

Name _____
AP Biology

Experimental Design

Adapted by G Cochrane



Brine Shrimp (*Artemia*)



A fundamental principle that is a focus of science courses are the skills of designing and modifying experiments. You must be able to recognize the components that make a design valid, reasons for error, and how to improve on the design to make results more conclusive. In this activity, you will work as a team to investigate a factor that may be significant in hatching brine shrimp. Read the background about *Artemia* and Great Salt Lake. Brainstorm variables you could test that may influence the hatching of the shrimp before you begin your experimental design.

Experimental Design

As a lab group, use the experimental design matrix sheet attached to outline the components of your experiment. Discuss the design and create an experiment that could lead to a valid conclusion. Each group will produce one report representing the best efforts of the group. Each student will monitor one of more trials of shrimp. In your lab report, be sure to include:

- Title of experiment (reflect description of experiment)
- Significant background research that lead to your research question.
- Identify your testable research question
- Identify your hypothesis with measurable prediction
- Clearly state both the independent and dependent variable
- Indicate the controlled factors
- Identify the experimental vs. control groups (if you have both)
- Number of trials, replicates, or sample size
- Collect and present data with labeled tables and graphs
- Analysis of data
- Conclusion reflecting hypothesis

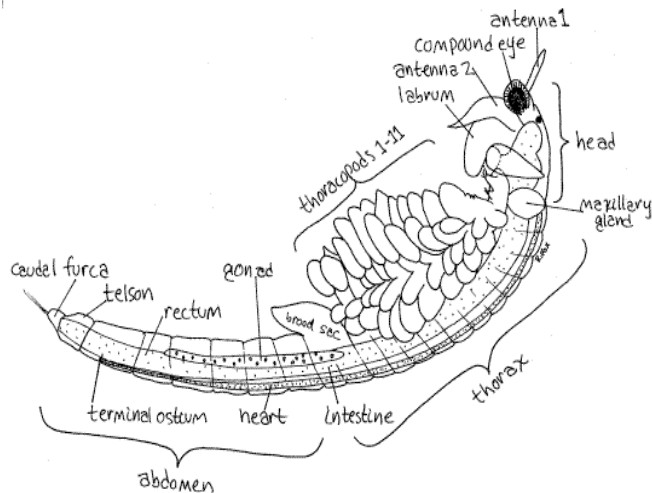


Brine Shrimp and Ecology of Great Salt Lake Brine Shrimp Lifecycle

The brine shrimp, *Artemia*, belongs to the phylum Arthropoda (joint-legged invertebrates), class Crustacea (shrimp, crab, lobster). There are several species of *Artemia* worldwide; *Artemia franciscana* is the species living in Great Salt Lake (and also in San Francisco Bay). Brine shrimp live in hypersaline lakes in which the salt content may be 25%, predators and competitors are few, and algal production is high. The life cycle of *Artemia* begins from a dormant cyst that contains an embryo in a suspended state of metabolism (known as diapauses or cryptobiosis). The cysts



are very hardy and may remain viable for many years if kept dry. Water-temperature and salinity changes in Great Salt Lake occur in about February and cause the cysts to rehydrate and open to release the first growth stage, known as a nauplius larva. Depending on the water temperature, the larvae remain in this stage for about 12 hours, subsisting on yolk reserves before molting to the second nauplius stage, which feeds on small algal cells and detritus using hair-like structures on the antennae known as setae.



Although the cysts are very small (about 200 micrometers in diameter; 50 could fit on the head of a pin) at times they become so numerous that they form large red-brown streaks on the surface of the lake. Under optimum conditions of food supply and lack of stress from increasing salinity or decreasing dissolved oxygen, fertilized female shrimp may produce eggs that hatch soon after emerging from the ovisac to produce nauplius larvae, which is known as ovoviparous reproduction. If conditions are perfect, the female can live as long as 3 months and produce as many as 300 live nauplii or cysts every 4 days. However, the cold spring-time temperatures and variable food supply in Great Salt Lake usually limit the population to two or three generations per year.

