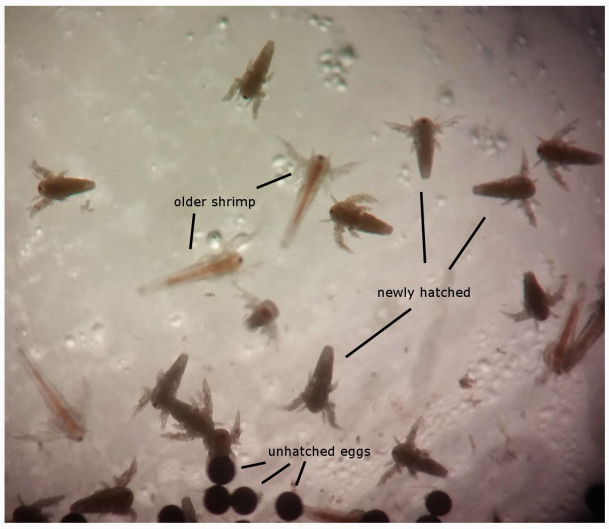
Name _____ **Class Period** _____

Experimental Design Using Brine Shrimp (*Artemmia*)

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 *Artemmia*. Brainstorm variables you could test that may influence the hatching of the shrimp before you begin your experimental design.

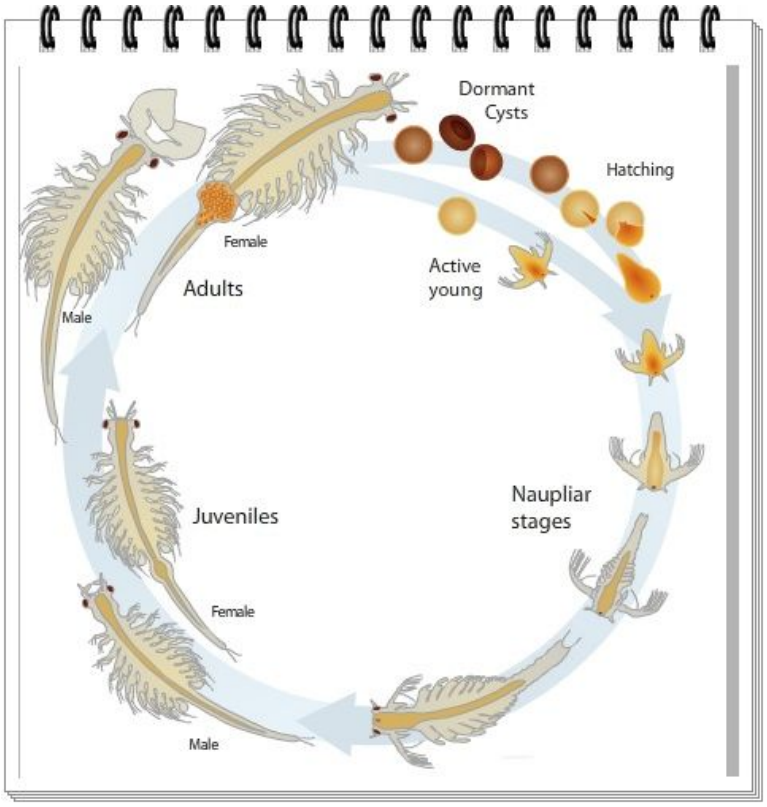
Brine Shrimp

Brine Shrimp (*Artemmia* sp.) are small crustaceans found in various saltwater lakes around the world, such as the Great Salt Lake. They inhabit other hypersaline bodies of water and can tolerate a salinity level of up to 25%. Their development is easy to observe with a microscope. A unique reproductive adaptation makes them an interesting model for studies of natural selection. Under ideal environmental conditions, female brine shrimp produce eggs that hatch quickly into live young; however, when conditions become less conducive, the shrimp instead produce cysts--encased embryos that cease development (enter diapauses) until conditions become favorable. When the temperature or the dissolved oxygen levels become too low or the salinity too high, each egg laid is covered in a hardened, brown chorion, which may keep the embryo viable for many years (in a dry, oxygen-free environment). The brine shrimp used in this activity have been stored in the dormant stage. Once the cysts are incubated in saltwater, the embryos quickly resume their development and hatch.



After the cyst breaks open, the brine shrimp remains attached to the shell, surrounded by a hatching membrane. This stage is known as the umbrella stage. The hatching membrane remains attached to the cyst for a number of hours until the young brine shrimp, known as a nauplius, emerges. During the first larval stage, the nauplius subsists on yolk reserved until it molts. During the second stage, the nauplius begins to feed on algae. The nauplius progresses through approximately 15 molts before reaching adulthood in 2-3 weeks.

Brine shrimp populations are greatly influenced by environmental factors such as salinity. Given the relatively short development time from cysts to nauplius (24-48 hours), the use of brine shrimp in this study is a fast and easy way to observe how some individuals of a population may be better adapted to develop and survive in different environmental conditions.



Materials Needed

5 Petri Dishes	Paintbrush	Salt Solutions of Varying
Double-sided Tape	Sharpie	Salinity (0%-2%)
Stereomicroscope	Brine Shrimp Eggs	Graduated Cylinder

Procedure: Day One

1. Obtain 5 petri dishes. Using the sharpie, label the bottom of each dish with the appropriate concentration of salt solution that will be placed into it.
2. Place a 2 cm piece of double-sided tape in the bottom of each dish.
3. LIGHTLY touch the paintbrush to the side of the container holding the brine shrimp eggs. Your goal is to collect **only approximately 20 eggs** on the brush. **DO NOT** cover the tip of the brush in eggs. Using a greater number of eggs complicates the counting sessions.
4. Carefully transfer the eggs to the first petri dish by dabbing the paintbrush to the tape inside it.
5. Repeat steps 3 and 4 for the remaining petri dishes.
6. Using a stereomicroscope, count the number of eggs in each petri dish. Record the values for each dish in the appropriate location in the data table under “0 hours.”
7. Once all your eggs have been counted and recorded, pour 10 mL of the appropriate saline solution in each dish and cover the dish.
8. Place the filled dishes in an undisturbed location at room temperature for 24 hours. You will need to return the next day to observe the eggs. You or someone in your group should allot about 15-20 minutes for this.

