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Selenium Burdens in Painted Turtles (Chrysemys picta)

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Selenium Burdens in Painted Turtles (*Chrysemys picta)*

by
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ABSTRACT

Burdens of Selenium in Painted Turtles (*Chrysemys picta)*

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Tile drain systems are a critical advancement in agriculture that move excess water from crop fields to streams, ditches, and wetlands. Selenium is a necessary mineral but is considered toxic at high levels. Previous research indicates that wetlands with tile drains are at a higher risk for elevated selenium concentrations. Selenium enters the aquatic ecosystem via these tile systems and us taken up by invertebrates and continues to bioaccumulate mainly via direct transfer in higher trophic level taxa such as fish, amphibians, reptiles, birds, and mammals. For this study, painted turtles (*Chrysemys picta)* served as a model organism to detect selenium levels in higher trophic level organisms. I hypothesized that tile site turtles' blood level selenium concentration would be higher compared to the control site turtles. During the summer of 2019, I collected water and turtle blood samples from four control (relatively pristine wetlands with no direct input of contaminants) and four tile wetland (wetlands with a direct input of contaminants from a tile drain) sites. Turtle blood samples were collected from fifteen control and nineteen tile sites (n=34). There was a positive correlation between water and blood selenium concentrations. I estimated a >99.99% probability that tile wetlands will have greater water selenium concentrations and a 99.8% probability that tile site turtles will have greater selenium concentrations in their blood compare to control site turtles. The average blood selenium concentration for tile turtles was 3.56 μg/L while control site turtles' average was 0.50 μg/L. Finally, there was also a positive correlation between turtle mass and blood selenium concentration. These elevated levels of selenium in tile wetlands are of great concern as they are potentially threatening the health of these wetland ecosystems and these turtles specifically.

Keywords: selenium, tile drains, painted turtles, wetland, bioaccumulation

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Globally, reptile and amphibian populations have been declining at an alarming rate. As of the year 2020, 2,442 amphibian species and 1,458 reptile species were considered threatened (ICUN, 2020). These population declines were first thought to be within natural fluctuations; however, anthropogenic activity is likely primarily responsible for declines over time (Tinke, 1979; Todd et. al., 2010). Habitat loss and degradation, introduction of invasive species, pollution, disease and parasitism, unsustainable use, and global climate change are the leading anthropogenic activities leading to reptile and amphibian population declines (Todd et al., 2010). Habitat loss and degradation are especially concerning as approximately 75% of Earth's land surface has been altered by human activity and is expected to be one of the leading causes of biodiversity loss over the next century (Cuarón, 2000; Hobbs et al., 2009).

Agricultural and pastoral systems are a specific type of land conversion that is especially concerning in eastern South Dakota (Johnston, 2013). A recent review concluded that agricultural and pastoral systems were a significant cause of species loss, and that habitat conversion to agriculture and livestock grazing fields most often lead to herpetofauna losses (Newbold et al., 2015; Sala et al., 2000; Thompson et al., 2016). Although some species have been able to adapt to these agriculturally altered landscapes, most now face chemical pressures from exposure to agrochemicals (Gibbs et al., 2009). One area that has been heavily converted into agricultural and livestock grazing fields is the Prairie Pothole Region (PPR) (Johnston, 2013).

The PPR spans from Alberta, Canada to central Iowa and is the result of Wisconsin-aged glaciers 12,000 years ago (Woo et al., 2019). These glaciers created depressions in the land that lead to the formation of wetland basins scattered throughout the PPR (Evelsizer & Skopec, 2018;). While these glaciers left behind fruitful soils, these wetland basins are problematic for the agricultural community. Wetlands keep the soil saturated and therefore prevent proper aeration for root development (Wright & Sands, 2001). Additionally, wetlands are physical barriers that make it difficult for fencing, moving machinery and infrastructure (Johnston, 2013). The agricultural industry turned to tile drainage systems as a solution to these problems. Tile drains are subsurface corrugated polyethylene pipes that are used to lower the water table below crop fields and drain excess water from root systems (McKenna et. al 2017). The efficiency of tile drains has made them a popular tool as 85% of croplands in the PPR utilize tile drains. Tile drains are installed either via digging a trench or plowing the ground and installing the pipes up to five feet below the surface (Wright & Sands, 2001).

Although there are many agricultural and economic benefits to the implementation of tile drains, there are also severe ecological consequences. The conversion of wetlands into tile drain catchment is one of the factors contributing to the 50-90% of wetland degradation that happens annually (Dahl, 2014). The physical disruption of the land due to installation increases the loading of naturally occurring elements and minerals to be deposited directly into wetland ecosystems. As tile drains move excess water from agricultural fields, they drain into wetlands, streams, rivers or ditches and bring heavy metals, pesticides and neonicotinoids into wetland ecosystems (Werner et al., 2016). One particular contaminant of concern for South Dakota wetlands is selenium. Selenium is an element that naturally occurs within the environment particularly in shale, coal and phosphate deposits, but can be released in elevated concentrations due to human activities such as the implementation of tile drainage systems (Massé et al., 2016). In South Dakota, tile drain permits are used almost exclusively in the eastern part of the state (Finocchiaro, R, 2014) (Figure 1).

