"Hands-on Science: Brine Shrimp Experiment"
Instructions

I. Question to be explored: Does alcohol damage a developing organism?

II. Hypothesis or prediction: Alcohol will decrease the number of brine shrimp that will hatch from their eggs.

III. Experimental Design

Materials

1 Brine shrimp egg pack
1 Screw-capped jar containing premeasured marine salt
4 60 mm Petri dishes with lids
3 1 ml Pipets
1 5 ml Pipet
4 Toothpicks
1 Ruler
1 Magnifying glass (dissecting microscope, optional)
Bottled spring water (recommended) or *tap water that is dechlorinated
1 ml of 100% Ethanol (alcohol; ethyl alcohol)***

***Important information regarding ethanol (alcohol) to be used for this experiment.

1. Absolute (100% also known as 200 proof), non-denatured grain alcohol is required. Do not use rubbing alcohol, or ethanol that is denatured with a contaminant such as benzene.
2. If you elect to substitute an over-the-counter alcoholic beverage, be sure to note the "proof" and adjust volumes accordingly. For example, should you utilize a 100 proof alcoholic beverage such as some vodkas or bourbons, simply double the volume of alcohol added to each dish. For the 1% condition use 0.2 mls; for the 2.5% use 0.5 mls and for the 5% condition use 1.0 mls.
3. In order to keep volumes reasonably small, we recommend that you use alcoholic beverages that are greater than 80 proof. Particularly useful is 190 proof grain alcohol (95% ethanol) which is readily obtainable and can be delivered at volumes that are virtually the same as those for 100% ethanol (For the 1% condition use 0.1 mls; for the 2.5% condition use 0.25 mls and for the 5% condition use 0.5 mls.)
4. Alcoholic beverages suitable for this experiment can be obtained by adults from local alcoholic beverage suppliers or from on-line liquor stores.

Procedures

Day 1. Preparation.

This experiment requires dechlorinated water. The day before the start of the experiment, fill a 500-1000 ml container with tap water and leave out overnight, uncovered. This will dechlorinate the water. *The effectiveness of this dechlorination method can be variable depending upon the chemical treatment of a given water supply. Alternatively, bottled spring water, available in most grocery stores, can be used successfully in this experiment. (Tip: To improve brine shrimp egg hatching, aerate the water overnight beginning on Day 1 by using an airstone attached to an aquarium pump.)

Day 2—Set up experiment.

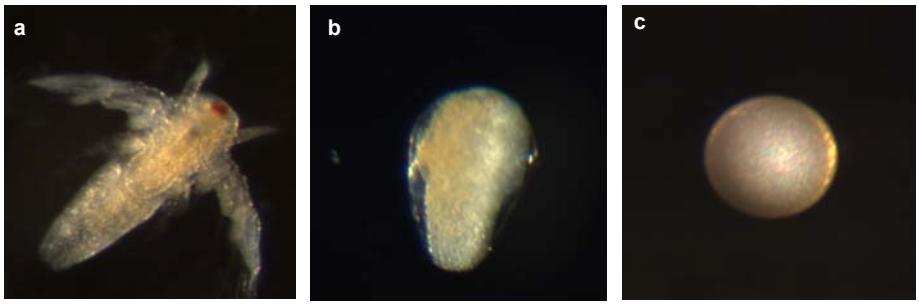
1. Add 500 mls of dechlorinated water to the screw-capped jar with the premeasured marine salt, or mix with 12g (1 Tablespoon) of marine salt. Secure the cap and shake vigorously to dissolve. Using a 5 ml pipet, transfer 10 mls of the marine salt solution into each of the four 60 mm petri dishes.
2. Label the bottoms (lids can get mixed up) of the petri dishes 1 through 4.

3. Petri dish #1 will have no ethanol added to it. This will be the control condition.
4. To petri dish #2, add 0.1 ml of 100% ethanol (final ethanol concentration of 1%).
5. To petri dish #3, add 0.25 ml of 100% ethanol (final ethanol concentration of 2.5%).
6. To petri dish #4 add 0.5 ml of 100% ethanol (final ethanol concentration of 5%).
7. Mark each of the toothpicks at an equal point (about 1/4 inch or 6 mm from the end) so that each can be used to transfer relatively equivalent numbers of eggs. Wet a toothpick in the marine salt solution and insert it up to the 1/4 inch mark into the packet of brine shrimp eggs. Release the adherent eggs by submerging the toothpick in the pertri dish. Repeat for each of the four petri dishes using a fresh toothpick for each petri dish.
8. Cover each petri dish with the correct lid.
9. Allow the dishes to sit 48 hours, undisturbed. Dishes may sit up to 72 hours, if necessary. To increase the number of hatched brine shrimp, place the petri dishes under an incandescent lamp for 48 hours, or double the number of eggs added to each petri dish.

Day 3. Wait

Day 4. Data collection and analysis

10. After forty-eight hours, examine each petri dish with a magnifying glass or dissecting microscope and record your observations. You may observe a) live brine shrimp, b) dead or immature brine shrimp and c) unhatched eggs, as shown in the picture below. *(See #1 below for possible extended activities.)



After 48 hours students may observe a) live brine shrimp b) dead or immature brine shrimp and c) unhatched eggs.

IV. Results and Conclusions

11. Summarize your results by making a table that shows what you observed for each condition, each day of the experiment or by filling out a data sheet provided by your teacher.
12. Answer the questions on the worksheet.

Assessment

Students submit the completed tables, their conclusions and completed worksheets for evaluation.

Extended Activities

*1. Students may quantify their results by sampling a small volume from each petri dish at two different time points; at 0 hours to determine the density of eggs (eggs per unit volume), and 48 hours later to quantify the ratio of live brine shrimp to other (which includes unhatched eggs, partially hatched eggs and dead brine shrimp) as a measure of viability. Students remove a 0.1 ml sample from each dish using a 1 ml pipet, and place the droplet on a slide. A fresh pipet should be used for each sample. Care should be taken to replace lids as soon as possible to avoid evaporation of the alcohol. Using the magnifying glass or dissecting microscope, the number of live brine shrimp, unhatched eggs, partially hatched and dead brine shrimp can be determined and recorded in the data sheet entitled “Quantification of Brine Shrimp Egg Hatching”. Graph the results.

2. For greater impact and with local approval, you may consider replacing 100% (200 proof) ethanol with a 100 proof alcoholic beverage such as some vodkas or bourbons. Simply double the volume of alcohol added to each dish. For the 1% condition use 0.2 mls; for the 2.5% use 0.5 mls and for the 5% condition use 1.0 mls.

3. Students can explore the influence of additional variables that may influence brine shrimp egg hatching. Some suggested variables to consider are a) temperature and b) other agents that may be dissolved in the salt water.