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Disease Prevalence and Selenium Bioaccumulation in Northern Leopard Frogs (Lithobates pipiens)

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Disease Prevalence and Selenium Bioaccumulation in Northern Leopard Frogs

(*Lithobates pipiens*)

By

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ABSTRACT

Disease Prevalence and Bioaccumulation in Northern Leopard Frogs (*Lithobates pipiens*)

Emily Eisenbraun

Director: Dr. Jacob Kerby, Ph.D.

Most amphibians are becoming imperiled in today's world via environmental contamination and emerging infectious diseases. This project's main objective was to determine the relationship between the prevalence of Ranavirus and selenium bioaccumulation in amphibians in South Dakota. I selected northern leopard frogs (Lithobates pipiens) to focus on specifically because they are susceptible to Ranavirus and are found ubiquitously throughout eastern South Dakota wetlands. The secondary objective of this project was to determine if disease prevalence was correlated with areas of agricultural runoff in South Dakota's prairie pothole wetlands. Intensive field sampling was conducted across five wetlands in Eastern South Dakota at three control sites with no agricultural runoff, and two experimental sites, where there is direct tile drainage. Northern leopard frogs were collected (n=21) from each wetland site via hand capture using dipnets and brought back to our laboratory. The livers of each euthanized individual were removed and processed for inductively coupled plasma mass spectrometry (IC-PMS) for selenium content analysis, and quantitative polymerase chain reaction (qPCR) analysis for detection of Ranavirus. I expected immunocompetence to decrease as the infection load of Ranavirus and bioaccumulation of selenium increase in northern leopard frogs. Additionally, I expected the viral load of Ranavirus in northern leopard frogs to be correlated with areas of agricultural runoff.

These hypotheses were rejected based on the limited amount of collected data, but important surveillance data was obtained.

Keywords: selenium, Ranavirus, Northern Leopard Frogs, bioaccumulation

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INTRODUCTION

Many amphibians are imperiled today due to a variety of stressors throughout their environments. As of 2020, 2442 species of amphibians were designated as threatened (ICUN, 2020). One species that is at risk of becoming threatened is the northern leopard frog (Lithobates pipiens). Since the 1990's northern leopard frogs have experienced large declines in population size and mass mortality events. There were petitions in 2006 to the U.S. Fish and Wildlife Service to add northern leopard frogs west of the Mississippi River as a threatened or endangered species. These have not passed but they are listed as a species of concern in Colorado, California, Arizona, and New Mexico (Palumbo, 2010). Currently, northern leopard frogs are listed as a secure status, but population declines have been noted due to habitat loss, Batrachochytrium dendrobatidis (Bd), Ranavirus, invasive species, chemical contaminants, UV-B radiation, and drought *Reptiles and Amphibians*," (n.d.). This research focuses specifically on Ranavirus and chemical contamination of northern leopard frog habitats with selenium.

Northern leopard frogs are amphibians that inhabit aquatic environments across the country. Historically the distribution was vast throughout North America, yet the population is becoming scarcer in the Western states of America (U.S. Department of the Interior, 2015). Northern leopard frogs are green, brown, and yellowish in color with identifiable spots along their dorsum (figure 1). Another distinguishable feature is the white dorsolateral folds that run down the back of northern leopard frogs ("Northern

Leopard Frog," 2022). Their mass typically ranges from 16 to 80 grams and their snout-vent length ranges from 2 to 11 centimeters with some smaller values depending on the stage of development ("Northern Leopard Frog", n.d.). They typically live in slow-moving or still water free of predators like fish or bullfrogs. Northern leopard frogs are semi-aquatic meaning they require a habitat that can provide land and water. Breeding, egg deposit, and early life typically occur in shallow aquatic environments. Adult frogs can be terrestrial but typically reside underwater over winter ("Northern Leopard Frog," 2017). These requirements make the wetlands of South Dakota an excellent habitat for northern leopard frogs. But the wetlands and northern leopard frogs could be in jeopardy due to stressors like, tile drainage systems, selenium bioaccumulation, and Ranavirus.

Figure 1: Pictures taken of northern leopard frogs found at the wetland sites used in this study**.**

Tile Drainage Systems

The Prairie Pothole Region (PPR) includes grasslands with wetlands throughout spanning Canada, the Dakotas, Minnesota, Iowa, and Montana (Johnson et al. 2008).

The prairie potholes were created around 12,000 years ago due to the ending of the Wisconsin Pleistocene glaciation changing the landscape and depositing water in the ground to create prairie pothole wetlands (Woo, et al., 2019). The abundance of nutrient-rich soils and natural wetlands from this Pleistocene glaciation make South Dakota and Iowa excellent for agricultural practices, such farming and ranching. The PPR is used heavily for agricultural development, cattle grazing, and small-game hunting. The wetlands spread across Eastern South Dakota create a problem for agricultural practices by oversaturating crops with water and creating physical barriers to agricultural practices (Johnston, 2013). The solution for this issue has been to drain wetlands or use them for tile drainage system waste (Tangen and Finocchairo 2017). Tile drainage systems have become increasingly popular in South Dakota due to their effectiveness in draining soils that have a propensity for retaining water (Tangen & Finocchiaro, 2017). Tile drains are subsurface pipes that carry water away from agricultural fields where the water, excess fertilizer, and metals are deposited into wetlands. Figure 2 shows the mechanisms in which many tiles drainage systems work. Around 85% of agricultural areas of the PPR began to utilize tile drainage systems by the end of the 20th century (Wright and Sands, 2001).

