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The Effect of Soil pH on the Integument of the Western Tiger Salamander (Ambystoma mavortium)

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THE EFFECT OF SOIL PH ON THE INTEGUMENT OF THE WESTERN TIGER SALAMANDER (*Ambystoma mavortium)*

By

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This study was done in an effort to better understand the multifaceted issues in amphibian decline, specifically within the eastern regions of Nebraska, to determine whether or not human-induced acid rain contributed to the decline. This is a heavily important topic of research as amphibians worldwide are declining related to many issues that stem from climate change and several other environmental issues of that nature. This study looked to use acidic pH within soil from western Nebraska to determine the reactionary impacts (primarily lesions) the western tiger salamander had after being subjected to it. It was found that pH did cause irritation in the skin among the test groups of the salamanders, but not lesioning. Over three months, the salamanders in the test groups observed had reddening of the skin occur on their ventral side and around their feet and toes. What was at the core of the cause of this reddening of skin was not known, and further studies will have to make sure that pH change does not trigger much more detrimental issues within salamander and amphibian decline, such as the deadly ranavirus or chytrid.

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Introduction

Western Tiger salamanders and amphibians in general are often less than appreciated by modern society today. Many adjectives have been used to describe these organisms, such as slimy, gross, ugly, and even poisonous. Though some amphibians are poisonous, others are quite harmless, and all contribute greatly to their habitat. But soon, their contribution may be gone. Throughout the world, amphibian populations are declining. This is only the results of our own doing. Much of our industrialization and growth has led to an increase in temperatures worldwide by way of climate change, and other related things of that nature. Anthropogenic climate change is causing drastic implications for many species all over the world. Among amphibian populations, the effect is more extreme than in some other organisms. Within Nebraska this is no different, and the western tiger salamander is a species that is proving that statement to be true. Populations of western tiger salamanders have begun declining in the eastern portion of Nebraska, but oddly enough, they are doing much better on the western side. It is not well understood as to why they are in decline in the eastern side of Nebraska, and this study looks to explore one of the possible causes of the decline, which is soil pH.

Among amphibians, there are three orders, salamanders, frogs and toads, and caecilians. Caecilians are not found within Nebraska, and mainly reside within much more tropical areas. A major characteristic of amphibians is their smooth, sometimes moist skin. This skin is semi-permeable, allowing flow of liquids and gases through it. Many salamanders can drink and even breathe through their skin, as some do not even have lungs. It is possible that much of the climate change effect is assisted by this semi-permeable membrane they have. Many of the factors inducing climate change, such as higher pollution rates can cause more frequent and severe problems for many species of amphibians. It is the fact that pollutants can change their environment so much, even to the extent of a pH change, that pH change is necessary to study among any affected species that inhabits a contaminated environment. When looking at how a species is declining within a distinct area over time, it is important to not rule out one factor over another, as something that seems like it may be disposable and play a small role, may play a larger role than previously thought.

Soil pH plays a vital role in in all ecosystems, due to its connection to the soil, as soil types and conditions determine much of the plant variation, as well as animal variation in an area. When soil can change an environment this much, soil pH can push that change even further. It can either poison the environment if too great of a change, or else become a necessity of some invasive organism that live in the area, or a preference of a certain animal that thrives in that location. In the case of amphibians, a change in pH in either water or soil can cause great distress for many, if not all of the species.

This project looks specifically at soil pH because most of the salamanders here in Nebraska are ambystomid salamanders, a genus of salamander commonly known to burrow into the soil. Soil pH will more greatly impact these salamanders as compared to other kinds of salamanders, as well as some other amphibians because of their ability to burrow. A good reason to study amphibians in general is because they are indicator species. Amphibians as indicator species can help in assessing poor environmental conditions if their population decreases abnormally. The reason for their being a good indicator species is because of their semi-permeable membrane that allows for passage of liquids and air through their skin.

In Nebraska, soil pH is a concern because of various pollutants that can be leached into the ground. Many common sources of changing soil pH within Nebraska tend to be pollutants from agriculture, or acid rain from coal-fired power plants. These pollutants contribute not only to a change in pH, but can also impact water quality greatly. On top of pH causing eutrophication through the contribution of algal blooms, it can also be detrimental to these salamanders as larvae. All salamanders' larval stages take place in water, usually in ponds. In fact, it has been found that poorer water quality has been linked to die-offs in some species of salamanders (Ryan, et al, 2013).

As stated earlier soil pH can cause varying impacts on an ecosystem, from completely changing the composition of plant species of an area to impacting the health of organisms. Considering health, amphibians can be heavily impacted by any environmental change, and pH is no different. Coupled with pollutants, pH changes among amphibians have caused reduced growth rates, reduced survival and hatching earlier than expected (Bradford, et al, 1992). Another study has found that atrazine has caused susceptibility to ranavirus (Forson and Storfer, 2006). Ranavirus is a deadly disease found in amphibians that has contributed to amphibian declines in recent years.

