The Effect of Ethyl Alcohol on the Hatching Success of *Artemia Salina* –

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INTRODUCTION

Artemia salina was selected as the subject in this experiment because of the overall similarities and characteristics with other crustaceans and the ability to apply the outcomes to other situations involving those related crustaceans. Previous experiments predicted the effect of this basic solution on the hatching success. These organisms were subjected to approximately 168 hours of exposure to a toxic solution known as ethyl alcohol. Different amounts of the toxin were added to a brine solution containing the eggs. Our results did agree with our hypothesis. The ethyl alcohol prevented the majority of the eggs from hatching. The class average data could not be related to the individual group data's due to the varied amounts; however, the trend was duly noted. The main goal of the experiment was to show the negative effect of a particular toxin on the hatching success of brine shrimp eggs.

Brine shrimp are crustaceans that live in extremely saline lakes and ponds, hence the scientific name, *Artemia salina.* These shrimp grow up to 1 centimeter on average and have a life span of approximately 1 year. The different species are given names based upon their habitat. The shrimp are most abundant in areas where salinity is high, predators are few, and there is a high production of algae for consumption. An example of this location would be the Great Salt Lake in Utah. Because these conditions cannot

be the same in every area, questions have been raised regarding other factors that may prevent brine shrimp from proliferating. In the past, these questions have been asked by scientists who have performed experiments to determine the structure of brine shrimp eggs and their ability to withstand certain toxins during the hatching process.

These particular eggs were chosen in this experiment over other organisms because of their culture, short life span, distribution, and availability. Many characteristics involving the structure and functions within brine shrimp make it easy to apply the conclusions and analysis of the experiment to processes in other living crustaceans. The almost microscopic size of the eggs and the small amount of money needed to purchase these organisms make them a hot commodity for researchers. The fact that they are so small makes the experiment more accurate because there are more eggs available for several trials. Due to the desire for the eggs, there are numerous opportunities to purchase them.

Anderson et al. wanted to observe the structure of the eggs during the hatching process. Most of the eggs these scientists looked at were found to be thick-shelled. The outer layer or shell is known as the tertiary envelope. The envelope goes through a series of stages, including being homogeneous, then porous, and once again homogeneous. Several different stains were used to view the various organelles and nutrients in the layers of the eggs, such as chromatin, glycogen, lipids, mitochondria, microtubules, Golgi complexes, and endoplasmic reticulum. The makeup of the shell of brine shrimp eggs is very complicated, due to the fact that it contains shell gland units with many cells within each layer and nucleoli to accompany them. In some cases, the hatching of the eggs depends on the amount of lipoprotein secreted from shell glands. Factors that can affect

the success of these eggs hatching include thickness, density, microstructure, or chemical composition of the different layers in the shell (Anderson et al.). The thickness of the shells, as a result of the many layers, is what we presumed would be the biggest barrier to the alcohol reaching the egg and having a successful hatching process.

Rao et al. wanted to determine the sensitivity of brine shrimp to different insecticides. Chlorpyrifos (CPP), monocrotophos (MCP), profenofos (PF), and acephate (ACEP) were the four organophosphates used to test the reaction of brine shrimp. The brine shrimp eggs were exposed to these toxins and were not given food. The number of survived eggs was determined 24 hours later. Another batch of eggs was soaked for an additional 24 hours and the number of eggs hatched was recorded. The different insecticides affected certain behaviors of the eggs even after hatching, such as speed of swimming. CPP was found to be the most toxic, while ACEP was the least toxic of all the organophosphates. CPP's toxicity indicates that it is highly effective in inhibiting the hatching of brine shrimp. The four organophosphates used in this experiment altered the morphology and locomotor behavior of *Artemia salina*, but did not have an effect on the hatching success of the shrimp (Rao, J. V., et al.). Chlorpyrifos is a weak acid ("Review of Chlorpyrifos"), which also has the ability to increase the velocity of the hatched shrimp and has no effect on the amount hatched, whereas a strong acid, such as ethyl alcohol, will not cause any changes in the hatching success.

