# Examining how Roundup affects the growth, development, and survivability of stream biota using snails as an indicator

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## Abstract

Roundup is the most commonly used herbicide on the planet. The key chemical in Roundup is glyphosate which frequently enters waterways through agricultural or residential runoff and overspray. A majority of these glyphosate-based herbicides are not regulated to enter aquatic environments. However, with its increased use, it is entering the environment rapidly. We suspect that glyphosate may have an impact on the rate at which eggs hatch and the survivability of stream biota such *Physa acuta*. Other literature has not focused on the glyphosate effects on the aquatic snail *Physa acuta* as our study does. This study aims to perform and investigate the toxicity of glyphosate on a species of aquatic snail in the city of Lynchburg. In this study, snails will be contained in different ten-fold roundup dilutions to monitor the rate at which collected eggs hatch. This research will be broadly applicable to the effects of glyphosate on other stream biota. Based on the literature, we expect snails to show delayed hatching and abnormalities. These results are anticipated to show the negative effects of herbicide overuse in the aquatic environment. Through our results, we hope to apply our findings to future research on other stream biota and adjust recommended glyphosate use to better serve local ecosystems.

## Introduction and/or Research Question

Glyphosate based herbicides such as roundup are the most used herbicides in the United States (Osteen, 2015). It is used on many crops grown locally such as corn and soy. Genetically modified crops (roundup ready) can be made resistant to roundup so only the weeds are killed. Glyphosate inhibits the creation of aromatic amino acids through the shikimic pathway, thus causing the death of the weeds (Saunders & Pezeshki, 2015). This boosts the product yield and health of the crops (Osteen, 2015). Glyphosate enters the aquatic environment via runoff originating from farms that receive herbicide treatments. Considerable bioaccumulation of glyphosate by aquatic plants has been linked to frequent use of the herbicide in the surrounding environment (Perez, 2017). Roundup use may have a negative effect on stream life. The objective of this study is to examine the effect roundup has on the hatch rate of *Physa* snails. The results will simulate what happens to stream biota when exposed to roundup.

## Methods

*Physa* snails reproduce at rapid rates, making them an ideal species for this study (Miyahira, 2023). *Physa* are abundant in Central Virginia, making this a localized study. We are studying the local snails found in a Liberty University Pond, Lynchburg VA. The *Physa* will be specifically drawn from the Liberty University pond. This pond is thought to be diverse and very close to the laboratory in which this study will be conducted. The Liberty University Pond is surrounded by university buildings and currently is next to a large construction project. The pond was originally built to be used as a retention pond for the University, described as a vegetated pond with wetland edges to help manage stormwater sustainability. The snail *Physa species* is commonly found in this pond year-round. They are being used in this study to serve as an indicator species of other aquatic biota within freshwater environments.

The following methodology will be replicated for a total of 12 beakers, three replicates of each concentration. Students will begin by acquiring adult *Physa* snails from the Liberty University Pond using dip nets and buckets. They are then transported to the lab to be set up in a cycled breeding tank. The breeding tank will include filtered media to keep ammonia levels down as well as 1 algae tablet per week for food. Glass microscope slides are lined at the bottom of the breeding tank for easy collection of egg masses. The breeding tank will be set up in a laboratory classroom near a windowsill with natural light. Breeding continues until a minimum of 12 viable egg masses are laid. Within 24-48 hours of egg masses being laid, they are moved onto a glass microscope slide to be placed in testing beakers. Before submerging the egg masses, a count of eggs is recorded. With one egg mass per slide, they are placed separately in four, 250ml beakers filled with 200ml of breeding tank water and roundup dilutions. Each beaker with a different environmentally relevant dilutions on a ten-fold scale- 0.25 mg/L, 2.5 mg/L, 25mg/L, and 0 mg/L (control). Any time roundup is being handled, proper PPE, gloves and goggles should be worn.

The hatching success of snails will be observed with a stereoscope every two days for a period of two weeks. Notes include hatching success (% hatched) and general observations. Along with feeding, each week the beaker water will be changed with the addition of new stock solution, at the same levels (Tate et al., 1997).

At the end of the study, an ANOVA test will be performed. A correlation and R-value show whether the roundup had a significant effect on the snails. Using an ANOVA test, it will be determined if roundup had a significant impact on the hatching success of young snails. With that, graphs are derived to explain the data (Figure 3 & 4).

At the conclusion of this experiment, using gloves and goggles, materials will be disposed of based on safety protocols of roundup usage. Any snails used in the experiment are to be euthanized.

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**Image 1.** *Visual setup of experimental design* (Source: McLean, Stephanie and Quinon Gresham 09 March 2023).

