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# Monitoring Selenium Bioaccumulation In False Map Turtles (Graptemys pseudogeographica)

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by

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# ABSTRACT

Monitoring Selenium Bioaccumulation In False Map Turtles (*Graptemys pseudogeographica*)

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Selenium is an element that becomes bioactivated in aquatic environments. Selenium bioaccumulation may threaten a South Dakota state-threatened species, the False Map Turtle (*Graptemys pseudogeographica*). Invertebrate filter feeder species like zebra mussels (*Dreissena polymorpha*) uptake selenium via exposure. Previous laboratory work has found that False Map Turtles consume large amounts of zebra mussels throughout the summer, leading to selenium bioaccumulation from selenium transfer from prey to predator. A myriad of adverse effects, like selenosis, reproductive infertility, and death, are associated with high selenium concentrations. Sampling was completed on False Map Turtles in the summer of 2022. Samples were taken once a month from July to September. A blood sample and several morphometric measurements were collected from each turtle. Data were collected from 15 male and 38 female False Map Turtles. The average selenium concentration present during each month appeared to peak in July with an average concentration of 0.94 ug/g and a maximum concentration of 6.24 ug/g. The relationship between sex and the average selenium concentration per month showed relatively equal concentrations among males and females, with similar concentrations during August and September. Yet, during the peak month of July, male turtles had a much higher selenium concentration (1.37 ug/g) than their female counterparts (0.73 ug/g). Further work needs to be done to determine why this difference exists. Differences in overall size might alter prey selection, where males are more likely to consume the smaller zebra mussels. Additionally, detoxification systems in the turtles themselves might differ between the sexes. Regardless, these findings highlight the importance of understanding the role of invasive zebra mussels in contaminant distribution in aquatic ecosystems.

*Key Words: Selenium, False Map Turtles, Selenosis, Selenomethionine*

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# Introduction

South Dakota's ecosystems are facing a negative impact from chemical pollution. Tong et al. (2022) review nine environmental thresholds humans have crossed and their associated health risks to ecosystems. One of these thresholds that is of concern in South Dakota is chemical pollution [1]. Chemical pollution can include naturally occurring elements as well as synthesized compounds. Selenium is a naturally occurring element in both the environment and in all species of animals. In trace amounts, selenium is an essential mineral in living species. However, at high concentrations, selenium can become toxic. Selenium accumulates in many living species via their diet as D,L-selenomethionine or sodium selenite [2]. Thus, selenium is easily moved and accumulated through the food web, with the highest concentrations occurring in top-tier trophic-level predators, such as turtles. When selenium is bioaccumulated in large quantities, it can cause a myriad of adverse health effects in all organisms.

The ability of selenium to become bioactive in aquatic environments is of great concern in South Dakota. South Dakota is known to have naturally high concentrations of selenium in soils due to glaciation during the Pleistocene Epoch [3]. The Pleistocene Epoch caused large selenium deposits and other elements to be left on South Dakota's topsoil. When selenium is bound in the soil, it is relatively harmless. However, it is easily mobilized into the aquatic environments.

The state of South Dakota is among the top 10 states in acres of wheat and soybeans planted by the farming and agricultural industry [4]. Many farmers often utilize agricultural irrigation practices and tile-drain systems to combat the wet and dry conditions produced by South Dakota's summer months. The installation of these draining tiles can lead to untapped deep deposits of selenium being released and bioactivated into the environment. This can lead to elevated selenium levels in wetlands and other aquatic environments [5]. Many bodies of water have become toxic due to high concentrations of selenium [6]. The target area for this study is Lewis and Clark Lake, the most downstream reservoir of the Missouri River, which was confirmed to have elevated selenium levels by researchers in 2010 [7].

One of pathways that bioactivated selenium can move throughout the food web begins with an invertebrate species called Zebra mussels (*Dreissena polymorpha*). Zebra mussels incorporate selenium into their bloodstream and the proteins of their tissues when they are consumed directly through the process of filter feeding. Zebra mussels were first reported in South Dakota in Lewis and Clark Lake in 2015 [8]. Work done in our laboratory has demonstrated that they have since become one of the food sources for False Map Turtles (*Graptemys pseudogeographica*) [9]. Female False Map Turtles have specifically been found to consume large amounts of mollusks [10]. Females have a greater propensity to consume mollusks like zebra mussels because of their larger head and body size than male False Map Turtles. Male turtles still have the ability to consume mollusks, but size disparity may alter prey selection quantity. This disparity in size may lead to a greater prevalence of selenium in female False Map Turtles since they are likely consuming larger quantities via their prey selection. One of the secondary goals of this research is to investigate this relationship between size and selenium concentration.

