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Ecology of freshwater turtles and other wetland wildlife in a north-central West Virginia watershed

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Ecology of freshwater turtles and other wetland wildlife in a north-central West Virginia watershed

Darien N. Lozon

**Thesis submitted to the Davis College of Agriculture, Natural Resources, and Design at
West Virginia University**

in partial fulfillment of the requirements for the degree of

Master of Science

in

Wildlife and Fisheries Resources

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Division of Forestry and Natural Resources

Morgantown, West Virginia

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Keywords: Acid mine drainage, anurans, biomonitoring, birds, common snapping turtle (*Chelydra serpentina*), compensatory mitigation, eastern painted turtle (*Chrysemys picta*), fish, heavy metal bioaccumulation, macroinvertebrates, methylmercury, mixed-use watershed, movement, plants, riparian wetlands, small mammals

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ABSTRACT

Ecology of freshwater turtles and other wetland wildlife in a north-central West Virginia watershed

Darien N. Lozon

The goal of wetland mitigation in the United States is to achieve a no-net-loss of wetlands; however, mitigated wetlands must be monitored to ensure wetland function is comparable to natural wetlands. In this study, relationships between land use practices and freshwater turtle abundance, wetland connectivity, and heavy metal bioaccumulation were investigated. In Chapter 1, a summary of pre-restoration species abundance and diversity is provided for anurans, birds, benthic macroinvertebrates, fishes, small mammals, plants, and turtles in riparian wetlands along Ruby Run, a tributary of Deckers Creek in north-central West Virginia, USA. These data provide a baseline for comparison after mitigation is completed to monitor effectiveness of achieving near-natural wetland function.

In Chapter 2, relationships between relative abundance of two common turtle species found in West Virginia (snapping turtles [*Chelydra serpentina*] and painted turtles [*Chrysemys picta*]) and habitat characteristics are investigated across 39 wetlands within the Upper Deckers Creek watershed in north-central West Virginia, USA. The effects of wetland and landscape characteristics on snapping turtle movement among wetlands are also assessed. Out of the 42 adult snapping turtles across 22 wetland sites (0.04–7.45 ha) that were equipped with radio transmitters, movement was documented among wetlands for 27 individuals (66%). Wetland perimeter, substrate depth, vegetative cover, and distance from roads were positively associated with snapping turtle relative abundance. Wetland perimeter and agriculture within 500 m were negatively associated with snapping turtle movement. Wetland vegetative cover and canopy cover were positively associated with painted turtle relative abundance. Landscape-level characteristics such as nearby farm ponds, wetlands, agriculture, and roads should be considered when developing wetland conservation plans to maximize turtle abundance. Wetland connectivity is important because snapping turtles and painted turtles regularly use a diversity of wetland types with abundant vegetation and natural surroundings.

In Chapter 3, the presence of cadmium, chromium, lead, mercury, selenium, and zinc concentrations is reported across sites within the Upper Deckers Creek watershed and relate landscape characteristics to concentrations found in wetland soil and turtle tissues. A total of 33 painted turtles and 24 snapping turtles across 22 sites were tested for metal concentrations through non-destructive tissue sampling (i.e. blood and nails). All metal concentrations were higher in nails than blood, but concentration differences between species varied across metals. Selenium levels in soil and turtle nails were positively correlated with proximity to mine land. Turtle nail lead concentrations were positively correlated with wetland soil Pb concentrations. Percent agriculture within 30 m of wetlands was negatively correlated with mercury in blood but positively correlated in nails, and all samples analyzed had mercury levels that exceeded U.S. Environmental Protection Agency consumption limits. Proximity to these land use practices should be considered when implementing and managing wetlands and associated buffer areas. Guidelines for turtle consumption limits should be investigated further to ensure human health and safety.

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CHAPTER ONE: Pre-restoration biodiversity monitoring of a riparian wetland in a mixed-use watershed in the Central Appalachians, USA

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ABSTRACT

Wetland mitigation efforts have increased in numbers over the past two decades to combat wetland loss in the United States. Data regarding wetland function such as biodiversity is required to be collected 5–10 years after a project is complete; however, pre-restoration data that can inform the effectiveness of mitigation is often not collected. We conducted pre-restoration surveys on various taxa at Ruby Run, a tributary of Deckers Creek in north-central West Virginia, USA, from 2016–2020 to determine baseline relative abundance and diversity within the riparian zone. In five years, we observed 178 species (95 plant, 56 bird, 13 fish, six small mammal, six anuran, and two turtle species) and 25 families of macroinvertebrates. The most common species were *Carex lurida*, *Melospiza melodia*, *Semotilus atromaculatus*, *Microtus pennsylvanicus*, *Pseudacris crucifer*, and *Chelydra serpentina* with oligochaetes as the most common macroinvertebrate taxon. Seasonal fluctuations in diversity were present, but mean diversity was relatively consistent among years across taxa except in anurans where there was a decrease each year. Wetland mitigation efforts should continue to be monitored for success using multiple taxa because land use change can affect taxa in different ways, resulting in well-rounded assessments that can improve wetland management practices.

Keywords: Anurans; Birds; Compensatory mitigation; Fish; Macroinvertebrates; Plants; Riparian wetlands; Small mammals; Turtles

Pre-restoration biodiversity monitoring of a riparian wetland in a mixed-use watershed in the
Central Appalachians, USA

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1. INTRODUCTION

Wetlands are considered among the most valuable ecosystems in the world (Costanza et al. 2006, Moreno-Mateos et al. 2012). Between 1986 and 1997, after the establishment of the Emergency Wetlands Resources Act of 1986, there was a recorded net loss of 256,600 ha of freshwater wetlands due to land use changes such as rural (21%) and urban development (30%) as well as agriculture (26%) and silviculture (23%) in the United States (Dahl 2000). The greatest losses of wetlands were in Louisiana, Mississippi, Arkansas, Florida, and the Carolinas since 1970 (Mitsch and Gosselink 1993). In West Virginia, 24% of historical wetlands have been lost due to anthropogenic change (Dahl 1990).

Most wetland losses in West Virginia have been due in part to agricultural land use change, where wetlands were drained and filled to make the land more suitable for crop production (Mitsch and Gosselink 1993). Since 1990, the U.S. Department of Agriculture Natural Resource Conservation Service initiated the Wetland Reserve Program that further evolved into the Wetland Reserve Easement component of the Agricultural Conservation Easement Program (ACEP) in 2014 (Rewa 2005, National Sustainable Agriculture Coalition

2019, Lewis et al. 2020). This program works with landowners to restore wetlands that were destroyed for agricultural purposes and passively manages them through efforts such as implementing upland buffers (Rewa 2005, Lewis et al. 2020). Compared to natural wetland systems, ACEP wetlands have similar characteristics (Lewis et al. 2020) and provide quality wetland habitat for wildlife such as birds and amphibians (Rewa 2005, Lewis et al. 2020).

Coal mining also has historically been one of the more common anthropogenic changes in the Central Appalachian region since World War II, where natural forests were cleared for surface mines (Townsend et al. 2009, Shapley 2011). When the Surface Mining Control and Reclamation Act was passed in 1977, impacted mining lands were required to be restored to near pre-mining conditions after a project was completed. The intent of this Act was to minimize the long-term degradation of lands that would have consisted of canceled or abandoned mines (Wooley 1979). As of 2011, over 7% of Central Appalachia—which consists of West Virginia, southeastern Kentucky, southwestern Virginia, southeastern Pennsylvania, and western Maryland—included active, reclaimed, or abandoned mines (Ross et al. 2016). Despite the reductions of critical processes including soil infiltration during the mining process (Simmons et al. 2008), post-mining topography can create wetlands (Atkinson and Cairns Jr. 1994). Because of the long-lasting impacts of coal mining (i.e. acid mine drainage), these wetlands do not provide the same habitat quality as reference wetlands for taxa such as macroinvertebrates and amphibians (Petty et al. 2013).

For landowners, managers, and consultants to gain a better understanding of wetland resources, the U.S. Fish and Wildlife Service (USFWS) developed the National Wetlands Inventory (NWI) according to classification criteria established by Cowardin et al. (1979). Riparian wetlands have been accepted as important migration corridors and stopovers and are

regularly used by many mammals, herpetofauna, fish, birds, and invertebrates throughout the world for breeding and foraging as well as safe havens for rearing their young (Cowardin et al. 1979, Ramsar 2013, USFWS 2018). Despite their ecological importance, riparian wetlands were not included in the NWI until 2009 (USFWS 2018). Land managers have been creating and restoring riparian wetlands through mitigation efforts over the last two decades to decrease the rate of wetland loss in the United States (Tiner 1995, Tiner 1996, Dahl 2000, Kentula 2002).

It is essential to periodically assess created and restored wetlands for their ecological diversity to ensure successful restoration practices. Vegetative community structure within created wetlands have been, on average, 26% lower than reference sites (Moreno-Mateos et al. 2012), and studies have found restored wetlands have significantly lower vegetative cover than natural wetlands (Campbell et al. 2002, Seabloom and van der Valk 2003). Studies have alternatively found that mitigated wetlands have higher diversity and abundance of wetland vertebrates (e.g. birds and anurans; Stevens et al. 2002; Balcombe et al. 2005a, b) and provide similar or better wetland functions than reference wetlands (Taylor and Middleton 2004, Spieles and Mora 2007, Gingerich and Anderson 2011, Strain et al. 2014, Bartholomew et al. 2018). Few studies have evaluated wetland structure and function before, during, and after restoration, which could assist managers in identifying possible challenges and improvements to wetland mitigation.

According to the National Environmental Policy Act, wetland mitigation is generally defined as a measure to avoid wetland impacts. When impact avoidance is not possible, mitigation may include minimizing or rectifying the impact either immediately or over time and compensating for the impacts (Ambrose 2000). The U.S. Army Corps of Engineers and Environmental Protection Agency implemented a compensatory mitigation planning hierarchy in

2008 with the goal of improving the quality of mitigated wetlands based on the likelihood of a mitigation project being sustainable and successful (USACE 2010).

By accounting for the classification and condition of wetlands that are being impacted, no-net-loss of wetland quality in a given area can be achieved (USACE 2010); however, simply replacing wetlands is not enough. Structural characteristics such as wetland size needs to be taken into consideration when creating and restoring wetlands. If mitigation is involved in a species management plan, decreased wetland sizes can negatively affect relative abundance of populations such as Blanding's turtle (*Emydoidea blandingii*; Attum et al. 2008), an endangered species according to the International Union for Conservation of Nature (van Dijk and Rodin 2011). Alternatively, larger created wetlands can lead to significantly different hydroperiods compared to natural wetlands (Cole and Brooks 2000). Species such as wood frogs (*Lithobates sylvaticus*) and spotted salamander (*Ambystoma maculatum*) depend on ephemeral systems to ensure they are fishless (Gamble and Mitsch 2009); however, created wetlands are commonly ponded to ensure hydrology for most of the year if not year-round (Cole and Brooks 2000, Gamble and Mitsch 2009).

Surrounding landscape can also influence the success of a mitigation effort. Houlihan and Findlay (2003) found that amphibian richness was positively correlated with nearby forest within 2 km of wetlands, while abundance decreased with increased road density. Similar relationships have been found with stream index of biological integrity (IBI) scores, where catchments with at least 7% of urbanization can result in poor or very poor stream quality (Snyder et al. 2003). Effects of agricultural land use on stream habitat quality are inconsistent. Snyder et al. (2003) found that agriculture did not have a significant effect on stream IBI scores when assessing relationships at catchment-level in West Virginia; however, Wang et al. (1997)

found that agriculture had a significant negative effect on IBI at watershed-level and within 100 m of streams in Wisconsin. Similarly, Genito et al. (2001) reported lower Ephemeroptera and Trichoptera richness with increased agriculture within the watershed, and Nichols et al. (2016) found significant differences in macroinvertebrate metrics such as taxa richness and Shannon diversity between rural and urban areas.

In 2017, a headwater dam of Deckers Creek in Preston County, West Virginia, USA, was renovated to increase the water capacity of the impoundment due to increased flooding problems in nearby residential areas. Because of the natural riverine wetland loss in this project, the West Virginia Conservation Agency planned a mitigation project on Ruby Run, a tributary of Deckers Creek that flows through cattle fields at the JW Ruby Research Farm in Preston County to compensate for the wetland loss. Six cover treatments, with two replicates of each (i.e., 12 treatment areas) were proposed within the riparian zone of Ruby Run (Figure 1); however, the details of the mitigation plan and when the treatments will be applied is uncertain.

The primary objective of this research was to establish a baseline of relative abundance and diversity for plant and animal taxa within the Ruby Run riparian wetland. These baseline data were used to quantify ecological response to varied restoration activities in Ruby Run and provide a characterization of diversity and abundance of species occupying a typical central Appalachian mixed-use watershed. Specific monitoring objectives included: 1) quantifying year-round relative abundance and diversity of birds; 2) determining annual relative abundance and diversity of wetland macroinvertebrates, small mammals, turtles, and anurans; 3) documenting annual plant diversity and relative abundance; and 4) identifying annual changes of in-stream community composition (i.e. fish and invertebrates). With these baseline data, we hypothesized there would not be a significant difference in biodiversity or vegetative composition after the

wetlands and riparian zones had five years to undergo succession. Once cover treatments are applied, post-restoration data can be used to relate observed biological response to patterns in physicochemical conditions and restoration treatments.

2. METHODS

2.1. Study Area

We conducted our study along Ruby Run, a tributary of Deckers Creek, at the JW Ruby Research Farm (hereafter “the Farm”) that falls within the Upper Deckers Creek watershed, a Hydrologic Unit Code 12 watershed that covers 7,778 ha in Preston County, West Virginia, USA, and encompasses 89.9 km of stream (Deckers Creek headwaters and its tributaries). Deciduous forests and wetlands (freshwater emergent wetlands, freshwater ponds, freshwater forested/shrub wetlands, and riverine systems) have been significantly fragmented from agriculture. Development of varying intensities also have fragmented the watershed with northern (Masontown [Lat 39.55056; Long: -79.79834], estimated population = 538 as of 2019 [USCB 2020]) and central (Reedsville [Lat: 39.51019; Long: -79.79799], estimated population = 604 as of 2019 [USCB 2020]) residential hubs within the watershed.

Ruby Run is an unnamed tributary of Deckers Creek that is 1.62 km in length with 65% of Ruby Run flowing through the Farm, and the remaining 35% of the stream flows through hardwood forest further upstream directly outside of the Farm boundary. In 2010, fencing was built around 679 m (0.73 ha) of Ruby Run that protects about 2.22 ha of palustrine emergent wetlands and upland riparian areas from constant cow disturbance, ranging from 22–91 m of riparian buffer area on either side of the stream, where most of the wetland area is located on the west side of the stream channel and continues into the pasture outside of the fenced area with some wetland area located on the east side. Cattle are allowed into the fenced area for limited

periods of grazing and access to water during the summer. Fall mowing around the fences also occurs to maximize access for repairs. Ruby Run is a first-order headwater stream of Deckers Creek, so the stream is narrow (mean $\pm$ SE width: 2.44 ± 0.32 m) and shallow (mean $\pm$ SE depth: 25.37 ± 4.23 cm) with increasing water depth and fine, mucky substrate further downstream compared to upstream where channel braiding is present and the majority of large riffles are located with larger rocks and cobble substrate.

This area is representative of similar mixed farm-woodland headwater streams and riparian habitat within the central and southern Appalachian regions that are the focus of the Appalachian Landscape Conservation Cooperative, a regional effort with the goal of promoting larger contiguous deciduous forest landscape and improve stream water quality (Landscape Partnership 2016). Agricultural land use makes up 26% of the region, and forested riparian areas are promoted as a best management practice; however, the effectiveness of this best management practice can vary based on factors such as adjacent land use, stream size, and topography (Landscape Partnership 2016, USDA 2021).

Stream habitat degradation within the Upper Deckers Creek watershed primarily results from coal mining (i.e. acid mine drainage; Denicola et al. 2014). Acid mine drainage from active and abandoned surface and underground mines lead to lower water pH and higher concentrations of contaminants such as iron oxide and selenium (USDA 2018), which directly affects organisms such as macroinvertebrates (Pond et al. 2008, Cianciolo et al. 2020). Treatments such as limestone channels and settlement ponds were applied since 2002 and the watershed has improved in terms of water quality and macroinvertebrate and fish diversity and abundance (Friends of Deckers Creek 2017).

2.2. Bird Surveys

To determine bird use of wetlands, we conducted five ten-minute 50-m radius point count surveys monthly from 2018–2020. We used removal sampling methods during each point count survey, where individuals were only accounted for at first detection (either visual or auditory) to avoid double-counting individuals (Farnsworth et al. 2002, McCallum 2005). Survey locations were about 250 m apart along Ruby Run to minimize counting the same individuals between surveys (Hanowski and Niemi 1995). We ensured all surveys were conducted no earlier than sunrise and no later than four hours after. We did not conduct surveys during periods of precipitation or on mornings with winds > 11 km/hr to minimize decreased detection rates (Ralph et al. 1995). We randomized the order of conducting point counts (starting upstream or downstream) to decrease time-of-day effects on detection.

We obtained detection and non-detection data using visual and auditory observations from the 50 m radius. Because of how narrow the wetland area was, the 50 m radius sometimes included birds using adjacent agriculture or forested areas. For each point count location, we recorded date, time, temperature (to the nearest degree Celsius), level of noise disturbance, wind, and sky condition (Appendix 1). We also classified birds as native or exotic to West Virginia (WVCA 1998). For each bird, we recorded whether it occurred within one of the 12 proposed vegetative cover treatment areas using cursory maps to elucidate wetland area used after the treatments are implemented (Figure 1).

2.3. Wetland Macroinvertebrate Sampling

We sampled benthic macroinvertebrates at five locations that generally contained standing water within the riparian wetland along Ruby Run from 2018–2020 using a 5 cm diameter handheld PVC core sampler (with samples taken at a depth of 15 cm; Anderson et al.

2013). At each visit, we collected five randomly located benthic core samples at each of the five locations between the stream and fence as determined by a random point generator in ArcGIS (v. 10.7, ESRI, Redlands, California). We collected samples quarterly (February, May, August, and November) to account for seasonal fluctuation. We refrigerated soil core samples until analysis and sorted samples within one week of collection. Any macroinvertebrates we found were preserved in 70% ethanol (Anderson et al. 2013). We identified individuals to family (except earthworms; Voshell 2002), and we recorded the quantity of each type for each soil core sample.

2.4. Small Mammal Surveys

We assessed temporal variation in small mammal relative abundance and diversity from 2017–2020 with Sherman live traps (5.1 x 6.4 x 16.5 cm, H.B. Sherman Traps, Tallahassee, Florida, USA) 10 m apart along a 20 x 50 m transect grid (3 rows of 6 traps each [18 traps total]) within each of the 12 proposed cover treatment areas (216 traps total). We placed the transect rows parallel to the stream at < 1 m, 5 m, and 15 m, and transect grids were placed 50 m from each other. We baited traps with an oatmeal and peanut butter mixture wrapped in wax paper (Edalgo and Anderson 2007) along with 20 dried mealworms to attract insectivores (e.g. short-tailed shrews [*Blarina brevicauda*]) and enhance survival of captured individuals (van Boekel 2013). Every 24 hours for 3 consecutive nights, we checked and rebaited traps if bait was missing. Trap sessions occurred once a month from May to September.

We marked shrews on their tails with unique color combinations of nail polish (Bergström 2004) and equipped all other captured animals with #1005-1 Monel ear tags (National Band and Tag Company, Newport, Kentucky, USA) to determine recapture rates. Recapture rates were calculated as the total number of new living individuals captured in a year divided by the number of unique individuals (living or dead) captured (Romairone et al. 2018).

With each capture, we determined if it was native or exotic to West Virginia (WVDNR 2001) and took note of date and time of capture, trap number, ear tag number or color combination, species, sex, and mass of each animal. We scheduled trap days according to weather forecasts provided by the National Weather Service to avoid trapping on severe cold, hot, or rainy days to reduce mortality (Manley et al. 2006, Natoli et al. 2016). We took proper precautions against Hantavirus according to Mills et al. (1995) and Kelt and Hafner (2010).

2.5. Turtle Surveys

We surveyed Ruby Run for turtles from 2017–2020 using four hoop net traps (0.91 m diameter; Flaherty et al. 2008, Gulette et al. 2019), where we checked traps every 24 hours for 3 consecutive days monthly from April to October. We equipped each trap with a flotation device to ensure traps were never fully submerged, allowing captured individuals to have constant access to air. We baited each trap with a half-can of sardines in containers that allowed the scent and oils to escape but did not allow the bait to be consumed. We replaced bait in each trap every 24 hours to ensure freshness. We determined the sex of turtles using secondary sex characteristics (Mosimann and Bider 1960, Bowne et al. 2006) and made unique shell markings with a hand file in the marginal scutes of each unique turtle to identify recaptures (Cagle 1939). We then photographed and released turtles near their capture site. For each species captured, we determined whether it was native or exotic to West Virginia (Pauley 2021).

2.6. Anuran Surveys

We evaluated anuran communities from 2017–2020 using nocturnal call count surveys. We followed standardized protocols developed by the U.S. Fish and Wildlife Service (outlined by Balcombe et al. [2005a]) when conducting surveys, where air temperatures could not be lower than 5.5 °C and the wind code could not exceed wind code 3 (17.7 km/hr, Appendix 1; Grant

2017). We conducted point count surveys once in late February; twice monthly during March, April, and May; and once in early June to account for temporal breeding differences among species. We randomized the order of conducting surveys (starting upstream or downstream) for each visit to account for species that call at different times in the evening.

For each survey, we allowed a two-minute acclimation period followed by a five-minute listening period, where we assigned a Wisconsin Index (WI) value (Mossman 1994) to each species heard according to call intensity (Balcombe et al. 2005a): 1 indicated non-overlapping calls and an exact count of individuals could be made, 2 indicated overlapping calls and only estimates of numbers could be made, and 3 indicated species that were calling in full chorus and were assigned a standardized estimate of 50 individuals (Crouch and Paton 2002, Balcombe et al. 2005b). For each species detected, we determined whether it was native or exotic to West Virginia (Pauley 2021). We recorded call locations within one of the 12 proposed vegetative cover treatment areas, and we used cursory maps to determine where they belong after the treatments have been implemented.