Selenium is often referred to as a ‘necessary toxin’ as trace levels are a necessary nutrient, but excessive levels can be toxic (Fan et al., 2002). Selenium plays an important role in antioxidant defenses; however, excessive levels of selenium can lead to the formation of radical oxygen species, resulting in oxidative stress with increased exposure (Janz et al., 2010). Oviparous organisms appear to be most sensitive to selenium toxicity. Reptiles primarily acquire excessive levels of selenium from their prey. Reptiles with elevated levels of selenium experienced histopathological abnormalities, lower hatching success and rates and maternal transfer (Janz et al., 2010).

Due to the naturally high concentrations of selenium in South Dakota soil and the disruption of the soil via tile drain installation, wetlands acting as tile drain catchments are at a higher risk for elevated selenium concentrations (Henry et al., 2020). A report by the United States Fish and Wildlife Service Environment Programs measured selenium concentrations in South Dakota wetland water, sediment, aquatic invertebrates, mallard eggs and various birds; however, no measurements were taken on reptiles. Selenium was found in 95% of tile wetland sites and tile outfall sites selenium concentrations often exceed South Dakota’s current chronic aquatic life criterion (Natural Resources Division, 2005). Selenium enters the aquatic ecosystems as primary producers incorporate inorganic selenium into their tissues where it is then consumed by invertebrates (Beaman, 2016; Gallego-gallegos et al., 2013; Ranz et al., 2011). From there, selenium can bioaccumulate mainly via dietary transfer in higher level tropic taxa such as fish, amphibians, reptiles, birds, and mammals (Janz et al 2010; Wu, 2004).

Many South Dakota reptiles (such as skinks, snakes, and turtles) depend on wetland ecosystems for survival. Wetlands serve as some reptilian species' primary habitat which supplies them with food, suitable breeding sites, and protection from predators (EC919 South Dakota State University et al., 2009). Many of these species reside within these wetlands regardless of whether or not the wetland is pristine or serves as a tile catchment. Thus, entire populations are potentially being exposed to elevated selenium concentrations.

The purpose of this study was to quantify selenium concentrations in painted turtles found in South Dakota wetlands, and compare those concentrations between control wetlands with no input of agricultural runoff and tile drain catchment wetlands, and tile wetlands, which have a direct input of agricultural runoff from a tile drain outfall. Painted turtles are omnivorous and feed on plants, algae, aquatic invertebrates, fish, and crustaceans, and thus can bioaccumulate selenium from a myriad of different sources and dietary pathways (Sherma, 2009; EC919 South Dakota State University et al., 2009.; Wu, 2004). Additionally, painted turtles can be found throughout South Dakota wetlands, rivers and lakes (Bandas, 2003). The distribution, habits and diet of painted turtles make them excellent model organisms to investigate the influence of tile drains on wetland ecosystems. I hypothesize wetlands that serve as tile catchments will have significantly higher levels of selenium concentrations in their ecosystems.

MATERIALS AND METHODS

Study Population & Sample Collection

In this study, painted turtles were collected from eight different Waterfowl Production Areas in Eastern South Dakota. Petri II, Habeger, Volker II, and Acheson are tile wetlands while Beck, Buffalo Lake, Pettigrew, and Lost Lake are control wetlands (Figure 2). Thirty-four painted turtles were collected between June and July of 2019 using partially submerged hoop nets and funnel traps that were baited with salted pig liver. Painted turtles of all ages and sexes were collected. Traps were set along vegetation for 24-48 hours and GPS coordinates were recorded. The traps were carefully set to ensure that any captured organisms would not fully submerge the net and caused accidental drownings; however, it was critical to ensure the bait was detectable in the water. If any unwanted organisms were captured a note was made and they were released immediately. The traps were checked and removed after the above interval and all painted turtles were processed on dry land to ensure accurate data collection. All data collection was done under an approved Institutional Animal and Use Committee protocol.

Blood samples were collected via the dorsal coccygeal vein using a comfort point 1 mL insulin syringe. 20-30 cc of blood were collected from adult individuals while ~10 cc of blood was taken juvenile individuals (for this study, juveniles were classified as an individual under 10 cm). All turtles were given a specific ID number using the numerical coding system for hard shell turtles (Ernest et al., 1974) (Figure 3). Captured individual's carapace length and width, plastron length, and mass were recorded, for recaptured individuals only additional blood samples were collected. Water samples were collected using 1 L glass amber bottles. The water was collected from undisturbed water 15 centimeters deep to the surface and nearer to the tile drain if applicable. Water samples were collected once in June and once in July. Blood, tissue, and water samples were stored on ice while in the field but were stored in a standard freezer at -10 degrees Celsius in the laboratory. Blood and water samples were sent to the University of Nebraska-Lincoln Water Sciences Laboratory for Inductively Coupled Plasma Mass Spectroscopy to estimate selenium content analysis. A Bayesian generalized linear model was used to analyze the differences in selenium content in painted turtle blood samples between tile and control wetlands. Using RStudio (with R v. 1.1.456), a Bayesian Generalized Linear Model with Site Type as a predictor variable was used to evaluate the probability of tile wetland water having a greater selenium concentration compared to control wetlands. This same analysis was used to evaluate the probability of tile wetland turtles having a great selenium blood concentration compared to a control wetland turtle.