It is necessary to understand that some amphibians are able to adapt to their environment better than others, allowing them to live in much more extreme environments than usual. This would allow them to live in areas usually known to cause them harm due to their semi-permeable skin. Some species of frogs are able to live in drier environments due to a pelvic patch, as well as their drier skin than other species of amphibians found in the world. This pelvic patch allows for absorption of liquids through a patch of skin around their pelvis to retain water. This is not found within salamanders, and being so, many salamanders cannot thrive in less moist areas like toad and frog species. This semi-permeable membrane also has it's setbacks as well. Although amphibians can more readily take in liquids and gasses, this means that any change within their environment could potentially cause them harm. As climate change brings about larger heat waves, the likelihood of drying out increases exponentially for amphibians, as well as issues with pollution. These pollutants and contaminants can cause their own impacts on amphibians. Many contaminants can seep into the skin of amphibians in the same way that water is absorbed.

This adaption that some have to survive better within a more extreme habitat than another species also means that the problem within this study is much more complex than it shows on the surface. Because there are possibilities of amphibians to survive better in some environments over other amphibians, it should not be ruled out that the same can be said for these salamanders. They may prefer areas that one would not think would be the best for them to inhabit. In fact, some western tiger salamanders often have higher population sizes in areas with agricultural production than in areas of their native habitat (Gray, et al, 2004). This may allude to the fact that western tiger salamanders may not be as fragile as we think. There may be some way this population has adapted to survive in areas that are not as natural as they could be, such as agricultural areas. It is evident that areas with higher agricultural production are impacted more by contaminants, such as an herbicide or a pesticide. Because of their semi-permeable skin, it can by hypothesized that salamanders should show some side-effect from living here. Though these areas are more polluted, and as decreased salamander abundance occurs within polluted agricultural ponds (Knutson, et al, 2002), it could be assumed that there may be adaption to the ponds occurring within this population. This may mean that western tiger salamanders may actually be more adapted to contaminants than previously thought. Their decline within Nebraska may not be because of contaminants or acid rain or a change in pH, but a much deeper reason than previously thought because they may have adapted to this change previously if Gray's (2004) study is applicable to Nebraska Populations.

Though some amphibians may be able to adapt more readily to a harsh environment, many understand that frogs have been greatly impacted by pollutants. Pollutants occurring within frog habitats are shown to cause malformations throughout growth processes, sometimes causing hermaphroditism and limb deformities. From the literature that was found, much of the study of how pollutants impact salamanders is through growth rates and survivalship rates. Pollutants, as well as pH changes, have been more specifically found to impact egg development as well as decrease reproductive activity in tiger salamander populations (Harte & Hoffman, 1989). But, one study did show that sewage effluents or pollutants contaminating habitats have been directly linked to lesioning in skin (Rose, 2006). It was assumed for this study that similar affects would occur when the salamander was subjected to a lower pH because many pollutants can decrease the pH within a habitat. Besides Rose's (2006) study, there does not seem to be a lot of information found on the integumental effects of pH on salamanders, and this study looks to explore the relationship between skin lesions & related effects of pH change as a cause for salamander decline within Nebraska.

Literature Review

The process to create this study was not overly difficult, but very time consuming. It had to be understood that no one else had a study that had been previously published that was done in this way as that would then make this study redundant. Because of amphibian decline as a whole and salamander decline within Nebraska, it was understood that decline among salamanders would be best to work with. There are many studies on the topic of the decline among amphibian populations, as well as the health of amphibians worldwide, and would ensure that literature could be found so that it was not difficult in building a reference list.

Upon determining that salamanders were to be studied within this research project, it was necessary to know some background information on the decline of salamander species and amphibian species within the United States or Nebraska. Because it was understood that Nebraska had use of coal-fired power plants to provide as a source of energy within the state, it was determined that acid rain could be studied. Literature was sought in order to determine what was not needed to be studied as deeply when discussing acid deposition, and what could have been studied more readily.

Because of what was found within the study done by Rose (2006), where it was found that lesions were caused in salamanders in effluent polluted areas, it was thought to be possible that other factors may be at play within the effluent polluted areas studied by Rose (2006). Studying how pH change can cause lesions like in this study would be important to studying how effluents impact salamanders. This is because as stated previously, there are many pollutants and contaminants that can change pH within an environment that they are contaminating. Studying pH within this study can assist in determining if pH is a factor of great importance when studying contaminants such as sewage effluents.