The nauplii molt about 15 times before reaching adult size of about 10 millimeters in length. Adult male shrimp are easily identified by the large pair of "graspers" on the head end of the animal. These are modified antennae and are used to hold onto the female during mating. The population of *Artemia franciscana* in Great Salt Lake includes both males and females and reproduces sexually, but some species of *Artemia* exhibit parthenogenesis, a reproductive mode in which only females are present that give rise to young females in the absence of males. Adult shrimp feed primarily on phytoplankton (algae) suspended in the water but can also "graze" on benthic algae such as blue-greens or diatoms growing on the bottom of Great Salt Lake in shallow areas. They also may reprocess fecal pellets excreted earlier in the year when large numbers of phytoplankton present in their diet were incompletely processed. A recent study showed that the shrimp can graze on diatoms that colonize shrimp exoskeleton parts released from their many molts. As the food supply becomes exhausted, salinity increases, dissolved oxygen decreases, or a combination of these conditions occurs, the female shrimp switch from producing live young to producing cysts through oviparous reproduction. In Great Salt Lake, the adult shrimp typically die from lack of food or low temperature during December. Although, live brine shrimp have been observed in the lake at a water temperature of 3 degrees Celsius (37 degrees Fahrenheit), it is unlikely they can reproduce at that temperature. The cysts, which in Great Salt Lake are lighter than the lake water, float on the water surface where they may be harvested or may overwinter to form the source of shrimp for the following year. Brine shrimp are also called "Sea Monkey"s and are raised in aquariums for their entertainment value.

