No effect of Roundup runoff on growth *Lymnaea stagnalis* juvenile growth and the LC50 of Roundup for the Chinese Mystery Snail *Cipangopaludina chinensis*

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Abstract

Roundup®, distributed by Monsanto, is an herbicide used worldwide for both agricultural and domestic purposes. It has been recently shown to be lethal to both aquatic and terrestrial amphibians, but aquatic snails, which are of similar size and inhabit similar locales, may also be at risk. In this study, juveniles of the large pond snail *Lymnaea stagnalis* were exposed to Roundup® at runoff concentrations. I hypothesized that higher concentrations of Roundup® would inhibit snail growth. Snails were placed in containers at 5 different Roundup® concentrations. No connection was found between Roundup®'s presence and the growth rate of snails, suggesting even juvenile snails can survive at herbicide concentrations frogs cannot survive.

A separate experiment aimed to delineate an LC50 for snails in general in the Wisconsin Northwoods. It is important to determine at what concentration Roundup® can become lethal to aquatic invertebrates. I used the Chinese mystery snail, *Cipangopaludina chinensis*, for this experiment, placing 10 snails apiece in containers with 8 Roundup® concentrations. Activity of the snails was monitored over the 60hr treatment period, and survivorship was determined at the end of the period. Snails were active only in the two lowest concentrations, 0 and 33mg glyphosate/L. The LC50 was determined to be 491mg glyphosate/L, higher than the maximum expected runoff concentration of 3.6mg glyphosate/L. At applied concentrations, Roundup® is harmless to snails when compared with its

effect on amphibians. It seems only an accidental spillage of Roundup® into stagnant water would pose a threat to snail life, an unlikely event.

Introduction

Roundup®, the second most frequently used herbicide in the United States (Relyea 2005a) has recently been under scrutiny for its effects on aquatic ecosystems. The herbicide is well-known for its use with "Roundup® Ready" crops. In these pairings of Roundup® with crop, the crops have genetically engineered resistance to the herbicide, which is sprayed for weed control. The initial concern with respect to widespread use of Roundup® was the danger to vertebrate mammals. This is no longer a concern, as studies have demonstrated the safety of Roundup® to terrestrial vertebrates (McComb et al. 2008) and invertebrates (Lindsey 2004). Aquatic systems near the fields where Roundup® is used are affected by the chemical runoff from those fields. According to Giesy et al., Roundup® runoff concentrations can only be as high as 3.6 mg glyphosate/L (only a fraction of a drop of Roundup® per L of runoff). At runoff concentrations, Roundup® has been shown to be highly lethal to amphibian species, both in the larval and adult stages (Relyea 2005a, b). Amphibian eggs are susceptible to the herbicide as well. The lowest concentration of a chemical at which the mortality of the population of a specific organism is 50% is called the LC50 for that chemical. The LC50 for Roundup® on the eggs of the frog *Xenopus laevis* is 12.5mg glyphosate/L (Perkins et al. 2000). Roundup® has not been shown to harm all aquatic vertebrates. At both 10x and 100x applied commonly applied dosages, rainbow trout are unaffected by the herbicide (Hidebrand and Sullivan 1982). Few studies have been done, however concerning the herbicide's effect on aquatic invertebrates, specifically snails.

One study (Tate 1997) observed the effects on the reproductive abilities of the snail *Pseudosuccinea columella* in the presence of Roundup®. Abnormalities were observed at different concentrations of Roundup®, including polyembrony and inhibited hatching. In order to build on the knowledge that Roundup® can adversely affect snail eggs but does not affect adult snails, I decided to run an intermediate study on the effects of Roundup® on juvenile snails of the species *Lymnaea stagnalis*, a model mollusc (Vehovszky 2007) native to the Wisconsin Northwoods. I specifically hypothesized that at higher concentrations of Roundup®, snail growth would be diminished when compared with that of control snails. It would also be important to understand at what concentration Roundup® is lethal to snails.

Although there have been plenty of recent experiments concerning the LC50 of amphibians when exposed to Roundup® (Relyea 2005c), there is a lack of LC50 experiments on different snail species. In addition to the experiment involving the juvenile growth rate for *Lymnaea stagnalis*, I performed an LC50 experiment of Roundup® on the invasive Chinese mystery snail, *Cipangopaludina chinensis.* An LC50 experiment from *Cipangopaludina chinensis* would be useful for two reasons:

1. A snail LC50 allows for comparison of an herbicide's effects on snails to other groups of organisms, including the recent LC50's of amphibians.