Researchers inquired as to how concentrations of cadmium, copper, and zinc in the Great Salt Lake affected the hatching success of brine shrimp: Contrary to previous research, Rao et al. found evidence that brine shrimp were hatching successfully in the Great Salt Lake despite levels of cadmium, copper, and zinc in the water. The researchers

determined that the increased amount of dissolved organic carbon found in the lake had a remarkable protective effect. They also noted that brine shrimp eggs have never been found to be affected when introduced to those specified metals, as proven by some previous experiments. This particular trial was performed using a higher concentration of the metals than was found in the lake. As a result, the scientists concluded that in normal concentrations, none of the metals had a dramatic effect on the brine shrimp. When rearranging the perspective of the experiment to focus on the hatching success of the eggs, it was found that their sensitivity to these concentrations was substantial.

The primary metal used to conduct this experiment was copper (Cu). In the end, the overall success of hatching was relatively high, ranging from 70% to 95%. The antibiotic in which the eggs were soaked prior to exposure to the metals had no effect upon their metal sensitivity. The Cu did have a significant effect on the hatching success of brine shrimp. This was only due to the speciation of the metal (Brix, K. V., et al.). Because ethyl alcohol is very different from the substances discussed above; we hypothesized that in our study, the alcohol would not yield as high a percentage of hatching success as did the metals.

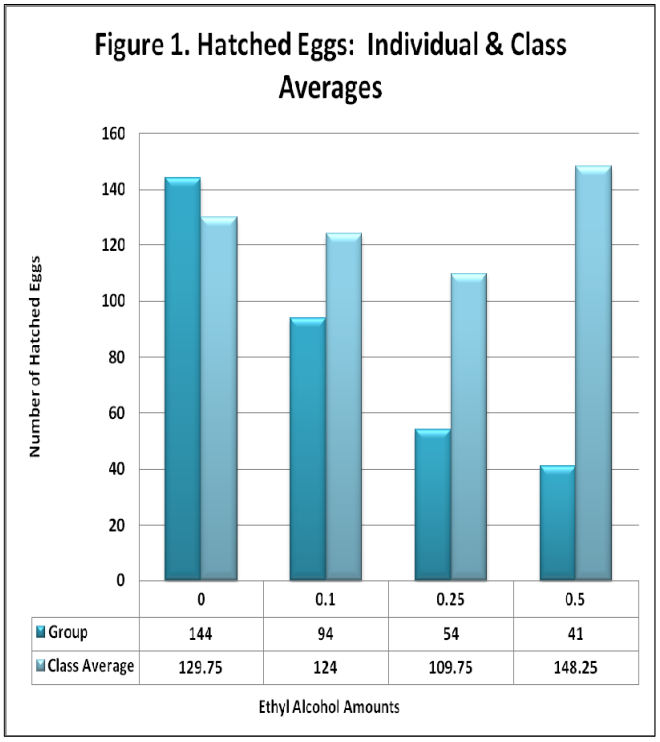
This paper describes our attempt to evaluate the effect of the toxin, ethyl alcohol, on the hatching success of brine shrimp eggs. I believed that the toxin would prevent some of the eggs from hatching. I predicted that the higher the concentration of toxin I used to soak the brine shrimp eggs in, the more difficult it would be for the eggs to hatch.

