Culturing Brine Shrimp Eggs

Introduction

Just add seawater to a small number of brine shrimp cysts and they seem to magically develop and hatch. Brine shrimp (*Artemia*) are amazing small organisms with the ability to survive severe environmental conditions. Watch nauplii larvae hatch and swim!

Concepts

- Cryptobiosis
- Environmental biology
- Larval development

Background

Taxonomy

Phylum: Arthropoda

Subphylum: Crustacea

Class: Branchiopoda (includes fairy shrimp, brine shrimp, daphnia, clam shrimp, tadpole shrimp)

Order: Anostraca (brine shrimp and fairy shrimp)

Genus and species: *Artemia franciscana* (the North American version of *Artemia salina*)

Reproduction

Typically, sexes are separate and adults are sexually dimorphic. Males have large graspers (modified second antennae) which easily distinguish them from females. In some species and populations of *Artemia* (for example, Europe), males may be rare and females reproduce by parthenogenesis.

During mating, males deposit sperm in the female ovisac where eggs are fertilized and covered with a shell. Eggs are then deposited and stored in a brood sac near the posterior end of the thorax (Figure M). Once fertilized, eggs quickly undergo cleavage and development through the gastrula stage (Figures A–E). After one or a few days, eggs are then released by the female (oviposition). Multiple batches of eggs may be released at intervals every few days by the same female.

Two types of eggs may be laid—(1) thin-shelled "summer eggs" that continue developing and hatch quickly, or (2) thick-shelled, brown "winter eggs" in which development is arrested at about early gastrula stage. Such "winter eggs," in their dried and encysted form, survive in a metabolically inactive state (termed cryptobiosis) for up to 10 or more years while still retaining the ability to survive severe environmental conditions. For example, *Artemia* eggs may remain viable after heating to 80 °C for 1 hour, cooling to –190 °C for 24 hours, or reducing air pressure to 0.000001 mm mercury for 6 months!

Embryology

Cleavage of the developing egg is total and yolk is equally distributed among blastomeres. While within the female brood sac, egg development proceeds rapidly through cleavage and blastula stages (Figures A–C). Eggs are then deposited in the environment where they may remain encysted, with embryonic development arrested at about early gastrula stage (Figures D–E). At this time, there are about 4,000 cells in the embryo and these are highly organized, but no organs are discernible.

When encysted eggs are exposed to more favorable conditions (rehydration), the eggs swell and rapid development of the embryo resumes, resulting in completion of the nauplius stage (Figures F-G). Hatching occurs in about 1–2 days, depending on temperature. For the first few hours, the nauplius stays within a hatching membrane that hangs beneath the cyst shell. This is also called the "umbrella stage" in which development of the nauplius is completed.

Larval stages and growth

Larval development of *Artemia* has been described in detail by several authors. Although basic interpretations of development are similar, there are differences among authors regarding the numbering of molts and the naming of various instar stages.

At hatching, the nauplius larva (instar #1) emerges as a free-swimming stage (Figure H). This stage is about 0.4–0.5 mm in length and brownish-orange in color, due to the presence of yolk material. In a sense, the body of the nauplius larva consists mainly of a head. It has three pairs of "head" appendages—a pair of small first antennae (antennules), a pair of well-developed second antennae, and a pair of mandibles. There is a large lip-like structure (labrum) covering a ventral mouth. A nauplius eye is present but it is not easily distinguished at this stage.

The posterior end of the nauplius consists of the future trunk—it is short, undifferentiated, and unsegmented (Figure H). The nauplius larva does not have a complete digestive tract and does not immediately feed. It relies on stored yolk as an energy

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source. Depending on temperature, it swims weakly for about 12–20 hours and then molts into the metanauplius larva (second instar).

The metanauplius larva is translucent in color and about 0.6 mm in length (Figure I). Its trunk region is noticeably longer, and this region continues to lengthen and differentiate through the next series of molts. The metanauplius swims vigorously using its second antennae which are now better developed. At this stage it starts filter-feeding. Its food consists mainly of microalgae, bacteria, and detritus.