2. An LC50 of *Cipangopaludina chinensis* most likely gives the highest LC50 of any freshwater snail in the Wisconsin Northwoods area, given its large size and effective operculum (trapdoor which allows the snail to effectively seal out chemicals). In general, a larger organism requires a higher chemical concentration to achieve a similar effect as with a smaller organism (Crowl 2009).

Methods

Initial Experiment: Growth Rate of *Lymnaea stagnalis*

Juvenile snails were collected from a vernal pond located in Ottawa National Forest. All 250 snails used were initially measured and weighed. Shortly following collection, 5 snails were haphazardly allotted to each of fifty 400mL opaque plastic containers. In each container, two maple leaves, 1.5 oz. of vernal pond sludge, and 200mL of vernal pond water were added to each container, with the pond water containing the allotted amount of Roundup® to achieve 5 different concentrations: 0 (control), 1.2, 2.4, 3.6, and 4.8mg glyphosate/L (3.6mg glyphosate/L is the maximum concentration of Roundup® expected to be in runoff).

Mean lengths and weights of the 5 snails in each container were taken at days 4, 9, and 14 after Roundup® was added. Analysis of data was performed using both SYSTAT 12 data analysis program and Excel.

Secondary Experiment: LC50 of *Cipangopaludina chinensis*

Three hundred adult *Cipangopaludina chinensis* were collected from a single 2m x 5m area in Brown Creek on UNDERC-East property. Snails were immediately placed 10 apiece in thirty 2L Tupperware containers. One liter of Roundup®-treated water was added to each container at the following concentrations: 0 (control), 33, 100, 300, 900, 2700, 8100, and 24.300mg glyphosate/L, corresponding to 0, 0.18, 0.55, 1.66, 5, 15, 45, and 135mL of Roundup® per L of treatment water. The lowest concentration (other than the control) was purposely chosen to be approximately 9 times maximum runoff concentration, based upon the results of the initial experiment. Activity of the snails was checked and recorded at 18hr, 36 hr, and 60hr. I considered a snail "active" if its body was visible. After 60hr of treatment, snail mortality was recorded, and a curve was generated with Roundup® concentration along the x-axis and survivorship along the y-axis. Graphed data was then natural log-transformed (x-axis became "ln(Roundup® concentration)," and y-axis became "ln(survivorship)"). This generated a line of data from which the LC50 could be determined.

Results

Results from the initial experiment testing Roundup®'s effect on the growth rate of juvenile snails were largely statistically insignificant. Graphs of both snail length and snail weight over time show no consistent trends relating growth rate to Roundup® concentration (Figures 1 and 2).

In the second experiment involving *Cipangopaludina chinensis*, activity was only seen at the two lowest concentrations, 0 and 33mg glyphosate/L. An average of 5.25 and 2.1 snails respectively were active at any given time over the 60hr observation period at those concentrations. The results of an ANOVA indicate a statistically significant difference between these two means ($F = 13.41, df = 1, p < .002$). Mortality was only observed at the end of the 60hr treatment period. The curve generated from plotting mortality (Figure 3) against Roundup® concentration gives an inversely proportional relationship. Natural log-transforming the data on both axes gave a line with equation (Figure 4):

Ln(Survivorship) = -1.119Ln(mL's of Roundup® in 1L of Treatment) + 5.406

($F = 63.86, df = 1, p = 0.00000003218$)

By setting survivorship at 50%, the concentration of Roundup® was found from this equation to be 2.73mL/ L. This translates to an LC50 of 491mg glyphosate/L after 60hr of treatment.

Discussion

Results from the initial experiment indicate a lack of effect of Roundup® on the growth rate of juveniles of *Lymnaea stagnalis*. This lack of change indicates that Roundup® herbicide is safe for even young snails at concentrations more than 1.3x maximum runoff concentrations. Roundup® should be considered safer for snails in general than amphibians at runoff concentrations. I include adults in this conclusion, as adults of a species are generally hardier than juveniles (Crowl 2009). These results are consistent with one of few previous studies of Roundup® and snails, in which the effect of Roundup® on adult snails was not even mentionable in the paper, let alone specifically studied (Relyea 2005b).