False Map Turtles are not the only species affected by high selenium levels in aquatic environments. A study was conducted on mallards to determine the effects of selenium exposure in diets such as D, L-selenomethionine, and sodium selenite. The first part of the study focused on the reproductive effects of selenium. The study's findings indicated a 40-44% decrease in hatchling survival when diet items consisted of 10 ug/g D, L-selenomethionine, or 25 ug/g of sodium selenite. 13.1% of surviving hatchlings suffered malformations from D, L-selenomethionine, and 3.6-4.2% suffered malformations from sodium selenite dosed food items [11]. This indicates that selenium exposure to D, L-selenomethionine, or sodium selenate has led to concerning mortality rates in offspring and decreased overall health of offspring. The study also looked at the effects of selenium on the development of day-old and adult mallard ducklings. This study indicated that day-old ducklings' growth decreased when 20 ug/g of D, L-selenomethionine, or sodium selenate was consumed for six weeks. Male adult mallards began to accumulate selenium in their liver and experienced adverse effects when consumed for 14 weeks at 32 ug as D, L-selenomethionine [11]. These findings indicate that selenium bioaccumulation significantly impacts the survival and development of mallard offspring. The results of this research study may give insight into the effects of selenium exposure on other species like turtles.

Selenium's short impact on individual organisms raises concerns about the long-lasting effects on the survival of entire species and aquatic ecosystems. When selenium enters aquatic environments, it can take years to leave these environments [12]. Selenium can be recycled from sediment into organisms or vice versa for large amounts of time, causing selenium to be trapped in these environments. Even when selenium is alleviated from the water source itself, it persists in the inhabitants of the aquatic environment as it progresses through the food chain to top-tier trophic-level predators. This means that organisms like aquatic birds and turtles may experience high amounts of selenium exposure for extended periods. Leading to many of the negative effects associated with selenium bioaccumulation, impacting several generations of these species. Should these effects persist for extended periods of time, they may cause species like the False Map Turtle to become endangered or even extinct. Changes like this alter the balance of entire ecosystems, especially when species loss occurs in top-tier trophic predators, leading to a cascade of downstream effects that permanently change the ecosystem.

Ecosystems with the False Map Turtle may already be in jeopardy of the consequences associated with species loss. False Map Turtles have already become a state-threatened species in South Dakota. The species experienced significant habitat loss and variation during the implementation of four dams along the central portions of the Missouri River [13]. The implantation of these dams has caused lotic riverine habits once preferred by the False Map Turtle to become lentic reservoir habits that are not viable habitats for turtles [9]. Thus, a species once found all along the Missouri River in South Dakota [13] is only found in much smaller riverine portions between the reservoirs created by the dams [9]. The already alarming population decline of species makes the findings of this research even more critical. Toxic selenium levels in the turtle would only amplify the severity of the False Map Turtle population loss.

This research aims to determine the blood selenium concentrations of the South Dakota state-threatened False Map Turtle species. A secondary goal of this research is to determine if the turtle's carapace length has a significant relationship with blood selenium concentration. Investigating the relationship between selenium and the False Map Turtle is important in understanding the prevalence of selenium the False Map Turtle and its' ecosystem. Understanding this relationship will also allow for educated steps to be taken on conserving the species and its ecosystem.

## Material And Methods

To determine if selenium bioaccumulation occurs in top trophic level predators, we conducted field sampling in Lake Francis-Case and the 59-mile stretch of the Missouri National Recreational River. Sampling specifically targeted False Map Turtles. False Map Turtles were captured at each site using partially submerged, baited hoop net traps. Upon capture, the following measurements and samples were collected from each turtle: carapace length and width, plastron length, mass, age, sex, blood, tissue, and diet sample via gastric lavage. This sampling process was completed three times over the summer months, once in July, August, and September, to gather data on temporal differences in selenium concentrations. After all blood samples were collected, they were sent to the University of Nebraska Lincoln Water Quality Laboratory to be analyzed for selenium content through inductively coupled plasma mass spectrometry (IC-PMS). Samples were compared across time points to determine how selenium concentrations in top-tier trophic-level predators vary across space and time and will allow us to infer if selenium bioaccumulation is a factor in population decline.

An Analysis of Covariance (ANCOVA) tests was used to individually evaluate the influence of sex (male vs. female) and time (month) on selenium concentrations in turtles. The dependent variable, selenium concentration, was treated as continuous, while sex was treated as a categorical independent variable. Data were analyzed using R software (v 4.3.3), ensuring that assumptions of normality and homoscedasticity were met prior to analysis. A similar ANCOVA was performed to examine carapace length.