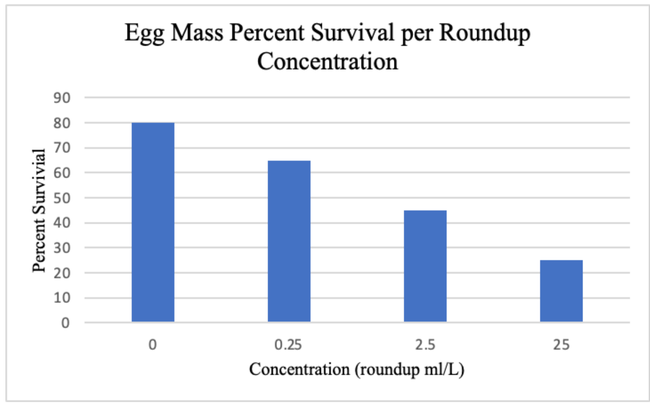


**Figure 1.** A graph (predicted) describing the percent survival of the eggs based on their beaker's roundup concentration

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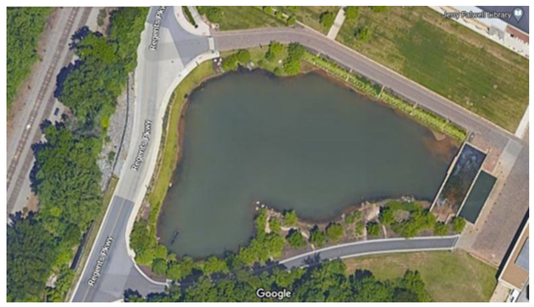


**Image 2.** *Aerial view of Liberty University Library Pond* Map Data ©2023 Google

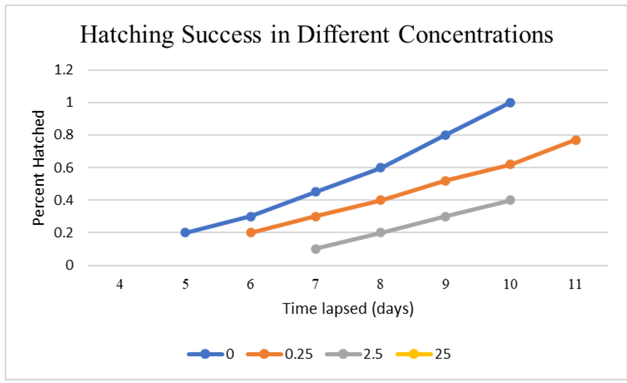


**Figure 2.** A graph displaying hypothetical data on the hatching success between different Roundup concentrations.

## Results and Broader Impacts

**Results**

We expect the eggs to take longer to hatch as the concentration of Glyphosate increases, as well as some eggs not hatching in the highest concentrations (Figure 4). A similar result was found in a study by Tate et al., with the hatching significantly lower in the highest concentration for the third generation (figure 2). It should also be noted that the embryos within this study also experienced various other side effects like deformities. In the test with the highest concentration of Roundup, we expect none of the snails to hatch. Juvenile snails will be more sensitive to higher concentrations of Glyphosate which may cause higher mortality (Prosser et al., 2016).

**Broader Impacts**

The widespread use of glyphosate in Roundup across the globe has had an impact on freshwater ecosystems. This study looks to improve the knowledge base available for those using Roundup on their farms and lawns by showing the relationship between stream biota health, and herbicides. Roundup is immensely useful for agricultural purposes to maximize crop yield and diminish the growth of invasive species that compete for resources. Additionally, using glyphosate-based herbicides can minimize the need to till large lots of farming land before planting. However, it is important to be aware of how it affects non-target organisms when it is released into outside areas (Saunders & Pezeshki, 2015). Understanding how it impacts other organisms can prevent further release and impact to the environment.

Glyphosate's powerful effect has not only been observed in plants but has been linked to various responses in other creatures. Endocrine disruption has been observed in terrestrial snails treated with glyphosate as well as levels of genital dysmorphia (Druart, 2017). Similarly, glyphosate has been shown to produce significant effects on the reproductive, survival, and body morphology of *Daphnia exili* (Rodríguez, 2021). Negative health risks are not reserved only for the smallest of creatures but are present in larger species, including humans. Upon exposure to glyphosate, some men have experienced a decrease in sperm count (Torres, 2022). In addition, glyphosate does not break down easily and can remain in the environment for extensive periods of time making discernment of its use critical (Bonansea, 2018).

## Future Work

Future studies in this area could examine the growth and development of the young snails' post hatch in similar environmentally relevant concentrations of roundup. This would provide greater insight into the unintended effects of roundup on stream biota.

## References and/or Acknowledgments

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