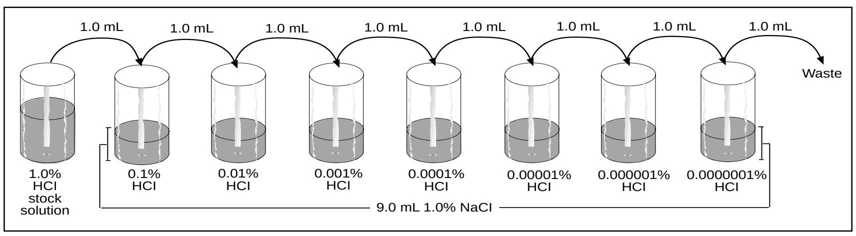


Figure 10. Diagram of dilution solutions.

3. Dispense 1.0 mL of brine shrimp into each dilution.
4. Incubate the containers in light at room temperature for 24 hours.
5. Count the living brine shrimp using the procedures in the Core Experiment listed in Step 7.
6. Determine the survivorship of brine shrimp in each acid concentration. Calculate the percent mortality by dividing the number of dead brine shrimp by the total number of brine shrimp counted. If brine shrimp have died in the control (1.0% NaCl without acid), subtract the percent mortality of the control from the percent mortality of each treatment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 15. Mortality of brine shrimp subjected to various concentrations of hydrochloric acid.

Percent HCl	Number of live brine shrimp	Number of dead brine shrimp	Percent survival = number live/total
1.0	0	50	0
0.1	0	50	0
0.01	0	50	0
0.001	38	12	76
0.0001	50	0	100
0.00001	50	0	100
0.0	50	0	100

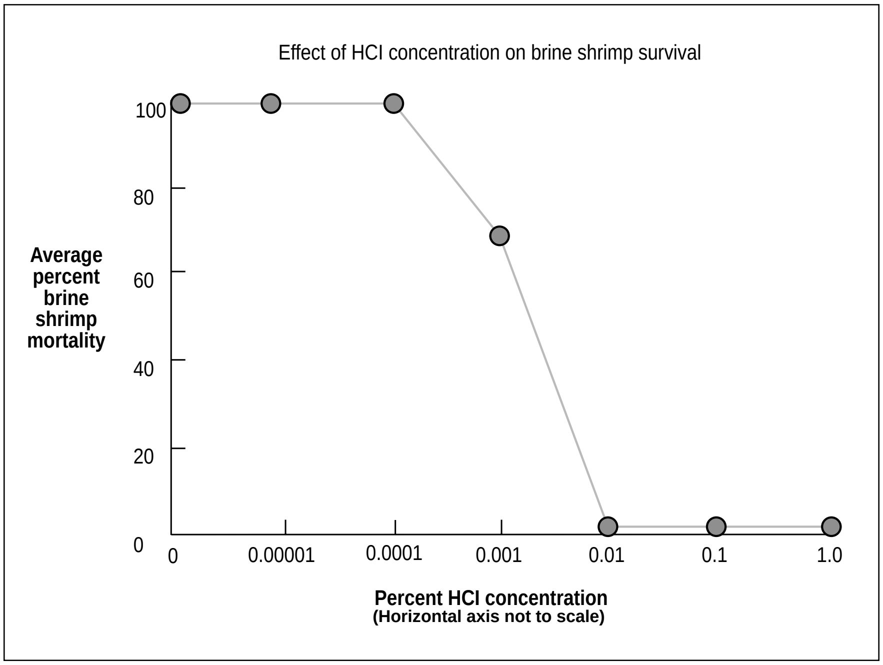
TEACHER'S NOTES

Interpretation

The hypothesis is supported by these data. The number of living brine shrimp decreased rapidly as the concentration of the acid increased from 0.0001% to 0.01%. Brine shrimp appear to be sensitive to changes in acidity.

Answer to Test Question

An increase in the concentration of acid causes an increase in the mortality of the brine shrimp. In many aquatic organisms the gills can be injured and respiration may be reduced or stopped.



Graph M. Survivorship in solutions of various acid concentrations.

TEST QUESTION

Discuss the effect of increased acid concentration on brine shrimp.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

• See Variation 9.

Tolerance Testing With A Hairless "Guinea Pig"

Directions for Students

INTRODUCTION

Have you ever had a pet guinea pig? Did you know that they became common in the United States as experimental subjects in biology laboratories before they were popular as pets? Even earlier, they were dinner fare and farmed for that purpose in South America. Maybe you had a pet called a sea monkey, or more likely, you had many of them. Today sea monkeys, also called brine shrimp (*Artemia franciscana*), as shown in Figure 1, have replaced guinea pigs and mice in many labs for toxicity testing of materials. So, brine shrimp are pets and experimental subjects, but are they farmed for table fare also? No, you will not encounter these small creatures on your dinner table, and they are not farmed. You might, however, use them to feed your tropical fish. They are aggressively and competitively harvested for sale to aquarists.