The next three stages (each terminated by a molt) are also classified as developmental stages. Examples are shown in Figures J–K. Some metanauplius trends during these later stages include more developed mouth part appendages (maxillules and maxillae) and a longer thoracic region, with some definition of thoracic segments.

Next, there are seven postnaupliar stages—one example is shown in Figure L. During these stages, the antennae begin to undergo a reduction in size and paired thoracic appendages begin forming. With each stage, these appendages become more numerous, larger, and functional. In addition, the compound eyes become more fully developed, the labrum is reduced in size, and abdominal segments become defined.

Then, there are a series of five postlarval stages (not illustrated) involving further reduction in the antennae, multiplication of ommatidial facets in the compound eyes, lengthening of the eyestalks, and formation of sexual organs.

Materials

Brine shrimp dried cysts, 2 g per liter of culture
Marine salts, 36 g per liter of culture
Water, distilled, deionized or spring
Aquarium air pump
Aquarium or other large, clean container
Aquarium tubing and air stone
Petri dishes, 60 × 15 mm or other small dishes
Pipets, Beral-type
Stereo microscopes

Safety Precautions

Follow normal laboratory safety rules during this laboratory. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Prepare a viable culture of living brine shrimp. Do this several days or more prior to the laboratory testing. Brine shrimp must be hatched in a clean container (rinsed completely with deionized water to remove any chemicals, soap, residues, etc.). Hatch the brine shrimp in numerous small containers instead of one large container if an aerator is not available. A small, clean fish bowl with an aerator works very well. If all of the cysts are used at one time, thousands of brine shrimp will hatch all at once! When the hatch occurs, conduct the lab within several days.

To mix the hatching salt solution, add 36 g of the marine salts to one liter of deionized or spring water. Do not use dechlorinated tap water as even trace amounts of copper are toxic to brine shrimp. Add the brine shrimp cysts to the hatching salt solution in the aerated container using approximately 1–2 g of brine shrimp cysts per liter of salt solution. Feed brine shrimp algae. Culture at 68–78 °F (room temperature). Do not place culture vessel in direct sunlight. Algae culture should be placed in direct sunlight.

Procedure

1. Using a pipet, place 10 hatched brine shrimp into a Petri dish.
2. Using a stereomicroscope, view the behavior and features of the brine shrimp nauplii.
3. Repeat daily for several days as the brine shrimp proceed through larval stages to the final adult stage.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Never release living specimens into the local ecosystem. Hatched brine shrimp larvae make excellent freshwater tropical fish food (after they are transferred briefly to spring water to diminish salt concentration). Use as fish food in a fish tank. If no fish tank is available, use Flinn Scientific Biological Waste Disposal Method I, heat or chemical sterilization.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Systems, order, and organization
Evolution and equilibrium
Form and function

Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard C: Life Science, structure and function in living systems, regulation and behavior, diversity and adaptations of organisms

Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard C: Life Science, matter, energy, and organization in living systems, behavior of organisms

Tips

- Use brine shrimp to study LD_{50} or to study crustacean development from cyst to adult. Flinn Scientific, Inc. offers a complete LD_{50} kit (Catalog No. FB1627) and a development kit (Catalog No. FB1605).
- Use brine shrimp to study phototaxis. Brine shrimp exhibit positive phototaxis and will move toward bright light and away from a dark area.
- Use brine shrimp and *Daphnia* when studying the feeding behavior of hydra. Brine shrimp are more easily caught by hydra. *Daphnia* and hydra evolved together and *Daphnia* are less sensitive to the nematocyst toxin of the hydra.
- Use brine shrimp to study fish feeding behavior.
- Use brine shrimp to study metamorphosis.

