

### FA176

# Teach Aquaculture Curriculum: Dancing with Brine Shrimp<sup>1</sup>

Elisa J. Livengood, Cortney L. Ohs, Amber L. Garr, R. Leroy Creswell, Carlos V. Martinez, Craig S. Kasper, Brian E. Myers, and Frank A. Chapman<sup>2</sup>

This is Activity 14 in a series of 25 in the Teach Aquaculture Curriculum. The introduction to this series is available at http://edis.ifas.ufl.edu/FA176.

### Abstract:

Brine shrimp live in and are harvested primarily from natural salt lakes and solar salt operations around the world. Two natural sources of brine shrimp in the United States are the Great Salt Lake in Utah and San Francisco Bay in California. Adult brine shrimp are also called "sea monkeys" and are used for entertainment or to feed aquarium fish. Brine shrimp can be used to observe, control, and manipulate a life cycle under laboratory conditions during a period of less than 2 weeks. They can be an excellent model for exploring the life cycle of an arthropod in the classroom as well as a useful tool to teach the scientific method through an inquiry-based experimental approach. The following activity will have students decapsulate and hatch brine shrimp cysts. This process is commonly used in aquaculture as well as for experiments that use brine shrimp as a model organism.

### **Objectives:**

Students will be able to:

- 1. Hatch and culture brine shrimp.
- 2. Observe the behavior of brine shrimp at different developmental stages.

### Grade Level:

5-12

The Institute of Food and Agricultural Sciences (IFAS) is an Equal Opportunity Institution authorized to provide research, educational information and other services only to individuals and institutions that function with non-discrimination with respect to race, creed, color, religion, age, disability, sex, sexual orientation, marital status, national origin, political opinions or affiliations. U.S. Department of Agriculture, Cooperative Extension Service, University of Florida, IFAS, Florida A. & M. University Cooperative Extension Program, and Boards of County Commissioners Cooperating. Millie Ferrer-Chancy, Interim Dean

This document is FA176, one of a series of the School of Forest Resources, Program in Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. First Published July 2010. Please visit the EDIS Web site at http://edis.ifas.ufl.edu.

<sup>2.</sup> Elisa J. Livengood, graduate student, School of Forest Resources and Conservation, Gainesville, Florida 32611; Cortney L. Ohs, assistant professor, School of Forest Resources and Conservation, Program in Fisheries and Aquatic Sciences, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, Florida 34945; Amber L. Garr, research associate, Harbor Branch Oceanographic Institute at Florida Atlantic University Center for Aquaculture and Stock Enhancement, 5600 U.S. 1 North, Fort Pierce, Florida 34946; R. Leroy Creswell, marine Extension agent, St. Lucie County Cooperative Extension, Fort Pierce, Florida; Carlos V. Martinez, assistant in Extension, School of Forest Resources and Conservation, Program in Fisheries and Aquatic Sciences, Gainesville, Florida 32611; Craig S. Kasper, aquaculture program manager, Hillsborough Community College, 10414 East Columbus Drive, Tampa, Florida, 33619; Brian E. Myers, associate professor, Department of Agricultural Education and Communication, Gainesville, Florida 32611; and Frank A. Chapman, associate professor, School of Forest Resources and Conservation, Program in Fisheries, Gainesville, Florida 32611.

# **Subject Area:**

Biology, Aquaculture, Agriculture, Chemistry

# Time:

24 hours total for hatching

Day 1: 50-minute class period to decapsulate cysts and prepare for hatching

Day 2: 50-minute period to observe hatched brine shrimp

# Student Performance Standards (Sunshine State Standards):

*11.03* Identify and describe the basic structures and external anatomy of crustaceans (LA.910.1.6.1, 2, 3, 4, 5; LA.910.2.2.2).

*11.10* List and describe the major factors in the growth of aquatic fauna and flora (LA.910.1.6.1, 2, 3, 4, 5; LA.910.2.2.2; SC.7.L.17.1, 2, 3).

*12.01* Recognize and observe safety practices necessary to carry out aquaculture activities (LA.910.1.6.1, 2, 3, 4, 5).

12.03 Safely operate aquaculture machinery and equipment (LA.910.1.6.1, 2, 3, 4, 5).

*14.01* Identify factors to consider in determining whether to grow an aquaculture species (LA.910.1.6.1, 2, 3, 4, 5; LA.910.5.2.3, 4, 5; LA.910.2.2.2; SC.7.L.17.3).