## Results

All sampling was completed during July, August, and September of 2022 (Figure 1). We captured 38 female False Map Turtles and 15 male False Map Turtles. The mass of the female False Map Turtles ranged from 120 - 1720 grams. Meanwhile, the male False Map Turtles were recorded with masses between 80 - 490 grams. The carapace length of female False Map Turtles ranged from 9.9-23.4 cm, and the male carapace length ranged from 10.2-15.4 cm. All turtles were estimated to be at least three years of age or older. These ranges in sex, mass, size, and age are aimed to be representative of the False Map Turtle population within the Missouri River.

The ANCOVA revealed no significant effect of sex on selenium concentrations ($F_{1, 50}$ = 1.820, p = 0.183), suggesting that male and female turtles have similar selenium levels when effects of time are controlled. In contrast, the time of sample collection (month) significantly affected selenium concentrations ($F(_{1, 50}$ = 7.629, p = 0.008). This indicates a substantial variation in selenium levels over different months, pointing to potential seasonal or monthly environmental influences affecting selenium bioaccumulation (Figure 2). During July, the average blood selenium concentration was 0.941 ug/g. Male False Map Turtles averaged slightly higher blood concentration levels (1.37 ug/g) than their female counterparts (0.732 ug/g). The standard deviation among the sampled population in July was ± 1.12 ug/g. The average blood selenium concentration in August was 0.383 ug/g. Male and female False Map Turtles appeared to have relatively equal blood selenium concentrations of 0.355 ug/g and 0.389 ug/g, respectively. The standard deviation for the sampled population in August was ± 0.13 ug/g. September yielded similar results between male and female False Map Turtles. The average blood selenium concentration was 0.232 ug/g. Female False Map Turtles had an average blood selenium concentration of 0.204 ug/g and male False Map Turtles had an average of 0.272 ug/g (Figure 2). The standard deviation of sampled individuals in September was similar to August (± 0.11 ug/g).

For carapace length, there was again an effect of time on measured selenium concentrations ($F(_{1, 50} = 6.280$, $p = 0.0155$). Yet when controlled for differences by month, the relationship between blood selenium concentration and carapace length was not significant ($F(_{1, 50} = 0.753$, $p = 0.3897$); Figure 3. The slope values show insignificant differences on a month-by-month basis. Thus, showing carapace length is not associated with blood selenium concentrations. Similar non-significant results were found for highly correlated measures of plastron length, carapace width, and mass signifying the size of the turtles had no effect on measurements of selenium concentrations found in their blood.

## Discussion

The results from this study show a peak average blood selenium concentration during July. The overall trend of blood selenium concentrations during the study was found to be gradually declining throughout the summer months. A similar study was done on another species of turtle and found an average blood selenium concentration of 0.27 ug/g in uncontaminated bodies of water and an average blood selenium concentration of 1.37 ug/g from turtles in bodies of water contaminated by coal combustion residues [14]. Comparing these values to the findings of this study makes it apparent that the False Map Turtles were experiencing significant selenium bioaccumulation during July. Our data reflect slightly elevated blood selenium concentrations in August and similar blood selenium concentrations in September compared to the uncontaminated blood selenium concentrations stated in the previous study (Figures 2, 3).

Elevated selenium levels during July is concerning for the conservation of an already state-threatened species. Research on other aquatic species have found that the packed cell volume (PCV) is significantly decreased when selenium levels in the blood and tissues are increased [15]. Decreased PCV leads to anemia which affects the organism’s carrying capacity for oxygen and carbon dioxide. Both of which are needed to for an organism to have healthy tissues [15]. In cases where organisms have experienced extreme selenium exposure, organ systems begin to fail, and the overall fitness of the organism is dramatically decreased [15].  Understanding the effects of selenium and the stressors it creates on organisms is essential in advocating for steps to protect this species

One potential explanation for the peak in blood selenium concentrations in July could be linked to farming tile drains and the amount of selenium dissolved in the aquatic environment. If this is the case, it raises important questions about the potential impact of these practices on the health of False Map Turtles and other aquatic species. The possibility that more selenium was drawn from the soil and pushed into the Missouri River via drainage systems in July, due to increased irrigation, is a concerning prospect that should be considered as a potential cause elevated selenium levels in the Missouri River.

The relationship between carapace length and blood selenium concentrations did not depict a significant relationship during the study. We hypothesized that female False Map Turtles would have higher selenium concentrations than males due to their size disparity. The contrast between our hypotheses and results indicates that several other factors need to be considered such as varying prey preferences and the detoxication systems in False Map Turtles.