RESULTS

Concentrations of selenium in the water ranged from 0.055 μg/L to 1.63 μg/L with the highest concentrations in tile sites and the lowest in control sites. Blood selenium concentrations in painted turtles ranged from 0.112 μg/L to 64 μg/L with the highest levels of selenium being in tile turtle blood (Table 1).

Tile wetland sites were estimated to have a >99.99% probability of having greater water selenium concentrations compared to control sites (Figure 4). On average, tile wetlands had higher levels of selenium in their water. The average water selenium concentration for tile wetlands was 0.7 μg/L and the average control selenium concentration was 0.1 μg/L. These findings support the hypothesis that subsurface tile drains are bringing excessive levels of selenium into wetland ecosystems. There was a 99.8% probability that tile wetland turtles will have a greater blood selenium concentration compared to control site turtles' blood (Figure 5). The average selenium concentration for tile site turtles was 4.5 μg/L while control site turtles was 0.6 μg/L. The positive correlation between water selenium concentrations and turtle blood selenium concentrations demonstrates how the excessive levels of selenium in the wetland ecosystems are being up taken by painted turtles (Figure 6). Additionally, there was a positive relationship between turtle mass and blood selenium concentrations (Figure 7). Mean selenium concentrations between turtle blood and water are compared in Figure 8 and summarized in Table 1.

DISCUSSION

This study confirms previous work that tile drains are linked to increased selenium concentrations in South Dakota wetland ecosystems (Henry et al., 2020). On average, tile wetlands measured a 6x higher water selenium concentration compared to control wetlands (Figure 4). Previous studies examined selenium levels in invertebrates, birds and amphibians, but this is the first study specifically looking at turtles in the Prairie Pothole Region. The elevated levels of water selenium concentrations in these wetlands corresponds to higher levels in painted turtle blood (on average 7x greater concentrations) (Figure 5).

Estimated selenium levels in tile site water and blood from turtles at tile sites were higher than those at control wetland sites. The disruptive nature of tile drain installation followed by drainage into wetlands is likely responsible for the elevated selenium concentrations we found in tile water and painted turtle blood. Selenium naturally exists in the soil; therefore, we were not surprised by trace levels in control wetlands. Subsurface pipes are collecting excess water from agricultural fields and draining directly into wetlands or ditches (Woo et al., 2019) and therefore could be accounting for increased concentrations in these wetlands. My data and previous work in the Kerby laboratory supports the idea that tile drains are primarily responsible for increased selenium levels in wetland ecosystems.

This study and previous work demonstrate how selenium is bioaccumulating in tile wetland ecosystems. Selenium bioaccumulation occurs primarily through diet rather than exposure via water (Chapman et al., 2010). Tile drains bring selenium from the soil into the water column where aquatic plants transform and enrich the selenium. Phytoplankton and periphyton ingest selenium from feeding on aquatic plants. Selenium then enters macroinvertebrates that serves as food sources for lower level trophic organisms. Selenium finally reaches top level predators as they feed on the lower trophic organisms (Beaman, 2016). Our study and previous work concluded that tile wetlands have significantly higher selenium concentrations in their water column (Henry et al., 2020). One study determined that invertebrates on average had two times greater selenium concentrations compared to control and runoff invertebrates. Selenium concentrations in tile invertebrates were high enough to cause reproductive impairment in birds (Henry et al, 2020). The increased levels of selenium in tile water columns and invertebrates correlates to selenium bioaccumulation in painted turtles, a top-level predator in wetland ecosystems. Juvenile painted turtle primarily feed on invertebrates while adults use aquatic plants as their main diet (Sherma, 2019). Painted turtles therefore are likely bioaccumulating selenium throughout various life stages.

There are several reasons why this increase in selenium is of grave concern. Oviparous organisms are highly sensitive to selenium exposure and bioaccumulation. Selenium toxicity occurs primarily through maternal transfer in to eggs that can lead to embryotoxicity and teratogenicity in egg laying vertebrates (Chapman, 2010). A study examined the toxicokinetics of selenium in slider turtles (*Trachemys scripta)* in a controlled lab setting. Slider turtles were fed varying amounts of Seleno- L- methionine as it readily bioaccumulates throughout the food web. The study concluded that the highest concentration of selenium accumulation in the kidneys followed by muscles, blood, and finally in the carapace then scuts (Smith et al., 2016). Additional studies indicate maternal selenium transfer in snakes, lizards, alligators, and turtles. Turtles and alligators passed 7.5 mg Se/Kg dry weight to their eggs while snakes ~22 mg Se/Kg dry weight to their eggs. Snakes with high exposure to selenium also exhibited abnormally high respiration rates in addition to decreased hatching success. Lizards transferred 33% of their excessive selenium into their yolk follicles (Janz et al., 2010). To my knowledge, no study has examined maternal transfer of selenium in painted turtles, but given the data above this seems an important concern.