In Rose's (2006) study, it was found that the effluents in the sewage lagoons could be potentially carcinogenic. In many cases, coupled with lesioning it was found that these effluents could create cysts and neoplasms within the salamander population. Determining that there was a myriad of toxins within the sewage lagoons that these salamanders inhabited in Rose's (2006) study, it is possible that there is potential that pH change may also attribute to these effects as well. In the case of Nebraska, we are studying pH changes in amphibians as determined by contaminants such as acid rain from coal-fired power plants and other possible contaminants that could potentially cause the same outcome as Rose's (2006) study. These contaminants could be any pH changing contaminant that is used for agricultural or horticultural purposes as these can leach through the ground the salamanders burrow in.

Even though agriculture could be a possible problem for Nebraskan populations of western tiger salamanders, it may not necessarily be the main contributor. Considering that even though eastern Nebraska is more populated than western Nebraska, which means heavier usage of land, this does not determine agriculture as a culprit in the case of the salamander decline. In western Nebraska, agriculture occurs as well. As previously determined, western populations of salamanders are fine. Many agricultural sites and ponds can potentially support salamander populations and have actually had higher salamander populations than more natural areas (Knutson, et al, 2002) (Gray, et al, 2004). While this may be true within the studies, this is likely because many agricultural areas have ponds that, in a salamander's case, look to be relatively good for a mating and breeding grounds. These agricultural ponds could be potential habitat because of similarities to vernal pools, where many amphibian larvae are found to occur.

Many agricultural ponds are smaller lake-like structures that would provide habitat for salamanders. It is likely that there is less predation on the salamanders occurring here. This is either because of the use of the pond within the agricultural area, or because there would be less species diversity within agricultural areas due to many farmers managing the area so that pests stay out of the farmland. Therefore, because of some of the different usages of these agricultural ponds, it is possible for many amphibians to call them home within the larval stages of their life, as they may provide better habitat for salamanders. At this same time, it is also likely that some of these ponds contribute to a decrease in larval stage amphibian populations, as some ponds are used by livestock (Knutson, et al, 2002). This use by livestock accounts for an increase in contamination by manure as well as urine. Having livestock walk through the agricultural pond could also be detrimental as well, causing increased turbidity and contaminant rates. But, because acid rain can impact a much larger area than agricultural ponds, agricultural ponds probably do not contribute heavily to the decline of salamanders within Nebraska. As acid rain is likely to impact the salamanders more than agricultural ponds due to an overall increase in area that it can contaminate when compared to agricultural ponds, it is necessary to state that the impact that acidic pH changes have on western tiger salamanders due to acid rain is much more of a focus within this study than agricultural ponds.

Major players within the amphibian decline are pathogens and fungus. There are two that are of acutely pertinent importance within amphibian populations at the moment. These two are chytrid, a fungus that has devastated many amphibian populations and is primarily found in frogs, and ranavirus, a virus that causes inflammation and reddening around the extremities. This is found in many different amphibian species, and is just as bad for the amphibian populations as chytrid is. The study done by Forson and Storfer (2006) observed ranavirus within amphibian communities that were subjected to atrazine. In the case of this study, it is important to understand what this means when concerned with a change in pH. As atrazine is a well-known herbicide and contaminant, it is of main concern when studying pH change. In order to change pH, a contaminant that is highly acidic will need to be used. Because atrazine has been found to cause ranavirus it is possible that other contaminants, including ones that are used in this study, may be contributing to the ranavirus epidemic found within amphibian populations. This means that during the study, observations will have to be made for any symptoms of ranavirus and care will have to be taken in order to identify these symptoms.

Methodology

The larval stage salamanders were collected in the spring of 2015, in Cherry County, Nebraska from the wild. They were kept over the summer and the beginning of the school year, through research preparations, until research began. Larval transformation started early in the school year, and they were fed waxworms and cut in half red worms after they morphed into adults. Larval salamanders were fed tubifex worm cubes that were broken into a powder and scattered into the water until the larvae transformed. Upon metamorphosis, the new adult salamanders were placed in a large container that had other new morphs in it. They were fed two to three wax worms or cut in half earthworms to determine if they were fully functioning as adult salamanders. Once fully functioning as adults, they were moved to another container that was filled with sand and a large water pool until experimentation was started.

