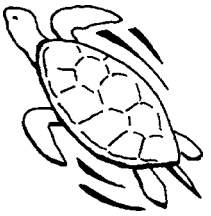


Activity #2

Salinity Tolerance Lab

● ● ● In Advance *Obtaining Lab Supplies*

- Order brine shrimp cysts (eggs) from a science supply house such as Carolina Biological Supply, Sargeant-Welch, Flinn, or Frey. Other sources include local pet stores and online aquaculture suppliers. (If you are unfamiliar with any of these sources, an Internet search will quickly help you identify a supplier.)

● ● ● Class Period One *Brine Shrimp Lab*

Materials & Setup

For each student

- Student Page "Lab Background and Procedures: Brine Shrimp Salinity Tolerance" (pp. 31-33)
- Student Page "Lab Worksheet: Brine Shrimp Tolerance for Fluctuating Environmental Conditions" (pp. 34-38)

For each lab group of three to four students

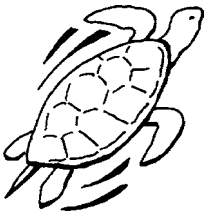
- Brine shrimp cysts
- 1/8 tsp measure
- Four test tubes (150 ml) with stoppers
- Labels or labeling pens for the test tubes
- Test tube rack
- Sea salt or noniodized salt
- Graduated cylinder (more than 100 ml)
- Triple beam or electronic balance
- Distilled water or tap water that has been aged 24 hours in an open container to dechlorinate it

Instructions

1) Divide students into lab groups of three to four students. Hand out the student pages and make sure each group has a complete set of equipment and supplies.

2) Conduct the "Brine Shrimp Salinity Tolerance" lab using the procedures outlined in the student page.

3) If more than 24 hours will elapse between the two lab sessions, feed the shrimp on the second day by placing a tiny amount of brewer's yeast or dried spirulina algae (available in pet stores) in each test tube.



● ● ● **Class Periods Two and Three** *Brine Shrimp Lab, Continued*

Materials & Setup

For each lab group of three to four students

- Hand lens or dissecting microscope
- Sampling pipette (transparent 1 ml or larger)
- Four petri dishes

Instructions

1) Continue the brine shrimp lab, covering questions as the lab proceeds.

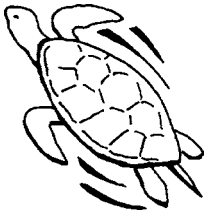
2) At the end of the lab, discuss groups' findings. Bring up the following questions and points in this discussion:
   - What is the advantage of brine shrimp, like hypogeal shrimp, being able to tolerate a range of salt concentrations?
   - What is the difference between a *tolerance* of environmental conditions and habitat *preference*? (In other words, brine shrimp may be able to survive and even reproduce in certain environmental conditions but may not congregate in those conditions if there is a more favorable option.)
   - At low salinity levels and optimal food levels, female brine shrimp can produce 75 free-swimming larvae per day. At salinities above 150 percent and low oxygen levels, the female produces non-developing cysts. In such unfavorable conditions, the female can release 75 cysts. These cysts float and eventually may drift ashore. Development will not continue until the cysts are washed back into the water and reach an area of favorable salinity and oxygen levels.
   - Shrimp reproduction did not factor into this experiment because brine shrimp do not reach adulthood until they are about eight days old.
   - Brine shrimp can live in extremely harsh and variable conditions with temperatures ranging from 43° to 95° F and salinities usually in the range of 28–70 parts per thousand. They can survive in salinities up to 340 parts per thousand.

Journal Ideas

- Explain what a variable is and what is meant by "controlling" a variable. Why is controlling variables important to scientific investigations? Use the brine shrimp experiment to illustrate your definitions and ideas.
- If you were going to study the habitat preferences of the *ʻōpae ʻula* (endemic Hawaiian shrimp found in anchialine ponds), what variables would you test? Why?

Assessment Tools

- Conduct during lab
- Student Page "Lab Worksheet: Brine Shrimp Tolerance for Fluctuating Environmental Conditions"
- Journal entries



Lab Background and Procedures: Brine Shrimp Tolerance for Fluctuating Environmental Conditions

Introduction

Brine shrimp are crustaceans, like lobsters, crabs, and crayfish. A hard exoskeleton supports their bodies and protects them from injury. Brine shrimp are found throughout the world and are adapted to live in harsh, changing environments. These are not exactly the same environmental conditions as the red shrimp that we've been studying inhabit. However, conditions for both shrimp can vary widely in "salinity" (salt concentration) and temperature.

In this lab, you will test the tolerance of brine shrimp to variations in environmental conditions. The measure of tolerance that you will use is the hatching rate of brine shrimp eggs. The environmental condition that you will vary is salinity.

Brine shrimp eggs are initially housed within a structure called a "cyst," which is similar to an eggshell. Brine shrimp cysts can dry out and the egg inside them will remain viable (able to hatch) for many years. As the egg develops, the cyst bursts. For the first few hours after the cyst bursts, the embryo hangs beneath the cyst in the "umbrella" stage. The embryo continues to develop and will emerge as a free-swimming larva.

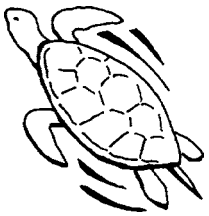
Brine shrimp tend to be brownish-orange in color in this first larval stage. After about 12 hours, they molt (shed their exoskeleton) and begin feeding on tiny algae, bacteria, or nonliving debris. They continue to feed and grow, molting 15 times before reaching adulthood in about eight days.

