Grey treefrog tadpole development and behavior altered by dicamba herbicide exposure

Honors Thesis
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"I affirm that my work upholds the highest standards of honesty and academic integrity at Wittenberg, and that I have neither given nor received any unauthorized assistance."

Abstract

Glyphosate has been shown to impact not only amphibian survival, but also their development, phenotypic response to predators, and overall behavior. Dicamba, another herbicide, was permitted for use by the EPA in 2016. However, less is known about its potential environmental impact. Being an extremely volatile chemical, dicamba poses a risk to aquatic organisms in areas that may experience runoff or overspray. It can also harm yields of neighboring crops, leading the EPA to ban dicamba use part way through this experiment in June 2020.

We investigated the effects of an environmentally relevant dosage of two forms of dicamba on grey treefrog tadpole development and behavior. We also investigated how dicamba impacted the response of tadpoles to predator cues. Herbicide treatment significantly decreased growth compared to the control. The presence of predator cues also significantly decreased growth, and there was no interaction between herbicide treatment and predator treatment. Tadpoles exposed to predator cues generally had wider tails, and the introduction of dicamba caused similar morphological changes. The presence of commercial dicamba also significantly reduced startle responses, potentially increasing the risk for predation. Dicamba has the potential for sublethal impacts on the development and behavior of tadpoles, and therefore should be further studied.

Introduction

Dicamba is a benzoic acid that was permitted for use as an herbicide in agricultural fields by the EPA in November 2016 (EPA, 2020). Dicamba is very volatile in warmer weather, allowing it to drift over long distances (Turner, 2020). Dicamba can spread to neighboring non-dicamba-ready crops and harm yields (Charles, 2020). Because of this, the EPA reversed its decision, banning the use of dicamba part way through this experiment in June 2020 (Charles, 2020). Because dicamba is especially motile, it is also easy to have concentrated runoff in a rain event. This runoff can wind up in ponds, creeks, and other fields. All of this said, the herbicide is likely present in ponds and small pools where many amphibians breed and develop throughout the spring and summer. Dicamba can also persist in the water for at least a couple months. Dicamba has a half life of 72.9 days in aerobic water and 423 days in anaerobic water (Minnesota Department of Agriculture, 2021). It lasts much less time on land, with an aerobic soil half life of just 18 days.

Amphibians are commonly used in studies involving environmental pollutants because of their ability to metamorphose, phenotypic plasticity, and unique physiology (Burggren and Warburton, 2007). Amphibians have the ability to respire across their skin, allowing the uptake of more pollutants (Burggren and Warburton, 2007). They also need to keep their skin moist, leading to more contact with pollutants in the water and on land. Most amphibians also spend a great deal of time in both water and land as they generally live in the water as larvae and move to land as adults. Another interesting factor with amphibians is that they have a complex life cycle involving a larval stage and a metamorphosis into the adult stage. They also have flexibility in how quickly they metamorphose and their size at metamorphosis. These traits are influenced by environmental conditions. Adverse environments where pollutants are present can increase levels of corticosteroid hormones, resulting in quicker metamorphosis at a smaller size (Rollins-Smith, 1998). Temperature can also effect metamorphosis speed. Generally, warmer temperatures cause faster metamorphosis, but extreme cold and extreme hot can halt metamorphosis (Goldstein et al., 2017). Amphibians also pose an interesting study because of their wide abundance and geographic distribution. There are over 5,000 species and they are found on every continent besides Antarctica (Lineback, 2017). Their habitat ranges from wetlands and streams to forests to deserts. Being that they are so widely dispersed, they are a key component of many ecosystems. Their populations have also been rapidly declining over the last few decdades, with an average decline of 3.79% each year (U.S. Geological Survey). Adult amphibians consume mostly insects and small fish but can even eat small mammals and birds (Burke Museum, 2021). They are also an important food source for fish, snakes, birds, turtles, and various mammals. Tadpoles will eat plant matter in the water, plankton, and detritus (BioExplorer, 2019). They are also eaten by a variety of fish, insects, and insect larvae. Because of their wide range, importance within ecosystems, and unique physiology, amphibians are an important animal to study in toxicological investigations.