Prediction

Prior to collecting your remaining data, **predict** which saline solution will yield the greatest hatchling viability. Be sure to **justify** your prediction. Write your prediction in the space below.

Procedure: Day Two (24 Hours Later)

1. Using a stereomicroscope, examine each petri dish and count the number of swimming brine shrimp. Record the number of swimming shrimp in the data table for the appropriate time period.
2. Count the number of dead or partially hatched shrimp and record this number in the data table for the appropriate time period.
3. Count the number of unhatched eggs and record this number in the data table for the appropriate time period.
4. Repeat steps 1-3 for each of the petri dishes.

Procedure: Day Three (48 Hours Later)

5. Using a stereomicroscope, examine each petri dish and count the number of swimming brine shrimp. Record the number of swimming shrimp in the data table for the appropriate time period.
6. Count the number of dead or partially hatched shrimp and record this number in the data table for the appropriate time period.
7. Count the number of unhatched eggs and record this number in the data table for the appropriate time period.
8. Repeat steps 1-3 for each of the petri dishes.
9. When you have collected all your data, dispose of the eggs/hatchlings according to your teacher's instructions.

Data: Record the data you collect for this lab on these data tables.

Table 1

		0 Hours	After 24 Hours			After 48 Hours		
Dish	**% NaCl**	**# eggs**	**# eggs**	**# dead or partially hatched**	**# swimming**	**# eggs**	**# dead or partially hatched**	**# swimming**
1	**0**							
2	**0.5**							
3	**1**							
4	**1.5**							
5	**2**							

Analysis

Now you will calculate the hatching viability percentage. To do this, you will perform the following calculation:

Hatching Viability = $\frac{\text{number of brine shrimp swimming at 24 hr + number of brine shrimp swimming at 48 hours}}{\text{Total number of eggs placed in petri dish initially}}$

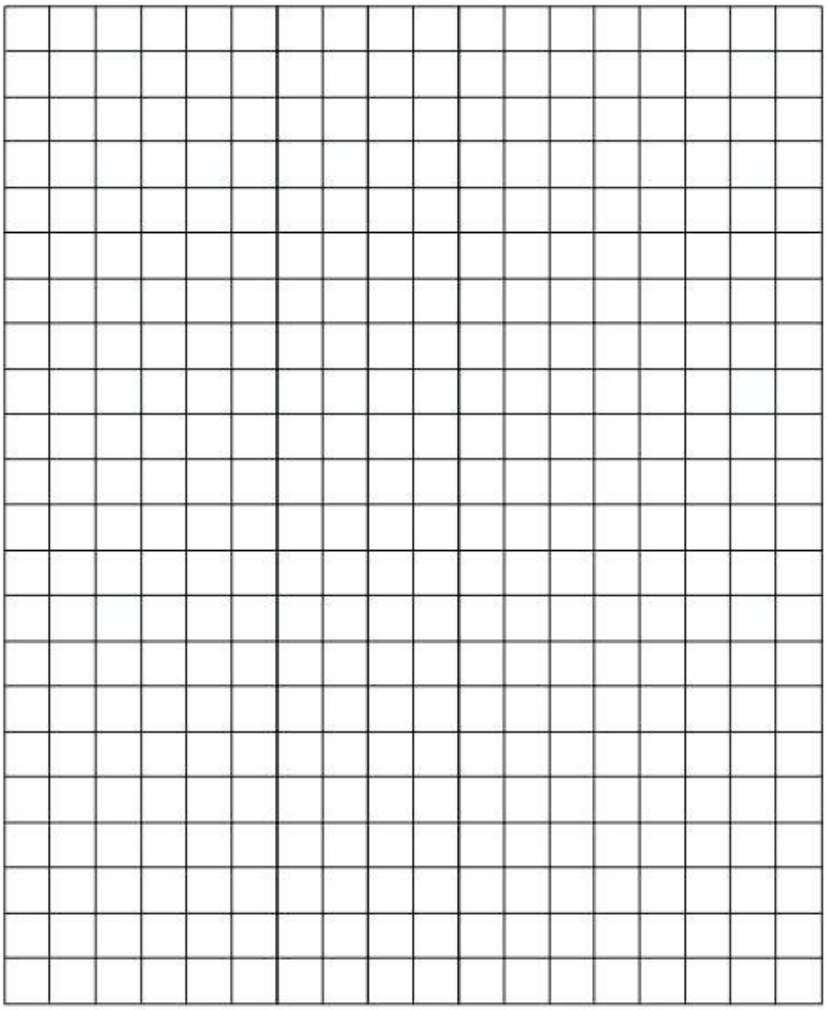
Multiply this by 100 to get a percentage. Enter the values in the table below.

Table 2

Dish	% NaCl	# Eggs	Hatching Viability %
1	**0**		
2	**0.5**		
3	**1**		
4	**1.5**		
5	**2**		

Plot the data from **Table 2**. Title the graph and label the axes after identifying the independent and dependent variables.

- The independent variable is _____.
- The dependent variable is _____.



Conclusion Questions

1. In which petri dish did you observe the highest hatching viability? Did the results support your prediction?

2. Describe two variables that are not controlled in this experimental procedure.

3. If brine shrimp were transported on shorebirds' feet from one hyper-saline lake to a less salty one, what might happen to them? Explain.