2.7. Vegetation Surveys

We assessed riparian wetland vegetation on the west side of Ruby Run in September 2016, June and September 2017 and 2018, and September 2020. We visited twice in 2017 and 2018 to improve species identification as all plants do not flower at the same time (Veselka et al. 2010a). We identified plants within 1 m² quadrats spaced every 10 m along a continuous transect placed parallel (1–5 m away) to Ruby Run. We used the midpoint of the Daubenmire (1968) cover classes (1 = 0–5% [2.5]; 2 = >5–25% [15]; 3 = >25–50% [37.5]; 4 = >50–75% [62.5]; 5 = >75–90% [82.5]; and 6 = >95% [97.5]) to record percent cover of bryophytes, woody debris, rock/barren/open water, plant litter, and each plant species at each quadrat. There were limited

trees or shrubs along Ruby Run (e.g. blackberry [*Rubus* sp.], alder [*Alnus* sp.], and multiflora rose [*Rosa multiflora*]), so we only used quadrats sized for herbaceous plants (Rentch et al. 2015). We used Radford et al. (1968), Strausbaugh and Core (1977), and Gleason and Cronquist (1991) to identify plants to species.

2.8. In-stream Community Assessments

We surveyed fish communities in Ruby Run once annually between August and November from 2017–2020 in the summer according to single-pass shocking methods by Huntsman and Petty (2014) with a Smith-Root LR-24 electrofishing unit. We separated Ruby Run into seven 100 m reaches and measured conductivity before shocking to ensure we were using proper wattage. After a reach was shocked, we identified each captured fish to species and recorded weight (to the nearest 0.1 g) and length (mm) according to standard length (caudal-peduncle). For each species captured, we identified if the species was native or exotic to West Virginia (WVDEP 2021). After processing, we returned each fish to the stream near their capture location.

We sampled stream macroinvertebrates once annually in May from 2017–2020 by obtaining kicknet samples (net dimensions 335 × 508 mm with 500 µm mesh) from four representative riffles (WVDEP 2014). We preserved all samples in 70% ethanol in the field, and we identified individuals to family for most individuals (except earthworms; Voshell 2002).

2.9. Statistical Analysis

Mean annual diversity for each taxon was calculated using R statistical software (R Core Team 2020). Because the primary objectives of this study were to establish baseline data prior to restoration and to document biodiversity in a typical mixed-use Appalachian watershed, we did not conduct any formal temporal statistical comparisons.

We calculated bird species diversity using a Shannon-Weiner Diversity Index (H' ; Tramer 1969) for each month, including all individuals detected visually and audibly among all point count locations and transects between point counts, and calculated the number of birds per 50-m radius plot. We calculated number of individuals per m^2 and H' of soil core samples for macroinvertebrates for each of the five wetland locations seasonally (Wilhm and Dorris 1966, Brooks 2000).

For small mammals captured at each trapline, we calculated monthly relative abundance by species and H' based on survey data collected for each year. We also calculated species richness for each trapline per month according to the number of species captured within the year. We defined catch-per-unit effort (CPUE) as the number of captures per 100 trap nights, and we calculated CPUE as a percentage of individuals captured divided by number of trap-nights for each survey period (Nicolas and Colyn 2006). We determined the number of trap nights as the number of traps set at each trapline times the number of nights set each month ($n = 54$ possible trap nights/trapline/month). For each capture, falsely snapped trap, or trap with missing bait, we subtracted a half trap-night from the total (Hannon et al. 2002).

We calculated species abundance and H' for turtles and anurans based on surveys at Ruby Run. We used WI metrics for each anuran species to calculate relative abundance. Similarly, we calculated annual fish and stream macroinvertebrate H' and abundance. We also calculated plant H' and species richness and coverage based on plants identified to species during surveys. We calculated evenness (J') using Pielou's evenness index (Pielou 1966):

$$J' = H' / H'_{\max} \quad [\text{Eq. 1}]$$

where H' = Shannon-Wiener diversity index and $H'_{\max}$ = the maximum Shannon-Wiener diversity index.

3. RESULTS

3.1. Birds

Fifty-eight bird species were observed between 2018 and 2020 (Appendix 2). Out of the 2,880 observations, 80% of the birds were detected within the fenced wetland area, while the remaining 20% of observations were in the adjacent forested or agricultural areas. The most common species across years were song sparrows (*Melospiza melodia*) that made up 45% of all observations (3.26 ± 0.28 birds per 50 m radius plot per month) and red-winged blackbirds (*Agelaius phoeniceus*) that made up 10% of all observations (0.73 ± 0.17 birds per 50 m radius plot per month; Table 1).

Highest Shannon diversity was observed in the summer months (May–July) due to the presence of less common migratory birds such as the Nashville warbler (*Leiothlypis ruficapilla*) observed in 2018 and even lesser common out-of-range observations such as the western meadowlark (*Sturnella neglecta*) found in May 2020 (Figure 2, Appendix 2). Song sparrows were observed to favor more central sections of the wetland and were less likely to be observed in edge point counts. The point count furthest upstream was directly adjacent to a wooded area across a two-lane road, so most species detected were most likely attracted to the forested edge habitat.

Birds such as red-bellied (*Melanerpes carolinus*) and downy woodpeckers (*Picoides pubescens*), chickadees (*Poecile* spp.), and white-breasted nuthatches (*Sitta carolinensis*) were regularly observed flying between trees within the upstream riparian wetland area and the wooded area across the road. Other species more associated with the surrounding open fields such as bobolink (*Dolichonyx oryzivorus*) used the wetland area for foraging across all years in early summer (May–June) and early fall (September) months. Late-season mowing in different

locations within the wetland area negatively affected the presence of birds each year. All birds observed were considered native (e.g., migratory and resident) species to West Virginia and the Appalachian region except European starling (*Sturnus vulgaris*).

3.2. Wetland Macroinvertebrates

Wetland soil core samples revealed 11 families across 7 orders between 2018–2020 (n = 275 soil samples across 5 wetlands; Table 2, Appendix 3), where earthworms (Oligochaeta) were the most abundant taxon out of the 89 individuals collected across years (47.0%), followed by mosquito (Chironomidae; 15.7%) and biting midge larvae (Ceratopogonidae; 13.5%). Mean annual H' ranged from 0.27 to 0.34 (Figure 3).

3.3. Small Mammals

Six species of small mammals were captured from 2017–2020 (Table 3, Appendix 4), where most captures were meadow voles (*Microtus pennsylvanicus*) and short-tailed shrews. Meadow voles, short-tailed shrews, and deer mice (*Peromyscus maniculatus*) were captured in all years, while white-footed mice (*Peromyscus leucopus*) were captured in 2018 and 2020. Meadow jumping mice (*Zapus hudsonius*) were only captured in 2020, and one eastern chipmunk (*Tamias striatus*) was captured in 2018 (Appendix 5). All observed small mammals were native species to West Virginia.

Number of trap nights in a session for a trapline varied from 27.5–54 per month and was primarily affected by bait loss (likely from raccoons [*Procyon lotor*]). Damage and loss of traps also affected the number of trap nights, due to the occasional presence of cattle (i.e. stepping on and smashing traps) and raccoons (i.e. removing pins or stealing entire traps) in the wetland. There were also issues of ants or slugs on bait that potentially affected trapping success. The

highest CPUE reported was 49.2 captures per 100 trap nights in September 2020 (Figure 4). Recapture rates were 10.87% in 2017, 10.67% in 2018, 2.80% in 2019, and 15.15% in 2020.

3.4. Turtles

One painted turtle (*Chrysemys picta*) and 6 snapping turtles (*Chelydra serpentina*) were captured between June and August from 2018–2020 (Table 4). Both of which were native species to West Virginia and the central Appalachian region. Shannon diversity was zero during 2018 and 2020 because of only snapping turtles being captured (Table 4). One female painted turtle was captured in 2019. The recapture rate in 2018 was 33%, while 50% of turtles in 2019 and 100% of turtles in 2020 were recaptures, indicating high site fidelity.

3.5. Anurans

Six species were detected across four years of surveys—spring peeper (*Pseudacris crucifer*), Cope’s gray tree frog (*Hyla chrysoscelis*), wood frog (*Lithobates sylvaticus*), American toad (*Anaxyrus americanus*), American bullfrog (*Lithobates catesbeianus*), and green frog (*Lithobates clamitans*)—where spring peepers were the most common species detected (Table 5, Appendix 6). Leopard frogs (*Lithobates pipiens*) were also detected within the riparian zone but not during surveys. Almost half of the 19 total visits (47.3%) had no individuals detected, and the highest Shannon diversity was observed in May 2017 ($H' = 1.22$). Annual variation in H' decreased from 0.94 in 2017 to 0.45 in 2018, 0.27 in 2019, and 0.26 in 2020. All anurans observed in Ruby Run were native species to West Virginia.

3.6. Vegetation

The number of surveyed quadrats varied from 63 in 2016 to 82 in 2020 ($n = 445$ plots across 6 visits in 4 years; mean $\pm$ SE: 74.17 ± 2.91 quadrats) with 154 total plant species observed, where 67% ($n = 103$) of the plants observed were native (Appendix 7 [Table 1]). Plant

diversity among years ranged from 0.22–0.26 (Table 6). The most abundant species (as determined by mean percent cover across all quadrats) in 2016 was sallow sedge (*Carex lurida*; $12.73 \pm 2.92\%$) followed by soft rush (*Juncus effusus*) in 2017 ($18.48 \pm 3.11\%$) and rough-leafed goldenrod (*Solidago rugosa*) in 2018 and 2020 ($14.59 \pm 2.32\%$ and $15.27 \pm 2.50\%$, respectively; Table 7).

3.7. In-stream Communities

Between the seven reaches across four years, 13 species of fish were captured (Table 8, Appendix 8). All fish species sampled were native to West Virginia. Relative abundance varied across years as only three species were captured in 2017, while five species were captured in 2018, four were captured in 2019, and ten were captured in 2020 (Appendix 8). Shannon diversity ranged from 0.69–1.11 (mean $\pm$ SD: 0.87 ± 0.03 ; Table 9).

From the four stream riffles assessed from 2018–2019 (no macroinvertebrates were found in 2020), 16 invertebrate families across 8 orders were represented (Table 10) with ceratopogonids as the dominant species (19%) followed by earthworms and chironomids (12%). Shannon diversity varied from 2.21 in 2018 to 1.24 in 2019. The primary taxa that were surveyed in stream riffles in 2018 were earthworms, dipterans (Simuliidae), and plecopterans (Perlidae) that accounted for 50.7% of all samples. Ceratopogonids were the majority taxon represented in 2019 (56%; Table 10).

4. DISCUSSION

4.1. Bird Diversity

We found over 50% of the bird assemblages consisted of song sparrows and red-winged blackbirds consistently across years. Red-winged blackbirds are obligate wetland birds and were observed using the riparian wetland area for foraging, courtship, and nesting, but they are

tolerant of human-induced habitat change (Veselka et al. 2010b), and most observations of red-winged blackbirds were singing males on fence posts near the wetland edge. Some females were flushed during surveys, but no nests were found. Song sparrows alternatively are facultative wetland birds as they more commonly utilize open meadow habitat as ground-foraging omnivorous birds.

Some of the remaining species detected were considered wetland specialists such as green heron (*Butorides virescens*) and spotted sandpiper (*Actitis macularia*; Veselka et al. 2010b). Most of the remaining birds detected across years such as American goldfinch (*Carduelis tristis*) and yellow warbler (*Dendroica petechia*) were habitat generalists that only occasionally use wetlands (Veselka et al. 2010b), which could be due to increased edge effects from the small size of the wetland area. Increased wetland size and vegetative diversity and heterogeneity are associated with riparian wetland bird richness and abundance in general (Lewis et al. 2019, Elliott et al. 2020). Moreno-Mateos et al. (2009) found opposite relationships between bird species abundance and wetland size; however, they attributed this to inconsistent surveying effort to account for larger wetlands.

4.2. Macroinvertebrate Assemblages

The current study indicated stream macroinvertebrate diversity was higher than benthic macroinvertebrates in riparian wetlands surrounding Ruby Run. Macroinvertebrate assemblages are expected to be different based on the wetland type (Battle and Golladay 2001), where characteristics such as hydroperiod and water and soil chemistry can influence wetland macroinvertebrate taxa. Oligochaetes were most abundant across years in soil core samples, which indicates that although standing water was not consistently present across the riparian area during the study, moist soil conditions were met to make the area sufficient for earthworms to

reside (Tripathi and Bhardwaj 2004). Ephemeropterans, plecopterans, and trichopterans (EPT) were present within stream samples, which indicates Ruby Run has improved stream quality since an upstream segment was identified as low priority (iron load: 0.59 kg/day as of 2014) and management was implemented for acid mine drainage in the Deckers Creek watershed (Denicola et al. 2014). EPT richness is negatively related to percent sand in streams (USEPA 2017), so monitoring sedimentation from runoff and channel degradation is necessary to ensure stream habitat quality.

4.3. *Small Mammal Communities*

Catch-per-unit effort for small mammals within the riparian area of Ruby Run was much lower compared to a similar study elsewhere in Preston County, West Virginia (Osbourne and Anderson 2002). Despite using similar trapping methods, Osbourne and Anderson (2002) had higher CPUE for taxa such as *Peromyscus* spp. that could have been due to trapping near field-forest transition zones that were not abundant in our study. Our low CPUE is also potentially due to ants and slugs that were a problem in Sherman traps. Slugs were noticed when there were lower dewpoints in the morning, while ants were mostly associated with areas with minimal vegetative ground cover. It is possible that small mammals avoided traps infested with ants, slugs, or both.

Variations in average diversity between years for this study could be due in part to mowing that occurred within the wetland at different times each year. Capture effort was also affected by the presence of cattle during summer months in 2019 because of smashed traps leading to a loss of trap nights. Some species were more abundant in certain traplines as well, where *Peromyscus* species favored more upstream areas and meadow voles were captured more frequently in downstream areas. The road that runs directly adjacent to the upstream riparian

zone could have been a factor as increased road density has been reported to negatively affect species richness for plants, reptiles, birds, and mammals (Findlay and Houlihan 1997).

Alternatively, Bissonette and Rosa (2009) found that road distance did not affect diversity or density of small mammals in Utah. Other factors such as vegetative density and diversity could be a factor in the inconsistent use of the wetland area by species.

4.4. Turtle Abundance

Turtle abundance and diversity was low during our study; however, this was not surprising because neither snapping turtles nor painted turtles in particular are commonly found in streams unless they are using stream habitat as a corridor between larger wetlands or for hibernation (Strain et al. 2012). Both species were found in a nearby wetland (0.9 ha; 105 m from Ruby Run and connected via culverts) that was surveyed at the same time as Ruby Run in 2019 with higher abundances of both species (Chapter 2, this document). A study in the Mississippi River found snapping turtles and painted turtles were least abundant in open river habitat compared to slough and backwater wetlands (Anderson et al. 2002). Similar relationships were observed with higher snapping turtle abundance in backwaters of the Ohio River (Zadnik et al. 2009). All snapping turtles captured were smaller than average (mean straight-line carapace length = 136.08 ± 5.05 mm; unpublished data) and hatchling snapping turtles were observed floating down the stream in 2019, which could be an indication that the riparian wetland provides refuge for younger individuals.

Although there were low turtle counts, evidence of turtles using the wetland were observed. Female snapping turtles nest near Ruby Run along a nearby road (Lozon pers. obs.), possibly due to loose gravel that provides easy digging for nest placement (Haxton 2000). An unmarked adult female painted turtle was captured in 2020, and a juvenile snapping turtle was

observed floating downstream in 2019 (Lozon pers. obs.), which could be an indication that the riparian area is a quality movement corridor (Burbrink et al. 1998). Habitat characteristics such as sediment composition, canopy cover, and basking sites could explain the lower abundance of turtles in Ruby Run (DonnerWright et al. 1999). Sediment transitions from rock and cobble substrate upstream to fine and mucky substrate downstream, and a concurrent study found an increase in substrate depth in a wetland increases snapping turtle CPUE (Chapter 2, this document). Because of the narrow channel, large stretches of the stream are almost completely covered by alder, multiflora rose, and *Rubus* sp., which reduces the ability to access sunlight to bask.

4.5. Anuran Diversity

Most anuran detections were found in wetland areas in the cattle field. Species such as American bullfrogs, leopard frogs, and green frogs were observed (anecdotally and bycatch during fish surveys) in the stream channel throughout the year. It is possible they relocated to shallow flooded areas in adjacent pastures at night to minimize predation because of fish being present in Ruby Run; however, most fish observed in Ruby Run may have been too small to prey on adult frogs (fish size range: 7–855 mm; mean length $\pm$ SE: 76.83 ± 2.12 mm; unpublished data), and these species are tolerant of fish presence (Smith et al. 2016, Swanson et al. 2019). Babbitt et al. (2009) compared tadpole abundance in pasture, prairie, rangeland, and wooded wetlands and found pasture wetland abundance to be lowest likely due to the lack of proximity between breeding and upland habitat. Evidence of anurans using the Ruby Run riparian area for foraging and laying eggs were present with tadpole bycatch during macroinvertebrate surveys as well as leopard frog and green frog bycatch during small mammal surveys.

4.6. Wetland Vegetation Diversity

Wetland vegetation diversity and evenness did not deviate significantly among years despite the inconsistent sampling effort. Relative wetness of each quadrat was calculated, and the mean weighted average indicated the plant assemblages fulfilled the hydrophytic vegetation requirement for the area to be considered a wetland (Atkinson et al. 1993, USEPA 2015; Appendix 7). The dominant plant species such as soft rush and sallow sedge were consistent with what has been found in previous studies in West Virginia wetlands (Balcombe et al. 2003). Other plant species such as glossy-leafed aster (*Symphotrichum puniceum*) and Allegheny blackberry (*Rubus allegheniensis*) were not detected by Balcombe et al. (2003), which could be because both species are facultative upland species.

Temporal variation in the riparian wetland vegetation between 2016 and 2020 indicated consistent percent cover of facultative upland species comparable to obligate or facultative wetland species (Appendix 7 [Figure 2]), and the probability of facultative upland species occurrence in wetlands is relatively low (1–33%; Reed 1996). Ho and Richardson (2013) found that wetland-dependent species (obligate or facultative wetland species) increased in richness over time with higher water inundation, and because the riparian wetland is occasionally inundated for short periods of time (e.g. flooding from snowmelt or significant rain events), upland species can establish within the riparian area.

The presence of cattle or heavy mowing equipment can drastically alter microtopography in wetlands, which can alter natural pooling within a wetland (Moser et al. 2007); however, the effects of microtopographic change in wetland plant diversity is inconsistent. Hong et al. (2021) found that nutrient runoff has a stronger effect on plant diversity than microtopography, and the effects that cattle have on microtopography is not certain (Morris and Reich 2013). Alternatively,

wetlands managed by disking, which is considered as the most intense form of wetland management (USFWS 2016), had microtopography comparable to natural wetlands and exhibited the highest plant diversity compared to natural and non-disked wetlands (Moser et al. 2007).

4.7. Fish Assemblages

The only fish species sampled in 2017 were largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*) and creek chub (*Semotilus atromaculatus*; Appendix 8), which are considered species tolerant of disturbance in stream habitats according to the Maryland and Mid-Atlantic Highlands indices of biological integrity (Snyder et al. 2003). Leonard and Orth (1986) found that green sunfish and creek chub were comparable in being used as tolerant species for indices of biological integrity in southern West Virginia. Similar assemblages were found in subsequent years, with increased abundance in tolerant species such as bluegill (*Lepomis macrochirus*) in 2018 and pumpkinseed (*Lepomis gibbosus*) in 2020.

Surveys in 2020 had noticeably more young-of-year fish than previous years with increased presence of disturbance-intolerant species such as spottail shiner (*Notropis hudsonius*) and central stoneroller (*Campostoma anomalum*), which could be due to the survey being conducted later in the year (November rather than August–September). Stream characteristics such as water temperature influence fish abundance and diversity (Smith and Kraft 2005) and should be compared with more frequent sampling to elucidate possible seasonal habitat use of Ruby Run throughout the year as either core habitat or a corridor.

5. CONCLUSIONS

Overall, we documented 237 species (78% of which were native: two turtles, six anurans, six small mammals, 13 fish, 55 birds, and 103 plants) and 25 families of macroinvertebrates in

this mixed-use watershed. Wetland mitigation efforts should continue to be monitored for success using multiple taxa because land use change can affect taxa in different ways, resulting in a well-rounded assessment (Yamanaka et al. 2020). Taxa within Ruby Run such as benthic macroinvertebrates and plants did not have noticeable annual change in diversity; however, anurans exhibited overall decreases in annual diversity, and the cause of this decline is currently unknown.

Continued monitoring of plant and animal abundance and diversity post-restoration is important to determine restoration success (Woodward and Hollar 2011). Efforts from government organizations such as the U.S. Army Corps of Engineers and the Environmental Protection Agency are clear in that they want to reach a goal of “no net loss” of wetlands; however, the quantity of wetlands alone will not fix the problem of wetland loss in Central Appalachia. Monitoring the effectiveness of mitigation projects is necessary to ensure that high-functioning natural wetlands are not being replaced with lower quality mitigated wetlands. Without careful monitoring, wetlands can become habitat sinks, especially for organisms such as macroinvertebrates and amphibians who depend on water quality, nutrient cycling, and hydrology for majority of their life cycle (Faulkner 2004). Wetland size reduction up to 50% has been predicted to reduce richness by 10–16% in any taxa (Findlay and Houlihan 1997). Hydroperiod of mitigated wetlands is also important to consider as some amphibians depend on semipermanent wetlands to breed and safely undergo metamorphosis (Gamble and Mitsch 2009).