Grey treefrogs (*Hyla versicolor*) are a common frog species found in much of the Eastern United States and parts of Southern Canada (IUCN, 2015). They are found in forests and wetlands and fall in the IUCN category of least concern (IUCN, 2015). Because of their wide range and high abundance, they can serve as a model species when investigating the effects of environmental pollutants on waterways near agricultural land. Grey tree frogs pose an interesting case study as environmental conditions like the presence of predators and pollutants in the water can affect their metamorphosis. Another interesting thing about this species is that they exhibit phenotypic plasticity. This means that they can change their morphological phenotype based on the environment they are in, particularly in the presence of predators (Van Buskirk and McCollum, 1999). Some typical anti-predator responses are a quicker time to metamorphosis and a change in tail shape (Cauble and Wagner, 2005; Relyea, 2002). Previous studies have investigates the sublethal impacts of other herbicides, such as glyphosate, on amphibians. Glyphosate was found to cause morphological changes similar to the phenotypic plasticity predator response in some amphibians (Relyea, 2012). Glyphosate has also been shown to decrease time to metamorphosis and lead to smaller individuals (Cauble and Wagner, 2004). These sub-lethal effects can compound on each other to make it very difficult for amphibians to persist in their environments.

Dicamba has generally been regarded as more safe than other herbicides since it has a similar mechanism to 2,4-D, imitating the plant growth hormone auxin causing abnormal growth and death of the plant (National Pesticide Information Center, 2012). Glyphosate (another common active ingredient in herbicides) works differently, blocking shikimic acid enzyme pathway to prevent plant growth (National Pesticide Information Center, 2019). Since dicamba has a different mechanism than glyphosate, we were interested to see if some of the same changes in development would be observed in tadpoles treated with dicamba. Prior studies have compared the lethality of both herbicides and found glyphosate to be more toxic and have a lower lethal concentration (Soloneski et al., 2016). The LC50 of dicamba in other amphibians was found to be 358.44 mg/L (Soloneski et al., 2016). Dicamba has an even higher lethal concentration for tropical fish, at 1639 mg/L (Ruiz de Arcaute et al., 2014). While the lethality has been studied before, very few studies have investigated the sublethal effects of dicamba on amphibians. We studied a simulated one-time runoff event that is similar to what grey tree frog tadpoles experience in ponds near agricultural land. We will investigate how the presence of dicamba affects various aspects of tadpole development and behavior. In particular, we recorded growth, tail shape plasticity, the emergence of back legs, and startle responses. We are interested to see the impact that allowing the use of dicamba could have had on amphibian populations and their interactions in the aquatic ecosystem.

Methods

General

We set up tanks containing two liters of aged tap water each in a laboratory. There were 48 tanks and each tank contained five wild caught grey tree frog tadpoles collected from cattle tanks at an agricultural field site in Springfield, OH. Because this is agricultural land, adults were likely exposed to low levels of herbicide previously. The tadpoles ranged in snout vent length from 6.31mm to 11.48mm and were in Gosner Stage 25 when the experiment started. We applied six treatments to equal numbers of tanks, assigned using a randomization software. Each tank received two treatments in this multi-factorial designed experiment. The first factor was herbicide type. This factor describes the type of herbicide being added to the tanks. Herbicide treatment has two levels: pure dicamba and commercial dicamba. The control is no herbicide added to the tank. Dicamba is pure dicamba powder from the manufacturer Sigma that was diluted appropriately in the lab to reflect the concentration of dicamba that could be seen in streams from run off (1mg/L). While there is a lack of data on the concentration of dicamba seen in runoff, previous studies have varied glyphosate from 0.7mg/L to 7.6mg/L to reflect environmental concentrations in experiments involving amphibian tadpoles (Bolis et al., 2020). In a separate study, glyphosate concentrations of 1 mg/L and 2mg/L were used to test for sublethal effects (Burraco and Gomez-Mestre, 2016). So we chose a dicamba concentration toward the lower end of this spectrum to ensure sub-lethal effects. Both dicamba treatments were added to the tanks a single time in the beginning of the experiment to simulate a one-time run off event. Commercial dicamba is a commercially available Roundup formulation with 0.43% dicamba as the active ingredient and numerous other inactive ingredients. No glyphosate was present in this formulation of Roundup. 1 µg/L was added to each tank to reflect the percentage of active ingredient dicamba in the Roundup. We investigated the effects of the commercial dicamba version to see if the inert ingredients have any adverse effects on tadpoles as well.

The second factor was the presence of predator cues. Predator cues were included in our experiment to test the phenotypic plasticity response to predator cues and see if the different types of herbicides affected their ability to respond. Predator cues were collected from water from one of two tanks containing a single dragonfly larvae (*Anax junius*) that was recently fed tadpoles. This should contain chemical cues to elicit a fearful response. The control is aged tap water. We added equal amounts (300mL) of control and predator water to the tanks weekly so that the predator cues remained in the water.

Tadpoles were maintained by feeding 0.1g of rabbit food twice a week. The food was left in the tank for approximately 24 hours and suctioned out. 500mL of water was also replaced twice a week to represent a non-static environment and maintain water quality.

