LAB: Using the Scientific Method to Study Brine Shrimp Development

Objective: Students will use the scientific method to observe how a variable affects the hatching rate of brine shrimp.

Background: Read and highlight the following short article before making your hypothesis:

The brine shrimp (Artemia) is in the phylum Arthropoda, class Crustacea. Artemia are zooplankton, like copepods and Daphnia, which are used as live food in the aquarium trade and for marine finfish and crustacean larval culture. Artemia are extremely euryhaline, withstanding salinities from 3 ppt to 300 ppt. They can even survive short periods of time in freshwater, but cannot reproduce in it. Artemia survive temperatures ranging from 15 to 55°C (59 to 131°F). They have two modes of reproduction. Sometimes nauplii (first Artemia swimming stage) hatch in the ovisac of the mother and are born live. However, when the body of water where adult Artemia are living begins to dry up and salinities rise, embryos are encased in a hard capsule, or cyst, so that they are protected and can hatch later when conditions are better.

The optimal conditions for hatching Artemia are: 1) temperature above 25°C, with 28°C being optimum; 2) salinity of 5 ppt (1.030 density); 3) heavy, continuous aeration; 4) constant illumination (example: two 40-watt fluorescent bulbs for a series of four 1-liter hatching cones); and 5) a pH of about 8. Stocking density is set by adding no more than 5 grams of cysts per liter of water.

Within 15 to 20 hours after being placed in seawater at 28°C, the shell breaks and the prenauplius in E-1 stage appears. For the first few hours, the embryo hangs beneath the cyst shell in what is called the umbrella stage. The newly hatched Artemia relies on its yolk sac for nutrients because its mouth and anus are not fully developed. The pre-nauplius E-2 stage is then released as a free-swimming nauplius called an Instar 1 nauplius. In this stage it is brownish orange because of its yolk reserves. It uses specially modified antennae for locomotion and later for food filtering. Approximately 12 hours after hatch it molts into the second larval stage (Instar II) and starts filter feeding on microalgae, bacteria and detritus. The Artemia nauplius can live on yolk and stored reserves for up to 5 days or through the Instar V stage, but its caloric and protein content diminish during this time. The nauplius progresses through 15 molts before reaching adulthood in approximately 8 days.

Hypotheses: *If brine shrimp cysts are exposed to ____________________________, then their hatching rate/success will ____________________________

Experimental Procedure:
1. *As a group, decide on an independent variable that you would like to test on hatching brine shrimp. Develop a hypothesis by completing the statement above.
2. Design a controlled experiment in the space below. Be sure to list the independent variable, the dependent variable, and all of the controlled variables.

3. Use a pencil to label your beakers with your class period, group number, and the variable that will be introduced to each beaker.

4. Set up your experimental beakers according to the directions decided upon by your group. Be sure to only modify the independent variable to be tested.

5. Allow the brine shrimp cysts to incubate for 24 hours.

6. After 24 hours, transfer one milliliter of water from each beaker to a counting slide. Count the number of hatched brine shrimp in this milliliter. Repeat this two more times for each beaker and calculate the average. Record these data in the table below.

7. Estimate the total population of hatched shrimp in each beaker by multiplying your average counts by the total volume of water in milliliters that was in each beaker.

**Data Analysis:**

1. Complete your data table below.

<table>
<thead>
<tr>
<th>Beaker</th>
<th>Count 1 (# hatched in 1ml)</th>
<th>Count 2 (# hatched in 1ml)</th>
<th>Count 3 (# hatched in 1ml)</th>
<th>Average # hatched in 1 ml</th>
<th>Estimated Population of Hatched Shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Follow the instructions in the table below to calculate the chi square (χ²) for your resulting data. A chi square statistic is used to investigate whether distributions of categorical variables differ from one another. This is how you will determine if your hypothesis should be supported or rejected.

<table>
<thead>
<tr>
<th>Beaker</th>
<th>Observed Pop Hatched (from above)</th>
<th>Expected Pop Hatched (Total / 2)</th>
<th>(Obs-Exp)</th>
<th>(Obs-Exp)²</th>
<th>(Obs-Exp)² / Exp</th>
<th>χ² =</th>
<th>probability level (α)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Now determine the degrees of freedom (df) for your experiment. In this case, it is equal to the number of treatments (i.e. beakers) minus 1). So your group’s df = ________

4. Now find the critical value for your data by using the table to the right. Use a probability of 0.05. Your group’s critical value = ________

5. If your χ² is higher than your critical value, then your hypothesis is supported. Use this to make your conclusion in the section below.

**Conclusion:** Explain your hypothesis were either supported or rejected by the data: ________________________________

**Discussion Questions:**

1. How can your results be applied to the culturing of brine shrimp? ______________________________________

2. What would you do differently next time? ______________________________________