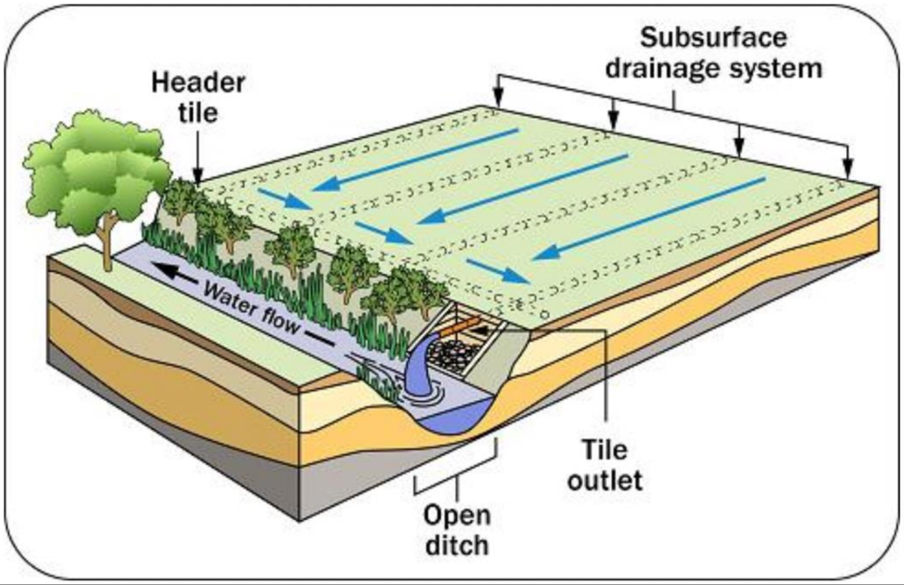

Figure 2: Pictorial representation of tile drainage system by Vander Veen, S. (2010).

Although tile drainage systems are beneficial for agricultural purposes there are negative consequences associated with native wildlife and water health. The wetlands are important habitats for amphibians, reptiles, and insects in South Dakota (Johnson et al. 2008). The wetlands serve as nutrient sinks and water storage sites for agriculture drainage and runoff (Johnson et. al. 2008; Tangen & Finocchiaro, 2017). This deposition can lead to increased contaminant concentrations which can have negative effects on biota (Martin & Hartman, 1987; Euliss & Mushet, 1999; Main, et al. 2014). The deposit of agricultural runoff through the tile drainage system is responsible for 50-90% of wetland destruction each year (Dahl, 2014).

Selenium and Bioaccumulation

One contaminant that is of particular concern in South Dakota is selenium. This concern exists because of naturally high sediment selenium concentrations found in South Dakota due to prehistoric volcanic activity and glaciation (Masse et al., 2016; Sando & Neitzert, 2003). Selenium is a naturally occurring element in the environment and is required in trace amounts in most animals but can be detrimental at high concentrations (Masse et al., 2016). Selenium is naturally found locked into glacial deposits of shale, coal, and phosphate in the soil. The installation of the subsurface tile drains makes selenium available to flora and fauna in the wetlands by breaking up the soil and releasing heavy metals locked within (Daniels 1996). Additionally, due to South Dakota's naturally higher selenium concentrations and the propensity for selenium-laden sediment to be deposited in the Missouri River during the annual high-water cycle, many aquatic organisms are at risk of selenium toxicity (Masse et al., 2016; Sando & Neitzert, 2003).

Selenium has a propensity to bioaccumulate, and increased selenium concentrations in water is positively correlated with increased selenium concentrations in native wildlife (Schwarz et al., 2018). Heavy metals, such as selenium, are not easily biodegraded and can be transferred to high trophic levels of the food web (Ley-Quinonez et al., 2013). I am particularly concerned about selenium transfer to and subsequent bioaccumulation in higher trophic-level predators. Once selenium is released into the environment, it enters at the benthic level of energy in the food web including microbial organisms where it is consumed by invertebrates (Franz et al. 2011;

Gallego-Gallegos et al. 2013). From here, bioaccumulation can begin to higher trophic levels through dietary transfer up wildlife including amphibians, like northern leopard frogs, reptiles, or birds (Janz et al. 2010).

High concentrations of selenium may pose a risk to aquatic biota, yet the effects of selenium bioaccumulation in aquatic frogs have not been studied as intensively as the effects in fish, birds, or mammals (Sparling et al., 2010). High concentrations of selenium can be detrimental to an organism's health and reproductive success due to the body's inability to distinguish sulfur from selenium in biochemical pathways. The selenium over sulfur mechanism is not well understood, but researchers believe it is due to the similar chemical nature of Sulfur and Selenium (Collins et al., 2012). This substitution of selenium for sulfur in proteins can lead to several negative health effects including immune system suppression, selenosis, decreased reproductive success, and acute toxicity ("Aquatic Life Criterion," (n.d.)).

Ranavirus

Ranavirus is another stressor for northern Leopard frogs that has a high mortality rate and can cause mass mortality events in amphibians (Hedrick et al. 1992). Ranaviruses belong to the Iridoviridae family, a group of large double-stranded DNA viruses that affect amphibians, reptiles, and fish. Symptoms of Ranavirus include lethargy, edema, hemorrhages, and abnormal swimming patterns ("Ranavirus," 2018). ("Ranavirus," 2018). The most well-known Ranaviruses that affect frogs include Frog Virus 3 and Tadpole Edema Virus, two serious and life-threatening diseases. Mortality typically occurs 3 days to 3 weeks following infection following direct contact with the virus through ingestion or exposure to infected soil or water ("Ranavirus", 2020). Mass mortality events of amphibians infected with Ranavirus have been documented in Cedar and Dixon counties of Nebraska (Davis and Kerby 2016). These counties border South Dakota and provide reasonable concern that mass mortality events could happen in South Dakota. It is pertinent to understand the factors that put northern leopard frogs at risk to prevent further population decline from pathogens such as Ranavirus.