Once mitigation plans have been implemented on the Ruby Run riparian zone, pre-restoration data will provide a baseline for abundance and diversity for each taxon. Comparing baseline data to post-restoration monitoring data can provide more information on time requirements for riparian wetland succession. Usual wetland monitoring continues 3–5 years

post-restoration to determine restoration success; however, time required to monitor post-restoration can vary based on the wetland hydrology and structure (e.g. 10–15 years to monitor bogs or fens; Christie et al. 2015). With each following year post-restoration, abundance and diversity can be calculated to determine if mitigation efforts need to be modified using an adaptive management framework to ensure improvement of wetland condition.

AUTHOR CONTRIBUTIONS

Author Contributions: Conceptualization, J.T.A, J.A.H.; methodology, J.T.A.; formal analysis, D.N.L.; investigation, D.N.L., J.T.A.; data curation, D.N.L.; writing—original draft preparation, D.N.L.; writing—review and editing, J.T.A., J.A.H.; visualization, D.N.L.; supervision, J.T.A. and J.A.H.; funding acquisition, J.A.H., J.T.A. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

This study was approved by the Institutional Animal Care and Use Committee Review Board of West Virginia University (Protocol #1702005283_R1).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from J.T.A. upon request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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FIGURES

Figure 1. Proposed cover treatment sites along Ruby Run at the JW Ruby Research Farm, Reedsville, West Virginia, USA. Drone imagery was provided by Paul Kinder, West Virginia University Natural Resources Analysis Center. Proposed cover treatments were taken into consideration when surveying birds, anurans, and small mammals and are as follows: 1) control, which is defined as only straw mulch being applied; 2) wood mulch; 3) riparian herbaceous species; 4) riparian woody species; 5) bioenergy species (i.e. switchgrass [*Panicum virgatum*]); and 6) wet meadow herbaceous species.

Figure 2. Monthly Shannon diversity of birds within the riparian area of Ruby Run at the JW Ruby Research Farm, Reedsville, West Virginia, USA, from 2018–2020. Birds were recorded in five equidistant 50 m radius point counts along the stream as well as along transects between each point count area. Lines are plotted to show trends in diversity from downstream to upstream.

Figure 3. Seasonal variation in benthic macroinvertebrate Shannon diversity (H') across five wetlands within the Ruby Run riparian zone at the J.W. Ruby Research Farm in Preston County, West Virginia, USA during 2018–2020. Error bars indicate standard error for each mean value.

Figure 4. Variation of small mammal catch-per-unit-effort (CPUE; calculated as the number of captures per 100 trap nights) among the 12 traplines surveyed along Ruby Run at the JW Ruby Research Farm, Reedsville, West Virginia, USA, across years (2017–2020). Six unique species were captured across the four years: white-footed mouse (*Peromyscus leucopus*), deer mouse (*Peromyscus maniculatus*), short-tailed shrew (*Blarina brevicauda*), Eastern chipmunk (*Tamias striatus*), meadow vole (*Microtus pennsylvanicus*), and meadow jumping mouse (*Zapus hudsonius*).

TABLES

Table 1. Bird species composition (number of individuals per 50 m radius plot per survey) listed within order of abundance within the Ruby Run riparian area at JW Ruby Research Farm in Preston County, West Virginia, USA, based on monthly point count surveys, 2018–2020.

Species name	Scientific name	Individuals per 50-m radius plot	
		Mean	SE
Song sparrow	<i>Melospiza melodia</i>	3.26	0.28
Red-winged blackbird	<i>Agelaius phoeniceus</i>	0.73	0.17
Canada goose	<i>Branta canadensis</i>	0.58	0.27
Barn swallow	<i>Hirundo rustica</i>	0.49	0.15
American goldfinch	<i>Spinus tristis</i>	0.29	0.08
American robin	<i>Turdus migratorius</i>	0.24	0.11
American crow	<i>Corvus brachyrhynchos</i>	0.19	0.10
Bobolink	<i>Dolichonyx oryzivorus</i>	0.11	0.05
Killdeer	<i>Charadrius vociferus</i>	0.09	0.04
Common grackle	<i>Quiscalus quiscula</i>	0.08	0.08
European starling	<i>Sturnus vulgaris</i>	0.08	0.03
Common yellowthroat	<i>Geothlypis trichas</i>	0.07	0.03
Mourning dove	<i>Zenaida macroura</i>	0.07	0.02
Northern cardinal	<i>Cardinalis cardinalis</i>	0.07	0.03
Savannah sparrow	<i>Passerculus sandwichensis</i>	0.07	0.04
Indigo bunting	<i>Passerina cyanea</i>	0.06	0.02
Mallard	<i>Anas platyrhynchos</i>	0.06	0.05

Table 1. Continued.

Species name	Scientific name	Individuals per 50 m radius plot	
		Mean	SE
Eastern bluebird	<i>Sialis sialis</i>	0.05	0.02
Eastern kingbird	<i>Tyrannus tyrannus</i>	0.04	0.02
Eastern phoebe	<i>Sayornis phoebe</i>	0.04	0.02
Red-bellied woodpecker	<i>Melanerpes carolinus</i>	0.04	0.01
Solitary sandpiper	<i>Tringa solitaria</i>	0.04	0.02
Blue jay	<i>Cyanocitta cristata</i>	0.03	0.01
Carolina wren	<i>Thryothorus ludovicianus</i>	0.03	0.01
Eastern meadowlark	<i>Sturnella magna</i>	0.03	0.01
Northern mockingbird	<i>Mimus polyglottos</i>	0.03	0.02
Ruby-throated hummingbird	<i>Archilochus coluris</i>	0.03	0.01
White-breasted nuthatch	<i>Sitta carolinensis</i>	0.03	0.01
White-throated sparrow	<i>Zonotrichia albicollis</i>	0.03	0.02
Baltimore oriole	<i>Icterus galbula</i>	0.02	0.01
Black-capped chickadee	<i>Poecile atricapillus</i>	0.02	0.02
Common raven	<i>Corvus carax</i>	0.02	0.01
Field sparrow	<i>Spizella pusilla</i>	0.02	0.01
Grasshopper sparrow	<i>Ammodramus savannarum</i>	0.02	0.02
Northern flicker	<i>Colaptes auratus</i>	0.02	0.01

Table 1. Continued.

Species name	Scientific name	Individuals per 50 m radius plot	
		Mean	SE
Yellow-rumped warbler	<i>Setophaga coronata</i>	0.02	0.02
Brown-headed cowbird	<i>Molothrus ater</i>	0.01	0.01
Brown thrasher	<i>Toxostoma rufum</i>	0.01	0.01
Cackling goose	<i>Branta hutchinsii</i>	0.01	0.01
Carolina chickadee	<i>Poecile carolinensis</i>	0.01	0.01
Chipping sparrow	<i>Spizella passerina</i>	0.01	0.01
Downy woodpecker	<i>Picoides pubescens</i>	0.01	0.01
Eastern towhee	<i>Pipilo erythrophthalmus</i>	0.01	0.01
Eastern wood-pewee	<i>Contopus virens</i>	0.01	0.01
Golden-crowned kinglet	<i>Regulus satrapa</i>	0.01	0.01
Lesser yellowlegs	<i>Tringa flavipes</i>	0.01	0.01
Nashville warbler	<i>Leiothlypis ruficapilla</i>	0.01	0.01
Northern rough-winged swallow	<i>Stelgidopteryx serripennis</i>	0.01	0.01
Orchard oriole	<i>Icterus spurius</i>	0.01	0.01
Rose-breasted grosbeak	<i>Pheucticus ludovicianus</i>	0.01	0.01
Tree swallow	<i>Tachycineta bicolor</i>	0.01	0.01
Tufted titmouse	<i>Baeolophus bicolor</i>	0.01	0.01
Turkey vulture	<i>Cathartes aura</i>	0.01	0.01

Table 1. Continued.

Species name	Scientific name	Individuals per 50 m radius plot	
		Mean	SE
White-crowned sparrow	<i>Zonotrichia leucophrys</i>	0.01	0.01
Western meadowlark	<i>Sturnella neglecta</i>	0.01	0.01
Willow flycatcher	<i>Empidonax traillii</i>	0.01	0.01
Wood duck	<i>Aix sponsa</i>	0.01	0.01
Yellow warbler	<i>Setophaga petechia</i>	0.01	0.01

Table 2. Benthic macroinvertebrate assemblages across five locations within the Ruby Run riparian zone at J.W. Ruby Research Farm in Preston County, West Virginia, USA, from 2018–2020. Relative abundance was calculated as number of individuals per square meter.

Class	Order	Family	Number of individuals	Individuals per m ²	
				Mean	SE
Oligochaeta			42	3565.07	640.85
	Coleoptera	Hydrophilidae	2	169.77	169.77
	Coleoptera	Ptilodactylidae	4	339.53	224.58
	Coleoptera	Staphylinidae	1	84.88	84.88
	Diptera	Ceratopogonidae	12	1018.59	640.85
	Diptera	Chironomidae	14	1188.36	662.95
	Diptera	Empididae	1	84.88	84.88
	Diptera	Psychoidae	2	169.77	169.77
	Diptera	Tabanidae	4	339.53	224.58
	Diptera	Tipulidae	1	84.88	84.88
	Poduromorpha	Neanuridae	1	84.88	84.88
	Trichoptera	Rhyacophilidae	1	84.88	84.88
	Trombidiformes		1	84.88	84.88

Table 3. Small mammal species assemblage of the riparian zone of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, from 2017–2020. Number of unique individuals from each species were combined across years to derive total number of individuals. Average annual number of individuals per 100 trap nights with standard error (SE) were calculated.

Species	Scientific name	Number of individuals	Number of captures per 100 trap night	
			Mean	SE
Meadow vole	<i>Microtus pennsylvanicus</i>	427	4.67	1.18
Short-tailed shrew	<i>Blarina brevicauda</i>	123	1.41	0.46
Deer mouse	<i>Peromyscus maniculatus</i>	33	0.35	0.12
White-footed mouse	<i>Peromyscus leucopus</i>	18	0.21	0.17
Meadow jumping mouse	<i>Zapus hudsonius</i>	2	0.04	0.04
Eastern chipmunk	<i>Tamias striatus</i>	1	0.01	0.01

Table 4. Shannon diversity (H') for turtles in Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, from 2018–2020. Two species were captured: snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*). Captured individuals were given a unique notch for future identification. Recapture rates were based on within-year and between-year recaptures divided by total captures.

Year	Snapping turtle	Painted turtle	Recapture rates	H'
2018	6	0	0.33	0.00
2019	3	1	0.50	0.56
2020	3	0	1.00	0.00

Table 5. Anuran assemblage from nocturnal call surveys conducted within the riparian zone of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA from 2017–2020. Average individuals per year is an estimate according to Wisconsin Index values given to each detection (1 = non-overlapping calls and an exact count of individuals could be made; 2 = overlapping calls with estimates of individuals made; 3 = calling in full chorus and assumed 50 individuals; Balcombe et al. 2005b).

Species	Scientific name	Wisconsin Index Values		
		Mean	SE	Median
Spring peeper	<i>Pseudacris crucifer</i>	1.63	0.13	1
American toad	<i>Anaxyrus americanus</i>	1.19	0.10	1
Cope's gray treefrog	<i>Hyla chrysoscelis</i>	1	0	1
Wood frog	<i>Lithobates sylvaticus</i>	1.67	0.33	2
American bullfrog	<i>Lithobates catesbeianus</i>	1	0	1
Green frog	<i>Lithobates clamitans</i>	1	0	1

Table 6. Mean ($\pm$ SE) annual variation of Shannon diversity (H') and Pielou's evenness (J) of plant species observed at Ruby Run, Preston County, West Virginia, USA. Surveys were conducted in September 2016, June and September 2017 and 2018, and July 2020. The number of plots varied between 63 in 2016 and 82 in 2020 ($n = 445$ plots across 6 visits in 4 years).

Year	Number of species	H'	J
2016	59	0.235 (0.007)	0.709 (0.022)
2017	87	0.258 (0.004)	0.748 (0.011)
2018	103	0.244 (0.003)	0.782 (0.011)
2020	90	0.216 (0.005)	0.721 (0.016)

Table 7. Dominant plant species surveyed within the riparian area of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, during September 2016, June and September 2017 and 2018, and July 2020. Dominant species were identified as contributing > 10% cover combined across all four years. Total mean plant cover was the combined mean percent cover for each species within quadrats across years, while mean plant cover was the estimated percent cover within quadrats per survey. All values are reported as percentages.

Species	Total mean cover	Percent cover per survey	
		Mean	SE
<i>Juncus effusus</i>	54.354	9.059	2.300
<i>Carex lurida</i>	39.324	6.554	1.590
<i>Impatiens capensis</i>	39.091	6.515	1.977
<i>Solidago rugosa</i>	35.509	5.973	3.659
<i>Dichanthelium clandestinum</i>	32.790	5.465	1.013
<i>Carex alopecoidea</i>	32.052	5.342	2.207
<i>Vernonia noveboracensis</i>	30.615	5.102	1.367
<i>Agrostis gigantea</i>	23.345	3.890	2.937
<i>Symphyotrichum puniceum</i>	15.998	2.666	1.536
<i>Eleocharis tenuis</i>	15.058	2.510	0.530
<i>Rubus hispidus</i>	14.162	2.360	0.790
<i>Lycopus virginicus</i>	14.102	2.350	0.865
<i>Aster novae-angliae</i>	12.558	2.092	2.053
<i>Rubus allegheniensis</i>	11.310	1.885	0.563
<i>Carex scoparia</i>	11.182	1.864	0.641
<i>Poa</i> spp.	11.159	1.859	0.995
<i>Polygonum sagittatum</i>	10.984	1.831	0.503

Table 8. Species assemblage of fishes surveyed by single-pass electroshocking in Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, from 2017–2020.

Species	Scientific name	Number of individuals	Mean per year (SE)	Mean proportion per year (SE)
Creek chub	<i>Semotilus atromaculatus</i>	499	125 (45)	0.62 (0.13)
Largemouth bass	<i>Micropterus salmoides</i>	82	21 (11)	0.12 (0.05)
Green sunfish	<i>Lepomis cyanellus</i>	77	19 (8)	0.19 (0.12)
Spotted bass	<i>Micropterus punctulatus</i>	12	3 (2)	0.017 (0.01)
Spottail shiner	<i>Notropis hudsonis</i>	12	3 (3)	0.015 (0.015)
Bluegill	<i>Lepomis macrochirus</i>	11	2.75 (2.75)	0.02 (0.02)
Smallmouth bass	<i>Micropterus dolomieu</i>	5	1.25 (1.25)	0.006 (0.006)
Yellow bullhead	<i>Ameiurus natalis</i>	2	0.5 (0.5)	0.001 (0.001)
Central stoneroller	<i>Camptostoma anomalum</i>	2	0.5 (0.5)	0.003 (0.003)
Pumpkinseed	<i>Lepomis gibbosus</i>	2	0.5 (0.5)	0.003 (0.003)
Blacknose dace	<i>Rhinichthys atratulus</i>	2	0.5 (0.5)	0.002 (0.002)
Brown bullhead	<i>Ameiurus nebulosus</i>	1	0.25 (0.25)	0.001 (0.001)
Longnose dace	<i>Rhinichthys cataractae</i>	1	0.25 (0.25)	0.001 (0.001)

Table 9. Temporal variation in species richness and Shannon diversity (H') of fish species electroshocked during surveys in August 2017, August 2018, September 2019, and November 2020 along a 700 m stretch in Ruby Run, a tributary within the Upper Deckers Creek watershed in Preston County, West Virginia, USA. Creek chub (*Semotilus atromaculatus*) followed by green sunfish (*Lepomis cyanellus*) was the most abundant species across years. Species richness values for each reach were calculated as the number of species present out of the total number of species surveyed for each year ($n = 3, 5, 4$, and 10 species in 2017, 2018, 2019, and 2020, respectively).

Year	Species richness		H'	
	Mean	SE	Mean	SE
2017	0.86	0.07	0.76	0.07
2018	0.60	0.12	0.63	0.21
2019	0.61	0.11	0.63	0.21
2020	0.34	0.05	0.49	0.18

Table 10. Macroinvertebrate assemblages within stream riffles surveyed in Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, from 2018–2019. Relative abundance was determined as number of individuals per square meter.

Class	Order	Family	Individuals per m ²	
			Mean	SE
Oligochaeta	Anisoptera	Gomphidae	0.03	0.03
			0.16	0.16
	Decapoda	Astacidae	0.09	0.09
	Diptera	Simuliidae	0.16	0.16
	Ephemeroptera	Heptageniidae	0.02	0.02
	Plecoptera	Capniidae	0.03	0.03
	Trichoptera	Hydropsychidae	0.13	0.13
	Diptera	Ceratopogonidae	0.25	0.19
	Diptera	Chironomidae	0.02	0.02
	Isopoda	Asellidae	0.08	0.08
	Plecoptera	Chloroperlidae	0.03	0.03
	Plecoptera	Perlodidae	0.16	0.16
	Decapoda	Cambaridae	0.06	0.06
	Diptera	Tipulidae	0.03	0.03
	Trichoptera	Philopotamidae	0.06	0.06
	Plecoptera	Perlidae	0.02	0.02

APPENDICES

Appendix 1. Measuring parameters for sky, wind, and noise disturbance used in bird and anuran surveys within the Ruby Run riparian area at J.W. Ruby Research Farm in Preston County, West Virginia, USA. Wind conditions follow the Beaufort scale.

Numerical Code	Sky	Wind	Disturbance
0	Clear sky	Smoke rises vertically	No noise disturbance
1	Partly cloudy	Smoke drift indicates wind direction, still weathervane	Slight noise disturbance; does not prohibit the observer from detecting birds
2	Completely overcast	Wind felt on face, leaves rustle	External noises moderately prohibit the observer from detecting birds
3	Drizzle	Leaves and small twigs constantly moving	External noises severely prohibit the observer from detecting birds
4	Raining	Dust, leaves, and loose paper lifted; small tree branches move	N/A

Appendix 2. Species assemblages of birds detected from 2018 to 2020 within the riparian wetland boundary of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA. Number of individuals and the proportion of detections for the entire year are reported for each species.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2018	Song sparrow	<i>Melospiza melodia</i>	145	0.403
	Red-winged blackbird	<i>Agelaius phoeniceus</i>	27	0.075
	American robin	<i>Turdus migratorius</i>	25	0.069
	American crow	<i>Corvus brachyrhynchos</i>	22	0.061
	Barn swallow	<i>Hirundo rustica</i>	19	0.053
	American goldfinch	<i>Spinus tristis</i>	18	0.05
	Canada goose	<i>Branta canadensis</i>	13	0.036
	Bobolink	<i>Dolichonyx oryzivorus</i>	11	0.031
	Killdeer	<i>Charadrius vociferus</i>	10	0.028
	Eastern bluebird	<i>Sialis sialis</i>	8	0.022
	Indigo bunting	<i>Passerina cyanea</i>	6	0.017
	Carolina wren	<i>Thryothorus ludovicianus</i>	4	0.011
	Common yellowthroat	<i>Geothlypis trichas</i>	4	0.011
	Eastern kingbird	<i>Tyrannus tyrannus</i>	4	0.011
	Mourning dove	<i>Zenaida macroura</i>	4	0.011
	White-throated sparrow	<i>Zonotrichia albicollis</i>	4	0.011
	Yellow-rumped warbler	<i>Setophaga coronata</i>	4	0.011
	Eastern meadowlark	<i>Sturnella magna</i>	3	0.008
	Northern cardinal	<i>Cardinalis cardinalis</i>	3	0.008
	Northern mockingbird	<i>Mimus polyglottos</i>	3	0.008

Appendix 2. Continued.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2018	Eastern phoebe	<i>Sayornis phoebe</i>	2	0.006
	Eastern wood-pewee	<i>Contopus virens</i>	2	0.006
	European starling	<i>Sturnus vulgaris</i>	2	0.006
	Field sparrow	<i>Spizella pusilla</i>	2	0.006
	Northern flicker	<i>Colaptes auratus</i>	2	0.006
	Red-bellied woodpecker	<i>Melanerpes carolinus</i>	2	0.006
	Savannah sparrow	<i>Passercules sandwichensis</i>	2	0.006
	Solitary sandpiper	<i>Tringa solitaria</i>	2	0.006
	Cackling goose	<i>Branta hutchinsii</i>	1	0.003
	Chipping sparrow	<i>Spizella passerina</i>	1	0.003
	Eastern towhee	<i>Pipilo erythrophthalmus</i>	1	0.003
	Nashville warbler	<i>Leiothlypis ruficapilla</i>	1	0.003
	Rose-breasted grosbeak	<i>Pheucticus ludovicianus</i>	1	0.003
	Ruby-throated hummingbird	<i>Archilochus coluris</i>	1	0.003
	White-breasted nuthatch	<i>Sitta carolinensis</i>	1	0.003
2019	Song sparrow	<i>Melospiza melodia</i>	222	0.445
	Red-winged blackbird	<i>Agelaius phoeniceus</i>	67	0.134
	Canada goose	<i>Branta canadensis</i>	54	0.108
	Barn swallow	<i>Hirundo rustica</i>	30	0.06
	American goldfinch	<i>Spinus tristis</i>	16	0.032
	Common grackle	<i>Quiscalus quiscula</i>	15	0.03
	American robin	<i>Turdus migratorius</i>	9	0.018