While this study focuses specifically on painted turtles, other non-target species may be in danger of selenium toxicity. Within South Dakota, five turtle species out of the seven native species are considered endangered, threatened or rare by the South Dakota Department of Game, Fish & Parks. Streams, lakes, rivers and wetlands are home to six out of seven turtle species in South Dakota; however, these habitats are being threatened due to South Dakota's agricultural expansion (Sherma, 2009). Blanding's turtle, snapping turtles and false map turtles also inhabit South Dakota wetlands and are likely experiencing the same threats of painted turtles regarding selenium exposure.

The introduction of excessive levels of selenium in turtle habitats is adding a new stressor. Specifically, painted turtles are constantly having to adapt the addition of selenium being a new stressor. Painted turtles feed, reproduce and spend the majority of their life in these now contaminated waters (Cagle, 2018). This study is a proactive measure to assess selenium concentrations in wetland ecosystems specifically looking at painted turtles. Being proactive is essential as these habitat changes and contaminants may be having serious implications before the scientific community is even aware. Turtles serve as excellent wetland ecosystem level indicator as they are top-level predators and are highly sensitive to environmental changes (Sherma, 2009).

Worldwide, reptile species are being threatened. Habitat loss and degradation, introduction of invasive species, environmental pollution, unsustainable use and global climate change are the main contributors to the decline of amphibian and reptile populations (Todd et al., 2010). There have been extensive conservation efforts focused on amphibians; however, more efforts are need for reptile conservation. Reptile conservation is vital as these species serve important roles within their specific ecosystems. They can serve as predators or prey within their respective food webs and some even serve as keystone species. Their interactions within the environment are important as their activities alter the environment that later benefit other organisms. Some reptiles even serve as pollinators and consume rodents and pests that ultimately benefit agricultural activities. Thus, understanding ecotoxicological impacts of anthropogenic modifications to the landscape, such as the implementation of tile drainage systems, is crucial to conserving global native reptile diversity.

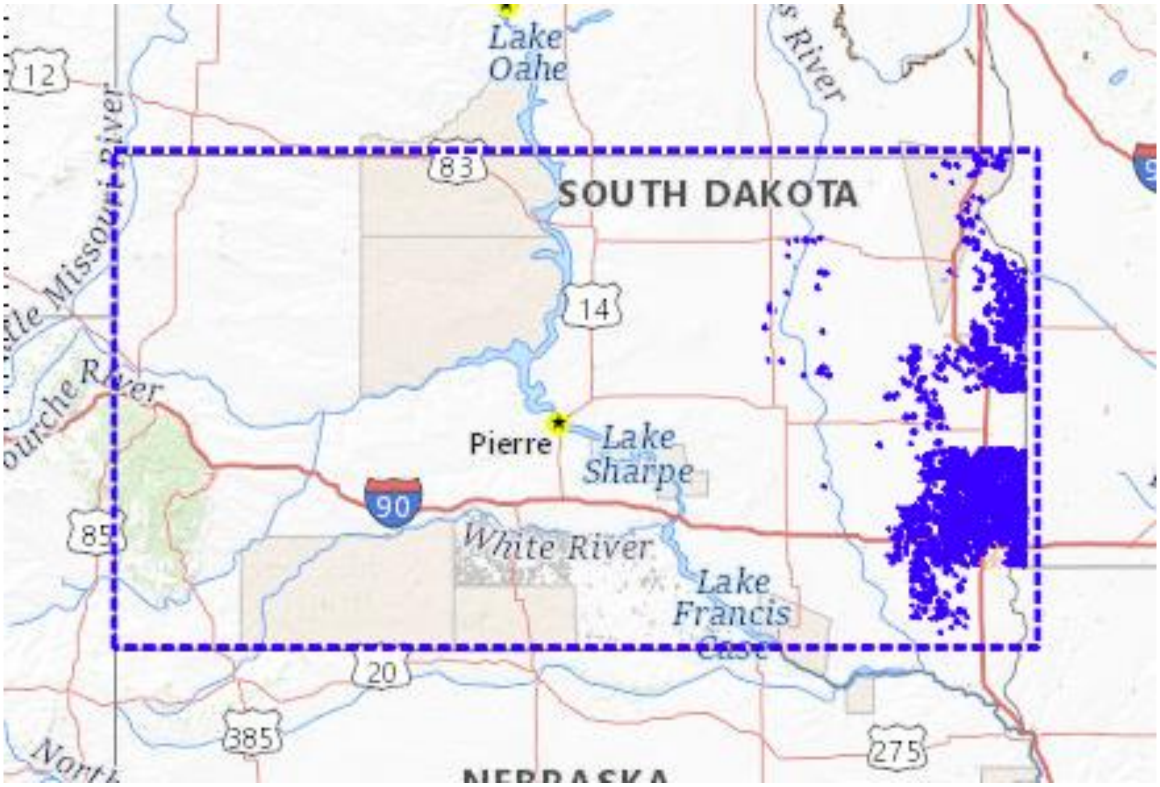

Figure 1: Distribution of tile drain permit sites throughout South Dakota. This map may have gaps due to some counties not requiring tile drain permits. Blue triangles represent a tile drain permit. (Finocchiaro, RG, 2014).