METHODS AND MATERIALS

The experiment was performed in a lab at room temperature. To begin with, I collected several brown-shelled brine shrimp eggs. Four Petri dishes were numbered one through four and marked so that four quadrants were visible. Each dish was given 10 milliliters of brine solution from a saltwater fish tank using a large pipette. Then a separate amount of 70% concentrated ethyl alcohol solution was added to three of the four dishes using three small pipettes. The dish labeled #1 was given 0.1 mL of the ethyl alcohol and each after that consecutively, 0.25 mL and 0.5 mL. The control dish was the one labeled #4; it contained no ethyl alcohol. The brine shrimp eggs were then added to the dishes using a toothpick. The toothpick was marked at 0.3 cm from the end. It was then wet with the brine shrimp solution and dipped 0.3 cm into the canister containing the brine shrimp eggs. A fresh toothpick was used each time so that the amount of toxin in the Petri dishes wouldn't be modified. The number of eggs was estimated by counting the amount in one quadrant of each individual dish and multiplying by four. Each dish then sat covered for 168 hours. When I returned, I found that some of the eggs hatched and others didn't. A magnifying lens was used to count the number of eggs in the dishes. I then recorded this number in a table. The dependent variable for this experiment was the number of eggs hatched because that was the result I looked for to see if the amount of toxin, the independent variable, would affect the eggs hatched. The control was the brine shrimp eggs because the same type was used in each dish.

RESULTS

I was very satisfied with the results that I got from the experiment. The ethyl alcohol did prove to be a very strong toxin, as displayed by Figures 1 and 2. The alcohol prevented the majority of the eggs from hatching. The more alcohol that was present in a particular Petri dish, the more eggs didn't hatch in that dish. Not even half of the original eggs that were present in the Petri dishes hatched. Even the Petri dish with no alcohol had issues hatching eggs. This shows that other factors had an effect on the hatching success.



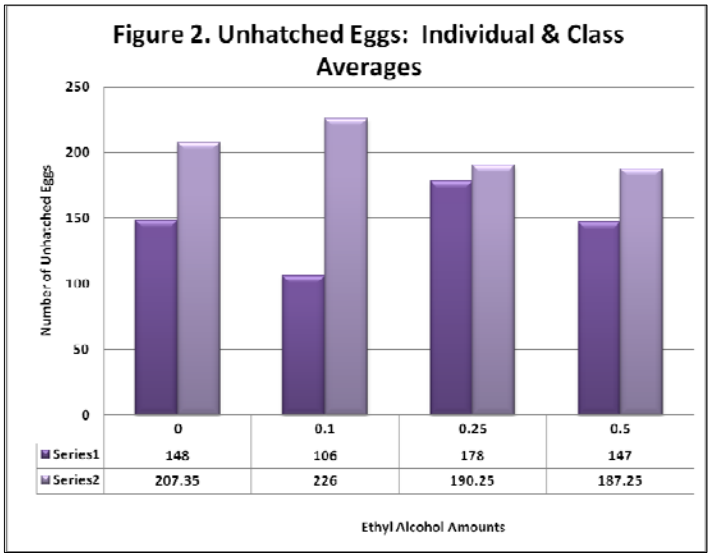
The highest number of eggs hatched was in the category that didn't have any alcohol for individual and in the solution containing 0.5 mL of ethyl alcohol for group averages. The lowest number of eggs hatched was in the Petri dish with 0.5 mL of alcohol for individual groups and in the Petri dish with 0.25 mL of alcohol for group averages. I

Table 1. Ethanol on Hatching Success

	Group Data		Class Average	
Amount of Ethyl Alcohol (mL)	Hatched	Unhatched	Hatched	Unhatched
0	144	148	129.75	207.35
0.1	94	106	124	226
0.25	54	178	109.75	190.25
0.5	41	147	148.25	187.25

expected this to happen — it is common sense that the more toxic solution present, the greater an effect it will have on the organism. The percentage of eggs hatched was very similar for individuals and averages. It was found that 36.5% of the eggs hatched in individual groups and 38.7% of the eggs hatched for group averages. The trend is very close for individual groups and averages. The graph shows that as the ethyl alcohol amounts increased, the number of eggs hatched decreased for individuals. There was no consistency with the number of eggs hatched as displayed by the group average; therefore, the highest and lowest numbers do not match up between group and class averages.