Variables Measured

The main measurement used to track growth was snout-vent length. This is the distance from the tip of the nose to the base of the tail, measured using calipers in millimeters. Snout-vent length was measured at the beginning of the experiment, towards the middle of the experiment (19 days post-exposure), and at the end of the experiment (29 days post-exposure). We then calculated the mean snout-vent length change for each tank. The percent change between each of these measurements was calculated per tank and used in analysis. We used the percent change in growth because the tadpoles varied in size at the start of the experiment.

We also measured the width at the widest part of the tail and the length of the tail using calipers at the end of the experiment. We then calculated a tail proportion by dividing the width by the length of the tail. A change to the tail structure is one of the phenotypic plasticity traits that tadpoles can vary depending on stressors in their environment.

We also recorded the number of tadpoles with emerged back legs in each tank at the end of the experiment. The presence of legs serves as a marker of how quickly the tadpoles are developing. We also calculated the proportion of tadpoles with legs in each tank since different tanks had different numbers of tadpoles by the end of the experiment.

We recorded startle data as a way to track the behavioral impacts of dicamba-based herbicides on grey tree frogs. We tapped the tanks twice on the side with a turkey baster and recorded the number of tadpoles that moved. This number was converted to a proportion as every tank did not have the same number of tadpoles as the experiment progressed and some tadpoles perished. We collected initial startle data before the treatments were administered and 5 more times over the course of the experiment.

Although we were concerned with sub-lethal effects in this experiment, there were mortalities and we tracked this too. The number of survivors in each tank was counted twice – midway at 19 days post-exposure, and at the end of the experiment 29 days post-exposure.

Statistical Analysis

We ran various analyses to determine the effects of dicamba on different aspects of grey tree frog development. All stats were carried out in R (Version 1.1.463 – © 2009-2018 RStudio, Inc.). We ran ANOVAs on each of the variables mentioned above using the type of herbicide, predator cue presence, and their interaction as the model terms. We used an $\alpha = 0.05$ threshold for significance. If an explanatory variable was not significant, it was removed from the model and run again. We also created Tukey intervals when an explanatory variable was significant in the ANOVA to test for differences between levels of the explanatory variable. We ran a repeated measures analysis on the startle data using trial number as a factor in the ANOVA and found no significance, suggesting that the startle response doesn't change over time.

Results

Growth

Tadpoles from the control treatment grew 19.6% over the entire experiment, and a growth rate of 17.7% in just the second half of the experiment. With most of the control growth happening in the second half of the experiment, this will give us the best picture of growth, and is the measurement we will focus on here.

Dicamba-based herbicides significantly reduced growth ($F= 3.76$, $p = 0.037$). In the second half of the experiment, pure dicamba treated tanks had 6.3% growth, and commercial dicamba had 5.0% growth. Predator cues also significantly reduced growth ($F = 6.64$, $p = 0.016$). Predator cue treated tanks had a percent growth of 7.08% whereas the control treated tanks had a percent growth of 22.3%. There was also no interaction between the herbicide treatment and predator cues ($F = 0.14$, $p = 0.72$) (Figure 1).