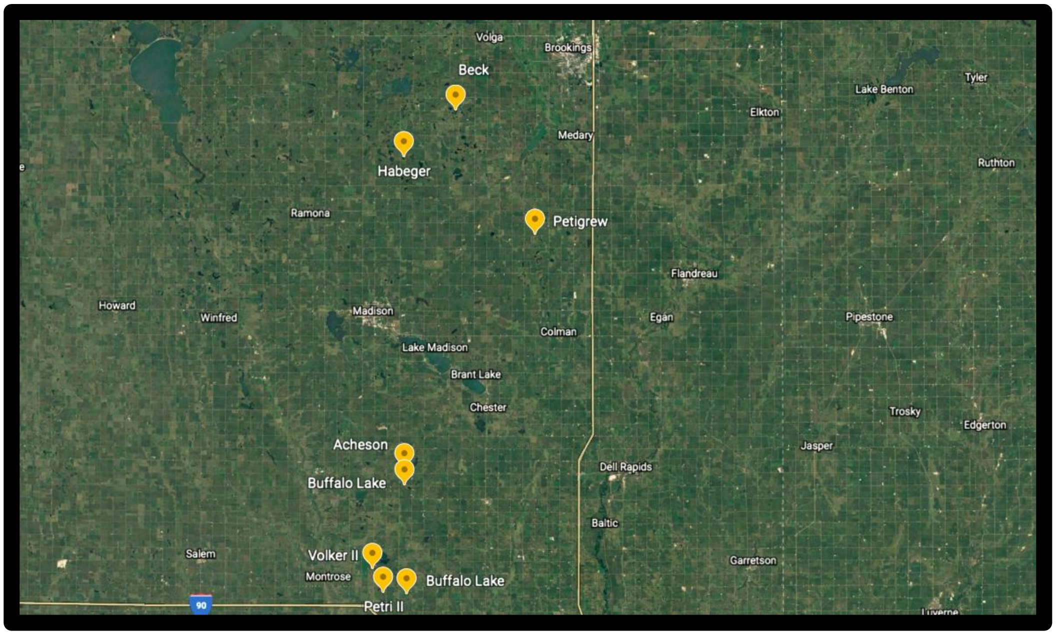

Figure. 2: Location of Waterfowl Production Areas in Eastern South Dakota. Control sites: Beck, Buffalo Lake, Pettigrew & Lost Lake. Tile sites: Petri II, Habeger, Volker II & Acheson.

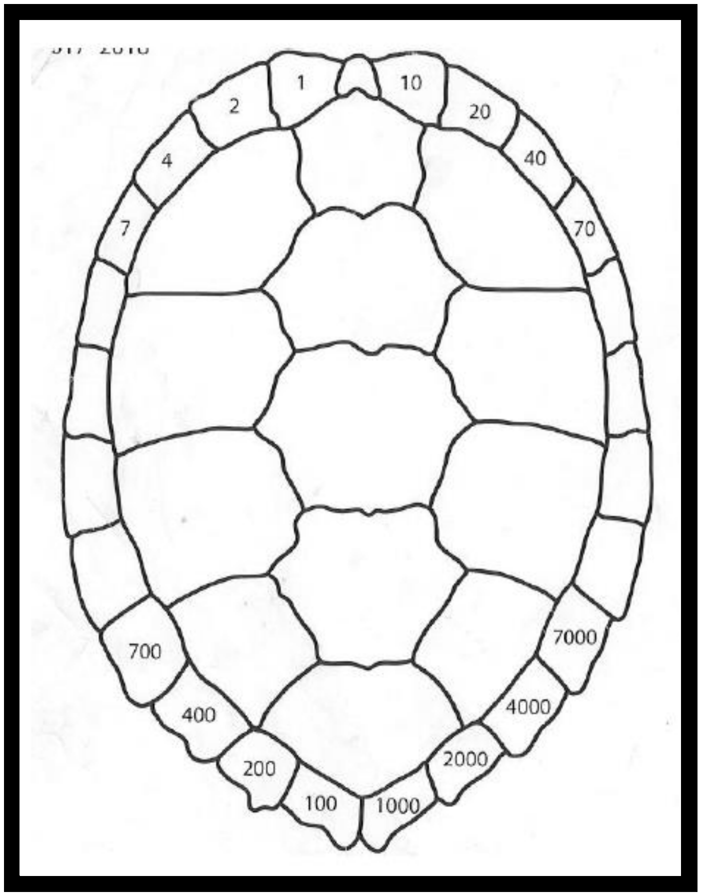

Figure. 3: Diagram of the numerical coding system for hard shell turtles used to assign individual identical numbers to capture individuals (Ernst et al., 1974).