One hundred pounds of soil was obtained from Thomas County, Nebraska in the Sandhills in late October. The containers were then set up with the soil from the Sandhills, also in late October. Mulch was also used in the container to keep the soil from drying out. Data had begun being recorded in November, after setting the experiment up. Three test groups were set up with four containers in each test group, with a single salamander being placed into each container a week after setting the containers up, with a water dish. After being placed into the new environment they were allowed to adjust for about two weeks. The pH of all the soils was then tested to determine the average pH for the Thomas County soil. The next week the soils were mixed up with mulch in the first group after animal removal, then the pH again to determine if pH had decreased at all. After determining pH was optimum, the animals were placed back into their container. The week after, this was done to the second group, and the week after that the same was done to the third group. All of the salamander’s snout-vent length and weight in grams were recorded. After this was all done, one test group was made the control, by using the Sandhills soil and not adjusting it. This pH remained around a pH reading of between 6 and 7. Test group two was made by using the same soil, but the pH of the soil was adjusted to a pH reading of between 5 and 6, using water mixed with hydrochloric acid that was a pH of about 3. The next test group was attempted to have a pH adjusted to a reading between 4 and 5, but due to mulch absorbing the acids, the pH was never brought below 5. This test group was brought to that pH reading by using water mixed with hydrochloric acid at a pH reading of about 2.5. The adjustments to the soil were all done after the salamanders were put into each container. Trials all started in early January. Every week the water was filled and a small amount of water was also poured on the soil in the control and test group containers. At the time water was poured in the control containers, acids mixed with water at a pH of either 3 or 2.5 were sprayed on the two other test groups after the salamanders were removed from each container. Every 2 weeks, the salamanders were checked for integument malformations and data was recorded on any deleterious-looking abnormality, as well as pictures taken.

Three months were used to observe & gather data, with three trials. Trials were all done in repetition of the first trial. Complete overview of every salamander's skin was done once every two to three weeks, with minimal observations being done in the weeks that they were not being observed. Measurements taken of the soil pH was done once per week minimum. At the end of the study, all salamanders were returned to proper health, and then returned to the site they were obtained at in Cherry County, Nebraska. Precautions were taken to ensure their safe transition from the lab to the wild.

Results

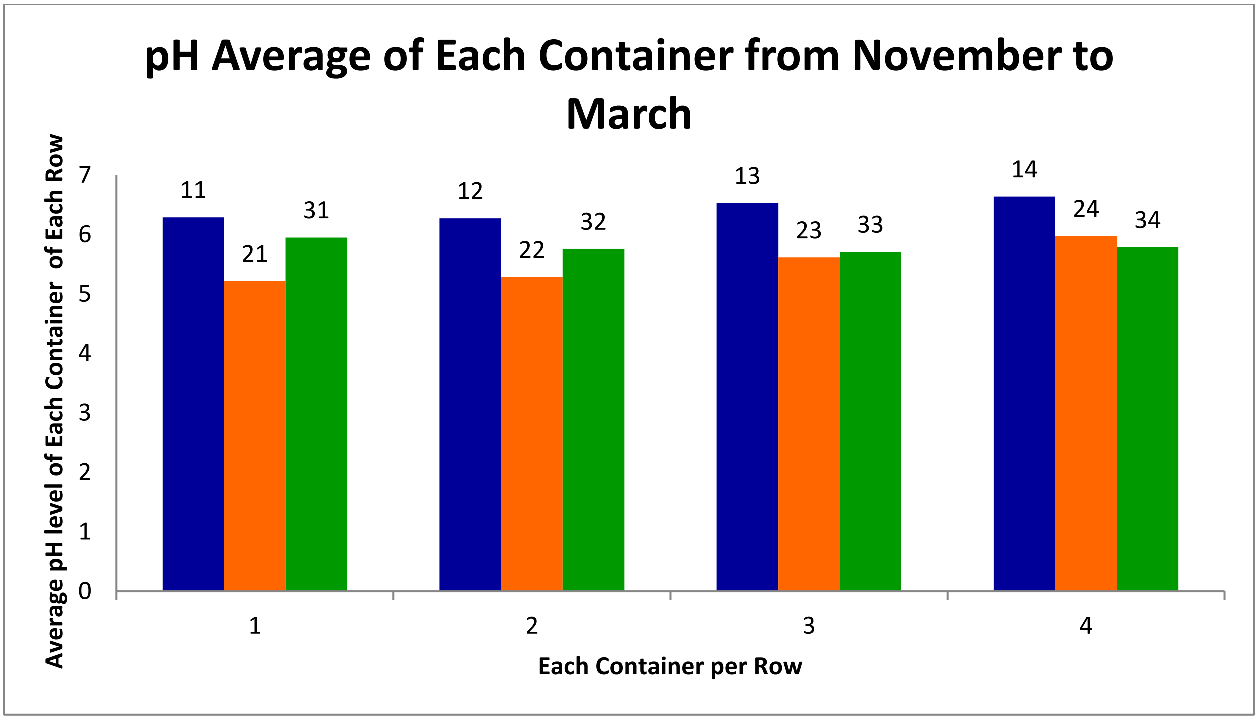

Figure 1: This figure shows the differences of pH among the average pH of each container per row from Nov. to March. Green represents row 3, orange represents row 2, and blue represents row 1, or the control within the study.