Study Objectives

The purpose of this study was to quantify the disease load of Ranavirus in northern leopard frogs of South Dakota wetlands and compare these values to the Selenium concentrations obtained from their livers. There is a relationship between presence of Ranavirus and accumulation of selenium because selenium puts stress on the organism, and this could make it harder to fight diseases. The secondary objective was to analyze whether disease loads were correlated with areas of agricultural runoff in South Dakota's prairie pothole wetlands. Agricultural runoff is another stressor that could make it harder to fight diseases like Ranavirus. These objectives were analyzed by determining the disease load of Ranavirus and liver Selenium concentration of northern leopard frogs from tile drainage sites or control sites. Tile drainage sites have direct agricultural runoff and control sites have no direct agricultural runoff. I selected northern leopard Frogs to focus on specifically because they are susceptible to Ranavirus and are found ubiquitously throughout eastern South Dakota wetlands. Adult and juvenile northern leopard frogs eat a variety of insects and other invertebrates as adults, while tadpoles are herbivores ("Nothern Leopard Frog"). Therefore, northern leopard frogs are at risk for Selenium bioaccumulation from lower trophic levels. Due to the vast abundance, distribution, and diet of the northern leopard frog, they were an excellent species to use in this study. I hypothesize infection load of Ranavirus to increase as bioaccumulation of selenium increases in northern leopard frogs. Additionally, I expected the viral load of Ranavirus in northern leopard frogs to be correlated with areas of agricultural runoff. This study is useful for our understanding of the interactions of chemicals and disease while doing so in a way that is safe to humans and still meaningful to South Dakota wildlife. This is because humans cannot become infected with Ranavirus.

MATERIALS AND METHODS

Data Collection

All data collection and procedures were completed under the approved Institutional Animal Care and Use Committee protocol. To complete the project's objectives, I conducted extensive field sampling in six wetlands within the PPR designated as Waterfowl Production Areas in the summer of 2021 (WPA). WPAs are grassland and wetland areas across the Prairie Pothole Region used for breeding and nesting of waterfowls ("Waterfowl Production Areas," 2004). Three of these wetlands were control sites with no agricultural runoff and three sites were tile drainage sites with direct agricultural runoff. The control sites include Lost Lake, Pettigrew, and Buffalo, while the tile drainage sites include Habeger, Petri 1, and Volker (Figures 3 &4) GPS locations were recorded (Table 1).

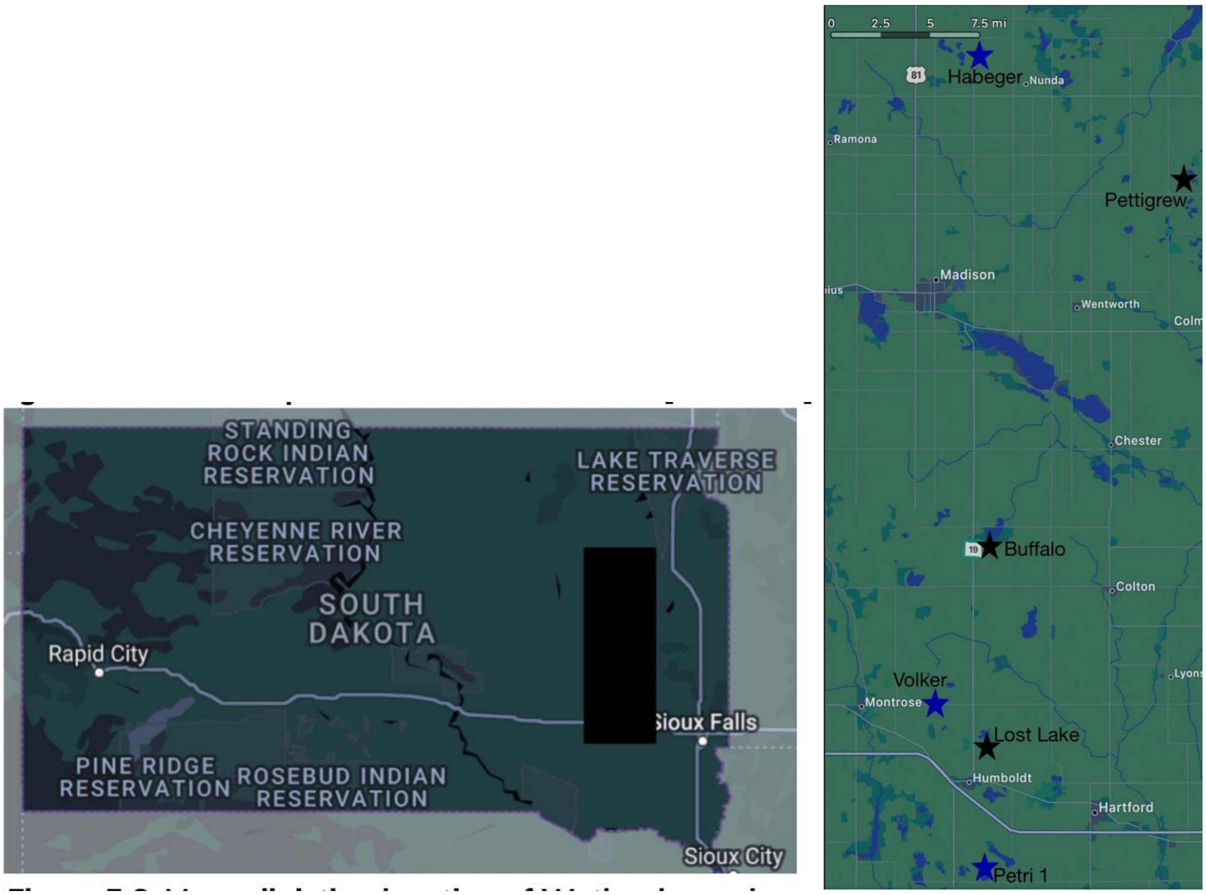

Figure 3 & Figure 4: Map of South Dakota and zoomed in map of Eastern South Dakota with wetlands sites used in experiment shown. Blue stars indicate tile drainage sites, while black stars indicate control sites.