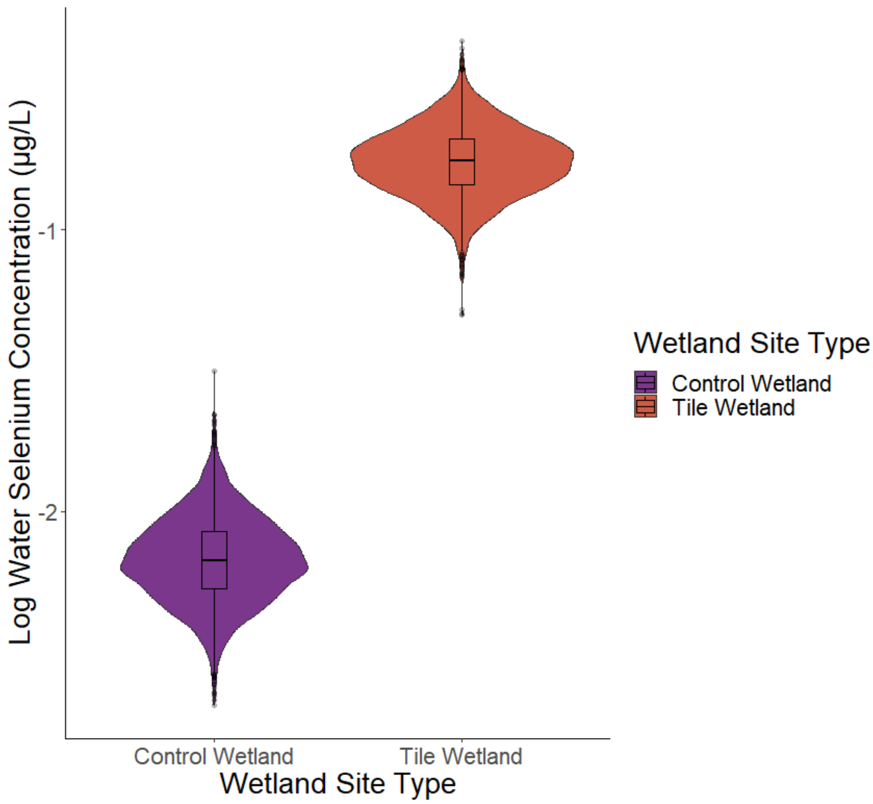

Figure 4: Violin plot comparing log water selenium concentration (µg/L) by site type. There is a >99.99% probability that tile wetland sites will have greater selenium concentrations.

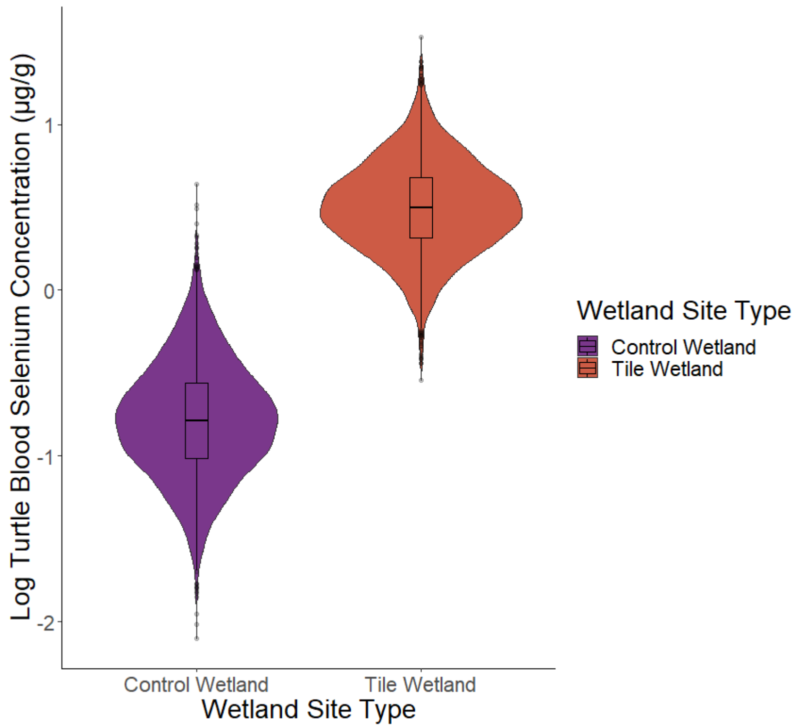

Figure 5: Violin plot comparing log turtle blood selenium concentration (μg/g) by site type. There is a 99.8% probability that tile wetland sites will have turtles with greater selenium blood concentrations.