Average pH was kept track of weekly within trials of the study starting in November. Throughout these trials, the pH was relatively balanced among row one, two, and three. Row one had very little change as the control, and row two was kept at a steady pH between 5 and 6. Row three was very complicated when decreasing the pH. As shown by the figure above, the pH of row three never decreased below a level between 5 and 6. This did lead to some skewed results and data, and in turn, produced lower quality data. It is believed that the reason the pH in row three did not decrease as it was supposed to, is because the mulch that was mixed in absorbed the acidity of the acid rain solution, causing the pH to stabilize at a much higher pH than expected. It is likely that if less mulch was used in row three the pH would have decreased. The reason this occurred in only row three and not in row two is because more mulch was used in row three rather than in row two. This was done in hopes to make the pH of the soil more acidic.

The problem with decreasing pH for row three could have been prevented first hand by carefully experimenting with different soil types from across western Nebraska before setting up the trials. As this study was time-limited, this would have been difficult to have done before trials would need to have started. This would have likely been the best choice, but it made sense to use soils from the same part of Nebraska that the salamanders were obtained from. These soils were in areas that had no issues with salamanders and had a relatively neutral pH. This allowed not needing to decrease the pH of the soil so much that it reaches the killing point of the salamanders used in the study.

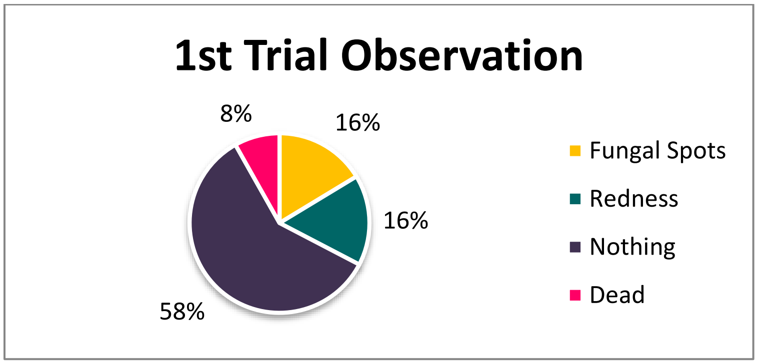

Figure 2: This represents all of the containers qualitative results for the first trial of observations of the integument of each Salamander. The control and trial groups are not shown separately within this graph.

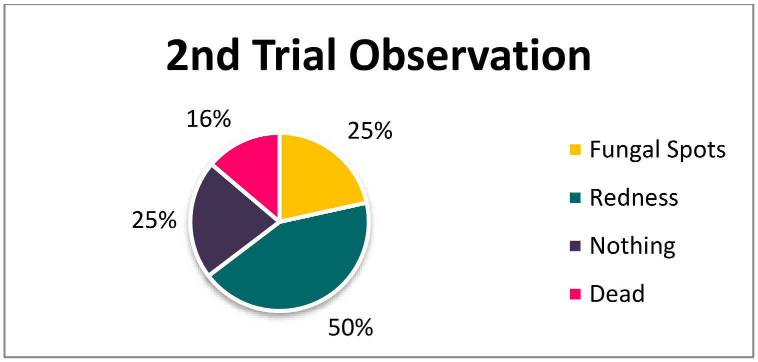

Figure 3: This figure represents findings similar to Figure 2, except this is the second trial, or the second repetition done within the study. There are several differences within the results of this graph from figure 2.

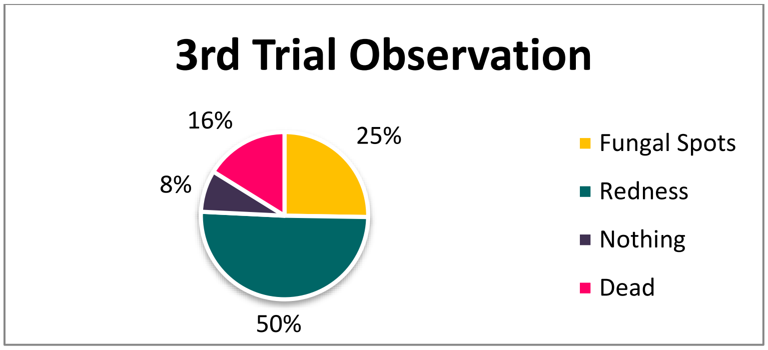

Figure 4: This figure represents the final trial or repetition done within the study and is very similar to figure 1 and 2 showing a decrease among salamanders with no results.

Figure 5: This is the overall set up of the lab, and shows the control, or row 1. It also shows the two test groups, row 2 and row 3.

Throughout the trials the salamanders were monitored very closely and inspected qualitatively and thoroughly every two weeks in February and March. Within the first trial fungal spots were identified within the control and redness of skin had occurred within the test groups around the extremities. By the second trial, many of the salamanders in the test group had become red, or become redder. This trend continued on in trial three. From the looks of figure 2, 3, and 4, this could have become a logistic trend over time, and could have possibly gotten more severe if the salamanders remained in the containers longer than they had for the three trials.