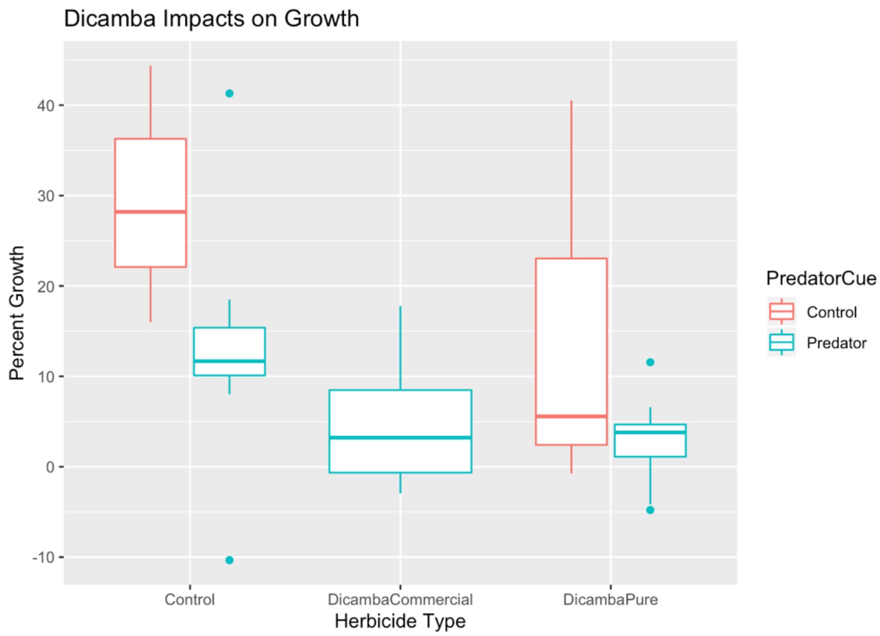


Figure 1. Dicamba herbicides and predator cues both decrease percent growth in tadpoles. Predator cue treated tanks had less percent growth on average than the control for tanks treated with pure dicamba and tanks that did not receive an herbicide. We are unable to compare the effects of predator cues in tanks treated with commercial dicamba, as there are no data points for tanks receiving commercial dicamba and no predator cues. There was more variation within control treated tanks. The outliers are: Tank 12 (predator cues and no herbicides) percent growth = 41.3%, Tank 33 (predator cues and no herbicides) percent growth = -10.3, Tank 39 (predator cues and pure dicamba) percent growth = - 4.8, and Tank 46 (predator cues and pure dicamba) percent growth = 11.6.

Survival

Survival was measured by counting the number of tadpoles alive per tank at the middle and end of the experiment. Around the middle of the experiment, there was a large die off that killed all tadpoles in each of the tanks on the right side of the room receiving control water, 11 tanks in total. This coincided with a test of a fume hood that occurred in the lab that morning. All the tanks on the right side of the room were closest to the fume hood. It is possible that the control water for that side of the room became contaminated, but we cannot know for certain. We excluded all tanks on the right side of the room and found no significant impact of herbicide or predator presence, on midway survival (F = 0.205, p = 0.817; F = 0.078, p = 0.783). Additionally, there was no interaction between these terms (F = 0.052, p = 0.949) on midway survival. There were unexpected results when looking at the final number of survivors excluding tanks on the right side of the room. Herbicide type was significant (F = 10.171, p = 0.001), with pure dicamba treated tanks having an average of 2.75 survivors per tank, commercial dicamba having 2.43 survivors per tank, and control having 0.89 survivors per tank. Predator cues were also significant (F = 48.001, p = 0.000), with predator cue treated tanks having an average of 3.55 survivors per tank and control tanks having just 0.62 survivors per tank. The interaction was insignificant (F = 0.855, p = 0.44183). This significance could be misleading due to the die off event and be a reminant of main effects.

Plasticity of Tail Shape

After removing outliers that were marked on the boxplots, the herbicide treatment had no significant effect (F = 0.426, p = 0.6587). However, both the predator cue (F = 4.206, p = 0.054) and the interaction of the two variables (F = 5.317, p = 0.032) had significant explainatory power. Tadpoles treated with predator cues had less streamlined tails (Tail Proportion = 0.34) than the control (Tail Proportion = 0.32) (Figure 2). The presence of dicamba reduces the difference in tail proportion between predator and control water, inducing the effect of less streamlined tails (Figure 3).

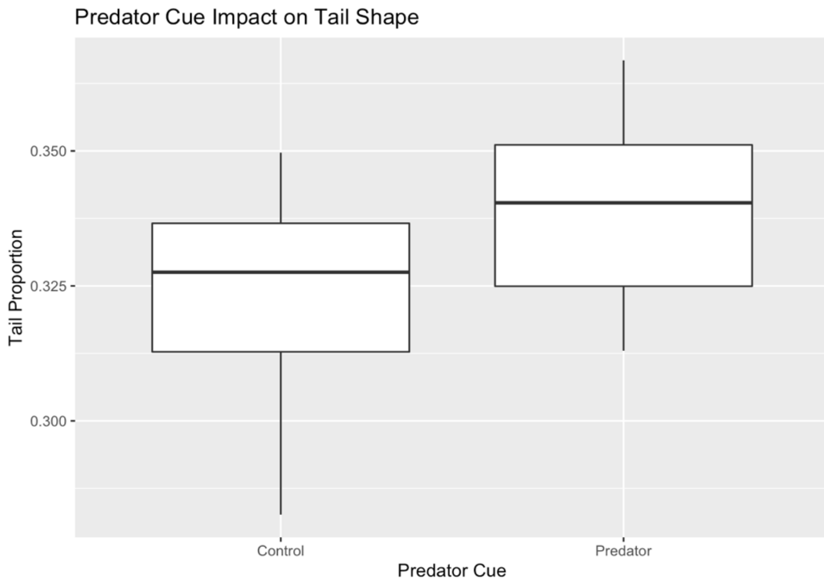


Figure 2. Predator cues induce less streamlined tails. Predator treated tanks have a mean tail proportion of 0.34. Control treated tanks have a mean tail proportion of 0.32. There was more variation in the control tanks.