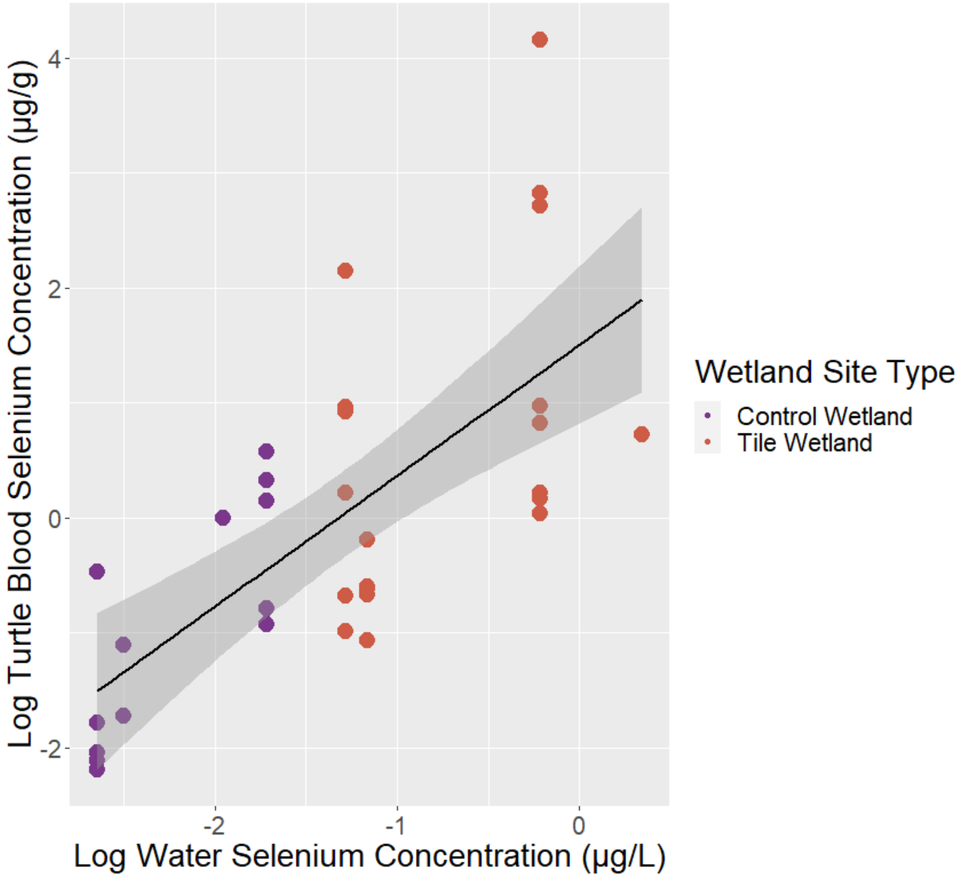

Figure 6: Regression plot comparing log water to log turtle blood selenium concentrations. The estimated slope is 2.97 µg/g with 95% confidence interval around estimated relationship (solid line).