Within the control, the unexpected had occurred. This was the fungal infection that was observed on two salamanders throughout the trials. At first sight, the fungal spots had appeared to be just retained skin that hadn't sloughed off the salamanders yet. Within the next two trials as the two salamanders' conditions worsened. It was apparent that there was some sort of fungal infection occurring within the salamanders skin, as the black dots that had appeared by the first trial had not gone away yet. It was not discovered within this study why two of the salamanders within the control had this fungal infection. It could possibly be conditions within the lab, though this would be highly unlikely, as many other amphibians with no similar issues were in the same room and they were the only two in the study that had this occur. Other causes could potentially be something occurring naturally within the soil in western Nebraska, where the soil was obtained. Their burrowing causing increased interaction with the soil could potentially lead to infection by a fungus within the soil in their natural environment, and this could make for another possible study in the future.

Among the test groups nothing extremely out of the ordinary occurred besides reddening of skin. Over time, more salamanders within the test groups had this occur to them, and they became redder. There were no other more detrimental results, and to pursue more of an understanding of what is occurring, further studies will have to be done. It is possible that this reddening could result in death as salamander number 22 in row 2 was subjected to extreme pH change, ultimately resulting in its death by the second trial of the study. Row two is shown in Figure 5 above, with container number 22 being the second container in the row.

Figure 6: This is salamander 22, its ventral side shown with bright red coloration along the underbelly and extremities

Deaths did occur within this study, the first one being accidental, and having nothing to do with the actual trials done within the study. This salamander was in container number thirty one and was waiting for transfer back to its original container. This was because after the first major pH change, the salamanders were removed for a week to allow the pH to stabilize. Within row 3, two weeks were used for this, as pH within the soils would not decrease. Upon returning a week later, the salamander that had meant to be returned to container number thirty one had died, and its death could possibly have been fungal related.

The second and last death to occur within the study happened before the second trial occurred. This death was very unusual, as the salamander was found in container number 22 with its arms and legs spread out across the top soil. Along with this, it was covered in orange mulch that had been mixed into the soil in November. Upon removal from the container, it was found that its ventral side was bright red. This is shown in Figure 6 above. This salamander's skin was even more bright red than when compared to other salamanders at the end of the third trial.

As this study looked for reactions, not deaths, having a death as a result of direct contact with acidic mulch should be looked at more closely, as the salamander that died was colored very bright red on its underbelly. This may be because of other more severely detrimental causes than just singularly pH changes within the soil. Being that there was only one death related to the actual study due to pH change the death could be looked at as an anomaly. It can be considered to not be a major concern within soil pH change in adults until further studies are done to explore the effect of pH change on adults and their skin. Further studies could show that there may be something more at the heart of this study causing the reddening of the skin of the salamanders. This would be done by the acidic pH triggering some sort of response within the body or on the exterior, such as a fungus or pathogen, causing the skin irritation that was shown in this study.

Discussion

Through the results of the findings in this study, it can be said that skin irritation among adult western tiger salamanders indefinitely corresponds to a change from a neutral to a more acidic soil pH within the habitat that these salamanders live in. In the case of the Western Tiger Salamander in Nebraska, it is unlikely that soil pH can attribute to being the whole cause of the decline of these salamanders within the state. This can primarily be because the salamanders did not have any major skin abnormalities, besides the fact that two of the control salamanders had fungal infections and the reddening of skin.

Stating that soil pH is not the entire cause of the decline does mean it is necessary to understand the multifaceted issue at hand when discussing salamander populations within the state. Soil pH may not a be main factor in the sense that it impacts their skin heavily, because from the study, we can only know that it attributes to reddening of the skin at this moment in time until further studies are done. Soil pH can possibly be connected to the decline because of the results of this study, but overall the decline within the state can be connected to other factors that are just as bad for the salamanders. These can be: any invasive species, fragmentation, and pesticide use (Bradford, et al, 1994). These are all things that are currently or have a higher possibility of happening in Eastern Nebraska than they do within the western part of the state. This is because there are larger cities in the eastern part, such as Omaha or Lincoln, and many of these cities continue to grow, which can lead to a decline of surrounding ecosystems and habitats.

When looking at how soil pH interplays with the other factors causing amphibian and salamander decline within the state, it is important to note that soil pH should be looked at as just as serious of an issue as any other factor involved in the decline. This is whether or not further studies show any other implications that result from the reddening of skin because of soil pH other than some irritation. This is because soil pH can change many things within the ecosystem that it impacts. Soil pH can change plant life, as well as animal life because of this. Using the western tiger salamander as an indicator species highlights the importance that they have as an indicator species, because with a pH decrease by only one, the salamanders had already began to show signs of skin irritation. When finding results like this, it is best to take action to make a management plan so that we can quickly work to mitigate any deleterious impacts any pH change may make on the environment that the salamander is living in, let alone the salamander itself.