Table 1: Wetland control and tile drainage sites with GPS coordinates listed.

	Site Name	GPS Coordinates of Site
Tile Drainage Sites	Habeger	44.15966 ° N, 96.99936 ° W
	Petri 1	43.575910 ° N, 97.061915 ° W
	Volker	43.70411 ° N, 97.10954 ° W
Control Sites	Lost Lake	43.67435 ° N, 97.05665 ° W
	Pettigrew	44.09303 ° N, 96.84885 ° W
	Buffalo	43.81972 ° N, 97.05972 ° W

Northern leopard frogs (n=21) were collected in the Summer of 2021 using hoop-net traps and hand capture with 15 being from control sites (n=5 Lost Lake, n=5 Pettigrew, n=5 Buffalo) and 6 being from tile drainage sites (n=5 Habeger, n=1 Petri 1, n=0 Volker). Despite many efforts to collect individuals, I had a challenging time finding any or finding live frogs at the tile drainage sites. No individuals were collected from the Volker tile drainage site, but multiple deceased individuals were noted in the field. The northern leopard frogs were transported back to the laboratory at the University of South Dakota and euthanized using benzocaine and decapitation. Individuals were euthanized on the same day as capture. Snout-vent length, total mass, liver mass, and sex, were collected for each northern leopard frog. A blood sample was collected using a capillary tube, and the liver of each euthanized individual was removed and processed for inductively coupled plasma mass spectrometry (IC-PMS) for selenium content analysis, and quantitative polymerase chain reaction (qPCR) analysis to detect

Ranavirus. Blood and tissue samples were stored in a standard freezer at -10° Celsius in the laboratory.

Sample Processing

All blood samples were sent to the University of Nebraska Lincoln Water Quality Laboratory for IC-PMS selenium analysis. qPCR tests were used to test for prevalence and the viral load of Ranavirus. Several variables were examined across the treatment groups via an ANOVA run with R statistical software (Version 4.2.2). A Tukey post hoc comparison was used to examine differences among control sites. I also used linear regression to compare selenium liver concentrations with estimated Ranavirus viral load.

RESULTS

Selenium

Selenium liver concentrations ranged from 0.001 to 8.96 µg/g and are listed in Table 2. The average selenium liver concentration for tile drainage sites was 4.710 µg/g +/- 2.633. Average selenium liver concentration for control sites = 0.812 µg/g +/- 0.588.

Table 2: List of the selenium liver concentrations from the 21 collected individuals.

Detection Limit: 0.020, *Below Detection Limit.

Site Type	Sample ID	Selenium (µg/g)
Control	PETTIGREW 1 - LIVER	0.323
Control	PETTIGREW 2 - LIVER	0.29
Control	PETTIGREW 3 - LIVER	0.364
Control	PETTIGREW 4 - LIVER	0.31
Control	PETTIGREW 5 - LIVER	0.386
Control	LOST LAKE 1 - LIVER	0.963
Control	LOST LAKE 2 - LIVER	1.14
Control	LOST LAKE 3 - LIVER	2.44
Control	LOST LAKE 4 - LIVER	1.27
Control	LOST LAKE 5 - LIVER	0.919
Control	BUFFALO 1 - LIVER	0.151
Control	BUFFALO 2 - LIVER	0.617
Control	BUFFALO 3 - LIVER	1.55
Control	BUFFALO 4 - LIVER	0.7
Control	BUFFALO 5 - LIVER	0.755
Tile	HABEGER 1 - LIVER	3.86
Tile	HABEGER 2 - LIVER	5.13
Tile	HABEGER 3 - LIVER	4.96
Tile	HABEGER 4 - LIVER	5.34
Tile	HABEGER 5 - LIVER	8.96
Tile	PETRI 1 - LIVER	*0.001