Further research needs to be conducted on the food preference choices among male and female False Map Turtles. The prey preference of female False Map Turtles may differ from male False Map Turtles because of their size difference. Females are more likely to be consuming larger quantities of larger prey items like zebra mussels since they have large head and body sizes. In contrast, male turtles may be stuck preying on smaller prey items due to their smaller size and are likely consuming smaller amounts. This may lead to varying amounts of selenium exposure.

Considering the information already known about the difference in prey selection among male and female turtles. Our results contrast the trend that would be associated with normal feeding patterns. One possible explanation for this may be that there is a difference among the short-term detoxification systems in male and female False Map Turtles. Differences in short term detoxification may explain why our data did not depict this relationship. Little research exists on the differences in the detoxification systems of turtles. Research must be conducted to determine the effect of prey selection and detoxification systems of male and female False Map Turtles to understand why the relationship between carapace length and blood selenium concentration is insignificant.

## Limitations

One practical limitation of this research is the area in which the samples were collected. The Missouri River is such a vast body of water that the sampled population may not be representative of blood selenium concentrations of False Map Turtles for the entire Missouri River. We were still able to have a broad and relatively large sample size with these limitations. This allowed us to generalize the blood selenium concentrations for this research.

Another limitation of this research was the ability to capture an adequate number of turtles to sample each month. The original plan for this research was to begin sampling in June instead of July. During the month of June, we were unable to capture a sufficient number of turtles to create an average blood selenium concentration for that month. This led to the first sampling group to be delayed by one month.

One final limitation of this research was the delay in sample processing. During May of 2023, a final set of samples were collected. Several of the turtles captured during this month were recaptures from August and September of the previous summer. By recapturing turtles from the previous summer, an analysis could be done to compare the blood selenium concentrations from the end of the turtle feeding season in 2022 to the beginning of the next feeding season in 2023. These results would have also helped understand the turtle's ability to detoxify selenium during its brumation period during the winter months.

## Future Work

In order to fully understand the effects of selenium bioaccumulation in False Map Turtles and other aquatic organisms, several steps must be taken to understand it. One of the first and most important steps is developing a standard indicating the thresholds at which the adverse effects of selenium impact reptiles. These thresholds exist for other taxa like mammals, but more research is needed to develop and understand these effects in reptiles. By developing these standards, more steps can be taken to protect species, like the False Map Turtles, from their rapid population declines.

Research should also be conducted on the relationship between aquatic selenium concentrations and the ability of zebra mussels to incorporate selenium into their tissues and bloodstream. By investigating this relationship, thresholds can be created to determine the concentration at which selenium dissolved in an aquatic environment leads to zebra mussels bioaccumulating toxic amounts of selenium. The Environmental Protection Agency (EPA) has determined that toxic selenium exposure occurs primarily through the consumption of selenium-contaminated food items and not necessarily through selenium dissolved in water [16]. Therefore, quantifying the water selenium concentration at which zebra mussels bioaccumulate large amounts of selenium would benefit this research since zebra mussels are a primary food source of False Map Turtles. Determining the selenium value at which this occurs would also be beneficial in protecting aquatic ecosystems. Water samples could be used as an indication of the overall health of aquatic environments since a threshold would be created to understand when selenium has reached toxic levels for aquatic inhabitants.

Further investigation must be conducted to understand if selenium bioaccumulation is impacting the ability of False Map Turtle populations to recover from rapid population loss. Quantifying the thresholds at which selenium begins to impact the False Map Turtle negatively is essential in protecting this species and other aquatic organisms.

Appendices


Figure 1: A map of the sampling area along the Missouri River.