Appendix 2. Continued

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2019	Common yellowthroat	<i>Geothlypis trichas</i>	7	0.014
	Bobolink	<i>Dolichonyx oryzivorus</i>	5	0.01
	European starling	<i>Sturnus vulgaris</i>	5	0.01
	Mourning dove	<i>Zenaida macroura</i>	5	0.01
	Savannah sparrow	<i>Passerculus sandwichensis</i>	5	0.01
	American crow	<i>Corvus brachyrhynchos</i>	4	0.008
	Indigo bunting	<i>Passerina cyanea</i>	4	0.008
	Baltimore oriole	<i>Icterus galbula</i>	3	0.006
	Blue jay	<i>Cyanocitta cristata</i>	3	0.006
	Common raven	<i>Corvus corax</i>	3	0.006
	Eastern kingbird	<i>Tyrannus tyrannus</i>	3	0.006
	Grasshopper sparrow	<i>Ammodramus savannarum</i>	3	0.006
	Killdeer	<i>Charadrius vociferus</i>	3	0.006
	Mallard	<i>Anas platyrhynchos</i>	3	0.006
	Northern cardinal	<i>Cardinalis cardinalis</i>	3	0.006
	Red-bellied woodpecker	<i>Melanerpes carolinus</i>	3	0.006
	Ruby-throated hummingbird	<i>Archilochus colubris</i>	3	0.006
	Eastern phoebe	<i>Sayornis phoebe</i>	2	0.004
	Field sparrow	<i>Spizella pusilla</i>	2	0.004
	Lesser yellowlegs	<i>Tringa flavipes</i>	2	0.004
	Yellow warbler	<i>Setophaga petechia</i>	2	0.004
	Brown-headed cowbird	<i>Molothrus ater</i>	1	0.002

Appendix 2. Continued.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2019	Carolina wren	<i>Thryothorus ludovicianus</i>	1	0.002
	Downy woodpecker	<i>Picoides pubescens</i>	1	0.002
	Eastern bluebird	<i>Sialis sialis</i>	1	0.002
	Golden-crowned kinglet	<i>Regulus satrapa</i>	1	0.002
	Northern flicker	<i>Colaptes auratus</i>	1	0.002
	Northern rough-winged swallow	<i>Stelgidopteryx serripennis</i>	1	0.002
	Tree swallow	<i>Tachycineta bicolor</i>	1	0.002
	Tufted titmouse	<i>Baeolophus bicolor</i>	1	0.002
	Turkey vulture	<i>Cathartes aura</i>	1	0.002
	White-crowned sparrow	<i>Zonotrichia leucophrys</i>	1	0.002
	Willow flycatcher	<i>Empidonax traillii</i>	1	0.002
	Wood duck	<i>Aix sponsa</i>	1	0.002
2020	Song sparrow	<i>Melospiza melodia</i>	219	0.489
	Barn swallow	<i>Hirundo rustica</i>	39	0.087
	Canada goose	<i>Branta canadensis</i>	37	0.083
	Red-winged blackbird	<i>Agelaius phoeniceus</i>	37	0.083
	American goldfinch	<i>Spinus tristis</i>	19	0.042
	American robin	<i>Turdus migratorius</i>	9	0.02
	American crow	<i>Corvus brachyrhynchos</i>	8	0.018
	Mallard	<i>Anas platyrhynchos</i>	8	0.018
	European starling	<i>Sturnus vulgaris</i>	7	0.016
	Northern cardinal	<i>Cardinalis cardinalis</i>	7	0.016
	Solitary sandpiper	<i>Tringa solitaria</i>	6	0.013

Appendix 2. Continued.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2020	Savannah sparrow	<i>Passerculus sandwichensis</i>	5	0.011
	Bobolink	<i>Dolichonyx oryzivorus</i>	4	0.009
	Eastern phoebe	<i>Sayornis phoebe</i>	4	0.009
	Killdeer	<i>Charadrius vociferus</i>	4	0.009
	White-breasted nuthatch	<i>Sitta carolinensis</i>	4	0.009
	Black-capped chickadee	<i>Poecile atricapillus</i>	3	0.007
	Mourning dove	<i>Zenaida macroura</i>	3	0.007
	Blue jay	<i>Cyanocitta cristata</i>	2	0.004
	Common yellowthroat	<i>Geothlypis trichas</i>	2	0.004
	Eastern meadowlark	<i>Sturnella magna</i>	2	0.004
	Northern mockingbird	<i>Mimus polyglottos</i>	2	0.004
	Orchard oriole	<i>Icterus spurius</i>	2	0.004
	Red-bellied woodpecker	<i>Melanerpes carolinus</i>	2	0.004
	White-throated sparrow	<i>Zonotrichia albicollis</i>	2	0.004
	Brown thrasher	<i>Toxostoma rufum</i>	1	0.002
	Carolina chickadee	<i>Poecile carolinensis</i>	1	0.002
	Carolina wren	<i>Thryothorus ludovicianus</i>	1	0.002
	Common raven	<i>Corvus carax</i>	1	0.002
	Eastern kingbird	<i>Tyrannus tyrannus</i>	1	0.002
	Grasshopper sparrow	<i>Ammodramus savannarum</i>	1	0.002
	Northern flicker	<i>Colaptes auratus</i>	1	0.002
	Ruby-throated hummingbird	<i>Archilochus colubris</i>	1	0.002

Appendix 2. Continued.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2020	Tufted titmouse	<i>Baeolophus bicolor</i>	1	0.002
	Western meadowlark	<i>Sturnella neglecta</i>	1	0.002
	Wood duck	<i>Aix sponsa</i>	1	0.002

Appendix 3. Taxonomic classifications for benthic macroinvertebrates identified from soil core samples in five wetlands within the riparian area of Ruby Run on JW Ruby Research Farm in Preston County, West Virginia, USA, from 2018 to 2020. Number of individuals from each taxon was used to calculate a proportion of the sampled individuals for each year.

Year	Class	Order	Family	Quantity	Proportion
2018	Oligochaeta			11	0.458
		Diptera	Chironomidae	9	0.375
		Coleoptera	Ptilodactylidae	1	0.042
		Diptera	Ceratopogonidae	1	0.042
		Diptera	Empididae	1	0.042
		Diptera	Tabanidae	1	0.042
2019	Oligochaeta			12	0.343
		Diptera	Ceratopogonidae	9	0.257
		Diptera	Chironomidae	5	0.143
		Coleoptera	Ptilodactylidae	3	0.086
		Diptera	Tabanidae	3	0.086
		Diptera	Psychoidae	2	0.057
		Trichoptera	Rhyacophilidae	1	0.029
2020	Oligochaeta			19	0.655
		Coleoptera		2	0.069
		Coleoptera	Hydrophilidae	2	0.069
		Diptera	Ceratopogonidae	2	0.069
		Trombidiformes		1	0.034
		Coleoptera	Staphylinidae	1	0.034
		Diptera	Tipulidae	1	0.034
	Collembola	Poduromorpha	Neanuridae	1	0.034

Appendix 4. Species assemblages of small mammals detected from 2017 to 2020 within the riparian wetland boundary of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA. Number of individuals and the proportion of captures for the entire year are reported for each species.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2017	Meadow vole	<i>Microtus pennsylvanicus</i>	79	0.705
	Short-tailed shrew	<i>Blarina brevicauda</i>	26	0.232
	Deer mouse	<i>Peromyscus maniculatus</i>	7	0.062
2018	Meadow vole	<i>Microtus pennsylvanicus</i>	63	0.708
	Deer mouse	<i>Peromyscus maniculatus</i>	11	0.124
	Short-tailed shrew	<i>Blarina brevicauda</i>	11	0.124
	White-footed mouse	<i>Peromyscus leucopus</i>	3	0.034
	Eastern chipmunk	<i>Tamias striatus</i>	1	0.011
2019	Meadow vole	<i>Microtus pennsylvanicus</i>	160	0.773
	Short-tailed shrew	<i>Blarina brevicauda</i>	45	0.217
	Deer mouse	<i>Peromyscus maniculatus</i>	2	0.010
2020	Meadow vole	<i>Microtus pennsylvanicus</i>	142	0.634
	Short-tailed shrew	<i>Blarina brevicauda</i>	51	0.228
	White-footed mouse	<i>Peromyscus leucopus</i>	15	0.067
	Deer mouse	<i>Peromyscus maniculatus</i>	13	0.058
	Meadow jumping mouse	<i>Zapus hudsonius</i>	3	0.013

Appendix 5. Small mammal species richness values for each trapline along Ruby Run, Preston County, West Virginia, USA, between 2017 and 2020. Values were calculated as number of unique species captured in a trapline divided by the total number of species captured in the year. Only eight unique species across three taxa were captured across the four years: white-footed mouse (*Peromyscus leucopus*), deer mouse (*Peromyscus maniculatus*), short-tailed shrew (*Blarina brevicauda*), eastern chipmunk (*Tamias striatus*), meadow vole (*Microtus pennsylvanicus*), and meadow jumping mouse (*Zapus hudsonius*). Three species were captured in 2017 and 2019, while five species were captured in 2018 and 2020.

Month	Trapline	2017	2018	2019	2020
May	1	0.33	0	0.33	0.2
	2	0	0.2	0.33	0
	3	0	0.2	0.67	0
	4	0	0	0.67	0.2
	5	0	0	0.33	0.4
	6	0.33	0	0	0
	7	0.33	0	0	0.2
	8	0.33	0	0.67	0
	9	0.33	0	0.33	0.2
	10	0	0	0.33	0.2
	11	0	0	0.33	0
	12	0	0.2	0.33	0.2
June	1	0.33	0.2	0.67	0.2
	2	0.33	0.2	0.33	0.2
	3	0	0	0.33	0.2
	4	0.33	0	0.67	0.4
	5	0	0	0.67	0.2
	6	0	0.2	0.67	0
	7	0	0	0.33	0.6
	8	0.67	0	0.67	0.4
	9	0.67	0	0.67	0.6
	10	0.67	0	0.67	0.4
	11	0.33	0	0.67	0.4
	12	0.33	0	0.33	0.2
July	1	0.33	0.2	0.33	0.2
	2	0	0.2	0.33	0.4
	3	0.33	0.2	0.67	0.4
	4	0.67	0	0.33	0.8
	5	0.33	0	0	0.4

Appendix 5. Continued

Month	Trapline	2017	2018	2019	2020
July	6	0	0	0.33	0.2
	7	0.33	0.4	0	0.8
	8	0.33	0.2	0.33	0.6
	9	0.33	0.2	0.67	0.6
	10	0	0.2	0	0.6
	11	0	0.2	0	0.6
August	12	0.33	0.2	0	0.4
	1	0.33	0.2	0.33	0.4
	2	0.33	0.2	0.33	0.4
	3	0.33	0.2	0.67	0.2
	4	0.67	0.2	0.67	0.8
	5	0.33	0.2	0.33	0.6
	6	0	0	0.33	0.6
	7	0.67	0	0	0.4
	8	1	0.6	0.33	0.8
	9	0.67	0.4	0.67	0.6
	10	0.67	0.2	0.67	0.6
	11	0.33	0.4	0.33	0.4
September	12	0	0.2	0.67	0.4
	1	0.33	0.2	0.33	0.4
	2	0	0.4	0.67	0.4
	3	0.33	0.4	0.67	0.4
	4	0.33	0.2	0.67	0.4
	5	0.33	0.4	0.33	0.2
	6	0	0	0.33	0.2
	7	0	0	0.67	0.8
	8	0.67	0.2	0.33	0.4
	9	0.67	0.6	0.67	0.6
	10	0.33	0	0.33	0.2
	11	0.33	0.4	0.67	0.4
	12	0	0.2	0.33	0.2

Appendix 6. Species assemblages of anurans detected between 2017–2020 within the riparian boundary of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA.

Proportion of individuals detected per year were calculated for each species.

Year	Species	Scientific name	Wisconsin Index values			Proportion of total detections
			Mean	SE	Median	
2017	Spring peeper	<i>Pseudacris crucifer</i>	1.44	0.12	1	0.750
	Wood frog	<i>Lithobates sylvaticus</i>	2	0	2	0.086
	American toad	<i>Anaxyrus americanus</i>	1.17	0.17	1	0.065
	American bullfrog	<i>Lithobates catesbeianus</i>	1	0	1	0.043
	Green frog	<i>Lithobates clamitans</i>	1	0	1	0.030
2018	Cope's gray treefrog	<i>Hyla chrysoscelis</i>	1	0	1	0.026
	Spring peeper	<i>Pseudacris crucifer</i>	1.64	0.19	1	0.882
	American toad	<i>Anaxyrus americanus</i>	1.25	0.16	1	0.079
	Cope's gray treefrog	<i>Hyla chrysoscelis</i>	1	0	1	0.031
2019	American bullfrog	<i>Lithobates catesbeianus</i>	1	0	1	0.007
	Spring peeper	<i>Pseudacris crucifer</i>	2.13	0.45	1.5	0.948
	American bullfrog	<i>Lithobates catesbeianus</i>	1	0	1	0.015
	American toad	<i>Anaxyrus americanus</i>	1	0	1	0.015
2020	Green frog	<i>Lithobates clamitans</i>	1	0	1	0.015
	American toad	<i>Anaxyrus americanus</i>	1	0	1	0.015

Appendix 6. Continued

Year	Species	Scientific name	Wisconsin Index values		Year	Species
			Mean	SE		
2019	Wood frog	<i>Lithobates sylvaticus</i>	1	-	1	0.007
2020	Spring peeper	<i>Pseudacris crucifer</i>	1.33	0.11	1	0.939
	Cope's gray treefrog	<i>Hyla chrysoscelis</i>	1	0	1	0.045
	American bullfrog	<i>Lithobates catesbeianus</i>	1	-	1	0.015

Appendix 7. *Is there hydrophytic plant presence within the riparian area of Ruby Run?*

METHODS

We assigned an origin to each plant as either native, introduced, exotic, or adventive according to the USDA Plant Index. Introduced plants were defined as plants native to elsewhere in U.S. and Canada and planted intentionally in West Virginia (e.g. seed mix). Exotic species were defined as plant species not native to U.S. or Canada, and adventive species were defined as species not native to West Virginia growing without known introduction that could have been native elsewhere in U.S. or Canada prior to European settlement.

Wetland indicator status (WIS) values were also assigned to each species based on the Checklist and Atlas of the Vascular Flora of West Virginia (obligate-wet [OBL] = 1, facultative-wet [FACW] = 2, facultative [FAC] = 3, facultative-up [FACU] = 4, and obligate-up [UPL] = 5; Atkinson et al. 1993, Reed 1996, Harmon et al. 2006). Coefficients of conservatism (0 = taxa well-adapted to high levels of disturbance and site degradation, not limited to a specific community; 10 = taxa restricted to a narrow range of habitat conditions and relatively intolerant of habitat degradation) were assigned to each plant species surveyed (Harmon et al. 2006, Rentch and Anderson 2006) to determine the range of species that are either tolerant or intolerant of site degradation.

We also calculated percent of hydrophytic vegetation sampled within quadrats following the basic rule in the 1987 U.S. Army Corps of Engineering Wetland Delineation Manual (USACE 1987). For each plant species, we used its origin and coefficient of conservatism to calculate a floristic quality index (FQI) for each survey period as:

$$FQI = \text{Mean } C \times \sqrt{N} \quad (1)$$

where C = coefficient of conservatism and N = total number of native species. As a measure of relative “wetness,” we calculated mean weighted averages for each quadrat (MWA_{quad}) using a formula proposed by Atkinson et al. (1993):

$$MWA_{quad} = (y_1u_1 + y_2u_2 + \dots + y_nu_n)/100 \quad (2)$$

where $y_1, y_2, \dots, y_n$ were the average cover estimates for species 1, 2, $\dots$ n, and $u_1, u_2, \dots, u_n$ were the WIS values of the respective species. We identified a wetness value of less than 3.0 on a scale of 1 to 5 as wetland vegetation, although a plant community with a value between 2.5 and 3.0 was considered to have marginal wetland vegetation cover.

RESULTS

Temporal shifts in relative wetness were observed within years and between years. The goal of relative wetness was for MWA_{quad} to be below 3, and 82.5% of surveyed quadrats were considered wet in September 2016, with subsequent visits reporting 50.70% and 97.18% in 2017, 97.43% and 61.25% in 2018, and 78.04% of quadrats within the relative wetness threshold in 2020. Floristic Quality Index scores ranged from 17.83 in June 2017 to 24.94 in September 2017 (21.21 ± 1.02).

FQI was relatively low, likely due to the mean coefficient of conservatism being 3.14 ± 0.10 out of 10, which means the species are not as habitat-specific and are tolerate disturbance such as cattle grazing and mowing. Upland species could have dispersed from adjacent fields through water, wind, or cattle and established that way as well.

Figure A7-1. Ruby Run wetland plant A) relative wetness (defined as mean weighted averages; Atkinson et al. 1993) and B) evenness (proportion of diversity to maximum diversity for each visit) for each survey period from 2016–2018 and 2020. The black dotted line indicates a relative wetness value of 3, which is the threshold of wetness determined by Atkinson et al. (1993). Gaps in evenness indicate no plant species were present in a quadrat

Figure A7-2. Mean total plant cover for each vegetative survey within the riparian wetland zone of Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, according to wetland indicator statuses (OBL = obligate, FACW = facultative wetland, FAC = facultative, FACU = facultative upland, UPL = upland, NI = no indicator).

Table A7-1. Origins (N = Native; I = Introduced, native elsewhere in North America north of Mexico and intentionally planted in WV; B = Both native and introduced in the lower 48 United States of America; E = Exotic, not native to North America north of Mexico; A = Adventive, native elsewhere in North America prior to European settlement, not native to West Virginia but now growing in the state without known introduction; U = Unknown), coefficients of conservatism (COC; 0–10), wetland indicator status (WIS) and index value (0 = WIS not reported; 1 = obligate wetland [OBL]; 2 = facultative-wet [FACW]; 3 = facultative [FAC]; 4 = facultative-up [FACU], and 5 = obligate-up [UPL]) for plant species observed around Ruby Run, Preston County, West Virginia, USA, from 2016–2018 and 2020. Years in which species were present is indicated with an X.

2016	2017	2018	2020	Scientific Name	WIS	Index value	COC	Origin
		X	X	<i>Abutilon theophrasti</i>	FACU	4	1	B
X	X	X	X	<i>Achillea millefolium</i>	FACU	4	3	B
		X		<i>Agastache scrophulariifolia</i>	FACU	4	9	N
	X		X	<i>Agrostis gigantea</i>	FACW	2	0	I
	X	X	X	<i>Alnus serrulata</i>	OBL	1	5	N
		X	X	<i>Alopecurus sp.</i>	FACW	2	2	B
X	X	X	X	<i>Ambrosia artemisiifolia</i>	FACU	4	0	B
X		X	X	<i>Andropogon virginicus</i>	FACU	4	3	N
	X	X	X	<i>Anthoxanthum odoratum</i>	FACU	4	0	I
		X		<i>Artemisia vulgaris</i>	UPL	5	0	I
X	X			<i>Aster novae-angliae</i>	FAC	3	8	N
X	X			<i>Aster prenanthoides</i>	FAC	3	9	N
			X	<i>Aster sp.</i>	FAC	3	0	U
	X			<i>Bellis perennis</i>	NONE	0	1	I
		X		<i>Bidens cernua</i>	OBL	1	3	N
		X		<i>Bidens discoidea</i>	FACW	2	6	N
		X		<i>Bidens frondosa</i>	FACW	2	1	N
X		X		<i>Bidens sp.</i>	FACW	2	0	U
	X			<i>Boehmeria cylindrica</i>	OBL	1	5	N
	X			<i>Bromus sp.</i>	FACU	4	0	B
			X	<i>Calystegia sepium</i>	FAC	3	0	B
X		X	X	<i>Carex alopecoidea</i>	FACW	2	7	U
		X	X	<i>Carex gynandra</i>	OBL	1	5	N
X	X	X	X	<i>Carex lurida</i>	OBL	1	4	N
X	X	X	X	<i>Carex scoparia</i>	FACW	2	8	N

Table A7-1. Continued.

2016	2017	2018	2020	Scientific Name	WIS	Index Value	COC	Origin
		X	X	<i>Carex sp.</i>	FACW	2	0	U
		X		<i>Carex stricta</i>	OBL	1	10	N
		X		<i>Cerastium sp.</i>	UPL	5	0	U
	X			<i>Chelone glabra</i>	OBL	1	7	N
X			X	<i>Chenopodium album</i>	FACU	4	0	B
X				<i>Cichorium intybus</i>	NONE	0	1	I
	X	X	X	<i>Clematis sp.</i>	FAC	3	0	U
			X	<i>Clematis virginiana</i>	FAC	3	4	N
	X	X	X	<i>Cornus amomum</i>	FACW	2	4	N
		X		<i>Crataegus sp.</i>	FACU	4	0	B
		X	X	<i>Cuscuta sp.</i>	NONE	0	0	B
	X			<i>Cyperus strigosus</i>	FACW	2	3	N
	X	X		<i>Dactylis glomerata</i>	FACU	4	0	I
X	X			<i>Daucus carota</i>	UPL	5	0	I
X	X	X	X	<i>Dichanthelium clandestinum</i>	FACW	2	3	N
	X	X	X	<i>Dichanthelium sphaerocarpon</i>	FACU	4	4	N
		X	X	<i>Digitaria filiformis</i>	NONE	0	7	N
X				<i>Digitaria ischaemum</i>	FACU	4	0	I
X				<i>Dulichium arundinaceum</i>	OBL	1	10	N
X	X			<i>Echinochloa crus-galli</i>	FAC	3	1	I
		X		<i>Echinochloa sp.</i>	FAC	3	0	U
		X		<i>Elaeagnus umbellata</i>	NONE	0	0	I
	X	X		<i>Eleocharis obtusa</i>	OBL	1	2	N
	X			<i>Eleocharis sp.</i>	OBL	1	0	U
X	X	X	X	<i>Eleocharis tenuis</i>	FACW	2	4	N
			X	<i>Eragrostis minor</i>	NONE	0	1	I
		X		<i>Erigeron philadelphicus</i>	FACU	4	2	N
X	X	X	X	<i>Eupatorium perfoliatum</i>	FACW	2	9	N
	X	X	X	<i>Euthamia graminifolia</i>	FAC	3	6	N
	X	X	X	<i>Eutrochium sp.</i>	FACW	2	0	U
X		X		<i>Festuca arundinacea</i>	FACU	4	2	I
	X			<i>Festuca obtusa</i>	NONE	0	10	N
X	X			<i>Fragaria virginiana</i>	FACU	4	4	N
		X	X	<i>Galium aparine</i>	FACU	4	0	N
	X			<i>Galium asprellum</i>	OBL	1	5	N
		X	X	<i>Galium mollugo</i>	FACU	4	0	I
X		X	X	<i>Galium obtusum</i>	FACW	2	10	N
X	X	X	X	<i>Galium tinctorium</i>	OBL	1	5	N
X	X			<i>Geranium columbinum</i>	NONE	0	0	I
X	X	X	X	<i>Geum sp.</i>	FAC	3	0	B
	X	X	X	<i>Glechoma hederacea</i>	FACU	4	0	I
		X		<i>Glyceria canadensis</i>	OBL	1	7	N
	X			<i>Glyceria sp.</i>	OBL	1	0	U

Table A7-1. Continued.