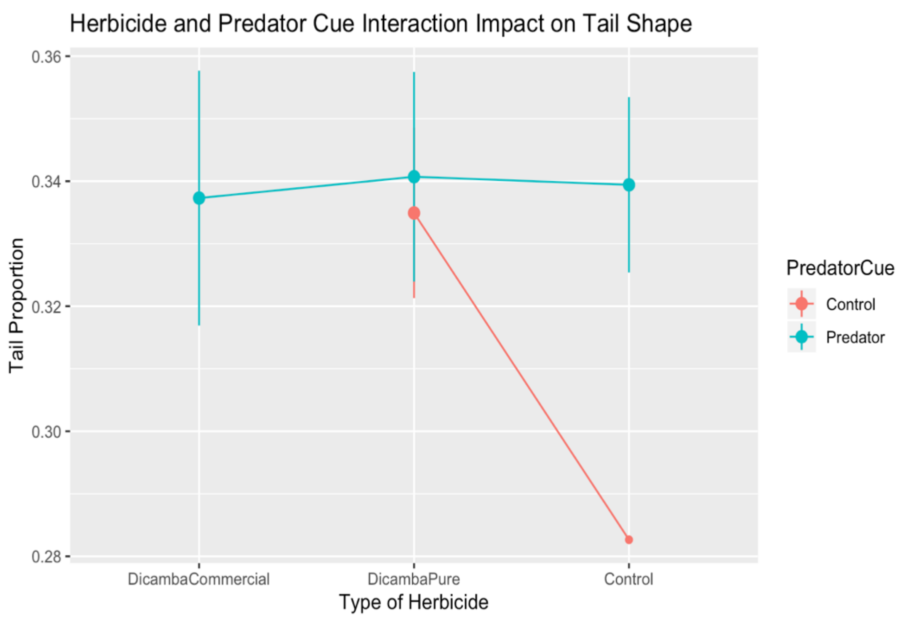


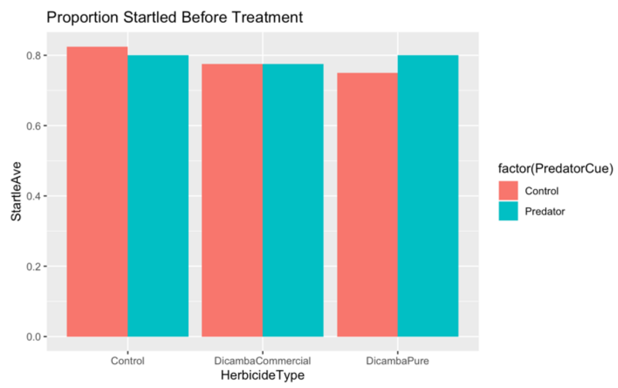
Figure 3. Dicamba induces less stream-lined tails, as seen with predator cues. Predator treated tanks have much wider tails than tanks not receiving predator cues when examining tanks that did not receive a dicamba herbicide. Tanks receiving pure dicamba still had predator treated tanks having wider tails, but the difference between predator treated tanks and control treated tanks was greatly reduced.

Leg Emergence

There was no significant difference in herbicide (F = 0.120, p = 0.887), predator cue (F = 0.002, p = 0.963), or their interaction (F = 2.394, p = 0.134) in explaining the proportion of tadpoles that developed legs in each tank. There was also was no significance in herbicide (F = 0.076, p = 0.927), predator cue (F = 1.419, p = 0.245), or their interaction (F = 0.355, p = 0.557) in explaining the total number of tadpoles that developed legs. There was a trend with the predator treatment, 11 of the tadpoles in predator treated tanks developed legs compared to 1 tadpole in a control tank that developed legs. This tank that did not receive predator cues was also treated with dicamba.

Startle Response

We ran an initial startle trial before treatments were administered and then five trials afterward. The herbicide treatment was significant only in the first trial after the treatments were administerd (F = 3.027, p = 0.0591). There was no significance in trial number (F = 0.412, p = 0.8001).Despite having no significance in trial number, the fact that herbicides are significant only in the first trial suggests that tadpoles recover from the effects of the herbicide in regards to their startle response. Pure dicamba and the control had the same average proportion startles (0.925). The startle response in tanks treated with commercial dicamba was decreased (0.825)(Figure 4). Predator cues (0.93) also significantly increased startle responses in trial 4 as compared to the control (0.73).