The highest number of eggs unhatched, as seen in Figure 2, was in the category with 0.25 mL of ethyl alcohol for our individual group and in 0.1 mL for the group average. The lowest amount of eggs to remain unhatched was in the solution containing 0.1 mL of alcohol for the individual and 0.5 mL of alcohol for the class average, which is what was expected to happen. These results for total unhatched eggs did not correlate with what I believed would occur because they did not show the opposite of what the hatched egg results showed. Even though the number of eggs hatched follows a specific pattern, the number of unhatched eggs does not because of the different number of eggs that were in each Petri dish. If the number of eggs had been the same in each dish, I



believe the unhatched eggs would have followed a specific pattern just as the hatched eggs did.

The percentage of eggs unhatched was relatively close for individual groups and class averages, 63.5% and 61.3%, respectively. There was no distinct trend that can be displayed by the graphs. Just as with the hatched egg graphs, the highest and lowest numbers do not match up between group and class averages.

I concluded that there must have been some unknown factor affecting the hatching success of the eggs because the trend in amount hatched for the class average data did not decrease with the increasing amount of ethyl alcohol as it did for our individual data. The unhatched values were significantly different in that they did not have any clearly visible trend.

There wasn't a blatant increase or decrease with increasing amounts of alcohol. Values in the different columns of the graph peaked and dropped significantly without following a specific arrangement. From this I can conclude that the unhatched and hatched values did not support one another for individuals or class average. The highest number in one column did not correspond with the highest in the other column; the same was true for the lowest numbers.

DISCUSSION

The hypothesis I stated for this experiment was supported. I first determined that the ethyl alcohol would have a negative effect on the hatching success of brine shrimp eggs, making it difficult for them to hatch. The number of eggs hatched decreased with increasing amounts of ethyl alcohol, mostly because of the alcohol's ability to diffuse across the shell and affect the role of the organelles. The first experiment looked at,

performed by Anderson and others, leads me to believe that the ethanol makes its way through the thick shell of the eggs and prevents the proper development of the brine shrimp fetus. The differences in group data and class average could be due to minor mistakes made in the different trials among groups. For example, group 1 may have had something occur that made the data collected in dish 3 inaccurate, while group 4 had inaccuracies in dish 2. These inaccuracies may have been due to the number of eggs counted, the amount of alcohol added, or contamination by an unknown substance.

The experiment I performed was very similar to the ones discussed earlier. Just as the organophosphate and the three metals in Rao's study did, our study showed that ethyl alcohol at increased concentrations is toxic to *Artemia salina.* Each one of these toxins causes a notable decrease in surviving shrimp when in contact with eggs during the hatching process. It can be concluded that these toxins do not improve hatching success of the eggs and can ultimately prevent the hatching process from occurring if high concentrations are present.

There are many differences between all of these experiments, including where the experiment takes place and the materials used. Brine shrimp species differ depending on the area where they are found; therefore, our brine shrimp were different from the ones used in the other researchers' studies. The location where the brine shrimp were found may have caused them to adapt to certain substances.

In the very beginning of the experiment, I placed a bundle of brine shrimp eggs in a Petri dish and estimated the number in each solution, and that estimation may be the cause of invalid data. Counting specifically, not estimating, the number of live shrimp after the experiment's conclusion may have taken some validity away from the

experiment. How long the ethyl alcohol solution sat before it was used for this experiment may have had an effect on its strength. Some bases do have the possibility of losing their strength when they remain idle for long periods of time. To get different and more precise results, next time the experiment can be repeated in a more contained room that is not subject to the usual particles found in the lab.

Our study, The Effects of Ethyl Alcohol on the Hatching Success of *Artemia salina,* is relevant to the larger scientific community because the results can be applied to other crustaceans and used to prevent their development from being harmed. Because ethyl alcohol has a pH of 7.33, scientists could use this experiment to determine the pH of a solution or medication that could protect development in eggs for humans as well as animals.

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Paris Anderson is a sophomore at Virginia Commonwealth University in Richmond, VA, majoring in criminal justice with a concentration in forensic crime scene investigation. After graduating, she plans to go to graduate school for a master's degree in clinical laboratory sciences, followed by medical school.