*14.02* Identify/describe facilities used in a grow-out operation (LA.910.1.6.1, 2, 3, 4, 5; LA.910.6.4.1).

*14.04* Determine the purpose and functions of a hatchery (LA.910.1.6.1, 2, 3, 4, 5; LA.910.6.4.1).

# **Interest Approach:**

Lead a discussion of the following items and how they relate to brine shrimp.

1. Water temperatures must be in the right range  $6-35^{\circ}C/43-95^{\circ}F$ .

2. Water must be high salinity: 70–240 parts per thousand (ppt).

a. The ocean is 35 ppt salt.

b. Brine shrimp have efficient systems to regulate salt (chloride, sulphate, and carbonate waters).

c. Brine shrimp naturally live in high salinity environments to avoid predators.

3. Adults are 2–3 millimeters in length. Cysts are very small: 200–300 micrometers in diameter.

4. Young embryos can live inside a capsule in a dry state and without oxygen.

5. Larve molt about 15 times as they grow to adulthood.

# **Student Materials:**

- 1. Air pump
- 2. Airline tubing
- 3. Salt
- 4. Measuring spoon
- 5. Thermometer
- 6. Cone-shaped container
- 7. Brine shrimp cysts
- 8. Bleach
- 9. Gloves
- 10. Magnifying glass
- 11. Funnel
- 12. Filter
- 13. Scale
- 14. Goggles

# **Teacher Materials: See Table 1**

# **Student Instructions:**

### **Decapsulation Instructions:**

- 1. Put on gloves and goggles for eye protection because you will be working with a caustic material.
- 2. Add brine shrimp cysts to 1L of fresh water and wait for one hour (hydrate).
- 3. Measure 1L of household bleach (5% solution) and add to container with hydrated brine shrimp cysts.
- 4. Record the time.
- 5. Set up an airpump by attaching an airline and airstone. Plug in pump. Place airline and airstone into solution.
- Brine shrimp cysts will change color from dark brown to gray to orange; this is the "shell" or capsule being dissolved off the brine shrimp cyst. Observe cysts for color change after about 3–12 minutes (do not go too long; when they are orange you need to stop).
- 7. Pour the suspension onto a fine mesh filter.
- 8. Rinse the cysts with fresh water by pouring water onto cysts on the filter.
- 9. Continue to rinse the cysts with fresh water until the smell of bleach can no longer be detected.
- 10. Rinse the cysts from the filter into a hatching container with fresh water. These are now decapsulated cysts.
- 11. You may now store the decapsulated cysts in a saturated brine solution in a refrigerator (See http://edis.ifas.ufl.edu/FA023 for specific directions) or proceed to hatching instructions.

### Hatching Instructions:

### DAY 1

1. Add 1L of freshwater to hatching container and add decapsulated brine shrimp cysts.

- 2. Place aeration tubing into the hatching container.
- 3. Weigh 25g of marine salt and add to hatching container.
- 4. Cysts will hatch in 24–36 hours depending on water temperature.

### DAY 2

- 1. After cysts hatch, turn off aeration. Wait for hatched brine shrimp to settle to the bottom of the hatching container.
- 2. Use a pipette to remove several mL of hatched brine shrimp from bottom of the container.
- 3. Place live brine shrimp in petri dish with some saltwater and observe different developmental stages with a dissecting microscope.
- 4. If the brine shrimp are fed yeast or microalgae, they can be observed over the next few days as the newly hatched brine shrimp nauplii grow and become adults. The students should observe the swimming behavior for each stage.

# Examples of additional experiments which can be conducted:

Brine shrimp are used extensively for experiments. NASA astronauts in space orbit assessed the effects of microgravity and cosmic rays on developing organisms using brine shrimp. A search on the Internet entering key words such as "brine shrimp experiments" will provide a list of potential activities that can be adapted for 5–12 students in any type of classroom setting. Like the astronauts, students can use brine shrimp to understand how environmental changes affect an organism throughout its life. It might be difficult to devise a classroom activity to measure the effects on brine shrimp of microgravity and cosmic rays, but fortunately there are many earthly variables to study:

- 1. Try examining the effect temperature, varying salt concentration, and light intensity have on hatching rates and survival of the nauplii.
- 2. Try feeding different types of foods (e.g., yeast and yeast + liquid highly unsaturated fatty acids, or HUFAs) and observe the effects on adult reproduction, and their offspring. HUFAs are an

essential nutrient for the rapidly developing young of both terrestrial and aquatic species. Brine shrimp are an excellent substitute for more traditional large laboratory animals because they allow students/teachers to quickly demonstrate the effects that such nutrients may have on the health, growth, or survival of organisms.