2016	2017	2018	2020	Scientific Name	WIS	Index Value	COC	Origin
			X	<i>Glyceria striata</i>	OBL	1	6	N
X				<i>Helenium autumnale</i>	FACW	2	4	N
	X	X	X	<i>Holcus lanatus</i>	FACU	4	1	I
X	X	X	X	<i>Hypericum mutilum</i>	FACW	2	5	N
			X	<i>Hypericum olympicum</i>	NONE	0	0	U
			X	<i>Hypericum sp.</i>	FAC	3	0	U
X	X	X	X	<i>Impatiens capensis</i>	FACW	2	4	N
	X			<i>Juncus brevicaudatus</i>	OBL	1	6	N
X	X	X	X	<i>Juncus effusus</i>	OBL	1	3	N
X	X			<i>Juncus filiformis</i>	FACW	2	9	N
X	X	X	X	<i>Juncus tenuis</i>	FAC	3	4	N
		X		<i>Lactuca canadensis</i>	FACU	4	6	N
X	X	X	X	<i>Leersia oryzoides</i>	OBL	1	2	N
	X			<i>Linum striatum</i>	FACW	2	5	N
		X		<i>Lolium perenne</i>	FACU	4	0	I
X	X	X	X	<i>Ludwigia palustris</i>	OBL	1	4	N
X	X	X	X	<i>Lycopus virginicus</i>	OBL	1	4	N
			X	<i>Lysimachia ciliata</i>	FACW	2	6	N
		X		<i>Lysimachia nummularia</i>	FACW	2	0	I
X				<i>Melilotus alba</i>	NONE	0	0	I
		X	X	<i>Melilotus officinalis</i>	FACU	4	0	I
	X		X	<i>Mentha spicata</i>	FACW	2	0	I
	X	X	X	<i>Mimulus ringens</i>	OBL	1	6	N
X	X	X	X	<i>Onoclea sensibilis</i>	FACW	2	10	N
X	X	X	X	<i>Oxalis stricta</i>	FACU	4	0	N
		X		<i>Panicum sp.</i>	FAC	3	0	U
	X			<i>Parthenocissus quinquefolia</i>	FACU	4	2	N
			X	<i>Pelargonium sp.</i>	NONE	0	0	I
		X		<i>Phalaris arundinacea</i>	FACW	2	0	N
	X	X	X	<i>Phleum pratense</i>	FACU	4	0	I
			X	<i>Phytolacca decandra</i>	NONE	0	0	N
X	X		X	<i>Pilea pumila</i>	FACW	2	4	N
X	X	X	X	<i>Plantago lanceolata</i>	FACU	4	0	I
X	X	X	X	<i>Plantago major</i>	FACU	4	0	I
		X	X	<i>Poa spp.</i>	UNK	0	0	U
	X			<i>Poa sylvestris</i>	NONE	0	9	N
X				<i>Polygonum erectum</i>	FACU	4	0	N
		X	X	<i>Polygonum hydropiperoides</i>	OBL	1	7	N
	X	X		<i>Polygonum pennsylvanicum</i>	FACW	2	0	N
X				<i>Polygonum persicaria</i>	FAC	3	0	I
X	X	X	X	<i>Polygonum sagittatum</i>	OBL	1	9	N
		X	X	<i>Polygonum sp.</i>	UNK	0	0	U
X	X	X	X	<i>Potentilla simplex</i>	FACU	4	4	N

Table A7-1. Continued.

2016	2017	2018	2020	Scientific Name	WIS	Index Value	COC	Origin
	X			<i>Prenanthes altissima</i>	FACU	4	5	N
	X		X	<i>Prunella sp.</i>	FAC	3	0	B
		X	X	<i>Prunus serotina</i>	FACU	4	4	N
X	X	X		<i>Pycnanthemum incanum</i>	NONE	0	0	N
		X	X	<i>Pycnanthemum pycnanthemoides</i>	NONE	0	0	N
		X	X	<i>Ranunculus repens</i>	FAC	3	1	I
			X	<i>Rorippa austriaca</i>	NONE	0	1	I
X	X	X	X	<i>Rosa multiflora</i>	FACU	4	0	I
X	X	X	X	<i>Rubus allegheniensis</i>	FACU	4	7	N
X	X	X	X	<i>Rubus hispidus</i>	FACW	2	5	N
	X	X		<i>Rubus sp.</i>	UNK	0	0	U
X		X	X	<i>Rumex crispus</i>	FAC	3	0	I
X				<i>Salix nigra</i>	OBL	1	4	N
	X			<i>Sambucus nigra</i>	FACW	2	5	B
		X	X	<i>Sambucus sp.</i>	FACW	2	0	B
			X	<i>Scirpus atrocinctus</i>	OBL	1	10	N
	X	X	X	<i>Scirpus cyperinus</i>	OBL	1	10	N
	X	X	X	<i>Scirpus polyphyllus</i>	OBL	1	5	N
		X	X	<i>Scirpus sp.</i>	OBL	1	0	N
		X		<i>Smilax glauca</i>	FACU	4	5	N
X	X			<i>Smilax rotundifolia</i>	FAC	3	4	N
	X	X	X	<i>Solanum carolinense</i>	FACU	4	0	N
	X			<i>Solidago canadensis</i>	FACU	4	1	N
X		X		<i>Solidago juncea</i>	NONE	0	0	N
	X			<i>Solidago patula</i>	OBL	1	8	N
X		X	X	<i>Solidago rugosa</i>	FAC	3	3	N
	X	X	X	<i>Solidago sp.</i>	UNK	0	0	U
		X	X	<i>Sparganium americanum</i>	OBL	1	6	N
	X	X	X	<i>Symphyotrichum dumosum</i>	FAC	3	5	N
		X	X	<i>Symphyotrichum puniceum</i>	FACU	4	6	N
	X	X		<i>Symplocarpus foetidus</i>	OBL	1	7	N
X	X	X	X	<i>Taraxacum officinale</i>	FACU	4	0	B
	X	X	X	<i>Thalictrum dasycarpum</i>	NONE	0	7	U
		X		<i>Toxicodendron radicans</i>	FAC	3	4	N
		X		<i>Tridens flavus</i>	UPL	5	6	N
		X		<i>Trifolium campestre</i>	NONE	0	1	I
X	X	X	X	<i>Trifolium pratense</i>	FACU	4	0	I
X	X	X	X	<i>Trifolium repens</i>	FACU	4	0	I
			X	<i>Tripsacum sp.</i>	FAC	3	0	U
	X	X	X	<i>Verbena hastata</i>	FACW	2	5	N
X	X	X	X	<i>Vernonia noveboracensis</i>	FACW	2	4	N
		X		<i>Viburnum recognitum</i>	FAC	3	6	N
	X	X	X	<i>Viola sp.</i>	UNK	0	0	U

Appendix 8. Fish assemblages in August 2017, August 2018, September 2019, and November 2020 within Ruby Run, a tributary of Deckers Creek at J.W. Ruby Research Farm in Preston County, West Virginia, USA.

Year	Species	Scientific name	Number of individuals	Proportion of total detections
2017	Green sunfish	<i>Lepomis cyanellus</i>	39	0.542
	Creek chub	<i>Semotilus atromaculatus</i>	17	0.236
	Largemouth bass	<i>Micropterus salmoides</i>	16	0.222
2018	Creek chub	<i>Semotilus atromaculatus</i>	92	0.643
	Green sunfish	<i>Lepomis cyanellus</i>	21	0.147
	Largemouth bass	<i>Micropterus salmoides</i>	14	0.098
	Bluegill	<i>Lepomis macrochirus</i>	11	0.077
	Spotted bass	<i>Micropterus punctulatus</i>	5	0.035
2019	Creek chub	<i>Semotilus atromaculatus</i>	227	0.767
	Largemouth bass	<i>Micropterus salmoides</i>	52	0.176
	Green sunfish	<i>Lepomis cyanellus</i>	15	0.051
	Yellow bullhead	<i>Ameiurus natalis</i>	2	0.007
2020	Creek chub	<i>Semotilus atromaculatus</i>	163	0.827
	Spottail shiner	<i>Notropis hudsonis</i>	12	0.061
	Spotted bass	<i>Micropterus punctulatus</i>	7	0.036
	Smallmouth bass	<i>Micropterus dolomieu</i>	5	0.025
	Central stoneroller	<i>Camptostoma anomalum</i>	2	0.010
	Green sunfish	<i>Lepomis cyanellus</i>	2	0.010
	Pumpkinseed	<i>Lepomis gibbosus</i>	2	0.010
	Blacknose dace	<i>Rhinichthys atratulus</i>	2	0.010
	Brown bullhead	<i>Ameiurus nebulosus</i>	1	0.005
	Longnose dace	<i>Rhinichthys cataractae</i>	1	0.005

CHAPTER TWO: Freshwater turtle relative abundance and movement based on habitat characteristics in north-central West Virginia wetlands

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ABSTRACT

Wetland characteristics and the surrounding landscape influences turtle abundance and movement among wetlands. We identified wetland and surrounding landscape characteristics that were related to painted turtle (*Chrysemys picta*) and snapping turtle (*Chelydra serpentina*) relative abundance and snapping turtle movement among wetlands. We surveyed turtles at 39 wetland sites (0.04–7.45 ha) in a mixed-use watershed in north-central West Virginia, where hardwood forests and wetlands have been heavily fragmented by agriculture and roads. We also applied radio transmitters to 42 adult snapping turtles (20 females and 22 males) across 22 of the wetlands. We documented movement among wetlands for 27 snapping turtles (66%), including 12 females and 15 males. Wetland perimeter and agriculture within 500 m negatively affected snapping turtle movement, where the likelihood of snapping turtles leaving their resident wetland decreased by 0.22% and 3.12% with each 1 m and 1% increase in wetland perimeter and agriculture, respectively. Snapping turtle relative abundance was positively associated with wetland perimeter, substrate depth, vegetative cover, and distance from roads. Painted turtle relative abundance was positively related to wetland vegetative and canopy cover. Landscape-level characteristics such as nearby wetlands, agriculture, and roads should be considered when developing wetland conservation plans to maximize turtle abundance. Wetland connectivity is important because snapping turtles and painted turtles regularly use a diversity of wetland types with abundant vegetation and natural surroundings.

Keywords: *Chelydra serpentina*, *Chrysemys picta*, Management, Painted turtle, Snapping turtle

Freshwater turtle relative abundance and movement based on habitat characteristics in north-central West Virginia wetlands

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1. INTRODUCTION

Many semi-aquatic turtle species use wetlands for annual cycle requirements such as foraging, mating, and hibernating (Burke and Gibbons 1995, Bodie and Semlitsch 2000, Semlitsch and Bodie 2003, Rizkalla and Swihart 2006). However, they also use upland areas for nesting (Obbard and Brooks 1980, Steen et al. 2012), basking (for some species), and migrating between wetlands throughout the year (Joyal et al. 2001, Milam and Melvin 2001, Attum et al. 2008, Ryan et al. 2008). Habitat characteristics such as wetland depth, velocity, and substrate influence abundance among species (Braun and Phelps 2016). Substrate composition is important as it is used for burrowing during hibernation and camouflage from predators and prey. Wetland habitat used by different subspecies of map turtle (*Graptemys geographica*, *G. ouachitensis*, and *G. pseudogeographica*) were distinguished based on different percent rock and muck (Fuselier and Edds 1994). Substrate preferences also vary by age as wetland composition preferences in different life stages have been observed in juvenile snapping turtles, where they favored sandy and mucky substrate rather than gravel (Froese 1978).

Canopy cover also influences turtle abundance in different ways depending on the species due to different life history requirements (Braun and Phelps 2016). Painted turtles regularly bask, leading them to favor habitats with decreased canopy cover (Cosentino et al. 2010). Common snapping turtles are considered habitat generalists (Ernst and Lovich 2009), so canopy cover has minimal direct influence in their habitat choice. Changes in habitat with increased canopy cover such as water temperature and wetland productivity (decreasing and increasing as canopy cover increases, respectively) could influence habitat preference in snapping turtles. Increased emergent vegetation in a wetland can be used as an indicator of quality nesting area as well as a possible food source for more herbivorous species (Rizkalla and Swihart 2006, Attum et al. 2008), and depending on species nesting habitat preference, this may be a driver for abundance in wetlands.

Upland habitat composition and wetland connectivity impact turtle abundance in wetlands. Correlations between patch size and population size have been observed across multiple taxa, creating sustainable populations less likely to become extirpated, as larger wetlands provide greater prey density and allow for more individual territories than smaller wetlands (Attum et al. 2008). Larger wetlands also provide greater species richness in birds, reptiles, amphibians, mammals, and plants (Findlay and Houlihan 1997); however, the increased abundance of fish in larger ponds significantly decreases abundance of taxa such as amphibians (Pilliod and Peterson 2001).

Landscapes surrounding turtle habitat can also have indirect effects on turtles because different landscapes influence soil hydrology (Zimmermann et al. 2006). Requirements necessary for suitable nesting locations for turtles such as soil moisture and cohesion are important characteristics of upland areas adjacent to wetlands. These characteristics fluctuate

depending on soil composition (i.e. percent of clay, silt, or sand; Christens and Bider 1987).

Painted turtles favor clay soils when nesting, and this benefits the eggs as they are less likely to dehydrate (Christens and Bider 1987). Alternately, snapping turtles nest in sandier soils (Thompson et al. 2017).

Connectedness between wetlands is just as important for ensuring population stability. Higher genetic diversity is observed in populations that travel between ponds compared to populations who stay in their original wetland (e.g. Berven and Grudzien 1990, Gibbs 1993). For turtles, wetlands of different sizes and types provide habitat for different life history stages. Adult snapping turtles (*Chelydra serpentina*), for example, spend their active months searching for mates and hunting in larger ponds, and they migrate to smaller streams to hibernate (Strain et al. 2012). Wetland connectivity is defined as the distance necessary for organisms to travel between wetlands (Attum et al. 2008), but the quality of habitat affects the success rate of an individual traveling between wetlands and must be considered (Attum et al. 2008).

Road density or road proximity to wetlands have inconsistent effects on movement, potential road mortality, and, ultimately, relative abundance of turtle populations. Crump et al. (2016) found 850 road mortalities of river cooters (*Pseudemys concinna*) and red-eared sliders (*Trachemys scripta elegans*) along a 3.5 km causeway that extended across an impoundment in Texas, USA. The presence of roads in particular skews sex ratios in populations due to females being more likely to succumb to road mortality when they move upland in search of nest sites (Aresco 2005, Reid and Peery 2014, Crump et al. 2016, Dupuis-Desormeaux et al. 2018).

Gibbs and Shriver (2002) specifically found that larger-bodied pond turtles (i.e., snapping turtles) were more likely to experience road mortality events due to their slower motility compared to smaller-bodied pond turtles (e.g., painted turtles) that experienced little to no effect

from high traffic and road density. Alternatively, Dorland et al. (2014) found no effect of roads on painted turtle relative abundance or sex ratio in Ontario, indicating a weak road mortality effect. Langen et al. (2012) concluded that painted turtles were affected by road proximity to wetlands but in limited hotspots, and relative abundance was not affected by the presence of roads in New York, USA. Studies have alternatively indicated that, although population dynamics are shifted, neither roads (Steen and Gibbs 2004, Attum et al. 2008) nor agricultural land use (Rizkalla and Swihart 2006) impact snapping turtle or painted turtle (*Chrysemys picta*) abundance.

The purposes of our research were to estimate the effects of environmental characteristics on abundance of painted turtles and snapping turtles and inter-wetland movement patterns of snapping turtles within a mixed-use watershed. We estimated turtle relative abundance based on habitat quality (e.g. substrate compaction and depth, vegetative cover, wetland size, and surrounding land use) and identified environmental characteristics that were associated with the likelihood of a turtle moving between wetlands. We hypothesized that: A) increased substrate depth would increase snapping turtle relative abundance; B) increased canopy cover would decrease painted turtle relative abundance; C) decreased distance between wetlands would increase the likelihood of snapping turtle overland movement; and D) average percent sand within 30 m of wetlands would decrease female snapping turtle overland movement.

2. METHODS

2.1. Study sites

We surveyed 39 sites (34 wetlands, two impoundments, and three stream segments) in the Upper Deckers Creek watershed. The watershed covers 7,778 ha in Preston County, West Virginia, USA, and encompasses 89.9 km of stream (Deckers Creek headwaters and its

tributaries; Figure 1). Deciduous forests and wetlands (freshwater emergent wetlands, freshwater ponds, freshwater forested/shrub wetlands, and riverine systems) have been significantly fragmented from agriculture. Development of varying intensities also have fragmented the watershed with northern (Masontown [Lat: 39.55056; Long: -79.79834], estimated population = 538 as of 2019 [U.S. Census Bureau 2020]) and central (Reedsville [Lat: 39.51019; Long: -79.79799], estimated population = 604 as of 2019 [U.S. Census Bureau 2020]) residential hubs within the watershed.

The multiform topography (Hartman 2000) due to the range in elevation (509–734; mean $\pm$ SE = 579.95 ± 0.05 m; U.S. Geological Survey 2018) promotes a variety of habitats available for wildlife within the watershed. Average annual precipitation was 107.52 ± 0.12 cm from 2019–2020 (mean $\pm$ SE, unpublished data). Soil composition predominantly consists of silt loam or loam types with average percent sand as 26.17% (SE: 0.01%) with an even representation of soil types that have high (e.g., well-drained sandy soils) or low water infiltration rates (e.g., clay soils with high water tables; Natural Resources Conservation Service 2016).

Sites varied in size (range: 0.04–7.45 ha; mean: 1.12 ha, SE: 0.17 ha) and had an even representation of wetlands surrounded by predominantly forested ($n = 14$; mean cover: 50.6%, SE: 2.64%) or agricultural ($n = 20$; mean cover: 48.98%, SE: 2.05%) land within 1 km, while five wetlands had an even combination of forested and agricultural land (mean forest: 36.56%, SE: 4.86%; mean agriculture: 44.01%, SE: 5.06%). Most sites ($n = 21$) were privately owned, 8 sites were associated with the West Virginia University J.W. Ruby Research Farm, and 10 sites were part of wildlife management area wetland systems (Figure 1). Vegetation management was both direct (i.e. algaecide/herbicide application and removal of emergent and floating plants) and indirect (e.g. mowing and cattle presence) depending on the site.

Wetlands within the Upper Deckers Creek watershed are predominantly created with estimated ages ranging between 23 and 43 years based on aerial imagery (Library of Congress 1980, Google Earth 1985–1997). Most sites ($n = 22$) were classified as palustrine unconsolidated bottom wetlands, while six sites were classified as palustrine emergent, three were riverine lower perennial, one was palustrine scrub/shrub, and seven were palustrine aquatic bed (Cowardin et al. 1979; Appendix 1).

2.2. *Abundance data*

We set ten aquatic hoop net traps of varied sizes (five 0.91 m diameter and five 0.76 m diameter; Flaherty et al. 2008, Gulette et al. 2019) and baited each trap with a half-can of sardines at each site across the Upper Deckers Creek watershed. We contained the bait so only the scent was able to escape and bait could not be consumed. We replaced bait after each trap-night to ensure freshness. We also included a flotation device in each trap to ensure captured individuals had constant access to air. Depending on the size of each site, we distributed traps throughout wetlands either in a straight line along one side of the wetland (for larger wetland sites) or around the entire perimeter of the wetland when accessible (for smaller wetland sites).

We set traps ≥ 10 m apart, depending on the size of the site, while we set traps in stream segments ≥ 25 m apart unless the channel was too narrow or water level was too shallow. Twenty-nine sites were surveyed in 2019, while 10 sites were surveyed in 2020. All sites were surveyed seasonally; we surveyed 2019 sites in spring (18 April to 19 May), summer (1–31 July), and fall (5 October to 1 November). Similarly, we surveyed 2020 sites in spring (11–29 May) and summer (30 June to 15 July), but we did not conduct fall surveys due to low capture rates in 2019. We visited sites for 3 consecutive days per session, and traps were checked every 24 hours.

Captured individuals were weighed to the nearest 5 g for individuals ≤ 600 g, to the nearest 100 g for individuals from 0.6–10 kg with Pesola hanging scales (Baar, Switzerland), and to the nearest 500 g for individuals > 10 kg with a Grainger hanging scale (Lake Forest, Illinois, USA). We measured straight-line carapace length (SCL) and width, plastron length and width, and body depth with calipers to the nearest 1 mm. Additionally, we measured pre-cloacal tail and posterior plastral scute lengths on snapping turtles to aid in determining sex with the pre-cloacal tail measuring less than the posterior plastral scute in females (Mosimann and Bider 1960, Ernst and Lovich 2009).

We individually marked each captured turtle using carapace notches and photographed every turtle for future reference (Cagle 1939, Ernst et al. 1974). We classified each captured individual in one of five age-sex classes as established by Bowne et al. (2006): adult male, adult female, sub-adult male, sub-adult female, and juvenile (Appendix 2). After we processed each turtle, we released them near their capture site.

2.3. Movement data

We tracked movement patterns by applying RI-2B transmitters (15.0 g, 18–30 month lifespan range; Holohil Systems LTD, Ontario, Canada) held by quick-setting epoxy to 42 adult snapping turtles captured weighing at least 300 g to keep transmitters at or below 5% of biomass to minimize impacts on mobility (Seburn 2012). We chose to track adults because sub-adult and juvenile movement can be influenced by the cost of traveling to distant ponds (i.e. predation) or they simply do not know distant wetlands exist due to their young age (Bowne et al. 2006). These age classes also do not have the movement requirements for locating nest sites or mates as adult conspecifics would.