The secondary experiment gives a number that allows for a determination of just how much Roundup® in an aquatic environment is dangerous to snails. An LC50 of 491mg glyphosate/L after 60hr of exposure is high when compared with 12.5mg glyphosate/L for the eggs of *Xenopus laevis* (Perkins et al. 2000) and 3.5mg glyphosate/L on average for five species of frog (Relyea 2005a, b). This gap between what amphibians and snails can tolerate allows me to suggest that an environmental disturbance due to Roundup® would need to be 40x stronger than one that would affect frogs in order to affect snails. I conclude that at all presently applied concentrations, Roundup® is safe for most aquatic snail species.

Perhaps only a spillage of Roundup® into stagnant water would be unsafe for snails.

At 33mg glyphosate/L, snails were actively participating in normal behavior, although this activity was significantly different from the control. The presence of activity and the absence of mortality at that concentration indicate the snails found it tolerable. This change in behavior could over time cause changes in amount of other behaviors, including mating and feeding. This could eventually have an impact on survivorship. Logically, this would require Roundup® at concentrations 10x that of maximum expected runoff concentrations for a period of many generations. Those conditions seem to be even more unlikely than an accidental spillage. The features of the Chinese mystery snail most likely had an effect on the snail's survivorship at different concentrations. *Cipangopaludina chinensis* possesses an operculum that allows it to seal itself from predators and chemicals. This allowed the snails to seal themselves from Roundup® from the start of the experiment. Perhaps snails lacking an operculum would need to be included in a second LC50 experiment to ascertain my conclusion that Roundup® is of little danger to snails at presently applied concentrations.

Acknowledgments

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Figures

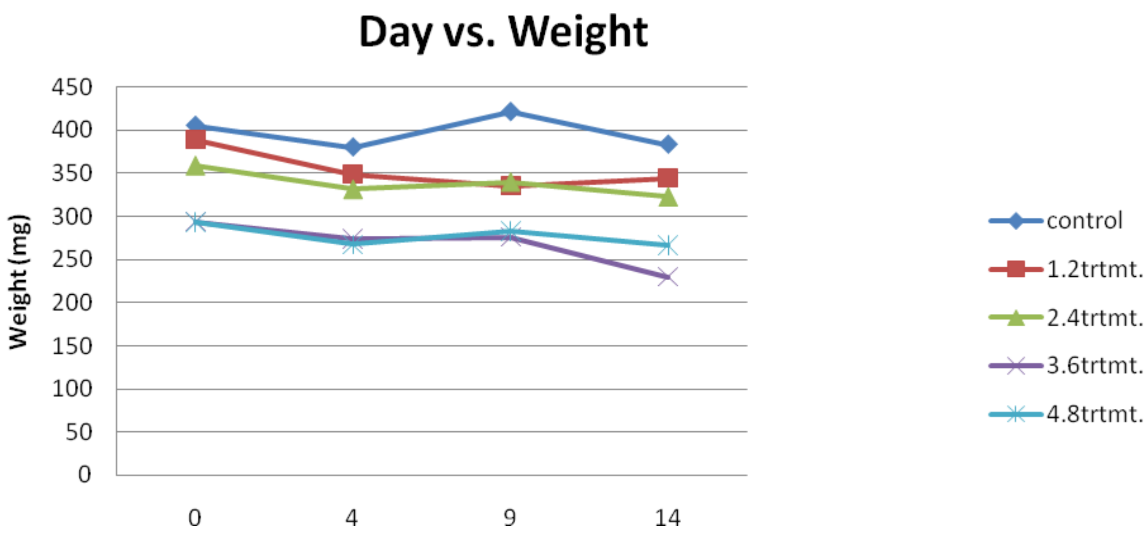

Figure 1: Change in mean weight of snails at each concentration over time. The slopes of the separate lines, which determine the change in weight over time, are not visibly different from each other.

