Landscape Effects on Freshwater Turtle Heavy Metal Bioaccumulation in West Virginia Wetlands

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INTRODUCTION

Increasing anthropogenic change of natural systems due to human population growth has created detrimental effects for wetlands in the United States. Agricultural land use practices introduce contaminants that would not naturally be found in the environment, and deforestation promotes surface runoff carrying contaminated sediment (Lowrance 1998). Road density concurrently increases with development and introduces other heavy metals such as cadmium, lead, and zinc from the natural wear of tire and brake lining during daily vehicle use (Croteau et al. 2008). Some heavy metals may be found naturally in the environment in trace amounts, but if they exceed a certain threshold, deleterious effects to wildlife may occur. Species that are long-lived and have higher trophic status such as turtles are especially vulnerable to harmful bioaccumulation of these heavy metals (Sparling et al. 2010; Turnquist et al. 2011). High levels of mercury and lead in turtles have been found to reduce fertility and increase embryonic mortality (Burger 1998; Thompson et al. 2018).

The relationship between heavy metal contamination and land use practices has been studied for decades (Cooper 1993; Tong and Chen 2002; Allan 2004; Croteau et al. 2008; Kellner et al. 2015). Yet results vary by the heavy metal in question, while other system variables can affect bioavailability (Driscoll et al. 2007; Turnquist et al. 2011). One method of monitoring land use effects on wetland health is testing for the presence of heavy metals in freshwater turtles. Using non-destructive methods by collecting external tissues (e.g., blood and toenails) eliminates the negative impacts on individuals or populations (Smith et al. 2016; Benjamin et al. 2018). These external tissues have been found to be positively correlated with concentrations found in internal, more invasive tissues such as liver and muscle (Smith et al. 2016).

OBJECTIVES

The primary purpose of my research is to collect data on levels of cadmium, chromium, lead, total mercury, selenium, and zinc accumulated in freshwater turtles at a series of wetlands and streams within the Upper Deckers Creek watershed in Preston County, WV. With these data, my objectives are to quantify the bioaccumulation of heavy metals among different age-sex classes of snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*), two common species found within the Upper Deckers Creek watershed (Figure 1). I will also determine how land use practices influence heavy metal bioaccumulation in turtles. I will test the following hypotheses to achieve these objectives:

1. Increased size of turtles will result in increased heavy metal bioaccumulation.
2. Adult females will have lower concentrations of heavy metals than adult males.
3. Snapping turtles will have significantly greater heavy metal bioaccumulation than painted turtles.
4. Concentrations of heavy metals found in turtles will be significantly different based on the dominant land use type within 1 km of the wetland.

FIGURE 1. The two target species used for this present study were (from left to right) painted (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*) because of their long lifespan, high trophic position, and abundance within the Upper Deckers Creek watershed in Preston County, West Virginia. Both species have been studied in ecotoxicology literature over the past decade in the United States. (Photo by Darien Lozon.)

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METHODS

In 2019, we set ten aquatic hoop-net traps of various sizes (0.91 and 0.76 m diameter; Gulette et al. 2019; Figure 2) from April 19—May 25 (spring), July 1—31 (summer), and October 8—November 1 (fall) at 29 sites within the Upper Deckers Creek watershed. The watershed covers 7,778 ha in Preston County, West Virginia, and agricultural land use (consists of grassland, cultivated crops, hay and pasture; 23.4%) and roads (3.2%) continue to fragment the deciduous forest (60.7%) that dominates the watershed. Wetlands were either predominantly surrounded by forest (n = 13) or agricultural land (n = 13) within 1 km, while three sites had an even combination of both (Figure 3). Traps were baited with a contained half-can of sardines so bait could be smelled but not consumed, and flotation devices were in each trap to ensure access to air for turtles captured. Bait was replaced after every 24 hours to keep bait freshness consistent over the three days traps were set out at each site per season. Individual turtles were given a unique mark filed in their marginal scutes for future identification (Cagle 1939; Figure 4) and classified once as either adult male, adult female, subadult male, subadult female, or juvenile, regardless of how many times the individual was captured (Bowne et al. 2006).