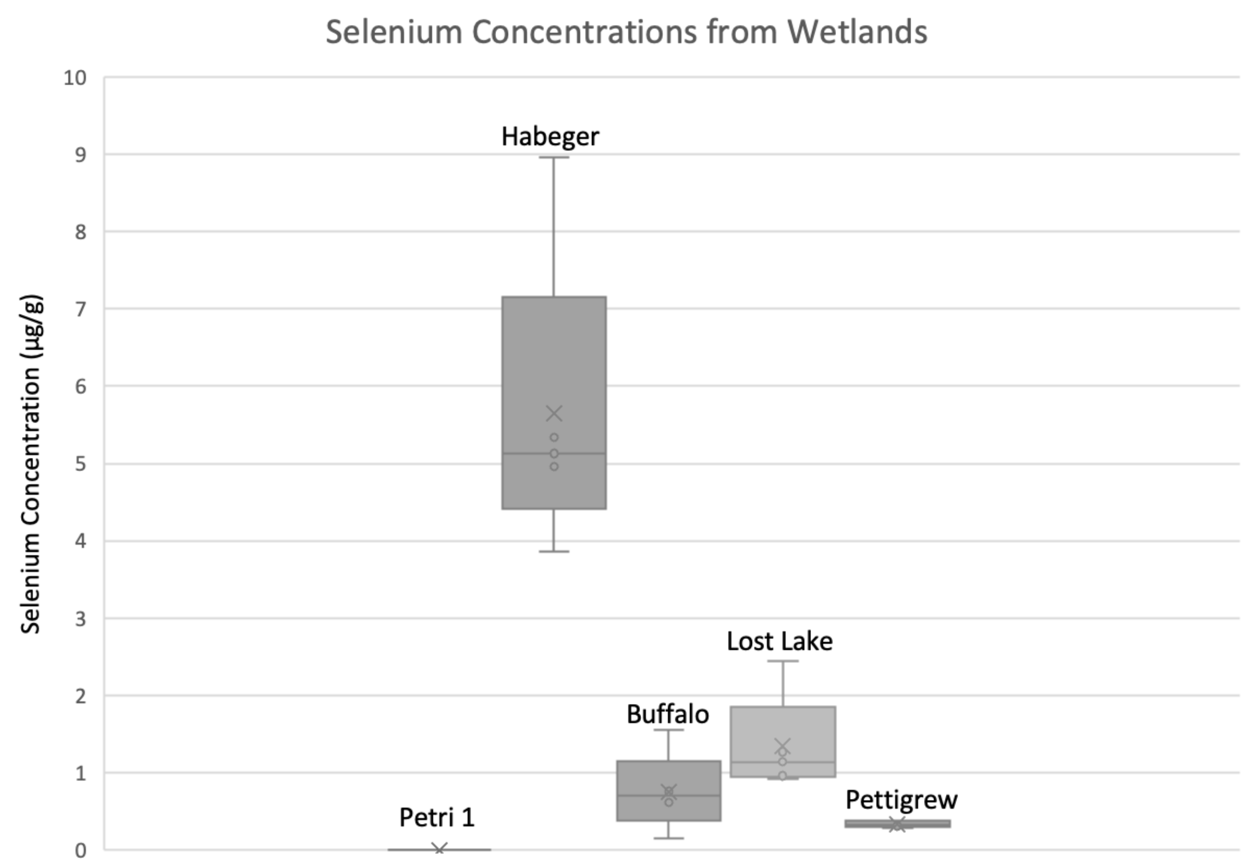

Figure 5: Selenium concentrations in µg/g for the five wetlands sites individuals were collected from. Shown is a box and whiskers plot. The X indicates the mean, the line indicates the median, and the different quartiles are shown.

I ran an ANOVA on the individual samples from each of the tile and control sites comparing liver concentrations without regard to the site. This approach was used due to low sampling success and the collection of only six individuals from tile drainage sites. The ANOVA showed a significant difference in selenium concentration in comparing livers from control and tile sites ($F_{1, 19} = 26.43$, $p < 0.0001$). The ANOVA test results are summed in Table 3 below. Figure 5 shows the average selenium liver concentrations of each site with error bars.

Table 3: Results of ANOVA test using individual samples from tile and control sites, without regard to specific site.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	1	65.07	65.07	26.43	5.81e-05 ***
Residuals	19	46.78	2.46		

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For control sites, I also ran an ANOVA to examine any differences among sites for selenium concentrations (table 4). A Tukey post doc test was performed to examine pairwise differences (Table 5).

Table 4: Results of ANOVA test using 3 control sites, using wetlands-specific sites.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	2	2.584	1.2920	5.965	0.0159 *
Residuals	12	2.599	0.2166		

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Table 5: Results Tukey post hoc using 3 control sites, regarding specific site.

	diff	lwr	upr	p adj
LostLake-Buffalo	0.5918	- 0.1934784	1.377078	0.1519946
Pettigrew-Buffalo -	-0.4200	-1.2052784	0.3652784	0.3587556
Pettigrew-LostLake	-1.0118	-1.7970784	-0.2265216	0.0126401

I detected a significant difference in liver selenium concentrations among control sites (F2, 12 = 5.965, p = 00159). A post hoc Tukey test exhibited a significant difference in concentration between Pettigrew and Lost Lake (p = 0.126), but not between Lost Lake and Buffalo (p = 0.151) or between Pettigrew and Buffalo (p = 0.3857).

Ranavirus

Ranavirus was detected in seven out of the twenty-one individuals collected. All seven of the positive individuals were obtained from control sites, two frogs from Buffalo and five from Lost Lake. The viral load of Ranavirus was determined from qPCR analysis and viral copies per sample were obtained. The highest viral load of Ranavirus was found in individual Lost Lake 4 with 70.986 viral copies per sample, while the lowest was in Lost Lake 5 with 3.043 viral copies per sample. The progression of the highest viral load to the least is shown in Figure 6.

In order from the highest viral load of Ranavirus to Lowest includes Lost Lake 4, Buffalo 5, Buffalo 3, Lost Lake 2, Lost Lake 3, Lost Lake 1, and Lost Lake 5. The seven positive Ranavirus individuals were compared to their liver selenium concentration. They were plotted in Figure 7 and linear regression was determined. A linear regression was performed to examine any relationship between selenium concentration and Ranavirus viral load. The results are indicated below in Table 7. I detected no significant relationship between liver selenium concentration on viral load (p = 0.7481)

Table 6: Results of linear regression test using positive Ranavirus individuals and their corresponding selenium concentration value.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	27.468	26.623	1.032	0.349
Conc	-6.479	19.092	-0.339	0.748
Residual standard error		26.62 on 5 degrees of freedom		
Multiple R-squared: 0.02251		**Adjusted R-squared**: -0.173		
F-statistic:		0.1152 on 1 and 5 DF		
p-value:		0.7481		