Artemia Development from Egg to Adult Stages

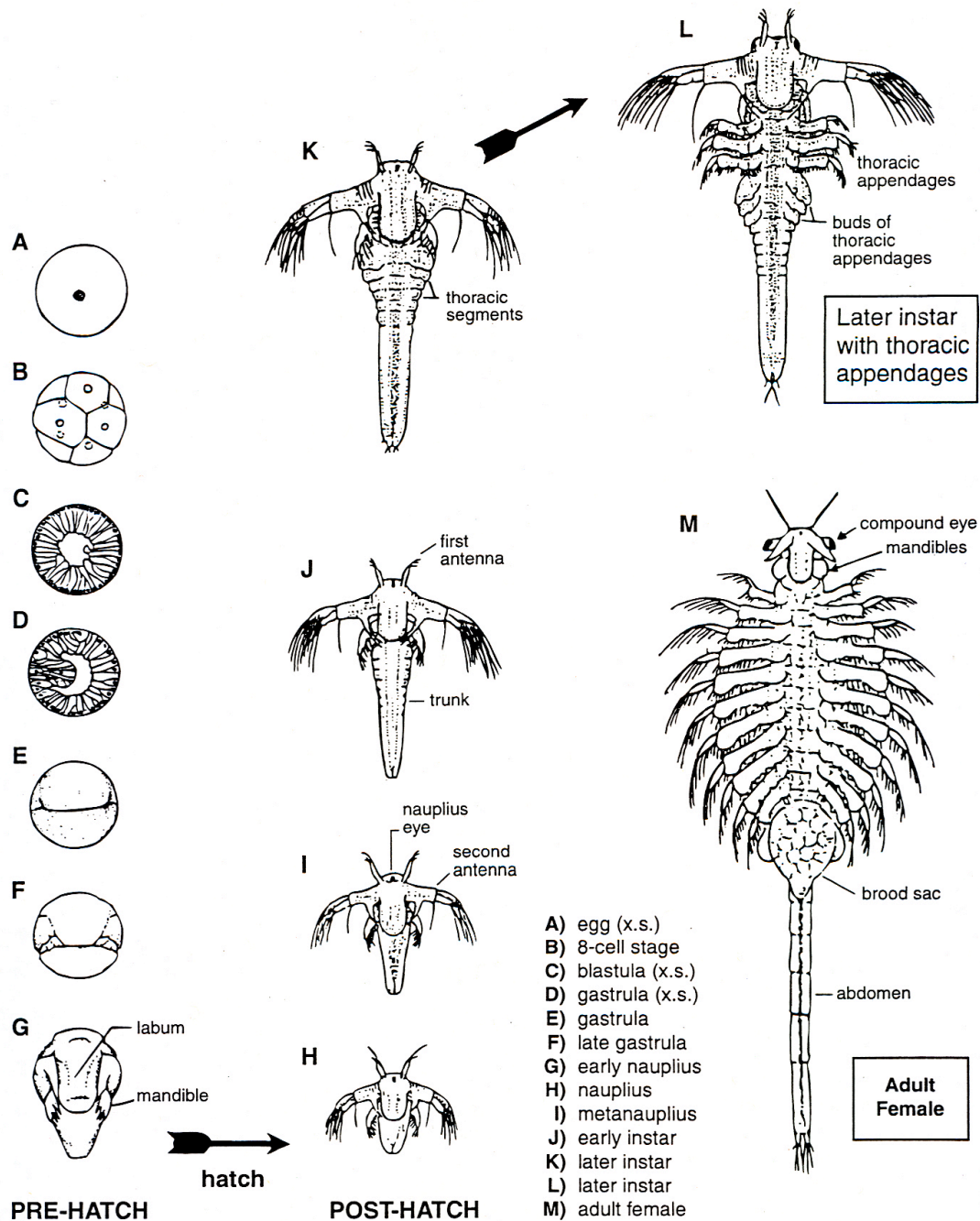


Diagram of *Artemia* development and life cycle.

Preparations and Procedures

We will use a method to quantify the hatching of the brine shrimp cysts described by C. Drewes at Iowa State University in his paper, Quantitative Investigations of Hatching in Brine Shrimp Cysts. It can be very difficult to quantify the hatching since the cysts are light-weight, tiny (about 0.25 mm diameter), and very susceptible to mechanical damage (crushing). Furthermore, it is difficult to follow the developmental progress of loose collections of cysts, especially if there is any slight movement of water. Lastly, if an overabundance of cysts is placed together in an unaerated container to observe mass hatching, the typical result is rapid and premature “crashing” of the newly hatched brine shrimp population.

Materials

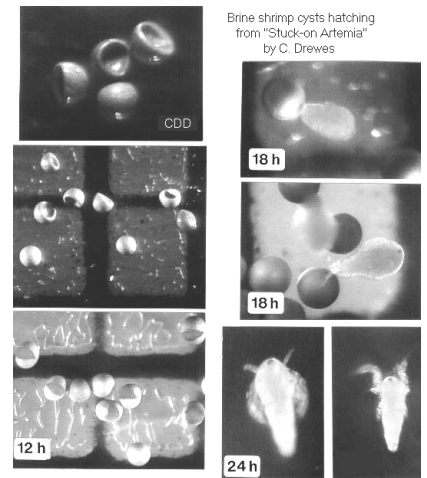
- 10 cm-diameter, disposable, plastic Petri dishes
- Artificial sea water [36 g sea salt/liter of spring water. About 20 ml of ASW will be used per Petri dish. Avoid exposing brine shrimp to chlorinated water which is toxic.]
- double-stick tape [3M Scotch brand double-stick tape is best]
- marking pen
- small, watercolor-type paint brush with soft, camel’s hair bristles
- fresh dried brine shrimp cysts [To prolong shelf-life, store cysts in a capped container in a refrigerator.]
- scissors
- small forceps
- small-bore plastic pipets
- hand-held metal paper punch
- clear acetate transparency sheets
- dissecting microscope
- food for larval and adult *Artemia* if continuing experiment with live shrimp

Procedure

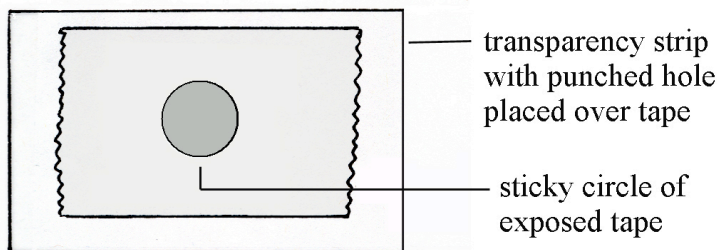
- (1) Make sure your hands and the paint brushes are clean and completely dry.
- (2) Use the scissors to carefully trim the extreme tips of the paint brush bristles so that the bristles are squared off at the end if necessary.
- (3) Use a forceps to affix a 2 cm length of double-stick tape to the bottom of a plastic Petri dish. Avoid making fingerprints on the tape.
- (4) Use a scissors to cut out a 2 cm x 4 cm rectangular strip of clear acetate transparency. Then, use the paper punch to punch a round hole in the center of the transparency strip.
- (5) Carefully place the punched strip over the tape strip in the bottom of the dish (see **Figure 1A**). Use the blunt end of a paintbrush or forceps to gently press against the transparency strip, thus securing it to the tape.
- (6) Next, very carefully touch just the bristled tip of the paint brush into the container of dried brine shrimp cysts. Touch the cysts so lightly with the tip of the bristles that only a few cysts attach to the bristles. If too many cysts attach, then gently tap the bristles against the lip of the container so that some of the cysts fall back into the container. Then, gently “paint” the cysts that are attached to the bristles onto the sticky circle in the bottom of the Petri dish. Brush gently back and forth to make

sure the cysts are secured to the tape. If necessary, repeat this procedure until a total of about 20-40 cysts are stuck to the tape within the grid area (see **Figure 1B**).