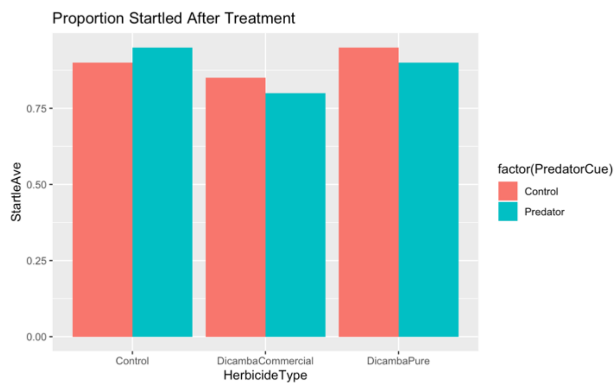


Figure 4. Commercial dicamba reduces startle response of tadpoles to a stimulus in first trial after treatment. Commercial dicamba has an average proportion startled of 0.825. Pure dicamba and the control each have an average proportion startled of 0.925.

Discussion

Since dicamba has been used as an agricultural herbicide, it is important to know the potential impacts of runoff in aquatic ecosystems. We aimed to investigate the impacts of dicamba on grey treefrog development and behavior and found several effects. Pure dicamba and commercial dicamba significantly reduced growth, altered the startle response, and made tails less streamlined. There was no significant impact of dicamba based herbicides on development, and we are inconclusive as to whether dicamba impacts survival.

Dicamba-based herbicides significantly reduced growth over the course of the experiment. There was also less variation in growth compared to the control. This suggests that tadpoles not exposed to herbicides had more flexibility to dedicate more or less energy to growth whereas tadpoles exposed to the herbicides had to dedicate their energy elsewhere. While not statistically significant, tadpoles treated with commercial dicamba had less growth than tadpoles treated with pure dicamba. This suggests that the inert ingredients in the commercial dicamba may be having an affect, and should be further studied. Slowed or reduced growth poses a potential problem for tadpoles because size at metamorphosis has the potential to impact their long-term survival. Smaller tadpoles are more susceptible to predators and have decreased abilitity to evade predators (Cabrera-Guzman et al., 2013). Cabrera-Buzman (2013) found that Cane Toads (*Rhinella marina*) that are larger at metamorphosis have increased fitness and a lower mortality rate compared to frogs that are smaller at metamorphosis. This could be due to an increased ability to find and consume resources, fewer predators, and improved locomotion. Roundup with glyphosate active ingredient has also been found to impair tadpole growth at 7.6 mg/L (Bolis et al., 2020). It is important to note that the concentration of dicamba used in this study was much lower, suggesting that dicamba could have a larger impact on growth than glyphosate.

Dicamba-based herbicides also significantly affected phenotypic plasticity responses in tadpoles, causing less streamlined tails. We also observed less streamlined tails in tanks treated with predator cues. This suggests that dicamba is inducing phenotypically plastic anti-predator responses. The shape of the tail can be an important factor in the ability of tadpoles to escape predators (Relyea, 2002). Deeper tails have been associated with tadpoles exposed to predator cues, and likely make them more able to escape a predator (Relyea, 2002). Glyphosate has also been found to induce morphological changes similar to that of an anti-predator response (Relyea, 2012).

We found no significant impact of dicamba-based herbicides on either the proportion per tank or the total number of tadpoles that had legs at the end of the experiment. The eruption of legs can act as an indicator of metamorphosis or development speed and is indicative of environmental conditions. While neither the herbicide nor predator treatment was a significant predictor of leg emergence, we did see most of the tadpoles with legs occurring in predator-cue treated tanks. The one case where there was a tadpole with legs in a tank not treated with predator cues was a tank that was treated with dicamba. Our experiment showed an overall slower time to metamorphosis than we would have predicted. This could be due to colder temperatures in the lab compared to what they would experienced in the field. Cooler temperatures have been demonstrated to decrease development speed (Goldstein et al., 2017). This trend should be further studied, as a quicker metamorphosis could be due to the tadpoles increasing development speed to exit a stressful environment quicker (Cauble and Wagner, 2005). If dicamba does cause a quicker time to metamorphose, this would be further evidence that the presence of dicamba is a stressor for them. Glyphosate can decrease time to metamorphose, with the tadpoles being smaller sized (Cauble and Wagner, 2005). Metamorphosizing at a smaller size can cause its own issues, such as an increased predation risk when they exit the water (Cauble and Wagner, 2005).

When investigating the behavioral impacts of dicamba, we found that commercial dicamba significantly reduces startle responses, but only in the first trial after treatment. Since this occurs only in the commercial dicamba, this suggests that the inert ingredients are causing the effect. Inert ingredients have been shown to be just as toxic as glyphosate (Mann and Bidwell, 1999). More research ought to be done on the effects of inert ingredients in this commercial dicamba product since we are seeing this trend. The fact that this occurs only in the first trial suggests that the effects wear off over time. The decrease of startle response poses an issue as it may leave tadpoles more susceptible to predation.