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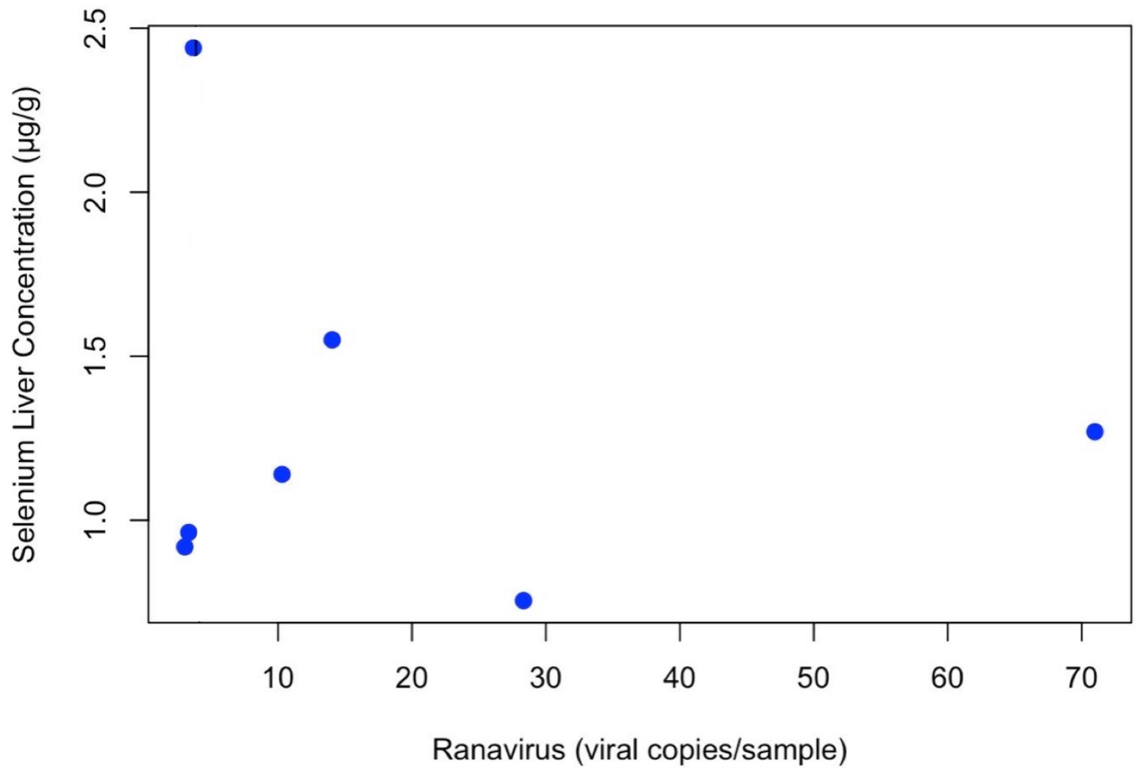

Figure 6: Ranavirus viral copies per sample plotted on the x-axis vs concentration of selenium. Linear regression found no significant relationship.

Mass and Length Data

Additional information was collected from everyone including total mass, the mass of the liver, and snout-vent length. The average mass of individuals was 15.50 grams +/- 20.71 with a range of 2.99 grams to 71.65 grams. The average masses of individuals from each wetland site are included in Table 3. Masses of the liver were collected immediately following dissection. The average mass of livers was 0.98 grams +/- 2.43 with a range of 0.06 grams to 2.80 grams. The average percentage body mass of the livers of collected individuals was 2.54% +/- 0.72 %. I ran an ANOVA on the individual samples from each of the tile and control sites comparing percent body mass liver without regard to site. The ANOVA did not show a significant difference in body mass percent liver in comparing control and tile sites ($F_{1, 19}$ = 1.72, p <0.204) (Table 8). The average snout-vent length was recorded for everyone with an average of 4.53 centimeters +/- 1.91 centimeters. Our sample size included 21 individuals with 8 males and 13 females. These findings are summarized in table 9.

Table 7: Results of ANOVA test using individual samples from tile and control sites, without regard to specific site looking at percent body mass of liver.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	1	0.97	0.97	1.72	0.204
Residuals	19	10.70	0.56		

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Table 8: Ranavirus-positive individuals for Ranavirus and average selenium concentration, mass, liver mass, and snout-vent length for each wetland site.

	Site Name	Positive Individuals	Total Individuals	Average [Selenium] (µg/g)	Average mass (g)	Average Liver Mass (g)	Average SVL (cm)
Tile Drainage Sites	Habeger	0	5	5.650	5.650	2.268	3.22
	Petri 1	0	1	0.001	50.21	1.06	7.80
	Volker	-	0	-	-	-	-
Control Sites	Lost Lake	5	5	1.346	41.73	1.388	7.04
	Pettigrew	0	5	0.335	5.86	0.146	3.58
	Buffalo	2	5	0.754	3.29	0.084	3.08