### **Teacher Instructions:**

### **Preparations:**

- Hydrate the brine shrimp cysts for each group 1 hour before class: it is essential for the cysts to be hydrated before the start of decapsulation. Begin by hydrating brine shrimp cysts in one gallon of fresh water and aerate. This will become your stock solution of hydrated brine shrimp cysts. Each group will need 3–4 g of cysts per container, so determine how many cysts to hydrate. A 1-pound (454-g) can of cysts will be a sufficient amount of hydrated brine shrimp cysts for 30 groups to hatch 4 containers per group (4 students per group or 120 students total). Then separate hydrated cysts into smaller 1-L containers for each participating group.
- 2. Acquire one gallon of household bleach (5% solution) for each pound of cysts.
- 3. Place all student materials at their stations.

### Activity:

1. Discuss safety and eye protection with students before starting since they will be working with bleach. Ensure that all students wear their safety goggles. If they get bleach on their skin they should immediately rinse it off in the sink with an excess of water. Bleach at this concentration can damage clothing. Closely observe the students as they decapsulate the brine shrimp.

### Post work/Clean-up:

1. After the brine shrimp have hatched and the activity is concluded, the remaining live brine shrimp can be fed to aquarium fish or poured down the sink drain.

### **Anticipated Results:**

The activity should result in successfully decapsulating and hatching brine shrimp cysts. Students should observe movement and growth of brine shrimp.

### Support Materials:

1. All about Brine Shrimp section

### **Explanation of Concepts:**

This activity demonstrates the science concepts of using observational skills, making experimental measurements, and following procedures. Students will also learn a critical aquaculture skill as they culture an organism from egg to adult. See teacher background for more of the science concepts covered.

### All About Brine Shrimp:

Brine shrimp, or sea monkeys, are crustaceans. Their scientific name is *Artemia salina* but they are commonly referred to as *Artemia*. Crustaceans are arthropods, just like arachnids or spiders, centipedes/millipedes, and insects. Crustaceans commonly cultured throughout the world include crabs, lobsters, and shrimps. Barnacles are also considered crustaceans, although they do not crawl but instead attach themselves to rocks. Most crustaceans are cultured for direct human consumption like marine and freshwater shrimps; but the brine shrimp (*Artemia* spp.), and more recently copepods, are popular for feeding the small larval and juveniles stages of other animals also destined for aquaculture purposes.

One of the major criteria for classifying crustaceans is that many have free-swimming, nauplius larvae (plural: nauplii), characterized by three pairs of appendages and usually a single eye (for pictures of the various life stages access the websites listed in the references). The use of brine shrimp was considered a breakthrough in larval feeding technology and facilitated the mass culture of many valuable species of freshwater and marine fish species (examples include flatfishes, sea bream, sea basses, carps). Brine shrimp are also commonly used

to feed many species of ornamental fish, as well as the larvae of edible shrimps, crabs, and lobsters.

Brine shrimp live in and are harvested primarily from natural salt lakes and solar salt operations around the world. The main commercial sources for brine shrimp in the United States are the Great Salt Lake in Utah and San Francisco Bay in California. Although some brine shrimp are harvested as adults (example: used for personal entertainment or to feed aquarium fish), the vast majority of the production is in the form of cysts. Hundreds of tons of brine shrimp cysts are harvested annually, packaged, and utilized by the aquaculture industry worldwide.

The optimal conditions recommended for hatching of brine shrimp cysts include:

- 1. Constant temperature between 25–28°C.
- 2. Strong illumination of 2000 lux.
- 3. Salinity of 15–35 g/L.
- 4. Dissolved oxygen levels above 2 mg/L

Decapsulated brine shrimp cysts do not have a chorion, or shell, leaving only the embryo surrounded by a thin membrane. Decapsulated brine shrimp cysts have several advantages compared to the normal dry packed cysts. Decapsulated brine shrimp have a higher energy content and higher hatch rates, and they are easier for larval and juvenile organisms to digest.