Acknowledgment

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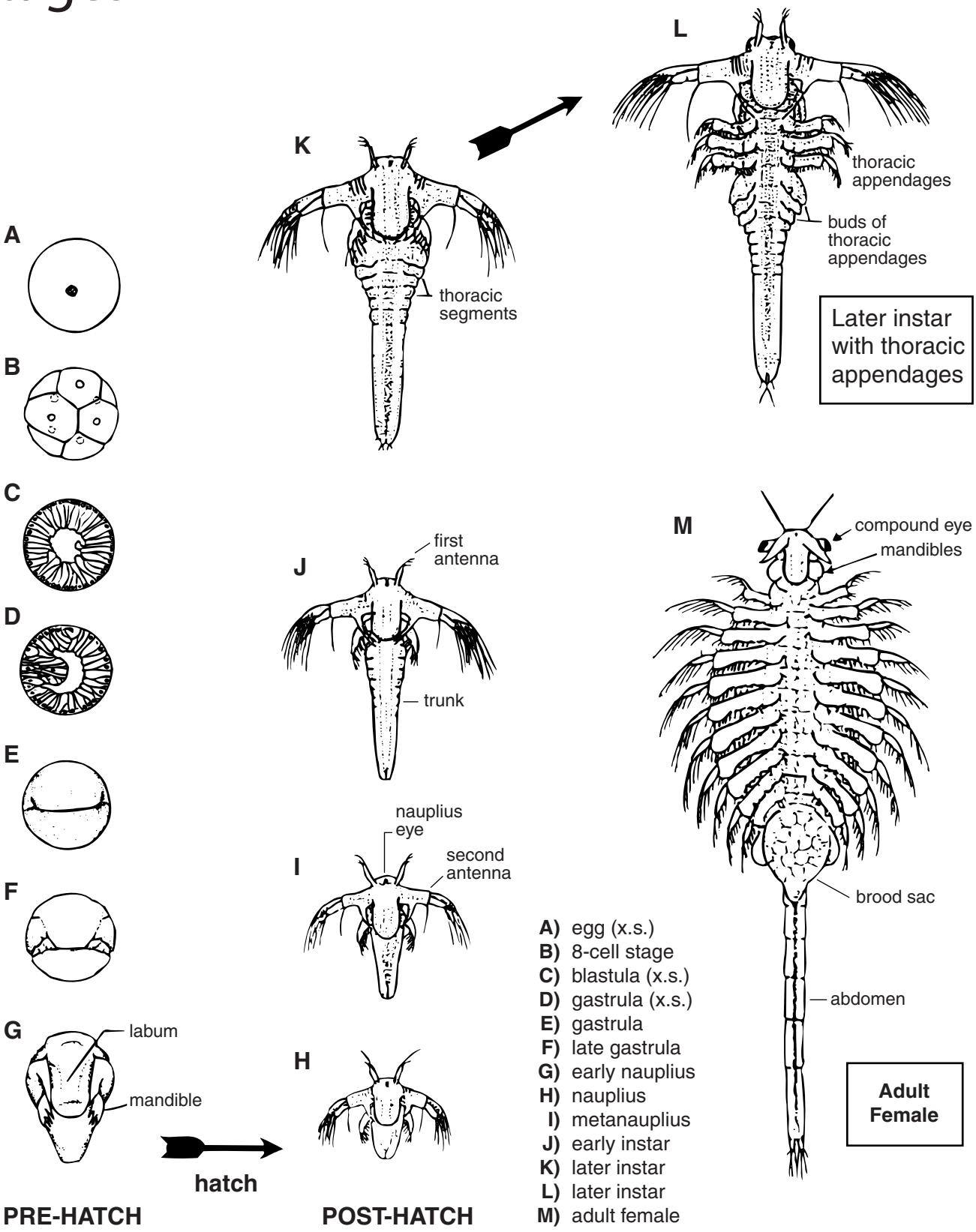
Materials for *Culturing Brine Shrimp Eggs* are available from Flinn Scientific, Inc.

Catalog No.	Description
FB0420	Brine Shrimp Eggs, 80 g
FB0421	Marine Salts—Instant Ocean®
FB0564	Brine Shrimp Eggs, 6 g
FB1605	Stuck-on *Artemia*
FB1627	LD_{50} Kit: Bioassay for Measuring Toxicity

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Artemia Development from Egg to Adult Stages



A) egg (x.s.)
B) 8-cell stage
C) blastula (x.s.)
D) gastrula (x.s.)
E) gastrula
F) late gastrula
G) early nauplius
H) nauplius
I) metanauplius
J) early instar
K) later instar
L) later instar
M) adult female