We relocated turtles every 2–5 days between April and October and once per month from November to March (Joyal et al. 2001, Strain et al. 2012) using an R2000 series receiver (Advanced Telemetry Systems, Isanti, MN) with an F152-3FB model antenna (AF Antronics, Inc., Urbana, IL). We estimated locations of individuals that were tracked in inaccessible locations (e.g. deeper water, private land) via triangulation and GPS point projection (Garmin eTrex Legend Cx model, Garmin, Olathe, KS). For each turtle relocation, we identified landscape type and turtle behavior, if possible, from several meters away so the natural behavior of the turtle was not disturbed (Baldwin et al. 2004). We also accounted for incidental movement through identifying inter-wetland recaptures.

Mean number of days in which snapping turtle transmitters were deployed and tracked was 269.04 ± 26.25 days (Appendix 3). The most common reason of no longer tracking a turtle was dead transmitter batteries or loss of transmitters due to faulty epoxy adhesive. One turtle at the Reservoir was removed from the analysis because he was only successfully tracked twice, which is possibly due to interference from construction equipment during the dam renovation.

2.4. Environmental characteristics

For each site, we measured substrate compaction (kg/cm^2) at both ends of each trap ($n = 2$ readings per trap location) with a soil compaction tester (Dickey-John Corporation, Auburn, Illinois, USA). We also measured water depth across each wetland using a metered weighted line (Doody et al. 2002), where depth at 15 equidistant points across the longest length of the wetland was measured to the nearest cm. We measured 15 additional points along a transect perpendicular to the first transect at the longest width of the wetland. Dimensions of each wetland were measured using ArcGIS (ver. 10.7; ESRI, Redlands, California). Depth in each

stream segment had 3 equidistant points measured across the channel at each trap location (n = 30 depth measurements for all sites).

We estimated percent emergent vegetation with 1 m² frames centered on each water depth location (n = 30; Marchand and Litvaitis 2004) and used the following categories: 1 (<5%), 2 (5-25%), 3 (26-50%), 4 (51-75%), 5 (76-95%), and 6 (>95%) (Daubenmire 1968, Cosentino et al. 2010). To calculate mean wetland emergent vegetative cover for each wetland across each trapping session, we used midpoint values for the respective category at each measurement location (i.e., 2.5, 15, 27.5, 62.5, 82.5, and 97.5). Wetland area (ha) and perimeter (m) were measured with ortho imagery in ArcGIS (version 10.7, ESRI, Redlands, CA, USA).

Because wetlands varied in size (0.04–7.45 ha), we standardized land use types by calculating percentages within 1 km of the perimeter of each wetland so they were easily comparable (Findlay and Houlihan 1997, Joyal et al. 2001, Marchand and Litvaitis 2004, Attum et al. 2008). We measured distance from nearby roads and wetlands with the Near function in ArcGIS. Land use data were retrieved from the 2011 National Land Cover Data Set (Strager 2012). Using the Soil Survey Geographic Database, we calculated average percent sand in soil within 30 m of each wetland (Natural Resources Conservation Service 2016) to quantify potential nesting area quality (Baldwin et al. 2004). To identify the type of each wetland, we used the U.S. Fish and Wildlife Service National Wetland Inventory (USFWS 2018) and wetland classification established by Cowardin et al. (1979).

2.5. Statistical analyses

We used linear regression models to quantify the influence of habitat characteristics and wetland type on relative abundance of snapping turtles and painted turtles. To quantify relative abundance, we used catch-per-unit effort (CPUE), which we defined as the number of unique

individuals per trap night at each site per season. Trapping effort ranged between 27 (one site was missing a trap when surveyed in Spring 2019) and 30 trap nights per site per season. We also identified the number of sites in which painted turtles and snapping turtles were captured as well as how CPUE differed between seasons and wetland types.

Rather than using the distinct number of sites used by each turtle, we used logistic regressions to determine which land use practices and wetland characteristics affected the likelihood of a snapping turtle leaving its resident wetland. We defined resident wetland of each turtle as the wetland in which the turtle was originally captured. For modeling this relationship, we accounted for the sex of the turtle; percent agriculture coverage within 1 km; minimum wetland distance from roads and other wetlands; average percent sand within 30 m; and wetland size (Attum et al. 2008, Patrick and Gibbs 2010). To determine if there was a difference in movement between sexes, we used a Kruskal-Wallis test as a nonparametric analysis of variance. We z-score standardized values prior to analyses and identified the most parsimonious models for relative abundance and movement with corrected Akaike Information Criteria (AICc) values, where the model with the lowest AICc value was the best fit model. Any models with $\Delta AIC < 2$ were comparable (Burnham and Anderson 2002). We considered predictors to have strong support if the 85% confidence interval did not overlap zero (Arnold 2010).

3. RESULTS

3.1. Wetland habitat characteristics

Mean percent canopy cover across sites was $28.72 \pm 3.49\%$ (SE) in 2019 and $16.48 \pm 4.85\%$ (SE) in 2020 with slight deviations within sites across seasons (Table 1, Appendix 4). Mean vegetative cover ranged from 2.5–68.5% (mean $\pm$ SE: $10.99 \pm 1.34\%$; Table 1, Appendix 4), where the dominant species present across sites were yellow pond lilies (*Nuphar lutea*),

duckweed (*Lemna minor*), and broadleaf cattails (*Typha latifolia*). Sites with agriculture as the predominant land use within 1 km were primarily associated with the J.W. Ruby Research Farm or private farms where the land was used for hay and pasture. Distance from roads ranged from 0 (Keener2) to 418.9 m (FPR7; mean $\pm$ SE: 98.05 ± 17.55 m; Appendix 5).

3.2. Wetland turtle relative abundance

We captured 522 turtles at least once over the course of the study: 111 snapping turtles and 267 painted turtles were captured in 2019, while 77 snapping turtles and 66 painted turtles were captured in 2020. One red-eared slider was captured in Ruby Pond in 2019. The number of sites in which painted turtles or snapping turtles were captured differed by season (Table 2). Seasonal differences in CPUE was observed, where spring had the highest average CPUE for both species (Figure 2A). Palustrine scrub-shrub (Anderson pond) was removed when comparing CPUE across wetland types due to the small sample size, and palustrine aquatic bed had the highest CPUE for both species (Figure 2B). The maximum snapping turtle CPUE was 0.27 at Laurel Lake in Spring 2019 and 0.63 at Vernon4 in Spring 2020. The maximum painted turtle CPUE was 0.70 at FPR5 in Spring 2019 and 0.37 at Hicks1 in Spring 2020 (Appendix 6).

3.3. Relative abundance model selection

The best performing model for snapping turtle CPUE included road distance, substrate depth, mean wetland vegetative cover, and wetland perimeter (model weight = 0.44; Table 3). Predicted CPUE increased from 0.04 to 0.06 when wetland distance to roads increased from 0 to 100 m (Figure 3A). A wetland with 0% vegetative cover was predicted to have 0.04 snapping turtle CPUE compared to 0.11 CPUE in a wetland with 50% vegetative cover (Figure 3B). Increased wetland substrate depth from 25 to 50 cm increased predicted CPUE from 0.05 to 0.07 (Figure 3C). Increasing wetland perimeter from 250 m to 500 m increased predicted CPUE from

0.05 to 0.06 (Figure 3D). Two candidate models were comparable to the top model given their ΔAIC values (Table 3). Road distance, mean vegetation, and substrate depth did not include zero within 85% confidence intervals; however, the confidence interval for wetland perimeter included zero (Table 4).

The most parsimonious painted turtle CPUE model included mean wetland vegetative cover and percent canopy cover (model weight = 0.46; Figure 4, Table 3). A wetland with 25% vegetative cover was estimated to have 0.14 painted turtle CPUE and was predicted to increase to 0.23 in wetlands with 50% vegetative cover. A similar increase in canopy cover (25–50%) increased painted turtle CPUE from 0.14 to 0.16, respectively. The 85% confidence intervals for both predictors did not overlap zero (Table 4). The vegetation-only model was comparable based on the ΔAIC and weight (Table 3).

3.4. Snapping turtle movement

Mean number of wetlands used did not differ between sexes (Kruskal-Wallis: $\chi^2 = 0.03$, $df = 1$, $p = 0.86$; males: 2.71 ± 0.23 wetlands; females: 2.92 ± 0.39 wetlands). Out of the 41 snapping turtles tracked, 16 (8 females and 8 males) stayed within their resident site. The 25 remaining turtles (12 females and 15 males) relocated to at least one other site, where 14 turtles used one additional site, five turtles used two additional sites, four turtles used three additional sites, and the remaining two turtles either used four or five additional sites (Table 5, Appendix 3).

The number of sites a turtle used differed based on the site in which it was first captured (Appendix 7). The most movement from one turtle between years was observed from a female residing from Ruby Pond, where she hibernated in a nearby flooded field (73.9 m). She returned to Ruby Pond in the spring, relocated to a culvert in a nearby cattle field for a week (251.7 m),

and ultimately relocated to a sediment pond nearby (141.9 m). She also relocated to a culvert under the road that connected the sediment pond to Ruby Run (n = 6 sites). The most movement from one tracked turtle within a year was a male snapping turtle residing from Vernon4. He relocated to a nearby tributary (33.7 m) and traveled 715 stream-m to another wetland 547 m away in 2020 (n = 4 sites). Incidental inter-wetland movement was observed with 29 painted turtles, where the farthest within-year movement was 166.8 m from Vernon1 to Vernon2 in 2020, while the farthest between-year movement was 1.3 km from Jesse pond in 2019 to UDC2 in 2020.

The best performing model for snapping turtle movement included wetland perimeter and percent agriculture within 500 m of wetlands (Table 6), where the likelihood of a snapping turtle leaving its resident wetland decreased by 0.22% and 3.12% with 1 m increased perimeter and 1% increased agriculture within 500 m of wetlands, respectively. The 85% confidence interval for wetland perimeter (-0.005—0.0005) did not include zero, but the confidence interval for agriculture within 500 m included zero (-0.07—0.002). The perimeter-only model was comparable because $\Delta AICc < 2$ (Table 6).

4. DISCUSSION

4.1. Habitat characteristics and relative abundance

Our study indicated that habitat characteristics correlated with CPUE differed between snapping turtles and painted turtles. Our hypothesis involving the relationship between substrate depth and turtle CPUE, for instance, was that increased substrate would have a positive effect on turtle CPUE because of increased ability to burrow in substrate as a hunting strategy or evasive maneuver. however, substrate depth only had a strong effect on snapping turtle CPUE.

4.1.1. Substrate depth

Our results showed that with each 1 cm increase in wetland substrate, snapping turtle CPUE would increase by 0.0008, which supports what was found in the St. Croix River in Minnesota and Wisconsin, USA, where increased percent muck had a strong positive correlation with snapping turtle abundance (DonnerWright et al. 1999). Although over 50% of the sites surveyed in this study were created as farm ponds, sediment catchments, or flood control impoundments, habitat characteristics are suitable for turtle use and are the primary habitat type in some parts of their ranges (Failey et al. 2007).

4.1.2. Vegetative structure and wetland type

Our study found painted turtle CPUE increased with increasing canopy cover, which was opposite to what Anthonysamy et al. (2014) concluded in that painted turtles favored less overstory canopy cover while snapping turtles favored more based on telemetry locations of six painted turtles and seven snapping turtles. Dudley et al. (2015) compared sites with different stream structure such as low (mean canopy cover $\pm$ SE: $27.2 \pm 7.3\%$) and high canopy cover (mean canopy cover $\pm$ SE: $73.1 \pm 4.4\%$), and abundance in both snapping and painted turtles was higher in sites with lower canopy cover, indicating a negative correlation. Our mean percent canopy cover ($26.39 \pm 3.01\%$) was comparable to the low canopy cover from Dudley et al. (2015), but our study was limited in that canopy cover was only measured at trap locations. Other characteristics related to canopy cover that were not measured in this study could be affecting relative abundance of snapping turtles in these wetlands such as primary productivity (Dudley et al. 2015). Although snapping turtles are not commonly associated with basking, they use fallen logs that would be used for basking to wedge themselves under the water to hide in the substrate (DonnerWright et al. 1999).

We found increased mean vegetative cover was associated with increased relative abundance for both snapping turtle and painted turtles, which is different from Gulette (2018) who found vegetation did not have a strong effect on snapping turtle or painted turtle abundance. Cosentino et al. (2010) found painted turtles were less likely to experience extirpation events if the colonized wetland had low canopy cover and high emergent vegetation; however, the same study also found occupancy probability to be higher in wetlands with lower vegetation.

Graves and Anderson (1987) found littoral zone aquatic vegetation was a strong predictor of wetland habitat viability for snapping turtles as a proxy for food availability, and Mitchell (1988) similarly attributed increased vegetation to be high quality habitat for painted turtles. Increased vegetative density along the shallow edge of wetlands can provide microhabitats for prey items such as small fish, tadpoles, and macroinvertebrates. Snapping turtles are seed dispersers for plant species such as mulberry (*Morus* sp.), barnyard grass (*Echinochloa crus-galli*) and curly dock (*Rumex crispus*; Kimmons and Moll 2010). Individuals surveyed from Ruby Run were found to have seeds in their feces (Lozon pers. obs.), which indicates turtles feed on parts or entire plants as well. Some plants species that in some circumstances are considered a nuisance in a wetland (i.e. *Typha latifolia*) are used as food for snapping turtles as well (Ernst and Lovich 2009), which should be considered when managing wetlands.

Wetland type was not a strong predictor for either species despite the relationship between wetland type and vegetative cover. Palustrine unconsolidated bottom wetlands tended to have lower vegetative cover (mean $\pm$ SE: $6.52 \pm 0.89\%$; Appendix 4), whereas palustrine aquatic bed wetlands were predominantly covered by lilies, which led to higher vegetative cover (mean $\pm$ SE: $30.51 \pm 4.63\%$; Appendix 4). Wetland type was likely not considered a strong predictor for snapping turtle CPUE despite mean vegetative cover being a strong predictor because they are

known as habitat generalists (Anthonysamy et al. 2014). Anthonysamy et al. (2014) also found that although painted turtles are macrohabitat generalists, they are more microhabitat specialists than snapping turtles.

4.1.3. Wetland size

Our study results did not indicate strong effects of wetland size parameters on painted turtle relative abundance even though there was a large difference in wetland sizes (0.04–7.45 ha). Other studies found wetland size to have stronger explanatory power of painted turtle and snapping turtle relative abundance, where relative abundance was higher in larger wetlands (Price et al. 2013, Winchell and Gibbs 2016). Cosentino et al. (2008) similarly found that painted turtle occupancy was higher in larger wetlands. Anthonysamy et al. (2014) found painted turtles favored deeper water (40–50 cm) compared to snapping turtles (20–30 cm) based on relocations of six and seven individuals, respectively, from May to September in Illinois, which is comparable to mean trap location water depth at sites in which painted turtles were captured (mean $\pm$ SE: 42.56 ± 1.69 cm; unpublished data) but slightly shallower than our sites for snapping turtles (mean $\pm$ SE: 42.34 ± 1.82 cm; unpublished data).

We found wetland perimeter was positively associated with snapping turtle relative abundance rather than wetland area. Our mean perimeter was 543.7 m ($\pm$ 49.5 m), but the wetland with the highest snapping turtles CPUE had a perimeter of 158.9 m, which is comparable to Hughes et al. (2016) who reported snapping turtles favored wetlands with perimeters > 150 m. Although painted turtles would be more likely to use the edge for basking, snapping turtles are more likely to nest directly along the edge of a wetland compared to painted turtles (Wirsing et al. 2012), so increased perimeter could be comparable to increased nesting quality for a site. Wetland perimeter could be a stronger indicator of more shallow edge in a

wetland that promotes vegetative cover. Vegetative cover was a strong predictor of turtle CPUE, and water depth thresholds exist for vegetative growth (e.g. 2.5 m for lilies and watershield [*Brasenia* spp.]; Obbard and Brooks 1981).

4.1.4. Surrounding landscape and percent sand

Our study indicated that relative abundance of painted turtles and snapping turtles were not strongly affected by the type of surrounding landscape. Similarly, Rizkalla and Swihart (2006) found snapping turtles and painted turtles were less affected by habitat fragmentation than red-eared sliders within the Wabash River basin in Indiana, where agriculture was the predominant land use type (about 88%) followed by forest (about 8%) and wetlands (e.g. marshes, impoundments, lakes, and streams; 2.5%). Quesnelle et al. (2015) found that freshwater turtle populations that depend on wetlands generally were not affected by distance to nearby wetlands or the quality of landscape between wetlands.

Gulette (2018) found wetland density (e.g. number of wetlands) within 1 km to promote painted turtle relative abundance in West Virginia. We found that distance to nearest wetland (range: 0.25–238.84 m; mean $\pm$ SE: 50.54 ± 5.64 m) was not a strong predictor for painted turtle CPUE. Complementary habitats such as open fields for nesting or nearby hibernation locations near wetlands in addition to the wetland characteristics promote turtle abundance (Quesnelle et al. 2015, Thompson et al. 2017). This would explain reports of higher turtle relative abundance in wetlands with more sandy soils compared to random locations (Petokas and Alexander 1980, Thompson et al. 2017, Gulette 2018). Our mean percent sand in soil ($20.7 \pm 1.09\%$) was slightly lower than sites surveyed by Gulette (2018; 24.7%), which could explain why we found no strong effects of percent sand in soil with painted turtle CPUE.

Similarly, percent sand not having a strong effect on snapping turtle CPUE could be due to low amounts of sand at our sites. Thompson et al. (2017) found snapping turtles nested in soil that was $> 50\%$ composed of fine sandy loam, which is much higher than the percent sand in soils we had across our sites (range: 9.1—34.65%; Appendix 5). Other characteristics that would not be accounted for in canopy cover measurements such as shrub density (height ≤ 1 m) or percent bare ground surrounding wetlands could be stronger predictors of nearby nesting area quality than soil composition (Thompson et al. 2017).

Road proximity was included in the most parsimonious model as a positive effect on snapping turtle CPUE, meaning wetlands farther from roads had higher relative abundance, which was expected. Similar negative correlations of increased road density on turtle abundance have been reported in Blanding's turtles (Attum et al. 2008). This result is different from Steen and Gibbs (2004) who found no effect on snapping turtle relative abundance between high (> 1.5 km/km²) or low (≤ 1.5 km/km²) road density within 1 km of wetlands. Winchell and Gibbs (2016) found snapping turtle relative abundance increased when the frequency of low-intensity (low-traffic) roads within 500 m of wetlands increased and distance to nearest road increased.

The best performing model for painted turtles did not include wetland distance from roads. Some studies indicated wetlands directly surrounded by residential land use had lower painted turtle abundance compared to golf course and agricultural land use types (Price et al. 2013). Mota et al. (2021) found painted turtle relative abundance had a weak negative relationship with landscape condition (i.e. ecological integrity) based on the presence of roads, development, and modified landcover within 2.5 km buffers surrounding West Virginia wetlands. Winchell and Gibbs (2016) also found positive relationships with roads, where increased wetland distance from roads and fewer low-intensity roads led to higher relative

abundance. Our study did not distinguish between varying road types, which could have different effects on relative abundance in turtle populations within a certain range.

4.2. Snapping turtle movement

4.2.1. Differences in movement patterns

There was not a difference in movement between sexes of snapping turtles, opposite of what was expected given different life histories that would result in a turtle leaving its resident wetland; however, other studies compared movement between male and female snapping turtles and found them to be similar (Paterson et al. 2012, Ryan et al. 2014). Other species such as western painted turtles in Saskatchewan similarly did not exhibit a difference in movement between sexes (Marchand et al. 2018). Alternatively, Pettit et al. (1995) found variations in movement between snapping turtles in Ontario, where females had home ranges almost four times greater than males. Paisley et al. (2009) found similar relationships where females ($n = 76$ individuals) had average home ranges 1.2 times larger than males ($n = 7$ individuals). Our study found that although there was not a difference between sexes in how many sites were used, estimated overland distance for females was 1.9 times larger than males.

These differences in movement patterns could be due to the different wetland types (e.g. streams, lakes, ponds; Ryan et al. 2014, Marchand et al. 2018). One male stayed within a 1.52 km stretch of Deckers Creek in the two years he was tracked, while a different male from Vernon4 started in a pond but used nearby streams as corridors to relocate to another pond about 533 m away (unpublished data). Anthonysamy et al. (2014) found that snapping turtles and painted turtles selected ponds over other wetlands such as cattail (*Typha* spp.) marshes, sedge meadows, and rivers. Paterson et al. (2012) found snapping turtles are habitat generalists in that they selected marsh and swamp wetlands followed by ponds lakes and fens.

4.2.2. Use of nearby habitat

We did not observe many upland relocations, which is likely because snapping turtles are nocturnal nesters (Wirsing et al. 2012) and all telemetry tracking occurred between sunrise and sunset. We expected that due to the proximity of sites within the watershed (mean distance between surveyed and non-surveyed sites = 48.79 ± 9.20 m), we would have increased inter-wetland movements; however, the mean number of sites used was one additional site to the one in which they were captured. Bowne et al. (2006) had a high inter-wetland recapture rate of painted turtles (46%) because they surveyed 9 wetlands that were the only wetlands in a 1.2 km agricultural area. Snapping turtles can move at least 1 km from their wetland and did not relocate more regularly within the 1.5 km stretch of wetlands in the Upper Deckers Creek watershed. This could be an indication of underlying landscape limitations that inhibit large upland movements such as the presence of large forested patches (Paterson et al. 2012).

Some studies have alluded to the importance of investigating wetland habitat characteristics relating to turtles relocating to another wetland (Bowne et al. 2006). Immediate change of the natural wetland such as drought or water drainage (Gibbons et al. 1983, Marchand 2002, Bowne et al. 2006) or application of herbicides (Parker 1984) could initiate turtle movement from wetlands. One female turtle from Jesse pond was in the same wetland until it was drained to dredge cattails. In this case, the transmitter battery could have died, or the turtle could have relocated too far for the receiver to read the transmitter once she moved. Other evidence of movement from drained wetlands was incidental recaptures of painted turtles and snapping turtles from the Reservoir that moved to the closest nearby wetlands, which were Farm3 and Farm4 (301 m and 330 m, respectively), after it was drained in 2017 to work on reconstructing the dam.