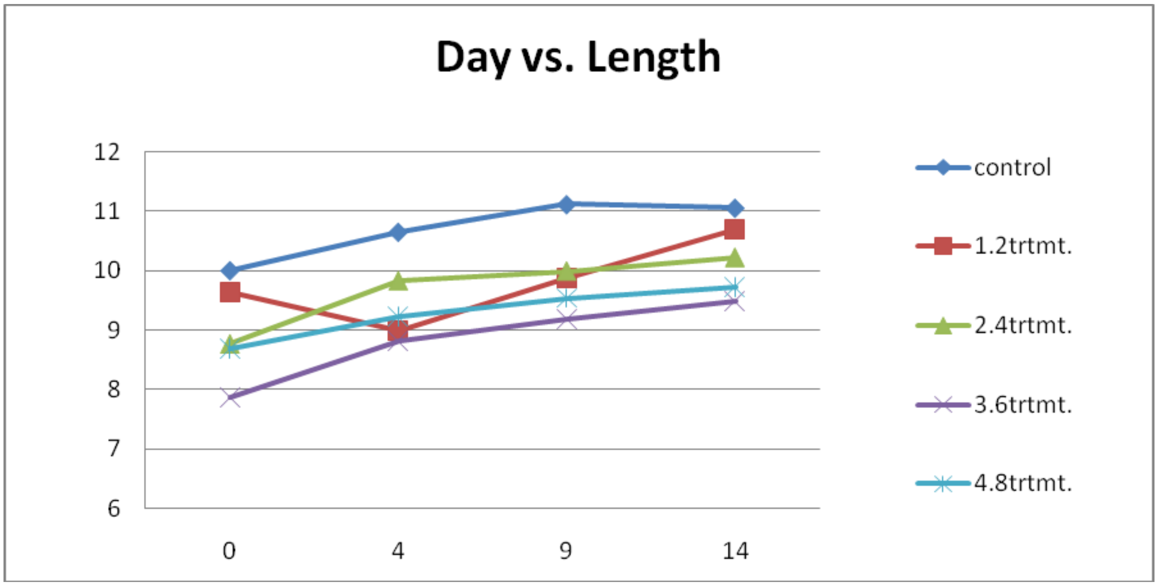

Figure 2: Change in mean length of snails at each concentration over time. The slopes of the separate lines, which determine the change in weight over time, are not visibly different from each other.

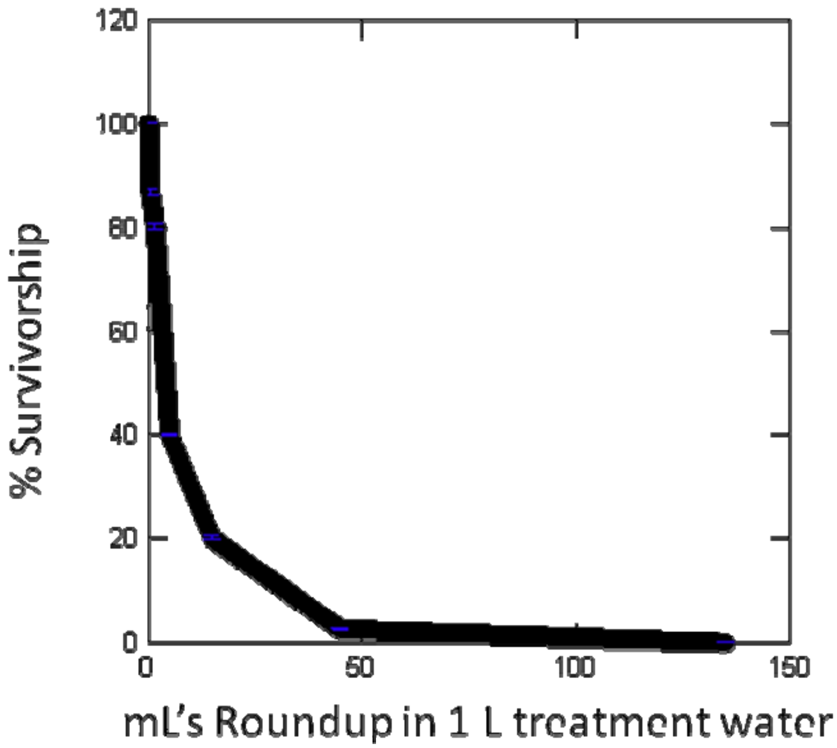

Figure 3: Curve demonstrating the decrease in survivorship as concentration of Roundup® in snail's treatment tanks was increased. Data points shown are means of each treatment concentration.