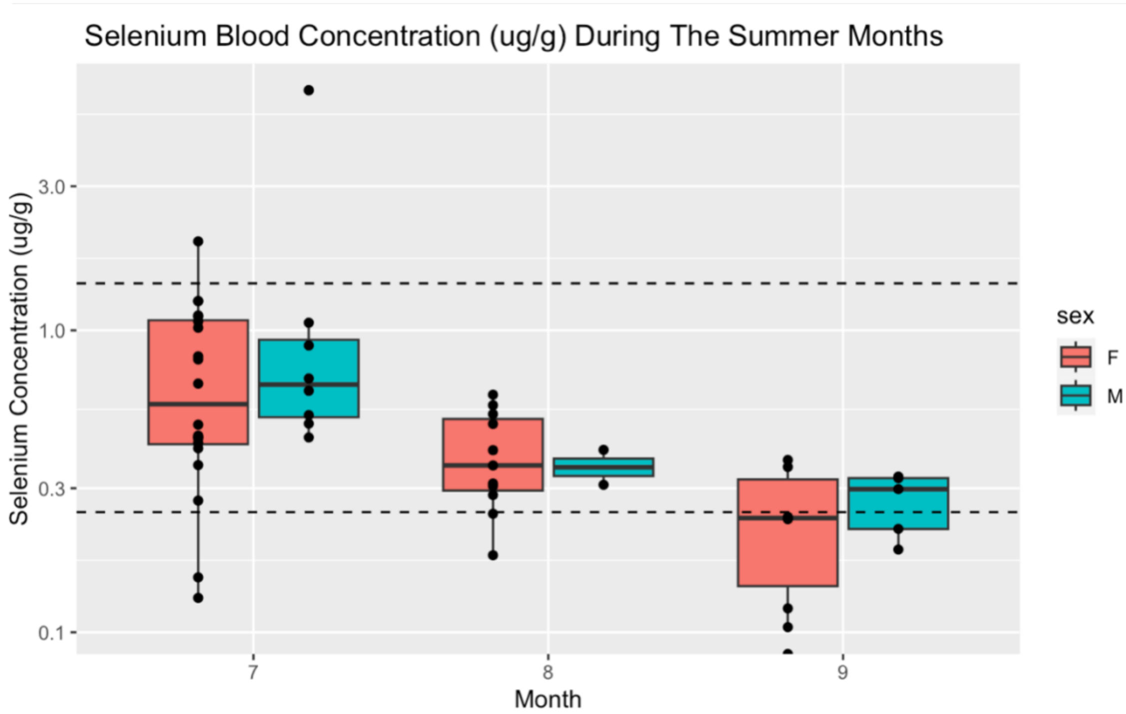


Figure 2: A graphical diagram comparing the blood selenium concentrations of male and female False Map Turtles from the months of July, August, and September. The graph also has two dashed lines representing the blood selenium concentrations from a similar study [15]. The top dashed line, at 1.37 ug/g, is the blood selenium concentrations from an area contaminated with coal contaminated residues, and the bottom dashed line, at 0.27 ug/g is the average blood selenium concentrations from an uncontaminated water source.

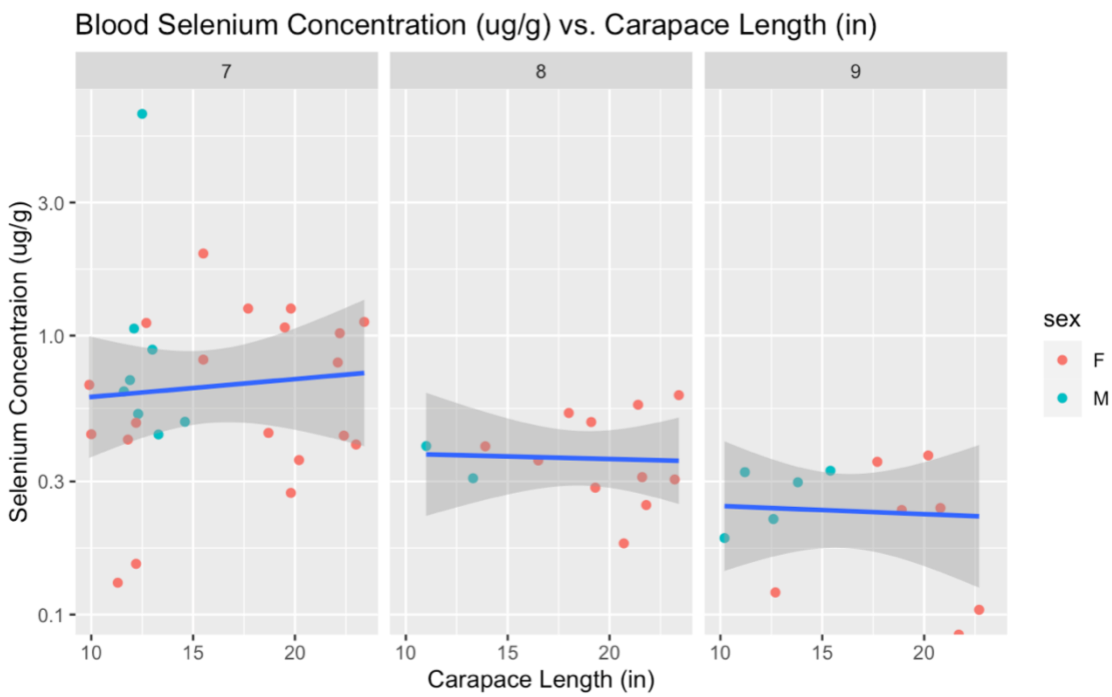


Figure 3: Diagram depicting the relationship between carapace length and blood selenium concentration of male and female False Map turtles.

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