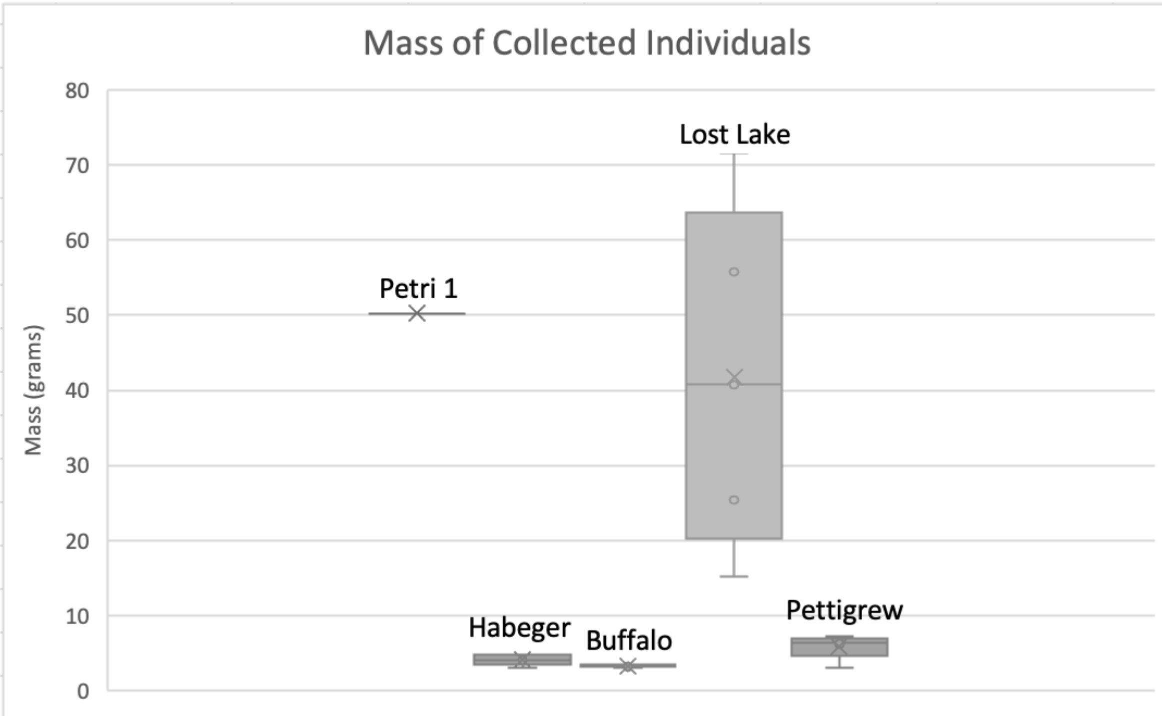

Figure 7: Average mass of collected individuals in grams for the 5 wetlands sites individuals were collected from.

DISCUSSION

Objectives

This project's main objective was to determine the relationship between the prevalence of Ranavirus and selenium bioaccumulation in amphibians in South Dakota. Our results did not show an apparent relationship between viral load and concentration of selenium, yet our sample size was small due to difficult environmental conditions. There is still a strong likelihood that stressors like metals can put stress on the bodies of wildlife, making it harder for them to combat other stressors. I need more sites and more collected individuals to further analyze this relationship.

I expected the infection load of Ranavirus to increase with higher levels of selenium bioaccumulation in northern leopard frogs. Additionally, I expected the viral load of Ranavirus in northern leopard frogs to be associated with areas of agricultural runoff. These hypotheses were rejected based on the limited amount of collected data, but important surveillance data was obtained. I added evidence to show livers in frogs of tile drainage sites contained significantly more selenium than those of control sites. With the increasing usage of tile drainage systems and increased exposure to selenium, we must understand how wildlife is affected (Wright and Sands, 2001). A normal range of selenium for most species is 0.1 to 0.38 µg/g and the maximum tolerable limit for 2 to µg/g (Bischoff, 2022). The selenium found in our individuals ranged from 0.001 to 8.96 µg/g. Our average liver selenium concentration fell below this with 0.812 µg/g average for individuals in control sites, but our tile drainage site individuals did not. The average liver selenium concentration of individuals collected from tile drainage was 4.710, which is in the range of the maximum tolerable limit.

Looking at two previous studies conducted in these wetlands during the summer of 2019 is useful to analyze my data collected in summer 2021. One study used adult American toad from identical control sites to my study and the Habeger tile site, where five out of the six individuals from tile sites were collected from in my study. This research found selenium liver concentration ranged from 0.16 to 5.82 µg/g and averaged 2.15 µg/g. This study found no significant difference in the liver selenium concentration of toads from controls site compared to tile drainage sites (Campbell, 2021).

The second study utilized identical wetland tile drainage and control sites to mine and studied selenium in Painted Turtles (Gerberding, 2021). The results of average blood selenium concentrations from this study are outlined in Table 9. Comparing this research to mine is interesting. The recorded selenium concentration was more than twice as high in Habeger and increased slightly for Lost Lake and Pettigrew in my data. Buffalo decreased slightly and Petri I decreased significantly. Yet, in my research Petri I only contained one individual and they were below the detection limit. Volker contained the highest blood selenium concentration in turtles in 2019 and in my field sampling we were unable to find individuals at Volker. It is a possibility the severely high selenium concentration in 2019 may have something to do with this.

Table 9: Blood selenium concentration in turtles in 2019 compared to liver selenium concentration in frogs in 2021 at the six wetland sites used in this study.

	Site Name	Average [Selenium] (μg/g) 2019 - Turtles	Average [Selenium] (μg/g) 2021 - Frogs
Tile Drainage Sites	Habeger	2.05	5.650
	Petri 1	0.46	0.001
	Volker	13.04	-
Control Sites	Lost Lake	1.03	1.346
	Pettigrew	0.19	0.335
	Buffalo	0.99	0.754

The secondary objective of this project was to determine if disease prevalence was correlated with areas of agricultural runoff in South Dakota's prairie pothole wetlands. Our results were unable to show this association due to individuals infected with Ranavirus only originating from control sites. Of the twenty-one collected individuals, fifteen were from control sites, and only 6 from tile drainage sites. This means out of the three tile drainage sites of Habeger (n=5), Petri 1 (n=1), and Volker (n=0), there were issues finding individuals. It is important to consider why there was trouble finding live individuals in Petri 1 and Volker tile drainage sites and why there were only Ranavirus-positive individuals in control sites.