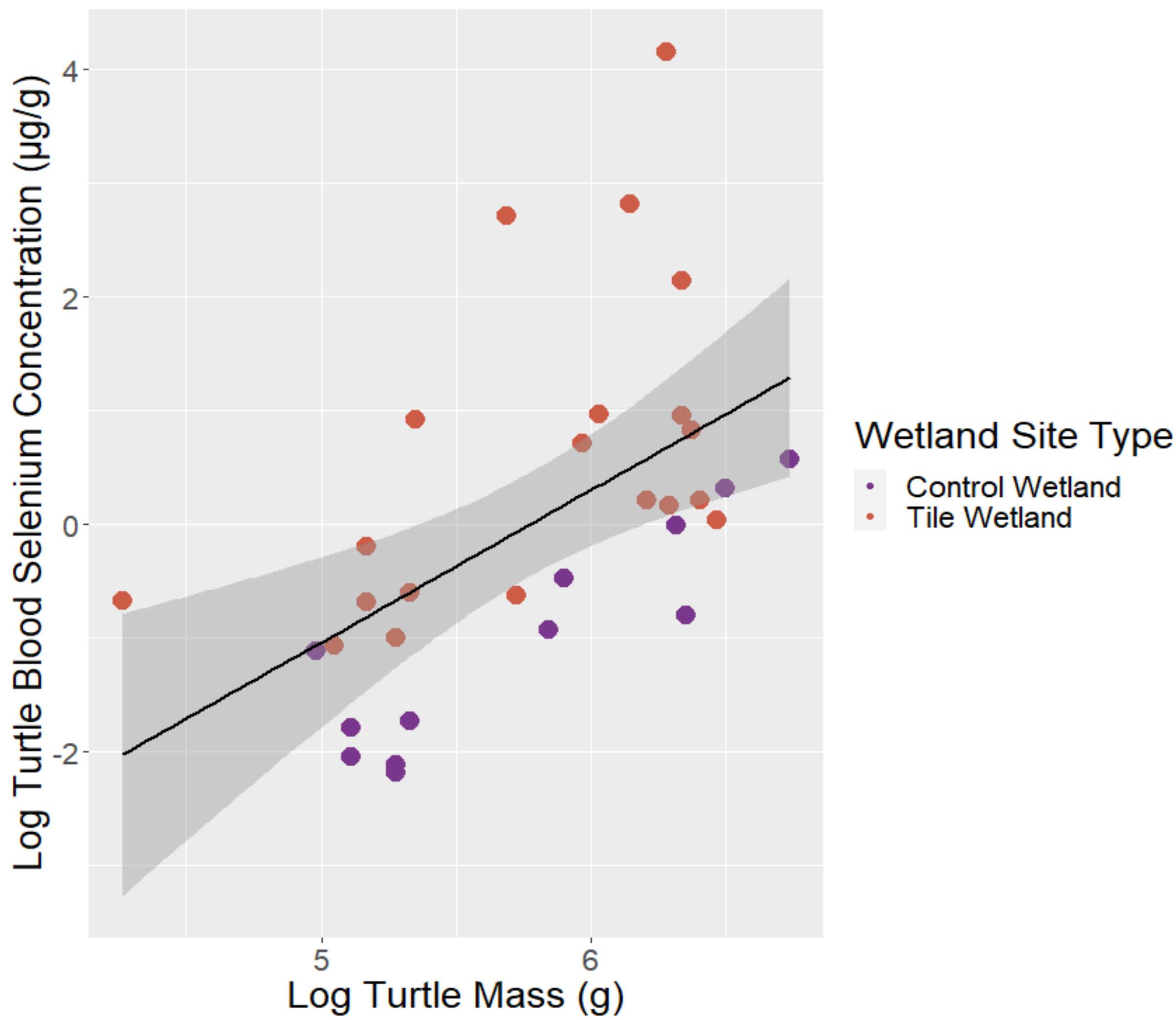

Figure 7: Regression plot comparing log turtle mass to log turtle blood selenium concentration. The estimate slope is 3.25 µg/g with 95% confidence intervals around estimated relationship (solid line).

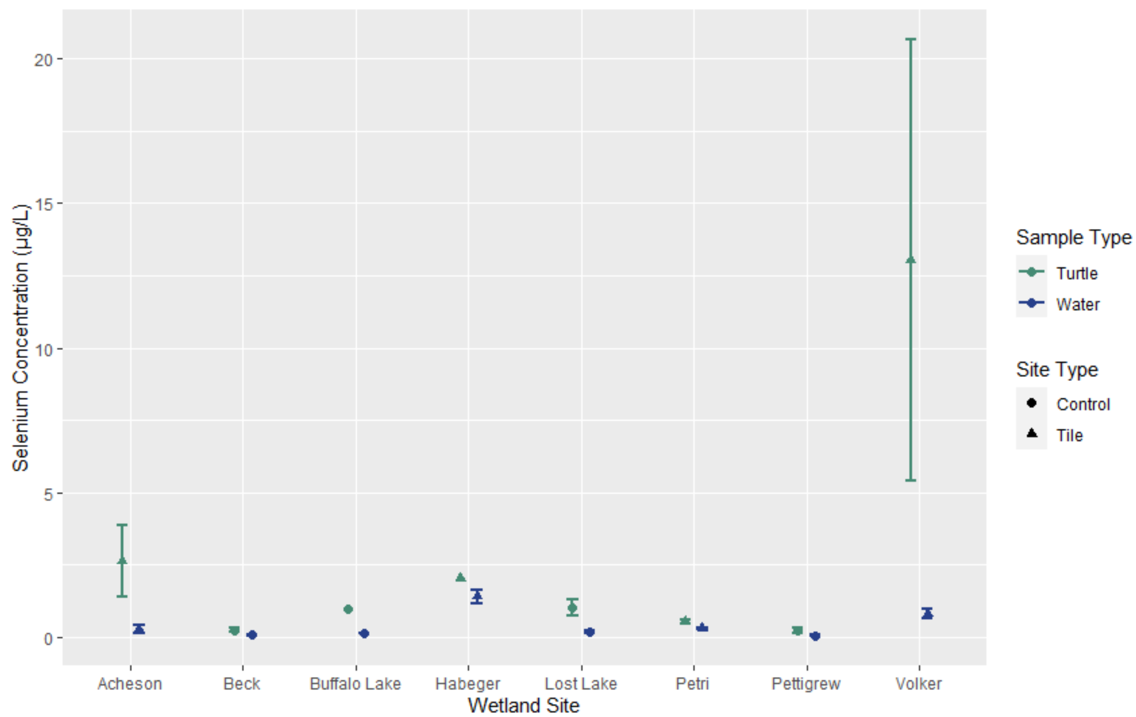

Figure 8: Dot plots comparing mean selenium concentrations between turtle blood and water for each wetland with ±1 standard error bars.

Table 1: Estimates of selenium concentrations by each sampled site

Site	Site Type	Average Blood [Se] (μg/L)	Average Water [Se] (μg/L)
Beck	Control	0.25	0.082
Pettigrew	Control	0.19	0.071
Buffalo Lake	Control	0.99	0.14
Lost Lake	Control	1.03	0.18
Acheson	Tile	2.25	0.28
Petri	Tile	0.46	0.31
Volker II	Tile	13.04	0.81
Habeger	Tile	2.05	1.41

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