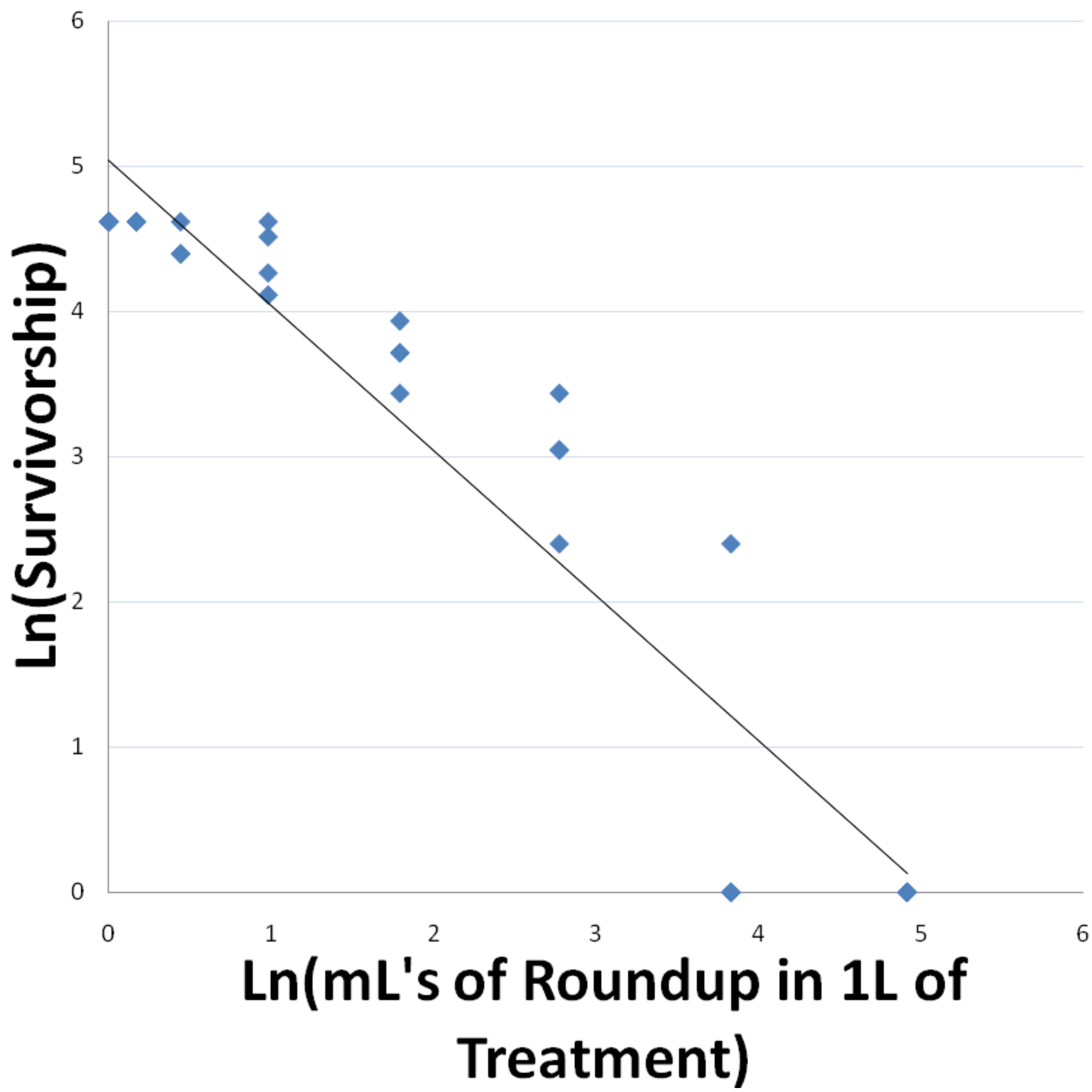

Figure 4: Graph of linearized Survivorship vs. Concentration in *Cipangopaludina chinensis* LC50 experiment. Points follow a least-squares line showing decreasing survivorship with increasing concentration, with equation:

Ln(Survivorship) = -1.119Ln(mL's of Roundup® in 1L of Treatment) + 5.406, R^2 =0.73, p-value: 0.00000003218