The environmental impact that pH change has, especially when acidic, can be explored even further within the ecological ideology of the salamander when it comes to interactions with other species. Die off within salamander populations could possibly cause some sort of ecological disturbance if an organism is required to switch to preying upon more of another organism because of it. This would inevitably contribute to the possible decline of the one species that gets preyed upon more by the predator that used to prey on both it and the Western Tiger Salamander. There is a correlation among amphibian decline, changing pH within a habitat and trophic cascades (Brodman, et al, 2010). In fact, there could be other impacts the amphibian decline may cause besides trophic cascade as well. In the case of western tiger salamanders, or any species of amphibian that burrows for that fact, many other animals that use their old burrows for a home may have a decrease in possible habitat, thus also contributing to a lower quality of ecosystem health.

Like all organisms, salamanders have preferences of a viable habitat they would best live in. Because of their semi-permeable skin, it could be thought that preference of habitat could be strictly regulated for salamanders and could also greatly impact their distribution. It could be believed to impact their distribution because in the case of a changing pH, if they are declining in areas of a changing, more acidic pH, they would eventually become non-existent within that area. In support of this idea, it was found that salamander density and distribution was decreased when subjected to a lower pH, and this may be attributed to death or just a sense that these salamanders had (Wyman & Hawksley-Lecault 1987). When looking at pH and how it affects distribution, it can be understood that the distribution of salamanders with a changing pH also interplays with other factors as well, such as the results that were obtained within this study. These factors coupled together might result in a response from the salamanders to either leave the area, or else have reduced survival, as some studies found such as Bradford's (1992).

This study did not look into how pH changes impact larval salamanders. This is because the study was only over how pH changes within the soil impact salamanders, therefore the study was only done on how it would affect adult populations. When looking at how pH impacts these salamanders, it would also be important to understand how they impact the larval stages. Salamander larvae can usually be found in relatively small lacustrine or riparian areas or vernal pools as well. These areas, though small, have a likelihood of being affected by contaminants such as acid rain within Nebraska, and in turn, a pH change will occur within these areas. The larval stage impacts of pH change seem to be relatively well studied, and it is known that pH changes can impact embryonic development, causing eggs of salamanders to hatch more slowly (Ireland, 1991). This can cause severe issues when discussing salamanders as a whole population. It can attribute to a decrease in the number of individuals that would reach adulthood because some of these eggs would be eaten by fish if the eggs are laid in a lacustrine or riparian area. This would cause a decrease in population overtime, as less and less individuals available to mate would decrease.

Because of the impact to larval salamanders that an acidic pH has, it is also possible to impact the interaction salamanders have with their prey as larvae, as well as when in the adult stage (Kiesecker, 1996). The reason for this is because salamanders in all of their life stages are gap limited predators. This means that they are only able to eat something that can fit in their mouths as wide as it can open. Their ability to prey on animals is directly correlated to their size, meaning the smaller they are, the less likely they will be able to eat something of larger size. As adults, salamanders already eat relatively small prey items, such as worms, and a change to an acidic pH would indefinitely result in many salamanders starving to death as they grew from larval stages because of their inability to eat.

In many areas that larval salamanders inhabit, adults could also be affected by an acidic change in pH. This is because many adults could potential come to that area for a drink, swimming through the contaminated water and absorbing this contaminated water or acidic pH through their skin, causing the problems seen within this study, or potentially more extreme impacts. Bioaccumulation could also occur for both larval and adult stage salamanders alike, if they ingest a prey item that has taken in the contaminated water or acidic pH, it could cause further implications for them as well.

Conclusion

This study was done because amphibians throughout the world are in extreme decline. This is related to issues involving climate change as well as other conservation issues, such as fragmentation, invasive species, habitat degradation, and pollution. As stated earlier, the issues surrounding amphibian decline are extremely complex, and for this reason, it may take many years to understand how to fix their decline. This is because it requires breaking down the main components of the issues at hand, solving them one by one. and finally taking action after each issue impacting amphibians is well understood. This study was looking primarily at how to assist western tiger salamanders within Nebraska, and has potential, with assistance of other studies, to quickly understand possible ways humans may intervene to fix the amphibian crisis here in the state. At a worldwide scale, this will be much more difficult to do, because of the multitudes of climates and various elevations that amphibians live in worldwide.

Each ecosystem that has any amphibian in it needs to be managed differently , as one thing that may be good for one ecosystem or habitat, may have an overall negative impact for another. One prescription for an amphibian may actually cause further harm for the habitat that it lives in and management plans need to be taken into deep consideration before actually being done. There is a possibility that in some habitats, some amphibians may have to be phased out because they will not be able to adapt to an evolving habitat anyways. This is because some amphibian species may be unable to conserve as the earth continues to change and, like the giant panda, may unfortunately already be on their way to extinction with very little that can be done to prevent it.