During field surveillance and attempted capture, very few individuals were observed. There was the observation of dead individuals at Volker and noticeable changes in habitat. At Volker, there was a significant amount of mud and less open water for the frogs to swim around in (figure 9). The two deceased individuals were observed in the mud. Additionally, there was noticeably less water than previous years at all wetland sites confirmed through observation of dried-up portions of wetlands. Figure 10 shows a portion of buffalo wetland that no longer contains water.

Figure 8 and Figure 9: Significant amounts of mud and little water at Volker wetlands and dried up portion of Buffalo wetland.

The seven Ranavirus-positive individuals were from Buffalo (n=2) and Lost Lake (n=5). Both locations were control sites, with no direct agricultural runoff. Ranavirus can cause mass mortality events, so all our individuals from Lost Lake being Ranavirus-positive is concerning (Hedrick et al. 1992). If 100% of the individuals we sampled from Lost Lake have Ranavirus, how many others in that wetland do as well? It is also interesting to look at the prevalence of Ranavirus between tile drainage and control sites. Zero of the six individuals from tile drainage sites were Ranavirus-positive, while seven of the fifteen individuals from control sites were Ranavirus-positive. This means none of our collected tile drainage site individuals and just over 50% of our control site individuals tested positive for Ranavirus.

Previous studies stressors can affect susceptibility to Ranavirus, but so can temperature and progression of metamorphosis ("Ranavirus," 2018). Although stressors, such as environmental contaminants have been found to increase susceptibility, this is not what we saw in this study. This could have been due to a low sampling number or the relationship not being strong in our findings. Either way, we are seeing a difference in the prevalence of Ranavirus between tile drainage sites and control sites. The trend in our data is opposite of what we would expect from a contaminant increasing susceptibility to Ranavirus. Another possibility is differences in metamorphosis. Infection has been found to occur in the summer seasons when the temperature is warmer, and most frogs are undergoing metamorphosis ("Ranavirus," 2018). Collection of individuals was done no more than a week apart, but the northern leopard frog undergoes metamorphosis in three to six months so a week could be significant (Amiotte, 2013). Frogs in metamorphosis and juveniles just finishing metamorphosis are found to be more suspectable to Ranavirus ("Ranavirus," 2018). We did not record the age of frogs in this experiment. This could be other reasonreasonhy we see the difference in Ranavirus-positive individuals between tile drainage and control sites.

Additional Collected Data

The mass of northern leopard frogs typically ranges from 16 to 80 grams and snout-vent length ranges from 2 to 11 centimeters depending on the stage of development ("Northern Leopard Frog", n.d.). The average values of our collected individuals fell within these ranges, but our sample population included smaller individuals. Sixteen of our twenty-one frogs weighed less than the typical lower range of 16 grams. Interestingly, all the individuals from Pettigrew, Buffalo, and Habeger were smaller than 16 grams. Yet, there was no significant difference between tile and control sites. The size of northern leopard frogs in this study falls in the range of the reported value, whether from a tile drainage or control site. According to the data, being in a wetland with direct tile drainage has not significantly decreased the mass of northern leopard frogs compared to control sites. This is good news, as a worry about being in a contaminated environment could be a reduced ability to thrive or grow.

Future Work

Further research is required to understand the effects of tile drainage on wildlife in Eastern South Dakota. This research is valuable to understand distribution of Ranavirus in South Dakota wetlands and show livers of individuals in tile drainage sites have significantly more selenium than those of frogs in control sites. If I were able to adjust methods and repeat this experiment, I would adjust the duration of capture and location. Although extensive fieldwork was conducted to obtain the individuals collected, there is room for improvement in our methods due to our inability to collect the goal number of individuals. Ideally, I would have more funding to pay for gas to allow for additional travel days to Eastern South Dakota wetlands. The closest wetland to the University of South Dakota was Petri 1, 68.5 miles away, while the farther was Habeger, 115 miles away. Typically, I would go to several wetlands each travel day and spend the entire day out, until we lose sunlight. Additional funding would allow for repeat days in wetlands I struggled to find frogs. Additionally, in the future, I do not suggest using Volker Wetland as an experimental tile drainage site. Because I was unable to collect live individuals, I did not find this location reliable enough for future wetlands. Fortunately, and unfortunately, many other waterfowl production areas have a direct agricultural runoff to replace Volker with. I may suggest swapping Petri 1 in the future, due to only one individual being collected from here.

Interesting future projects related to this study include studying other wildlife in the wetlands with similar methods, such as the False Map Turtle (Graptemys pseudogeographica). Additionally combining the false map turtle and the northern leopard frog in a single study of bioaccumulation or disease would be informative to add another level to bioaccumulation in a controlled environment. This is due to the predator-prey relationship between the false map turtle and the northern leopard frog. Future researchers could also collect individuals and run a study in the laboratory, gradually exposing northern leopard frogs to selenium, Ranavirus, or some combination of both and looking at bioaccumulation and disease.

Implications

Implications of this research provide further evidence of contamination of wetlands by agricultural runoff. Although tile drainage systems are helpful for agricultural practices, installation and methods of drainage release heavy metals and pesticides in the wetlands of Eastern South Dakota (Wright & Sands, 2001). These are the same wetlands that house much biodiversity and wildlife. This study also has major implications for hunters utilizing Eastern South Dakota wetlands for waterfowl harvest.

If we find that a proximal species is being negatively affected by selenium bioaccumulation in the form of reduced immunocompetence, humans that are consuming game from areas with high selenium levels could also be bioaccumulating selenium and compromising their immune health. Waterfowl and other avian game species rely on these wetlands for water and food and are exposed to these elevated levels of selenium which they can subsequently pass on to humans who hunt in these areas. Thus, we must understand how selenium is affecting native wildlife in these areas and how it can subsequently affect human health.

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