Additionally, each captured individual had 3-4 hind toenails collected using dog nail clippers, and 1 mL of blood was taken from the dorsal or ventral caudal vein using a non-heparinized syringe (Perpiñán 2015; Figure 5). Samples were

FIGURE 2. A snapping turtle (*Chelydra serpentina*) lingering after being released near the hoop net trap in which he was captured. The flotation device in this case was a pool noodle, and the sardines used for bait were in a storage container with punctured holes so the scent could escape. He learned his lesson and did not get captured a second time. (Photo by Darien Lozon.)

FIGURE 3. Nine of the 29 sites surveyed across the Upper Deckers Creek watershed in Preston County, West Virginia. Wetland sizes range between 0.04 and 8.41 ha. Three of the 29 sites surveyed were different segments of Deckers Creek, while the other 26 sites were public and private ponds and impoundments. Choice of wetlands to survey was limited by landowner permissions. (Photos by Darien Lozon.)

collected in the field and stored in a cooler until we could return from the field and store them in a -20°C freezer until analysis. Two samples of wetland soil were collected along the shore of each wetland between 0-10 cm of the surface for environmental heavy metal analysis. We used a PVC corer instead of a conventional steel soil corer to avoid potential sample contamination (Hubbart 2002). Soil samples were refrigerated until analysis. All tissue and soil samples were sent to the National Research Center for Coal and Energy Analytical Lab at West Virginia University and tested for cadmium, chromium, total mercury, lead, selenium, and zinc using the U.S. Environmental Protection Agency method 3050B (1996) with a Perkin Elmer NexIon 2000 inductively-coupled plasma mass spectrometer.

RESULTS

We captured 119 snapping turtles and 327 painted turtles over the course of the 2019 season. A subset of tissues from 62 painted turtles and 32 snapping turtles across 22 sites are being analyzed for cadmium, chromium, total mercury, lead, selenium, and zinc by sex, species, and surrounding land use.

NEXT STEPS

For landscape analysis, I will use a national land cover dataset from the West Virginia GIS Technical Center (Strager 2012). Digitization of wetlands will be conducted using ArcGIS (version 10.7, ESRI, Redlands, California), and land use types (focusing on forest, agriculture, and roads for analysis) will be quantified within 250, 500, and 1000 m of each wetland (Marchand and Litvaitis 2004; Attum et al. 2008). Values will be calculated as percent coverage within each buffer size to account for the range of wetland sizes (median = 0.55 ha, range = 0.04—8.41 ha; Marchand and Litvaitis 2004).

To investigate the relationships between heavy metal bioaccumulation in snapping and painted turtles, I will use generalized linear models with each individual heavy metal

FIGURE 4. An example of a marked painted turtle (*Chrysemys picta*) to determine if it is a recapture. This individual is marked as #579. (Photo by Darien Lozon.)

FIGURE 5. Turtle blood samples for heavy metal analysis were taken using a non-heparinized syringe from the dorsal caudal vein in painted turtles (*Chrysemys picta*) and the ventral caudal vein (pictured) in snapping turtles (*Chelydra serpentina*). (Photo by Darien Lozon.)

as a response. Candidate models will be determined using forward stepwise model selection (Burnham and Anderson 2002). Model predictors will represent environmental characteristics of each site (e.g., soil contamination and surrounding land use) as well as turtle demographics (e.g., species and sex), morphometrics (straight-line carapace length, hereafter CL), and tissue type. Interactions between land use and soil contamination, tissue type and soil contamination, tissue type and species, CL and species, and CL and sex will also be included *a priori* based on relationships previously found in the literature.

This research is part of a master's thesis that will be completed in May 2021. The findings will be submitted to a peer-reviewed journal for publication. Preliminary results will be presented virtually at The Wildlife Society annual conference (September 27—October 1, 2020). ■

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