- (7) Now, grasp the Petri dish in your fingers and invert it so that the sticky circle faces the floor. Then, use the finger and thumb on your other hand to gently flick the bottom of the Petri dish. The idea is to dislodge and discard any cysts that are not securely stuck to the sticky circle. Thus, your count of cysts within the circle should be an accurate count for the entire dish contents. *WHEN DOING THIS, BE CAREFUL NOT TO DIRECTLY TOUCH OR PRESS ON THE CYSTS BECAUSE THEY ARE VERY FRAGILE!*
- (8) Under a dissecting microscope, count the number of cysts that are stuck to the circle. Draw a map of the distribution of cysts in the circle. (Refer to circular templates in **Appendix B**).
- (9) If a particular dish is designated as an experimental treatment group in which *dry cysts will be exposed to some environmental extreme (e.g., freezing, microwave irradiation, heating, etc)*, then that treatment should be done now, before starting the next step.
- (10) Next, fill the Petri dish about half-full of artificial sea water (about 20 ml), making sure the sticky surface with attached cysts is fully immersed. Cover and label the container. Then, place it in continuous room light at room temperature.
- (11) If possible, inspect and make sketches of the cysts at 12, 18, and 24 hours after immersion begins. Each day for the next four days, continue to inspect and make close-up sketches of cysts.
- (12) Each day for the first four days, use a small-bore pipet to carefully remove and count all newly hatched nauplius larvae. After four days, few if any brine shrimp should be hatching.
- (13) Complete a table of result for each group of cysts. Then use graph paper to plot a hatching curve for each group. The graph should show the *daily cumulative percentage of hatched nauplius larvae*. The vertical coordinate should represent hatching success (*i.e.*, percent of cysts that hatched). A maximum of 100% hatching success would correspond to hatching of all cysts originally placed in the dish. The horizontal coordinate shows time increments: day 0, day 1, day 2, day 3, day 4, etc.



A



B

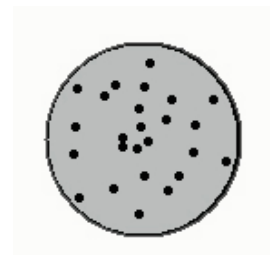
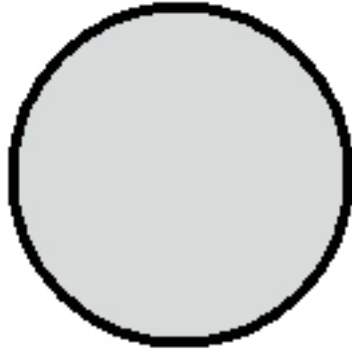


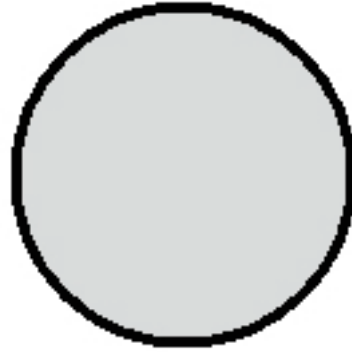
Figure 1. Panel A shows a sticky circle created by laying a punched transparency strip over double-stick tape stuck to the bottom of a Petri dish. Panel B shows 25 brine shrimp cysts stuck to the sticky circle.

Templates for mapping initial distribution of cysts on sticky circle.

The accompanying table is for daily recording of the numbers of hatched nauplius larvae.



Control group dish



Experimental group

Treatment Group (control or experimental) _____ Initial number of cysts in dish _____			
	# of newly hatched nauplius larvae removed	cumulative # of hatched nauplius larvae (day1 + day2 + day3...)	cumulative hatching percentage (cumulative number of hatched larvae / initial number of cysts X 100)
Day 0 start			
Day 1 (24 h)			
Day 2 (48 h)			
Day 3 (72 h)			
Day 4 (96 h)			

Experiment Design Planner

Research question					
Title of experiment					
Hypothesis					
Independent variable (include units)					
Levels of independent variable					
Number of repeated trials					
Dependent variable (include units of measurement)					
Controlled factors (include at least 5 and explain how they will be controlled)					
Control or explanation of why it is a controlled experiment					