While our experiment was designed to track the sub-lethal impacts of dicamba, lethality is another important factor to consider. We used a sublethal dose of dicamba and did not expect any differences in mortality. There were no deaths immediately after exposure (within two days) for any of the treatments. The first tadpole death was 4 days post-exposure in a pure dicamba treated tank. While we tracked the number of survivors at the middle and end of the experiment, an unexpected die-off event makes it impossible to draw conclusions on the lethality of dicamba. This die-off only happened on the right side of the room and disproportionately effected tanks that did not receive predator cues. When removing the side of the room where the die-off occurred from the data, neither dicamba treatment or predator cue treatment had a significant effect on survivors at the time of the die off. However, both of these factors were significant in determining the number of survivors at the end of the experiment. Those treated with predator cues had higher survival and those treated with dicamba also had higher survival, although there was no interaction between these two. The increased survival in tanks treated with predator cues may be due to tadpoles being scared and going to the bottom of the tank where herbicide concentration is lower (Relyea, 2012). However, there is no good biological explanation for increased survival in tanks treated with herbicides. Again, we are unable to draw conclusions on survival in this experiment. Other studies have examined the lethal impacts of various herbicides on amphibians. Glyphosate based herbicides have been found to induce a high mortality rate at environmentally relevant concentrations (Relyea, 2005). Dicamba has been regarded as a less lethal herbicide with a LC_{50}= 358.44 mg/L compared to an LC_{50}=78.18 mg/L for glyphosate (Soloneski et al., 2016).

We saw decreased growth, decreased startle response, and a change in tail shape associated with dicamba treatment. We also saw tadpoles erupting back legs sooner in tanks treated with predator cues. Each one of these sublethal effects has the potential to cause stress on the tadpoles and be energetically costly. The presence of herbicides causes tadpoles to devote energy toward avoidance mechanisms instead of using that energy for growth and to use in finding food. Stress increases levels of corticosteroid, the hormone that is also involved in metamorphosis (Rollins-Smith, 1998). So increased stress may cause quicker metamorphosis. Increased stress levels can also decrease immunity and lead to an increased risk of disease in amphibians (Rollins-Smith, 1998). The increased stress along with a reduction in growth and abnormal behavior has the potential to decrease fitness of amphibians. They are at more risk of predation, disease, and cannot forage for food as effectively. This is not something we want to see in populations that are already in decline. The sublethal effects on amphibians are also an indicator of broader ecosystem health. If we lose amphibian populations, we are losing a key component of many ecosystems since so many species rely on them as a food source. Because of their unique physiology, they can also serve as a general indicator of how these herbicides effect aquatic ecosystems and the animals within them. If we are seeing sublethal effects in tadpoles, it is likely that there are sublethal effects in other species as well. These effects can compound and change the entire ecosystem and how the species in it interact.

Dicamba has the potential for various sub-lethal effects on grey treefrog tadpoles. In this study, we tracked the effects relating to development, behavior, phenotypic plasticity response, and response to predator cues. We have found dicamba to reduce growth, decrease startle responses, and induce a phenotypically plastic response in tail shape similar to an anti-predator response. Knowing these impacts is just the start of understanding potential impacts of dicamba on grey treefrogs. We need to continue researching the effects of dicamba on amphibians and aquatic ecosystems. I recommend a follow-up study similar to this one with a larger sample size and where conditions are kept constant and at an ideal temperature to maximize growth. Multiple concentrations of dicamba should also be investigated. There should also be more research on the range of dicamba concentrations observed in waterways after a runoff event. While this study focuses on the sub-lethal effects, the lethal effects of dicamba should be further studied . Further studies should also compare the lethal and sublethal effects of dicamba to other herbicides. And finally, we should expand the conversation beyond amphibians and investigate other facets of aquatic ecosystem health. With the recent ban on dicamba, it is important to determine which herbicides have the least impact on aquatic ecosystems.

Work Cited

BioExplorer. 2019. Tadpoles Diet. Retrieved April 07, 2021, from https://www.bioexplorer.net/what-do-tadpoles-eat.html/

Burke Museum. 2021. All about amphibians. Retrieved March 30, 2021, from https://www.burkemuseum.org/collections-and-research/biology/herpetology/all-about-amphibians/all-about-amphibians

Burke Museum. 2021. What's endangering amphibians? Retrieved March 30, 2021, from https://www.burkemuseum.org/collections-and-research/biology/herpetology/all-about-amphibians/whats-endangering-amphibians

Battaglin, W. (n.d.). Glyphosate herbicide found in Many midwestern Streams, antibiotics not common. Retrieved March 30, 2021, from https://toxics.usgs.gov/highlights/glyphosate02.html

Bolis A, Gazzola A, Pellitteri-Rosa D, Colombo A, Bonfanti P, Bellati A. Exposure during embryonic development to Roundup® Power 2.0 affects lateralization, level of activity and growth, but not defensive behaviour of marsh frog tadpoles. Environmental Pollution. 2020;263(Part B). doi:10.1016/j.envpol.2020.114395

Burraco P, Gomez-Mestre I. Physiological Stress Responses in Amphibian Larvae to Multiple Stressors Reveal Marked Anthropogenic Effects even below Lethal Levels. Physiol Biochem Zool. 2016 Nov/Dec;89(6):462-472.