Another female snapping turtle in Wolfe pond relocated to a pond within an abandoned mining area 160 m away after an algaecide-herbicide was applied to her resident pond. Parker (1984) observed an increase in emigration of pond sliders (*Pseudemys scripta*) after herbicide was applied, seemingly due to a decrease in food availability. Wolfe pond was not the only wetland that had an algaecide or herbicide applied (i.e. Farm 3, Farm 4, Born 1), and no other sites had evidence of emigration immediately after, so it might not be the primary reason the Wolfe turtle left. Time of year in which application was applied did not appear to be a factor in how turtles responded as all sites that had algaecide-herbicide applied were in late spring (April–May).

4.2.3. Land use effects on movement

We found that road proximity to wetlands did not strongly affect snapping turtle movement, which is potentially because turtles require different wetland habitat types to support life history requirements (Strain et al. 2012), and these multi-source needs increase movement potential (Forman et al. 2003). Roadsides are particularly attractive to snapping turtles for nesting and basking (Haxton 2000, Forman et al. 2003, Lozon pers. obs.), and road mortality is related to increased activity during nesting periods (Haxton 2000). Langen et al. (2009) reported that road mortality hotspots were identified as road segments bordered by wetlands within 100 m of each side.

Although 67% of sites surveyed in our study are within 100 m of roads, road mortality appears to be relatively low in the study area. We observed two instances of road mortality from presumed inter-wetland movement between Turnley and Adams ponds (unmarked adult male) and near Ruby Pond (unmarked unidentifiable individual), where roads were within 140 m of wetlands (unpublished data). Landowners additionally reported mortalities between Keener1 and

Keener² as well as Vernon² and Vernon³, where wetlands ranged from 0–185 m from roads (personal communication).

Snapping turtles could also be intentionally avoiding wetlands near roads, which would explain the positive relationship between relative abundance and distance from roads. Not much is known on the physics of turtle hearing, but Bowles (1997) reports that testudines have weak hearing (40–50 dB). Red-eared sliders were found to be more sensitive to noise and vibrations under water than in air (Christensen-Dalsgaard et al. 2012). Vibrations and sounds at higher frequencies resulting from vehicle traffic can inhibit vocal cues that adult turtles use for finding mates (Colafrancesco and Gridi-Papp 2016) and hatchling turtles use in synchronized hatching (Lacroix et al. 2020). Like improving movement, relative abundance of turtle species will likely be improved through landscape-scale management and implementing buffers to reduce road effects on turtles.

Although percent agriculture within 500 m was included in the most parsimonious snapping turtle movement model, the predictor strength was weak. Most agricultural land use between wetlands that turtles did travel through were hay fields or pastures, which may be a more natural open field landscape than active crop cultivation and be more appealing for use in finding new wetlands or nesting (Gulette 2018). At least two snapping turtles (one tracked and one untracked) were observed to use adjacent flooded fields for hibernation spots. Mitchell (1988) found similar instances where painted turtles and musk turtles (*Sternotherus odoratus*) were mutilated by lawn mowers when upland. One wetland that seasonally has open field mowed around it had snapping turtles with injuries (i.e. cleft lips) that were presumably from a lawn mower.

4.3. Management implications

Habitat-level characteristics such as vegetative cover was positively associated with relative abundance for both species, and wetland vegetation should be managed to promote quantity as well as diversity of vegetation to create microhabitats for prey and improve overall primary productivity of the wetland. Other habitat characteristics that promote vegetative growth, such as wetland perimeter or water depth, should be considered when managing or creating wetlands for freshwater turtles as well. Developing proper buffer zones around wetland habitats for increasing snapping turtle relative abundance can be difficult as there are not regulatory requirements for buffers around wetlands, and requirements vary based on wetland type (e.g. ponds, rivers, emergent wetlands, forested/shrub wetlands) and characteristics (e.g. soil, hydrology, topography, and wildlife; Brown et al. 1990). Increased wetland perimeter not only had a strong effect on snapping turtle relative abundance but on their site fidelity, which could be implemented in wetlands found in more urbanized areas with higher road densities to decrease road mortality potential.

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

Data available by request to J.T.A.

Code availability

Not applicable.

Authors' contributions

Author Contributions: Conceptualization, J.T.A. and D.J.B.; methodology, J.T.A. and D.J.B.; formal analysis, D.N.L.; investigation, D.N.L. and J.T.A.; data curation, D.N.L.; writing—original draft preparation, D.N.L.; writing—review and editing, J.T.A. and D.J.B.; visualization, D.N.L.; supervision, J.T.A. and D.J.B.; funding acquisition, J.T.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval

All animal experiments were approved by the West Virginia University Institutional Animal Care and Use Committee, reference number 1807015968.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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FIGURES

Figure 1. Wetlands within Preston County, West Virginia, USA, where snapping turtles and painted turtles were surveyed to investigate habitat characteristics that affect relative abundance and snapping turtle odds to conduct inter-wetland movement.

Figure 2. Mean catch-per-unit effort (CPUE) across seasons (A) and wetland types (B; PAB = palustrine aquatic bed [n = 7], PEM = palustrine emergent [n = 6], PUB = palustrine unconsolidated bottom [n = 22], RUB = riverine unconsolidated bottom [n = 3]) for snapping turtles and painted turtles surveyed across 38 sites within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, from 2019–2020. CPUE was calculated as the number of unique individuals per trap night per session. Error bars indicate standard errors. Points without error bars indicate a standard error of zero. Only one palustrine scrub-shrub wetland was surveyed (CPUE = 0.63), so it was removed from the analysis.

Figure 3. Relationships between snapping turtle catch-per-unit effort (CPUE), which was calculated as the number of unique individuals per trap night per session, and habitat predictors for wetlands surveyed in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, from 2019–2020. Model selection using Akaike’s Information Criterion resulted in the top model including a) wetland distance from roads, b) mean wetland vegetative cover, c) wetland substrate depth, and d) wetland perimeter. Shaded gray areas indicate the 85% confidence interval.

Figure 4. Relationships between painted turtle catch-per-unit effort (CPUE), which was calculated as the number of unique individuals per trap night per session, and habitat predictors for wetlands surveyed in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, from 2019–2020. Model selection using Akaike’s Information Criterion resulted in (a) canopy cover and (b) mean wetland vegetative cover having a strong effect on CPUE.

TABLES

Table 1. Mean ($\pm$ SE) values for wetland vegetative cover (%), percent canopy, substrate depth (cm), substrate compaction (kg/cm^3), and wetland depth (m) by season across years in Preston County, WV, USA. Measurements were taken at each site per trapping session in 2019 ($n = 29$ sites) and 2020 ($n = 10$ sites).

Year	Season	Vegetative cover		Canopy cover		Substrate depth		Substrate compaction		Wetland depth	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2019	Spring	9.26	2.55	42.8	7.17	16.35	2.24	6.27	0.36	150.25	13.83
	Summer	15.67	3.31	25.65	5.15	24.19	2.23	8.7	0.51	145.45	13.4
	Fall	6.22	1.26	16.89	4.51	35.64	2.65	9.07	0.44	128.28	13.21
2020	Spring	9.69	2.27	12.72	5.38	69.09	3.62	8.93	0.47	138.16	25.92
	Summer	16.61	5.04	20.24	8.21	69.35	3.99	10.19	0.56	126.12	22.66

Table 2. Number of sites within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, where snapping turtles and painted turtles were captured from 2019–2020.

Turtles were surveyed from 26 wetlands and 3 stream sites in 2019 from 18 April to 19 May (spring) and 1–31 July (summer), and 5 October to 1 November (fall), while 10 wetland sites were surveyed from 11–29 May (spring) and 30 June to 15 July (summer). Two wetlands were not sampled in Fall 2019 due to low water levels prohibiting trapping.

Species	2019			2020	
	SP	SU	FA	SP	SU
Snapping turtle only	5	6	3	3	1
Painted turtle only	5	3	7	1	2
Both	12	15	1	6	5
None	7	5	16	0	2
Total sites	29	29	27	10	10

Table 3. Candidate models with listed predictors for painted turtle and snapping turtle relative abundance in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, with corrected Akaike's Information Criterion (AICc) values, change in AICc from the top model, and model weight. Competitive models had $\Delta\text{AICc} < 2$ and are bolded.

Model	AICc	ΔAICc	Weight
<i>Snapping turtle</i>			
Minimum distance from roads + Substrate depth + Mean wetland vegetative cover + Wetland perimeter	-204.62	0.00	0.44
Minimum distance from roads + Substrate depth + Mean wetland vegetative cover	-204.06	0.56	0.33
Minimum distance from roads + Mean wetland vegetative cover	-202.74	1.88	0.17
Minimum distance from roads	-200.48	4.15	0.05
Substrate depth	-195.18	9.44	0.00
Mean wetland vegetative cover	-195.18	9.45	0.00
Wetland area	-192.60	12.03	0.00
Null	-191.52	13.10	0.00
Wetland perimeter	-191.44	13.18	0.00
Percent canopy	-190.53	14.09	0.00
Percent sand within 30 m	-190.06	14.57	0.00
Percent ag within 1 km	-189.83	14.80	0.00
Percent ag within 250 m	-189.80	14.82	0.00
Mean wetland depth	-189.72	14.90	0.00
Substrate compaction	-189.57	15.06	0.00
Minimum distance to nearest wetland	-189.43	15.19	0.00
Percent ag within 500 m	-189.41	15.21	0.00
Wetland type	-189.12	16.50	0.00

Table 3. Continued.

Model	AICc	Δ AICc	Weight
<i>Painted turtle</i>			
Mean wetland vegetative cover + Percent canopy	-102.21	0.00	0.46
Mean wetland vegetative cover	-102.11	0.10	0.44
Wetland type	-97.89	4.31	0.05
Wetland area	-94.51	7.70	0.01
Percent canopy	-93.81	8.40	0.01
Null	-93.51	8.69	0.01
Substrate depth	-92.66	9.54	0.00
Substrate compaction	-92.53	9.68	0.00
Percent sand within 30 m	-92.35	9.85	0.00
Minimum distance to nearest wetland	-92.03	10.18	0.00
Wetland perimeter	-91.71	10.49	0.00
Mean wetland depth	-91.54	10.67	0.00
Percent ag within 250 m	-91.48	10.72	0.00
Percent ag within 1 km	-91.48	10.72	0.00
Percent ag within 500 m	-91.48	10.73	0.00
Minimum distance from roads	-91.41	10.80	0.00

Table 4. Coefficients for predictors within the most parsimonious linear models for snapping turtle and painted turtle relative abundance in wetlands within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, along with the upper and lower boundaries for the 85% confidence intervals (CI). Predictors were determined to have a strong effect if the CI did not include zero.

Predictor	Coefficient	85% CI	
		Lower bound	Upper bound
<i>Snapping turtle</i>			
Minimum distance from roads	0.0002	0.00009	0.00034
Mean wetland vegetative cover	0.0013	0.00004	0.0022
Substrate depth	0.0008	0.00026	0.0014
Wetland perimeter	0.00002	0.000003	0.00005
<i>Painted turtle</i>			
Mean wetland vegetative cover	0.0034	0.0019	0.0048
Percent canopy	0.0007	0.00002	0.001

^a Coefficients for wetland type are compared to painted turtle CPUE in palustrine aquatic bed wetlands;

^b Coefficients for wetland-vegetation interactions are compared to painted turtle CPUE in palustrine aquatic bed wetlands

Table 5. Telemetry data for 42 snapping turtles (*Chelydra serpentina*) that were surveyed across 22 sites and tracked between 2019 and 2020. The number of days in which snapping turtles were tracked varied from 19–480 days (mean $\pm$ SE: 269.04 $\pm$ 26.25 days). Seventeen snapping turtles stayed in their resident wetland for the duration of the study, while 25 snapping turtles used at least one other wetland. Overland movement distance (m) between wetlands were estimated as straight-line measurements between areas since exact routes of movement were unable to be determined in this study.

Sex	Number of turtles	Sites visited			Mean overland distance (SE)
		Mean (SE)	Min	Max	
Female	20	2.1 (0.32)	1	6	128.34 (53.91)
Male	22	2.05 (0.23)	1	4	67.29 (18.22)

Table 6. Candidate logistic regression models for describing the odds of a snapping turtle relocating from its resident wetland based on telemetry data from 42 snapping turtles that were tracked from 2019 to 2020 in the Upper Deckers Creek watershed in Preston County, West Virginia, USA. Competitive models (bolded) were considered if $\Delta AICc < 2$. The top model included wetland perimeter and percent agriculture within 500 m of the wetland, where a 1 m increase decreased the odds of a snapping turtle leaving its resident wetland by 0.22% and a 1% increase in agriculture within 500 m decreased the odds by 3.12%.

Model	AICc	$\Delta AICc$	Weight
Wetland perimeter + Percent ag within 500 m	54.10	0.00	0.34
Wetland perimeter	55.11	1.00	0.20
Minimum distance from roads	56.55	2.45	0.10
Null	56.95	2.84	0.08
Percent ag within 1 km	57.68	3.57	0.06
Minimum distance to nearest wetlands	57.78	3.67	0.05
Wetland area	57.88	3.77	0.05
Percent ag within 500 m	58.47	4.36	0.04
Percent ag within 250 m	59.11	5.01	0.03
Sex	59.15	5.04	0.03
Percent sand within 30 m	59.15	5.05	0.03

APPENDICES

Appendix 1. Wetlands and streams in Preston County, West Virginia, USA, that were surveyed for turtles in either 2019 or 2020. Wetland classifications are according to Cowardin et al.

(1979): palustrine aquatic bed with rooted vascular plants (i.e. yellow water lily, *Nuphar lutea*; PAB3), palustrine emergent (PEM), palustrine scrub/shrub (PSS), palustrine unconsolidated bottom (PUB), and riverine lower perennial with unconsolidated bottom (R2UB). Dominant land use surrounding wetlands were either forested (deciduous forest area dominated by trees generally > 5 m tall and > 20% of the total vegetation) or agriculture (areas of hay/pasture which consists of grasses, legumes, or grass-legume mixtures of vegetation that account for > 20% of the total vegetation) according to the U.S. Geological Survey (2011) and Strager (2012).

Year	Site	Ownership	Wetland type	Area (ha)	Dominant land use (1 km)
2019	Anderson	Private	PSS	0.21	Forested (46%)
	Born1	Private	PEM	0.13	Forested (45%)
	Born2	Private	PEM	0.04	Forested (43%), Agriculture (44%)
	Born3	Private	PUB	0.05	Agriculture (45%)
	Born4	Private	PUB	0.06	Agriculture (44%)
	Deckers Creek	Private	R2UB	0.33	Agriculture (42%)
	Farm1	Public	PUB	0.30	Agriculture (63%)
	Farm2	Public	PUB	0.55	Agriculture (53%)
	Farm3	Public	PUB	0.25	Agriculture (50%)
	Farm4	Public	PUB	0.12	Agriculture (51%)
	FPR1	Public	PUB	2.72	Forested (46%)
	FPR2	Public	PUB	1.71	Forested (48%)
	FPR3	Public	PEM	0.33	Forested (46%)
	FPR4	Public	PEM	1.21	Forested (53%)
	FPR5	Public	PAB	2.87	Forested (59%)
	FPR6	Public	PUB	7.45	Forested (69%)
	FPR7	Public	PUB	0.56	Forested (61%)
	FPR8	Public	PUB	0.77	Forested (66%)
	Hodges	Private	PUB	0.03	Forested (61%)
	Jesse	Private	PUB	0.45	Agriculture (42%)
	Keener1	Private	PUB	0.62	Agriculture (46%)
	Keener2	Private	PUB	0.34	Agriculture (47%)
	Laurel Lake	Private	PUB	3.17	Forested (44%), Agriculture (45%)

Appendix 1. Continued.

Year	Site	Ownership	Wetland classification	Area (ha)	Dominant land use (1 km)
2019	Reservoir*^	Public	R2UB	0.29	Agriculture (49%), Forested (43%)
	Ruby Pond	Public	PAB3	0.91	Agriculture (61%)
	Ruby Run	Public	R2UB	0.76	Agriculture (57%)
	UDC1	Public	PAB3	2.33	Forested (36%)
	UDC2	Public	PAB3	2.46	Agriculture (46%)
	Wolfe	Private	PUB	0.19	Forested (51%)
2020	Adams	Private	PUB	0.07	Agriculture (54%)
	Cedar Meadow	Private	PUB	0.08	Forested (51%)
	Hicks1	Private	PAB3	0.17	Agriculture (44%)
	Hicks2	Private	PAB3	0.15	Agriculture (43%)
	Hicks3	Private	PUB	4.91	Agriculture (57%)
	Turnley	Private	PAB3	0.22	Agriculture (60%)
	Vernon1	Private	PEM	0.13	Forested (30%)
	Vernon2	Private	PEM	0.07	Forested (28%), Agriculture (26%)
	Vernon3	Private	PUB	0.08	Agriculture (31%)
	Vernon4	Private	PUB	0.14	Agriculture (32%)

* Stream length and width was determined using 2017 aerial imagery due to the area being under construction.

^ Land use composition was determined using the historical reservoir border since this wetland has been dammed for the last 40+ years, and this habitat structure is what turtles are adapted to.

Appendix 2. Age-sex classification as modeled by Bowne et al. (2006) and criteria for painted turtles (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*).

Age	Painted turtle		Snapping turtle	
	Male	Female	Male	Female
Juvenile	≤ 2 annuli ^{a^}		CL ≤ 127 -152.4 mm ^{d^}	
Sub-adult	≥ 3 annuli ^a		Age	Age
			≤ 12 annuli ^b	≤ 12 annuli ^b
			CL > 152.4 mm ^d	CL = 158-232 mm ^c
			Sex	Sex
Adult			Precloacal tail length longer than posterior plastral scute ^d	Posterior plastral scute longer than precloacal tail ^d
	≥ 10 mm foreclaw ^a	PL ≥ 106 mm ^a	Age	Age
			>12 annuli ^b	>12 annuli ^b
			Sex	Sex
			Precloacal tail length longer than posterior plastral scute ^d	Posterior plastral scute longer than precloacal tail ^d

^aBowne et al. 2006, ^bCongdon et al. 1994, ^cChristiansen and Burken 1979, ^dMosimann and Bider 1960. [^]Juvenile turtles were not distinguished by sex due to their size.

Appendix 3. Telemetry data for 42 snapping turtles (20 females and 22 males) that were surveyed across 22 sites and tracked between 2019 and 2020 in Preston County, West Virginia, USA. The most common reason in which a snapping turtle was no longer tracked was transmitter loss or dead batteries. Because turtles were equipped with transmitters at different times in the season, the number of days in which snapping turtles were tracked varied from 19–480 days (mean $\pm$ SE: 269.04 $\pm$ 26.25 days). Seventeen snapping turtles stayed in the wetland in which they were captured for the duration of the study, with 25 turtles using at least one other wetland. Overland movement between wetlands are estimated as exact routes of movement are unable to be determined in this study.

Site	Sex	From	To	Days	Visits	Sites visited	Estimated overland distance (m)	Transmitter condition
Born1	F	4/25/2019	10/04/2019	162	49	1	0	Presumed dead battery
Born1	F	7/28/2019	8/12/2020	381	66	2	152.28	Fell off
Born2	M	7/22/2019	8/30/2020	405	72	3	121.59	
Deckers	M	5/8/2019	8/22/2020	472	118	1	0	Presumed dead battery
Farm1	M	5/8/2019	8/30/2020	480	90	1	0	
Farm1	M	5/8/2019	8/26/2020	476	91	3	241.91	Presumed dead battery
Farm2	F	5/8/2019	7/20/2019	73	23	1	0	Presumed dead battery
Farm2	M	5/8/2019	8/30/2020	480	105	3	249.18	
Farm3	F	4/29/2019	7/21/2020	449	77	2	14.70 ^a	Fell off
Farm3	F	4/29/2019	8/9/2020	467	86	2	25.40	
Farm3	F	4/29/2019	8/11/2019	104	34	2	38.85	
Farm4	F	4/29/2019	8/12/2020	471	91	1	0	
Farm4	M	4/29/2019	7/20/2020	448	74	2	107.25	Fell off
Farm4	F	4/29/2019	7/13/2020	441	71	2	38.85	Presumed dead battery

Appendix 3. Continued.

Site	Sex	From	To	Days	Visits	Sites visited	Estimated overland distance (m)	Transmitter condition
FPR3	F	5/8/2019	8/16/2020	466	91	2	17.42	
FPR3	M	5/8/2019	8/6/2019	456	24	1	0	
FPR4	M	5/10/2019	7/17/2019	68	21	2	44.00	
FPR4	M	5/10/2019	8/11/2019	93	29	2	44.00	
FPR4	M	5/17/2019	8/10/2020	451	92	2	22.00	Fell off
FPR4	M	5/17/2019	1/9/2020	237	44	4	49.23	Presumed dead battery
FPR5	F	5/10/2019	8/10/2020	458	95	5	852.33	
FPR5	M	5/10/2019	10/18/2019	161	48	3	73.24	
FPR6	M	5/10/2019	7/21/2019	438	23	1	0	Presumed dead battery
FPR6	F	5/15/2019	10/4/2019	142	43	4	133.97	Confirmed dead battery
FPR8	M	7/30/2019	7/15/2020	351	45	1	0	Presumed dead battery
Hicks1	F	5/18/2020	9/12/2020	117	41	1	0	
Hicks1	M	5/18/2020	9/10/2020	115	40	2	87.49	
Hicks2	F	5/21/2020	9/12/2020	114	40	1	0	
Hicks3	M	5/21/2020	9/14/2020	116	41	1	0	
Hicks3	F	5/21/2020	9/14/2020	116	41	1	0	
Hicks3	M	5/21/2020	7/10/2020	50	17	1	0	Fell off
Jesse	F	4/25/2019	9/12/2019	140	42	1	0	
Reservoir	M	7/23/2019	8/11/2019	19	10	1	0	Unable to find turtle
Ruby Pnd	F	8/5/2019	9/2/2020	394	77	2	0 ^b	
Ruby Pnd	F	8/1/2019	8/12/2020	377	70	6	592.08	
Turnley	M	5/15/2020	8/7/2020	84	32	1	0	
Vernon1	F	5/27/2020	9/21/2020	117	39	2	168.84	
Vernon4	F	5/27/2020	9/18/2020	114	38	1	0	

Appendix 3. Continued.