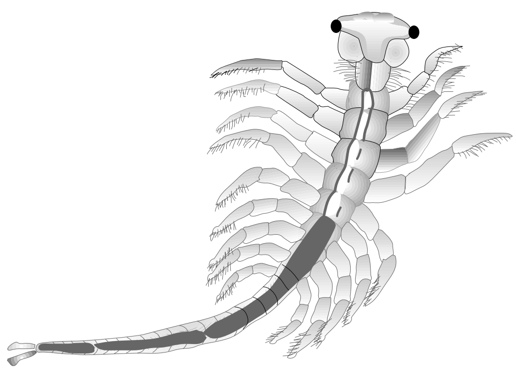


Figure 1. Brine shrimp.

Brine shrimp are found in salt ponds and lakes throughout the world. At the peak of harvest, small planes fly over Utah's Great Salt Lake—a very salty and slightly basic lake—spotting brine shrimp eggs for harvest teams. How can the tiny eggs be harvested with little contamination from other organisms? How many organisms do you suppose can tolerate the high salt concentration of the Great Salt Lake? Is the salinity of a salt pond constant? Are there other changes in an aquatic environment that would affect the ability of different species to survive?

The tiny eggs are really immature, dormant shrimp and will keep for months on the shelf. When put in salt water, they hatch in only 2 or 3 days. Brine shrimp larvae will survive another day or two without food. During this time, they are very sensitive to some materials, such as mining and manufacturing wastes that might pollute water. They also are sensitive to materials that might not be aquatic pollutants, but could be used as drugs to prevent tumor formation in humans. They are used in preliminary assays to screen for anti-cancer potential. These hairless creatures, brine shrimp, are truly modern guinea pigs.

OBJECTIVES

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

- Define and determine the tolerance limit of *Artemia* larvae to environmental variables, such as salinity.
- Describe how an environmental variable may be a limiting factor in an ecosystem and act as a selective pressure in the survival of a species.
- Develop the skill of using a serial dilution to prepare various concentrations of NaCl solutions.

SAFETY NOTES

- Always wear goggles and lab aprons when working with chemicals.
- Label all containers so solutions do not get confused.
- Always add acid to water when preparing acid solutions.
- Use caution when mixing the suggested pollutants during the introduction of the Core Experiment. If other "pollutants" are used in addition to those suggested, be aware of any potential for the chemicals to react with one another.
- Never mix bleach and ammonia or substances containing either substance; toxic fumes will result.
- Use only substances that can be poured safely down the sink.

MATERIALS NEEDED

For each group of two students, you will need the following:

- 1 10-mL disposable syringe or 10-mL graduated pipette with bulb
- 1 permanent marker
- 10 translucent film cans, 50-mL beakers, test tubes, or 1 divided box with 10 chambers
- 1 teasing needle
- 1 1-mL graduated plastic pipette
- 1 stereo microscope
- 10 mL 8.0% NaCl
- 40 mL distilled H_2O
- 1 dropping pipette
- 1 petri dish with counting grid

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of brine shrimp and the limiting factors in their ecosystem. 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. Why is controlling the 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. How do these data relate to the hypothesis?
2. What caused errors in your experiment?
3. How would you modify your experiment to reduce any errors?
4. What does your graph tell you about survival or hatching of brine shrimp?
5. What other important information did you learn from your experiment?
6. How does this laboratory relate to current class study?
7. What other questions come from your results?
8. To what other biology or science topics is this laboratory related?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

After you have discussed the results of the Core Experiment with your classmates, write down questions that occurred as you tested the various salt concentrations on brine shrimp tolerance. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

• How will increasing the pH of the water environment, i.e., making it more basic, affect brine shrimp?
• What is the effect of temperature on brine shrimp survival?
• Will *Artemia* show a preference for shallow depths?
• Will increasing phosphate concentration affect the survival rate of brine shrimp?
• What effect will the increase in nitrate concentrations have on brine shrimp?
• What effect do other metal ions have on brine shrimp larvae survival?
• What effect will the substitution of a halogen ion have on the survival rate of brine shrimp?
• What effect will combining a harmful cation with a "safe" anion have on brine shrimp survival?
• What effect will increasing the concentration of oil have on brine shrimp survival?
• What effect will an increase in acid concentration have on brine shrimp survival?