BIO LAB:
Hatching Brine Shrimp Eggs

TEACHER

LEVEL: Year 7 | TOPIC: Cells | TIME REQUIREMENT: 45 mins

CURRICULUM ALIGNMENT

- *Classification helps organise the diverse group of organisms. Grouping a variety of organisms on the basis of similarities and differences in particular features.*
- *Measure and control variables, select equipment appropriate to the task and collect data with accuracy*

BACKGROUND

Brine Shrimp live in saline environments and can be found in saline lakes on all continents except Antarctica. The global prevalence of Brine Shrimp is due to their ability to tolerate a wide range of salt concentrations. Commonly cultured as live food for fish, Brine Shrimp produce two different types of eggs; thin-shelled summer eggs and thick-shelled winter eggs. The thin shelled eggs develop steadily and then hatch; while the development of the embryo within the thick shelled eggs halts when it reaches a particular stage. The eggs, and the embryos within them, are then able to survive for months or even years until changes in the environmental conditions trigger the embryos to develop further and hatch. Brine Shrimp eggs hatch into larvae called nauplii.

In this practical, students study the hatching of dormant Brine Shrimp eggs and design an experiment to investigate factors that may influence hatching success. Students set up hatching chambers, prepare Brine Shrimp eggs for hatching and watch the living creatures develop. Students record hatching numbers and test their skills in statistics and data analysis. This is a great opportunity for students to form a hypothesis, test the hypothesis through experimentation and practice data collection. This practical provides students with an understanding of hypotheses and experimentation design.

PREPARATION - BY LAB TECHNICIAN

Preparing Salt Solution

1. To create a standard salt solution for hatching eggs you will need a solution of 3.5% or 35 ppt (parts per thousand) dissolved salts.
2. A day or two before the practical, dissolve 35 g of synthetic sea salt or non-iodized table salt in spring water to make 1 L of solution. 'Aged' tap water can also be used if you don't have any spring water. Simply fill a container with tap water and leave open to the 'air' for a few days to remove the Chlorine.
3. Store solution at room temperature and you may divide into volumes of 30 mL or allow students to measure the solution themselves.

METHOD - STUDENT PRACTICAL

Making a Hatching Chamber

1. Using your scissors and ruler, measure and cut a 2.5 cm long piece of double-sided tape and carefully adhere it to the inside bottom of a petri dish. Ensure the tape is not wrinkled or has any air bubbles.
2. Measure and cut a 2.5 cm long piece of transparency film to match the dimensions of the tape.

MATERIALS

- Brine Shrimp Eggs, Live
- Plastic Petri Dishes
- Pipette
- Markers
- Synthetic Sea Salt or Non-iodized Table Salt
- Double-sided Transparent Tape
- Paint brushes, Fine
- Clear transparency film
- Hole punch
- Scissors
- Ruler

SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- Wash your hands thoroughly before and after the activity.
- Do not release any organisms into the environment.
- Disinfect work areas before and after use.

SOUTHERN Biological

3. Using a hole puncher, puncture a hole towards the centre of the piece of transparency film.
4. Place the film over the tape strip. The film should entirely cover the tape, except for the hole near the centre. The small circle of exposed tape will create a sticky surface for the eggs to attach to.

Collecting the Eggs

1. Insert your brush into a container of dried Brine Shrimp eggs. Be slow and gentle when doing this to ensure the bristles of the brush only pick up a small amount of eggs. If the brush picks up too many eggs, simply tap the excess eggs off against the lip of the container.
2. Bring the brush to your petri dish and lightly brush back and forth across the small exposed circle of the tape. If required, you may repeat this step until there are approximately 20 to 50 eggs stuck to the tape.
3. Place the remaining eggs on the brush back into the container.
4. Using a microscope, inspect the eggs in your petri dish and conduct an initial egg count. Record the results in Table 1.
5. Gently pour 30 mL of salt solution into the petri dish and replace the lid.

Designing the Experiment

1. Once you have set up your Brine Shrimp housing, make a list of conditions you believe might influence the hatching of Brine Shrimp eggs. Briefly describe how and why you believe these factors will influence egg hatching.
2. Choose one specific potential influencer from your list and develop a question about the hatching of Brine Shrimp eggs. You should be able to answer this question through the experiment. Based on your question, form a hypothesis. State your hypothesis in the form of "if ...then ... because". Based on your hypothesis, design an experiment to test it. This may include creating a 'control' batch along with your "Experiment" batch, to compare the results. Your teacher will need to approve your procedure before you start your experiment.

	Description	Example
Question	Question regarding how a particlular conditions influence egg hatching.	"How does temperature affect the hatching of Brine Shrimp?"
Hypothesis	Hypothesis "if ...then ... because".	"If I raise the temperature 5 degrees then more eggs will hatch because brine shrimp eggs thrive in warmer temperatures".
Experiment Design	Experiment design that will test your hypothesis.	"I will set up 2 Brine Shrimp hatching chambers. One will act as a "Control" and will be prepared with a 3.5% salt solution and with a pH of 7. This "Control" will be stored under normal test conditions at 25 °C. The "Experiment" will be prepared using the same procedure but will be placed under a heat lamp at a temperature of 30°C".

3. Conduct your experiment and report the results.

Counting Hatchlings

1. Using a pipette, remove the hatched Brine Shrimp from the hatching chamber and transfer the hatchlings into a clean petri dish.
2. Examine them under the microscope and count the number of eggs hatched during the 24-hour period.
3. Repeat step 2 every day for 4 days and record the figures of hatched eggs in Table 1. Where possible, try and collect new hatchlings at the same time of day.
4. Calculate the cumulative number of larvae hatched and percentage of hatched larvae for each day and record the results in Table 1.
5. Analyze the results of your experiment and determine whether or not your hypothesis was proven correct. Provide evidence to support your claim.

OBSERVATION AND RESULTS

Results will vary depending on the experiment that is being conducted and the environmental conditions the eggs are subjected to. The number of eggs hatched is the main evidence that students will use to prove or disprove their hypotheses. Below is an example result of a batch of 46 eggs hatched in a standard 3.5% salt solution at 25°C with a pH of 7.

Counting Date	Number of Larvae hatched	Cumulative number of Larvae hatched	Cumulative percentage of Larvae hatched
Day 0	0	0	0%
Day 1	20	20	43%
Day 2	12	32	70%
Day 3	5	37	80%
Day 4	2	39	85%

EXTENSION EXERCISE

To further explore Brine Shrimp anatomy, you may like to guide students through an exercise in telling a male Shrimp apart from a female Shrimp. One main difference between the two, is that male Brine Shrimp possess whiskers under their chins; while females do not. Males Shrimps can be observed fighting over a female. When fighting, the two male Shrimp can be seen locked together. However, if you see a male and a female Brine Shrimp locked together, they are most likely mating. Another way to identify females Brine Shrimp is to look for a pouch. Female Brine Shrimp develop a pouch during pregnancy. Interestingly, female Brine Shrimp can fertilize their own eggs via a process known as parthenogenesis.