Brine shrimp females can produce either live nauplii or cysts, depending on environmental cues. Such reproductive adaptation permits the survival of the species during periods of high salinity, extreme temperatures, and drought or desiccation. Brine shrimp cysts are true embryos (not eggs) that are in a state of physiological dormancy or "diapause." In the wild, the cysts float at the surface of the water, where they are harvested either in the open water or along the shoreline as they are pushed ashore by winds and waves. Commercially available dry packaged brine shrimp cysts may be stored for months or even years, and will remain in dormancy as long as they are kept dry. The dormant embryo is encased by a membrane and an outer shell, or chorion, which is hard and protects the embryo from harsh conditions. Dry brine shrimp cysts are small (about 200 micrometers in

diameter) and when viewed under a microscope may have a large dimple on their side. Changes in water temperature and salinity usually cause the brine shrimp cysts to rehydrate and initiate the development of the embryo. As the cysts hydrate they become spherical. After the cyst hydrates for about 20 hours at 25–28°C, it ruptures and the embryo can be seen surrounded by a membrane. Shortly thereafter the embryo hatches and a free-swimming nauplius emerges. The nauplius molts (sheds its external skeleton) a couple of times while it feeds on its own yolk reserves. Nauplii at this stage of development have the highest energy content and nutritional value as a food source; therefore, they are the most widely used form of brine shrimp in aquaculture.

Brine shrimp are suspension feeders and feed continuously in the water column. Brine shrimp can be fed yeast, algae, or bacteria. Since brine shrimp are raised primarily to feed other aquaculture species, they can be enriched by feeding them lipids, proteins, and other nutrients beneficial to the animal that ingests them. The nauplii can also serve as a carrier of chemicals to treat or prevent diseases in the organisms that eat them. After 2 weeks of culture, the brine shrimp may be about 5 mm in length. Before reaching adulthood, the nauplii molt approximately 15 times and reach a size of approximately 8-10 mm. Adult male brine shrimp are easily recognized by the well-developed mandibles (or mouthparts) on their heads; females have a distinct brood pouch towards the end of the main body segment. Female brine shrimp can live up to 3–4 months and every 4-6 days release approximately 300 nauplii or cysts.

Year-round availability of brine shrimp cysts is necessary to supply aquaculture hatchery and nursery operations worldwide. As mentioned before, most of the world supply of brine shrimp is harvested from the wild. Most brine shrimp cysts are purchased, and their production in aquaculture operations is restricted to decapsulating, hatching, and harvesting the nauplii.

### References

Decapsulating brine shrimp eggs http://edis.ifas.ufl.edu/FA023

Manual on the Production and Use of Live Food for Aquaculture ftp://ftp.fao.org/docrep/fao/003/w3732e/ w3732e00.pdf

About brine shrimp http://saltlakebrineshrimp.com/about-brine-shrimp/

Use of *Artemia* (Biology, Tank and Pond Production), http://www.aquaculture.ugent.be/coursmat/ online%20courses/faoman/1st\_page.htm

The Culture and Use of Brine Shrimp, as Food For Hatchery-Raised Larval Prawns, Shrimps, and Fish http://www.fao.org/docrep/field/003/AB906E/ AB906E00.HTM

The Brine Shrimp Project http://www.ncsu.edu/sciencejunction/terminal/ lessons/brine.html

### Table 1. Teachers Materials

Material	Store	Estimated Cost
Air pump	Pet store, Walmart	\$10 and up
One liter bottles or similar containers (one/group of four)	Grocery store	\$1.50 and up
Brine shrimp ( <i>Artemia</i> ) cysts ~30 g/class, 3–5 g/group	Online (for example: www.aquacave.com)	\$14.95 for 4 oz. (72 g)
Marine salt (for _aquaria)	Online, pet store	\$2.95 and up
Dechlorinated tap water or fresh water (one gallon/group)	NA	NA
Bleach (5% solution)	Walmart, grocery store	\$2 and up
100- to150-µm mesh filter (one/group of four)	Aquatic ecosystems http://aquaticeco.com	\$2 and up
Digital scale that measures by grams or smaller units	Walmart, Carolina Biological Supply http://www.carolina.com/	\$10–20