Brine Shrimp (*Artemia salinia*) Inquiry Lab (Biotech Series)

Each year it is necessary to revisit the process of correctly applying the scientific method. This practice will continue throughout high school and college. In the Biotechnology pathway, labs and protocols will become extremely complicated and will necessitate the constant use of accurate, controlled, and methodical scientific conventions. In this lab, you will design your own experiment to discover conditions that influence hatching rates in brine shrimp. The common brine shrimp (Artemia) are closely related to zooplankton such as daphnia and are often used as live food for aquariums. The Artemia life cycle begins by the hatching of dormant cysts which are encased embryos that are metabolically inactive. The cysts can remain dormant for many years as long as they are kept dry. When the cysts are placed in salt water, they are rehydrated and resume their development. After 15 or 20 hours, the cysts burst and the embryo leaves the shell. For the first few hours, the embryo hangs beneath the cyst shell, still enclosed in the hatching membrane. The embryo will grow and progress through 15 molts before reaching adulthood in approximately 8 days. Adult Artemia average about 8mm long, but can reach lengths of 20 mm under ideal conditions. Brine shrimp require certain abiotic conditions in their environment to hatch and then grow, such as the amount of salt that is present. Brine is actually a word used to describe the amount of salt in water. After brainstorming with your partner and the class, you will start this lab investigation by writing a procedure in your Biotech notebook that you can follow to do the experiment and obtain valid conclusions. How would you design your experiment to determine the best conditions for brine shrimp eggs to hatch and survive? What would you need to do? How will you measure an effect? In this investigation, you and your partner will gather and statistically analyze data about how different factors may affect the hatching and development of brine shrimp. You will be required to analyze your data using inferential statistics, so you will need to follow the "Statistical Analysis Protocol". In addition, all students will have the opportunity to design an experiment, conduct an investigation using the scientific method, analyze the results using inferential statistics and share the results with the rest of the class. The purpose of this lab is to review and reinforce correct scientific methodology through the exploration of hatching rates in Artemia and to also use inferential statistics to analyze data.

Directions: By evaluating the information given, your group will design an experiment that tests the effects of various abiotic factors on brine shrimp hatching rates. Make sure in your experimental design to include a hypothesis, replications of your test, and a control. You must also make at least two data tables and one graph showing and analyzing your results using the "Statistical Analysis Protocol". Be detailed in your scientific design, as your grade will be on your methods and analysis not your results. Materials that will be available to you will depend on your experimental design; however, all students are required to quantify the cysts and the hatching rates using the protocol described below:

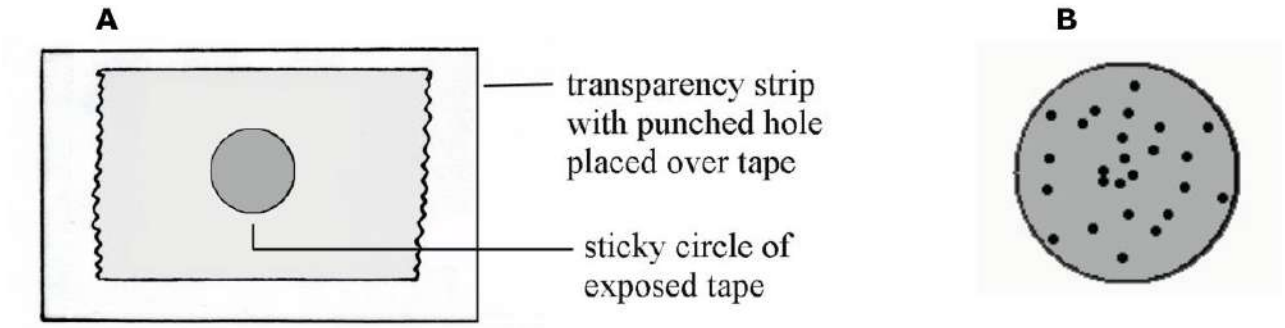


Figure 1. Panel A shows a sticky circle created by laying a punched transparency strip over double-stick tape stuck to the bottom of a Petri dish. Panel B shows 25 brine shrimp cysts stuck to the sticky circle.

(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.

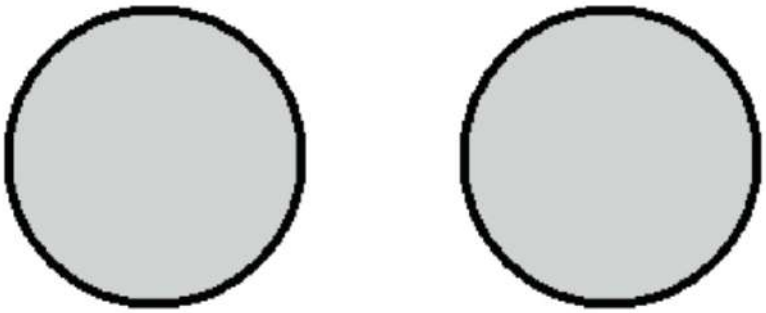
(5) Carefully place the punched strip over the tape strip in the bottom of the dish (see Figure 1A). Use the blunt end of a paintbrush or forceps to gently press against the transparency strip, thus securing it to the tape.

(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 below).

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**

(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. If your group has chosen to manipulate salinity or lighting, adjust accordingly.

(11) If possible, inspect and make sketches of the cysts at 12, 18, and 24 hours after immersion begins. Adjust these times according to your class schedule (traditional, AB block, etc.)

(12) Each day for the first four days, use a small-bore pipet to carefully remove and count all newly hatched nauplius larvae. Again, adjust this according to your class schedule (traditional, AB block, etc.).

(13) Complete a table of results for **each group** of cysts. Then use graph paper (or a computer program) 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. Adjust the times on your data tables according to your class schedule (traditional, AB block, etc.)

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)			

Notebook Entry: Your Biotech notebook entries should include at minimum: Observation/Background Research, Hypothesis, Experimental Design, Data Collection Tables, Analysis Graphs (Statistical Analysis Protocol) and Discussion.

Here are a few suggestions to guide you in your notebook entries:

1. Good place to start: write a "How does [independent variable] affect the [dependent variable] sentence so you know the problem you are studying and the variable you will be comparing.

2. Identify your variables: Independent variable, Dependent variable and controls

3. Write steps you will follow to do your experiment. You MUST say how you will change your independent variable, how you will measure your dependent variable, tell what you will keep constant, say how many trials you will do, and describe the materials you will need.

4. Follow the Biotech "Statistical Analysis Protocol" to analyze your data.

4. Discuss any sources of error. How could your experiment be improved? How could your experiment be extended?

***Artemia* Development Schematic Diagram (over) →**

Artemia Development from Egg to Adult Stages