In the sense of amphibian decline, the answer to the question that asks if acidic pH has an impact on the western tiger salamander is yes. This is because even though lesions were not found to be caused by acidic pH changes, reddening of skin was found. Further studies could be done to understand what is occurring when this reddening of skin takes place, as at the moment this study has found no other issues at hand. The deaths that occurred were found to be anomalies as one died from other reasons and another is assumed to have died from extreme exposure and direct contact to mulch buried within the soil. A deeper look into this may find much more deleterious effects occurring as the main culprit of the irritation of salamander integument when subjected to a lower pH, such as ranavirus or other severe pathogenic or fungal infections.

This impact that acidic soil pH has remains true as far as this study shows for much of Nebraska, and because of similarities within ecosystems, can hold true for most parts of the Midwest. There are areas such as the Appalachian Mountains where this is different, as many salamanders in that region have adapted to much more acidic soils. One could assume that salamanders in that region may be better adapted to acid rain issues than salamanders here in Nebraska. Because of their semi-permeable skin, it would also make sense that they would have similar problems as the western tiger salamander here if acid rain or pH changing contaminants made direct contact with their skin. It was found that frog populations with geographic variations like the salamanders in the Appalachian region and Midwest; were more tolerant in some areas than other species of frogs when subjected to a more acidic pH change (Glos, et al, 2003). Because of this reason all ecosystems should be looked at differently; just within the United States are two different ecosystems where amphibians live with two very different soil pH levels. A prescription for what is causing the amphibian decline within Nebraska would likely not work the exact same way if done within the Appalachian Mountain region, because the organisms living there have adapted to different living conditions.

Salamanders, like many organisms on the earth, are very complex ecologically, physiologically, and biologically. For the Amphibia class this can be attributed to the semi-permeable skin that is characteristic in all of the species. This semi-permeable skin allows them to be sensitive to their environment, and any change within it could alter any part of their life history for better or worse for the species. The same goes for the reasons of the amphibian decline. Many factors are at hand that attribute to the decline of the class, and there are even more issues within those factors causing those factors to occur. pH change is just one of these other factors involved in the decline, and because of the issues it causes for larval salamanders, it should be presumed that adult salamanders should or could be impacted just as greatly by them.

The reddening of skin that was found among the adult salamanders in this study shows that something was going on among the salamander population kept within the lab during this study. It should be assumed that this reddening of skin has the likelihood of being the side effect of something far greater than just acidic pH. This is because ranavirus causes reddening and irritation around the extremities and mouth like what had been seen in the study. This may or may not mean that ranavirus could be occurring within the population of salamanders that were used in the lab. Further tests should be done to prove whether or not there are any bacterial or pathogenic implications involving salamanders being subjected to a more acidic pH than their environment usually is. This could be done by swabbing the skin of the salamanders and allowing cultures to grow on petri dishes. This would be the most logical study to be done after this study, as this study was unable to delve into what was causing the skin's reaction to the soil pH due to financial capabilities.

Other studies could potentially be anything studying their behavior, such as decreased burrowing habits. Food studies have been done, showing that salamanders have impaired eating when subjected to a lower pH (Roudebush, 1988). This could possibly be attributed to Ireland's (1991), and Kiesecker's (1996) studies, as a lower pH also decreases the ability for larval stage salamanders to grow, as well as decreases their ability to capture prey items. Larval salamanders are usually diurnal feeders, but depending upon environmental circumstances, can become nocturnal (Leff & Bachmann, 1986 (as cited in Norris, 1989)). Studying the effect of pH acidification on western tiger salamanders would work and make sense for a study like Leff's and Bachmann's (1986 (as cited in Norris, 1989)), as pH change greatly alters environmental circumstances. This is especially true when taking into consideration the semi-permeable skin that a salamander has and the effects on it found in this study as well as other studies such as Roudebush's (1988).Reproduction could be studied as well, as acidic pH could possibly decrease sperm count among male salamanders, and this could cause severe detrimental impacts among any population of salamanders.

As climate changes and human population rises, we need to continue watching amphibian populations throughout the world and learn from them about the world we live in. Amphibians, as well as other known indicator species are our key to the future. Through these species we can possibly find one small component to a metaphorical equation to keep humans sustainable on the Earth. It is important to remember that conservation biology should be used as a tool to not allow us a place to live, and keep allowing us to bend the earth to our will as we grow, but to allow us to live in a balance and harmony with all species and life on Earth. As humans, we need to remember that we are part of Earth and we should use amphibians to assist us in a management plan for ourselves and the rest of life on Earth as their populations decline. If we act, there may be time to assist their populations and this may cause us to act more sustainably. Understanding amphibian decline may make humans realize that if we do not act like better neighbors on this earth; our time may be just as limited as we are making the amphibians.

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