Adult brine shrimp are generally around 8 mm long. Adult males can be identified by large claspers near their heads, and females by the brood pouch, where the cysts develop, just below the last of their legs. Their lifespan is typically several months.

Materials

Lab Period One

- Brine shrimp cysts
- 1/8 tsp measure
- Four test tubes (150 ml) with stoppers
- Labels or labeling pens for the test tubes
- Test tube rack
- Sea salt or noniodized salt
- Graduated cylinder (more than 100 ml)
- Triple beam or electronic balance
- Distilled water or tap water that has been aged 24 hours in an open container to dechlorinate it



Lab Periods Two and Three

- Hand lens or dissecting microscope
- Sampling pipette (transparent 1 ml or larger)
- Four petri dishes

Lab Period One Procedure

1) On the lab worksheet, write the question or problem investigated by this experiment and your hypothesis.

2) Using salt and the distilled or aged tap water, mix solutions of the following concentrations:
   1 percent salt solution
   5 percent salt solution
   10 percent salt solution
   20 percent salt solution

   The formula for creating solutions of a specific concentration is:
   X grams of NaCl (salt) per 100 ml H_2O (X=target percent concentration)

   Measure 100 ml of water into the graduated cylinder and pour it into a test tube. Weigh the salt and add it to the test tube. Place the stopper on the test tube and mix until the salt is dissolved. Place the test tube in the rack and label it. Mix all of the solutions before moving on to the next step.

3) Unstop all of the test tubes and add 1/8 teaspoon of brine shrimp cysts to each test tube. Leave the tubes open, unless you are moving your test tubes and rack to another part of the room.

4) Leave your test tube rack out of direct sunlight, preferably in a dim part of the classroom. All lab groups should leave their test tube racks in the same location in the classroom.

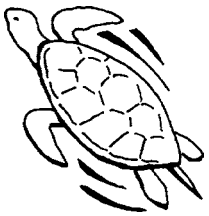
5) Answer question #3 on the lab worksheet.

Lab Period Two Procedure

1) Observe your test tubes. Write your observations on the lab worksheet (question #4, first row in the table)

2) Working with one test tube at a time, make a count of the brine shrimp in a sample volume of water. If the shrimp are not evenly dispersed throughout the water, gently swirl the test tube. Use a pipette to measure 1 ml of water and shrimp (if any have hatched) from the test tube and into a clean petri dish.

3) Using the hand lens or dissecting microscope, count the shrimp in that sample of water and record your findings on the lab worksheet (question #4, first row in the table).



4) Place the shrimp and water back into the test tube they came from.

5) Continue until you have sampled and counted shrimp from each of your four test tubes and recorded your observations on the lab worksheet (question #5, first row of the table). Replace your test tubes and rack to the location where you kept them previously.

6) Answer question #6 on the lab worksheet.

Lab Period Three Procedure

1) Observe your test tubes. Write your observations on the lab worksheet (question #4, second row of the table).

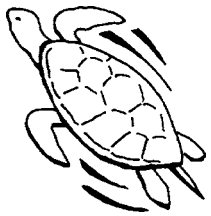
2) Working with one test tube at a time, make a count of the brine shrimp in a sample volume of water. If the shrimp are not evenly dispersed throughout the water, gently swirl the test tube. Use a pipette to measure 1 ml of water and shrimp (if any have hatched) from the test tube and into a clean petri dish.

3) Using the hand lens or dissecting microscope, count the shrimp in that sample of water and record your findings on the lab worksheet (question #5, second row of the table).

4) Replace the shrimp and water back into the test tube they came from.

5) Continue until you have sampled and counted shrimp from each of your four test tubes.

6) Answer questions #6-10 on the lab worksheet.



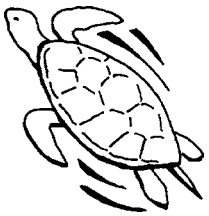
Lab Worksheet: Brine Shrimp Tolerance for Fluctuating Environmental Conditions

Lab Period One Date:

1) State the problem or question this experiment investigates.

2) State your hypothesis.

3) Why is it important for all lab groups to leave their test tubes in the same location? Use the concept of "variable" to explain your response.



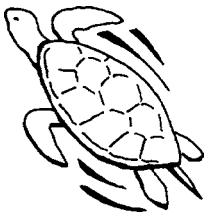
Lab Period Two (Date:) **and Three** (Date:)

4) Test tube observations

	1% solution	5% solution	10% solution	20% solution
Lab Period Two				
Lab Period Three				

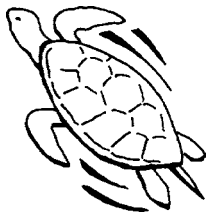
5) Shrimp count per 1 ml water

	1% solution	5% solution	10% solution	20% solution
Lab Period Two				
Lab Period Three				



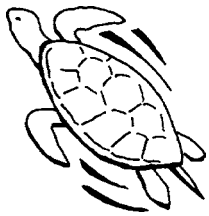
6) Did your group encounter any difficulties counting the brine shrimp in the samples? If so, how were you able to resolve them?

7) Summarize the results of your experiment.



8) Based on the results of your experiment, which solution was the best in which to hatch brine shrimp eggs? Explain your answer.

9) Design another experiment to measure the tolerance of brine shrimp to a different environmental variable such as light or temperature. Describe it here.



10) What types of field observations (made at the ponds, not in a laboratory experiment) could you conduct to test the habitat preferences of the shrimp that live in the anchialine ponds on Maui?