Cabrera-Guzmán E, Crossland MR, Brown GP, Shine R. Larger body size at metamorphosis enhances survival, growth and performance of young cane toads (Rhinella marina). PLoS One. 2013;8(7):e70121. Published 2013 Jul 29. doi:10.1371/journal.pone.0070121

Cauble K, Wagner RS. Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. Bulletin of environmental contamination and toxicology. 2005;75(3):429-435. https://search.ebscohost.com/login.aspx?direct=true&db=mnh&AN=16385946&site=eds-live. Accessed April 19, 2020.

Charles, D. (2020, June 09). Court ruling on popular weedkiller dicamba upends midwestern agriculture. Retrieved February 07, 2021, from https://www.npr.org/2020/06/09/872710977/court-ruling-on-popular-weedkiller-dicamba-upends-midwestern-agriculture

EPA registers dicamba formulation for use On DICAMBA Tolerant crops. (2020, October 20). Retrieved February 07, 2021, from https://www.epa.gov/pesticides/epa-registers-dicamba-formulation-use-dicamba-tolerant-crops

Goldstein JA, Hoff KVS, Hillyard SD. 2017. The effect of temperature on development and behaviour of relict leopard frog tadpoles. Conservation Physiology. 5(1)

IUCN SSC Amphibian Specialist Group (IUCN SSC). (2015, August 25). The IUCN Red list of threatened species.

Lineback, N. (2017, December 14). Geography in the news: Tragic deaths of amphibians. Retrieved March 30, 2021, from https://blog.nationalgeographic.org/2014/04/06/geography-in-the-news-tragic-deaths-of-amphibians/

Mann R, Bidwell J. 1999.The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australia frogs. Archives of Environmental Contamination & Toxicology. 36(2):193-199.

Minnesota Department of Agriculture. 2021. Dicamba - general information. Retrieved April 07, 2021, from https://www.mda.state.mn.us/dicamba-general-information

National Pesticide Information Center. 2012. Dicamba. Retrieved March 30, 2021, from http://npic.orst.edu/factsheets/dicamba_gen.html

National Pesticide Information Center. 2019. Glyphosate. Retrieved March 30, 2021, from http://npic.orst.edu/factsheets/glyphogen.html

Relyea Rick A. New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. Ecological Applications. 2012;22(2):634-647. Accessed February 24, 2021. https://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.41416788&site=eds-live

Relyea RA. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. Ecological monographs. 2002;72(1):77-93. doi:10.1890/0012-9615(2002)072%5B0077:LPDIPP%5D2.0.CO;2

Rick A. Relyea. The Lethal Impact of Roundup on Aquatic and Terrestrial Amphibians. Ecological Applications. 2005;15(4):1118-1124. Accessed February 24, 2021. https://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.4543422&site=eds-live

Rollins-Smith LA. Metamorphosis and the amphibian immune system. Immunol Rev. 1998 Dec;166:221-30. doi: 10.1111/j.1600-065x.1998.tb01265.x. PMID: 9914915.

Ruiz de Arcaute C, Soloneski S, Larramendy ML. Evaluation of the genotoxicity of a herbicide formulation containing 3,6-dichloro-2-metoxybenzoic acid (dicamba) in circulating blood cells of the tropical fish Cnesterodon decemmaculatus. Mutat Res Genet Toxicol Environ Mutagen. 2014 Oct;773:1-8.

Soloneski, S., Ruiz de Arcaute, C. & Larramendy, M.L. Genotoxic effect of a binary mixture of dicamba- and glyphosate-based commercial herbicide formulations on Rhinella arenarum (Hensel, 1867) (Anura, Bufonidae) late-stage larvae. Environ Sci Pollut Res 23, 17811–17821 (2016). https://doi.org/10.1007/s11356-016-6992-7

U.S. Geological Survey. (n.d.). Why are frog and toad populations declining? Retrieved April 07, 2021, from https://www.usgs.gov/faqs/why-are-frog-and-toad-populations-declining?qt-news_science_products=0#qt-news_science_products

Warren W. Burggren, Ph.D., Stephen Warburton, Ph.D., Amphibians as Animal Models for Laboratory Research in Physiology, ILAR Journal, Volume 48, Issue 3, 2007, Pages 260–269