Site	Sex	From	To	Days	Visits	Sites visited	Estimated overland distance (m)	Transmitter condition
Vernon4	M	5/27/2020	9/25/2020	121	40	4	254.41 ^c	
Vernon4	M	5/27/2020	9/18/2020	114	38	2	46.17	
Vernon4	M	5/27/2020	9/21/2020	117	37	4	139.82 ^d	
Wolfe	F	4/25/2019	7/13/2020	445	87	3	532.04	Confirmed dead battery

^a Captured in a nearby wetland; ^b Likely traveled 112.98 stream m from Ruby Pond to the Sediment Pond; ^c Traveled 308.38

stream m before crossing an open field to another part of the stream; ^d Traveled 787.5 stream m to a nearby pond.

Appendix 4. Mean percent canopy cover (PC), substrate depth (SD), and substrate compaction (SC) for each site surveyed within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, for painted turtles and snapping turtles in 2019 and 2020. Percent canopy cover was measured at each hoopnet trap location using a densiometer. Substrate depth (cm) and compaction (kg/cm^3) were measured in opposite corners of each hoopnet trap location (i.e. front-left and back-right). Born 2 and Anderson wetlands were only surveyed twice in 2019 because of inadequate water levels in the fall.

Season	Site	Percent canopy	Substrate depth	Substrate compaction
Spring 2019	Anderson	75.85	5.397	3.186
	Born1	6.46	9.271	6.229
	Born2	6.82	5.588	6.187
	Born3	47.49	9.271	6.24
	Born4	88.75	27.178	5.062
	Deckers	0	8.001	6.224
	Farm1	67.87	25.527	11.234
	Farm2	89.61	17.399	9.298
	Farm3	23.34	11.684	7.102
	Farm4	44.57	19.685	6.205
	FPR1	71.49	10.922	5.767
	FPR2	77.8	12.065	3.532
	FPR3	100	7.874	5.75
	FPR4	22.06	32.258	3.339
	FPR5	10.35	22.86	3.622
	FPR6	17.94	12.446	5.804
	FPR7	87.87	9.017	4.185
	FPR8	45.4	11.557	3.851
	Hodges	0	18.415	6.942
	Jesse	0	35.941	8.244
	Keener1	2.5	3.937	6.085
	Keener2	0	6.731	6.258
	Laurel Lake	76.25	2.921	6.4
	Reservoir	0	60.198	6.057
	Ruby Pond	0	14.351	6.31
	Ruby Run	0	9.398	6.311
	UDC1	83.96	19.05	8.665
	UDC2	94.9	17.78	7.558

Appendix 4. Continued.

Season	Site	Percent canopy	Substrate depth	Substrate compaction
Spring 2019	Wolfe	0	27.517	10.194
Summer 2019	Anderson	19.9	15.875	5.902
	Born1	14.2	13.462	8.965
	Born2	35.94	13.97	11.568
	Born3	76.94	14.097	8.929
	Born4	88.75	30.099	7.803
	Deckers	0	34.163	7.311
	Farm1	50.64	37.846	11.988
	Farm2	53.59	21.844	9.034
	Farm3	23.34	28.575	9.087
	Farm4	44.57	42.037	7.485
	FPR1	50.17	14.859	7.891
	FPR2	41.35	16.637	6.396
	FPR3	0.46	10.541	8.999
	FPR4	12.96	16.51	6.219
	FPR5	13.79	16.51	6.168
	FPR6	10	11.176	8.275
	FPR7	88.17	17.653	6.748
	FPR8	61.45	30.099	6.432
	Hodges	2.66	26.543	7.381
	Jesse	13.02	36.068	7.399
	Keener1	2.5	19.685	9.386
	Keener2	0	22.86	8.367
	Laurel Lake	13.39	31.623	11.529
	Reservoir	0	65.278	6.818
	Ruby Pond	0	38.608	9.526
	Ruby Run	0	17.399	20.248
	UDC1	14.63	14.097	7.03
	UDC2	0.94	21.717	9.28
	Wolfe	10.42	21.59	10.037
Fall 2019	Anderson	-	-	-
	Born1	20.75	21.209	9.544
	Born2	-	-	-
	Born3	50.57	12.192	12.075
	Born4	88.75	47.117	7.989
	Deckers	0	35.179	9.983
	Farm1	12.4	47.117	8.613
	Farm2	43.5	28.956	11.125
	Farm3	14.02	39.878	7.875
	Farm4	34.15	48.641	9.017

Appendix 4. Continued.

Season	Site	Percent canopy	Substrate depth	Substrate compaction
Fall 2019	FPR1	0	37.973	8.192
	FPR2	25.58	41.529	8.28
	FPR3	2.16	31.496	9.496
	FPR4	9.92	50.927	8.313
	FPR5	16.15	34.798	7.717
	FPR6	9.77	35.56	9.491
	FPR7	68.85	29.972	7.541
	FPR8	40.96	48.641	7.75
	Hodges	0	32.7025	8.227
	Jesse	0	62.865	6.592
	Keener1	0	13.208	9.175
	Keener2	0	23.114	7.612
	Laurel Lake	0	33.147	10.266
	Reservoir	0	71.628	6.575
	Ruby Pond	0	30.226	7.47
	Ruby Run	0	19.558	18.702
	UDC1	0	31.115	9.458
	UDC2	13.38	27.432	8.613
	Wolfe	5.18	26.035	9.193
Spring 2020	Adams	0	66.231	8.526
	Cedar Mdw	0	65.849	9.181
	Hicks1	0	74.168	11.585
	Hicks2	10.52	73.279	8.947
	Hicks3	42.35	58.039	8.647
	Turnley	41.15	73.533	7.946
	Vernon1	2.68	49.53	9.545
	Vernon2	0	60.071	7.577
	Vernon3	6.62	85.344	10.81
	Vernon4	23.91	84.8995	6.504
Summer 2020	Adams	0	73.025	11.779
	Cedar Mdw	0	71.374	12.232
	Hicks1	0.05	71.628	11.81
	Hicks2	16.05	80.899	7.083
	Hicks3	64.28	74.422	10.002
	Turnley	57.73	74.168	10.546
	Vernon1	6.87	52.578	10.475
	Vernon2	0	61.214	9.176
	Vernon3	9.41	46.228	11.285
	Vernon4	48.05	88.011	7.505

Appendix 5. Site-level measurements for each wetland surveyed within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, for painted turtles and snapping turtles during spring, summer, and fall in 2019 and spring and summer in 2020. Average water depth (cm) was measured in 30 equidistant locations across the longest length and width of each wetland. Average percent sand was determined using Web Soil Survey data (USGS 2019). Wetland area (ha) and perimeter (m) as well as wetland distance from roads (m) were digitized using ArcGIS 10.7. Percent agriculture within 1 km of each wetland was calculated using National Land Cover data in ArcGIS (Strager 2012).

Season	Site	Water Depth	Sand	Area	Ag (1km)	Mean Veg	Road Distance	Perimeter
Spring 2019	Anderson	178.7	32.6	0.21	37.396	2.5	127.922	601.217
	Born1	82	19.6	0.18	41.807	2.5	10.388	186.793
	Born2	126.833	29.4	0.04	43.756	2.917	235.085	85.391
	Born3	161.033	20.4	0.05	44.644	5.167	35.911	90.978
	Born4	124.5	20.4	0.06	44.295	2.5	45.858	112.257
	Deckers	69.333	26.23	0.33	42.011	2.5	183.794	754.917
	Farm1	125.667	16.95	0.3	63.606	5.417	69.818	268.612
	Farm2	187.833	15.93	0.55	53.989	3.333	88.566	351.302
	Farm3	182.759	22.4	0.25	50.832	4.655	0	197.209
	Farm4	116.267	17.26	0.26	51.062	14.833	68.818	297.741
	FPR1	187.833	9.1	2.72	27.902	2.917	41.632	921.474
	FPR2	273	9.1	1.71	22.156	2.5	0	1263.448
	FPR3	139.967	9.1	0.33	22.607	8.333	12.872	441.771
	FPR4	117.367	9.1	1.21	18.881	9.25	109.566	636.38
	FPR5	51.633	9.1	2.87	13.529	32.333	132.341	797.792
	FPR6	187.867	20.98	7.45	9.534	6.833	23.519	1322.995
	FPR7	359.9	9.1	0.52	10.675	2.5	418.864	529.565
	FPR8	202.767	16.35	0.97	9.027	2.5	252.408	931.507
	Hodges	216.233	22.7	0.04	16.917	4.5	58.785	70.512
	Jesse	97.333	20.87	0.45	42.053	4.833	17.621	285.461
	Keener1	112.467	26.3	0.62	46.085	10.667	50.114	184.981
	Keener2	234.167	23.95	0.34	47.367	7.667	0.1603	227.763
	Lrl Lk	252.2	18.88	6.75	44.039	2.5	55.471	1936.154
	Reservoir	22.183	22.46	8.41	48.323	2.5	0	597.903

Appendix 5. Continued.

Season	Site	Water Depth	Sand	Area	Ag (1km)	Mean Veg	Road Distance	Perimeter
Spring 2019	Ruby Pnd	118.433	31.08	0.91	61.506	19.333	34.653	459.502
	Ruby Run	27	27.75	0.76	61.411	2.5	0.331	1687.871
	UDC1	106.467	20.26	2.33	57.545	30.083	67.816	878.473
	UDC2	119.3	22.58	2.46	46.132	68.083	102.617	772.133
	Wolfe	176.3	18.2	0.19	33.593	2.5	32.563	178.589
Summer 2019	Anderson	83.4	32.6	0.21	37.396	8.75	127.922	601.217
	Born1	140.933	19.6	0.18	41.807	2.5	10.388	186.793
	Born2	110.833	29.4	0.04	43.756	39.833	235.085	85.391
	Born3	147.333	20.4	0.05	44.644	2.5	35.911	90.978
	Born4	116.333	20.4	0.06	44.295	2.5	45.858	112.257
	Deckers	29.041	26.23	0.33	42.011	2.5	183.794	754.917
	Farm1	143.167	16.95	0.3	63.606	14.083	69.818	268.612
	Farm2	196.067	15.93	0.55	53.989	4.083	88.566	351.302
	Farm3	193.667	22.4	0.25	50.832	21.5	0	197.209
	Farm4	160.333	17.26	0.26	51.062	29	68.818	297.741
	FPR1	163.667	9.1	2.72	27.902	27.083	41.632	921.474
	FPR2	363.167	9.1	1.71	22.156	10.333	0	1263.448
	FPR3	138.333	9.1	0.33	22.607	15.583	12.872	441.771
	FPR4	128.567	9.1	1.21	18.881	10.5	109.566	636.38
	FPR5	102.3	9.1	2.87	13.529	25.583	132.341	797.792
	FPR6	218.2	20.98	7.45	9.534	12.333	23.519	1322.995
	FPR7	306.5	9.1	0.52	10.675	2.5	418.864	529.565
	FPR8	151.333	16.35	0.97	9.027	33.417	252.408	931.507
	Hodges	161.967	22.7	0.04	16.917	3.333	58.785	70.512
	Jesse	86.833	20.87	0.45	42.05	2.5	17.621	285.461
	Keener1	133.133	26.3	0.62	46.085	2.5	50.114	184.981
	Keener2	193.4	23.95	0.34	47.36	2.5	0.1603	227.763
	Lrl Lk	168.333	18.88	6.75	44.039	5.333	55.471	1936.154
	Reservoir	15.917	22.46	8.41	48.323	2.5	0	597.903
	Ruby Pnd	141.9	31.08	0.91	61.506	26.083	34.653	459.502
	Ruby Run	31.75	27.75	0.76	61.411	9.75	0.331	1687.871
	UDC1	138	20.26	2.33	57.545	68.5	67.816	878.473
	UDC2	88.5	22.58	2.46	46.132	64.417	102.617	772.133

Appendix 5. Continued.

Season	Site	Water Depth	Sand	Area	Ag (1km)	Mean Veg	Road Distance	Perimeter
Summer 2019	Wolfe	165.267	18.2	0.19	33.593	2.5	32.563	178.589
Fall 2019	Anderson	-	-	-	-	-	-	-
	Born1	122.767	19.6	0.18	41.807	2.5	10.388	186.793
	Born2	-	-	-	-	-	-	-
	Born3	74.733	20.4	0.05	44.644	2.5	35.911	90.978
	Born4	104	20.4	0.06	44.295	2.5	45.858	112.257
	Deckers	14.139	26.23	0.33	42.011	2.5	183.794	754.917
	Farm1	157.17	16.95	0.3	63.606	4.5	69.818	268.612
	Farm2	152.033	15.93	0.55	53.989	5.667	88.566	351.302
	Farm3	144.467	22.4	0.25	50.832	17.583	0	197.209
	Farm4	158.133	17.26	0.26	51.062	6.5	68.818	297.741
	FPR1	113.533	9.1	2.72	27.902	2.5	41.632	921.474
	FPR2	280.8	9.1	1.71	22.156	4.0833	0	1263.448
	FPR3	95.733	9.1	0.33	22.607	2.5	12.872	441.771
	FPR4	96.067	9.1	1.21	18.881	6.5	109.566	636.38
	FPR5	68.933	9.1	2.87	13.529	2.5	132.341	797.792
	FPR6	176.567	20.98	7.45	9.534	10.417	23.519	1322.995
	FPR7	299.3	9.1	0.52	10.675	2.5	418.864	529.565
	FPR8	168.9	16.35	0.97	9.027	2.5	252.408	931.507
	Hodges	160.1	22.7	0.04	16.917	4.083	58.785	70.512
	Jesse	128.9	20.87	0.45	42.053	2.5	17.621	285.461
	Keener1	122.1	26.3	0.62	46.085	2.5	50.114	184.981
	Keener2	192.6	23.95	0.34	47.367	4.5	0.1603	227.763
	Lrl Lk	176.533	18.88	6.75	44.039	4.917	55.471	1936.154
	Reservoir	21.082	22.46	8.41	48.323	2.5	0	597.903
	Ruby	109.267	31.08	0.91	61.506	27.333	34.653	459.502
	Pnd							
	Ruby	17.357	27.75	0.76	61.411	2.5	0.331	1687.871
	Run							
	UDC1	55.61	20.26	2.33	57.545	15	67.816	878.473
	UDC2	80.767	22.58	2.46	46.132	21.75	102.617	772.133
	Wolfe	171.867	18.2	0.19	33.593	2.5	32.563	178.589
Spring 2020	Adams	123.7	24.45	0.068	54.681	2.5	11.891	99.979
	Cdr Mdw	110.833	18.2	0.078	19.719	2.5	6.266	121.707
	Hicks1	136.3	24.45	0.171	44.324	15	364.999	161.983
	Hicks2	113.4	23.85	0.147	43.512	14.417	227.457	193.707

Appendix 5. Continued.

Season	Site	Water Depth	Sand	Area	Ag (1km)	Mean Veg	Road Distance	Perimeter
Spring 2020	Hicks3	356.633	15.43	4.911	57.753	7.25	341.051	1977.547
	Turnley	90.533	23.1	0.22	60.463	9.333	48.858	213.773
	Vernon1	58.833	21.7	0.129	24.404	13.167	74.883	136.936
	Vernon2	121.6	20.3	0.065	26.373	24.667	55.914	105.113
	Vernon3	168.967	33.25	0.081	31.709	2.5	142.547	109.793
	Vernon4	100.833	34.65	0.144	32.105	5.583	272.673	158.894
Summer 2020	Adams	142.867	24.45	0.068	54.681	2.5	11.891	99.979
	Cdr Mdw	96.133	18.2	0.078	19.719	2.5	6.266	121.707
	Hicks1	103.5	24.45	0.171	44.324	29.167	364.999	161.983
	Hicks2	105.333	23.85	0.147	43.512	48.333	227.457	193.707
	Hicks3	314.717	15.43	4.911	57.753	21.333	341.051	1977.547
	Turnley	93.983	23.1	0.22	60.463	31.917	48.858	213.773
	Vernon1	56.85	21.7	0.129	24.404	8.083	74.883	136.936
	Vernon2	107.767	20.3	0.065	26.373	17.25	55.914	105.113
	Vernon3	153.767	33.25	0.081	31.709	2.5	142.547	109.793
	Vernon4	86.3	34.65	0.144	32.105	2.5	272.673	158.894

Appendix 6. Number of captures and catch-per-unit effort (CPUE), which was calculated as the number of unique individuals per trap night per session, for painted turtles (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*) surveyed across 39 wetlands within the Upper Deckers Creek watershed in Preston County, West Virginia, USA. Site trapping effort ranged from 27–30 trap nights per session. Two sites were not surveyed in Fall 2019 because of inadequate water levels for setting traps.

Season	Site	Captures		Trap nights	CPUE	
		<i>Chelydra</i>	<i>Chrysemys</i>		<i>Chelydra</i>	<i>Chrysemys</i>
Spring 2019	Anderson	1	2	30	0.017	0.033
	Born1	1	0	30	0.011	0
	Born2	0	0	30	0	0
	Born3	0	0	30	0	0
	Born4	0	0	30	0	0
	Deckers	1	0	30	0.011	0
	Farm1	3	4	30	0.033	0.044
	Farm2	4	13	30	0.044	0.144
	Farm3	4	7	30	0.044	0.078
	Farm4	4	16	30	0.044	0.178
	FPR1	0	1	30	0	0.011
	FPR2	0	1	30	0	0.011
	FPR3	3	17	30	0.033	0.189
	FPR4	8	2	30	0.089	0.022
	FPR5	7	21	30	0.078	0.233
	FPR6	3	4	30	0.033	0.044
	FPR7	0	0	30	0	0
	FPR8	2	0	30	0.022	0
	Hodges	0	2	30	0	0.022
	Jesse	1	12	30	0.011	0.133
	Keener1	1	0	30	0.011	0
	Keener2	0	0	30	0	0
	Laurel Lake	8	18	30	0.089	0.200
	Reservoir	0	0	30	0	0
	Ruby Pond	0	4	30	0	0.044
	Ruby Run	0	0	30	0	0
	UDC1	0	8	30	0	0.089
	UDC2	5	8	30	0.056	0.089
	Wolfe	2	0	27	0.023	0

Appendix 6. Continued.

Season	Site	Captures		Trap nights	CPUE	
		<i>Chelydra</i>	<i>Chrysemys</i>		<i>Chelydra</i>	<i>Chrysemys</i>
Summer 2019	Anderson	5	19	30	0.083	0.317
	Born1	2	0	30	0.022	0
	Born2	3	2	30	0.050	0.033
	Born3	1	0	30	0.011	0
	Born4	0	0	30	0	0
	Deckers	2	1	30	0.022	0.011
	Farm1	4	0	30	0.044	0
	Farm2	2	0	30	0.022	0
	Farm3	0	19	30	0	0.211
	Farm4	1	3	30	0.011	0.033
	FPR1	1	3	30	0.011	0.033
	FPR2	0	0	30	0	0
	FPR3	1	2	30	0.011	0.022
	FPR4	2	3	30	0.022	0.033
	FPR5	2	9	30	0.022	0.100
	FPR6	1	5	30	0.011	0.056
	FPR7	0	0	30	0	0
	FPR8	1	0	30	0.011	0
	Hodges	0	0	30	0	0
	Jesse	1	3	30	0.011	0.033
	Keener1	0	2	30	0	0.022
	Keener2	0	0	30	0	0
	Laurel Lake	1	2	30	0.011	0.022
	Reservoir	1	0	30	0.011	0
	Ruby Pond	1	3	30	0.011	0.033
	Ruby Run	1	2	30	0.011	0.022
	UDC1	5	6	30	0.056	0.067
	UDC2	4	4	30	0.044	0.044
	Wolfe	0	3	30	0	0.034
Fall 2019	Anderson	-	-	30	-	-
	Born1	0	0	30	0	0
	Born2	-	-	30	-	-
	Born3	0	0	30	0	0
	Born4	0	0	30	0	0
	Deckers	0	0	30	0	0
	Farm1	0	0	30	0	0
	Farm2	0	1	30	0	0.011
	Farm3	2	1	30	0.022	0.011

Appendix 6. Continued.

Season	Site	Captures		Trap nights	CPUE	
		<i>Chelydra</i>	<i>Chrysemys</i>		<i>Chelydra</i>	<i>Chrysemys</i>
Fall 2019	Farm4	0	2	30	0	0.022
	FPR1	0	0	30	0	0
	FPR2	0	0	30	0	0
	FPR3	0	0	30	0	0
	FPR4	0	0	30	0	0
	FPR5	0	1	30	0	0.011
	FPR6	0	0	30	0	0
	FPR7	0	0	30	0	0
	FPR8	0	0	30	0	0
	Hodges	0	0	30	0	0
	Jesse	0	3	30	0	0.033
	Keener1	0	0	30	0	0
	Keener2	1	0	30	0.011	0
	Laurel Lake	1	0	30	0.011	0
	Reservoir	0	0	30	0	0
	Ruby Pond	0	2	30	0	0.022
	Ruby Run	1	0	30	0.011	0
	UDC1	0	0	30	0	0
	UDC2	0	8	30	0	0.089
	Wolfe	0	1	30	0	0.011
Spring 2020	Adams	1	0	30	0.017	0
	Cedar Mdw	0	4	30	0	0.067
	Hicks1	8	11	30	0.133	0.183
	Hicks2	2	0	30	0.033	0
	Hicks3	9	2	30	0.150	0.033
	Turnley	1	3	30	0.017	0.050
	Vernon1	3	10	30	0.050	0.167
	Vernon2	1	2	30	0.017	0.033
	Vernon3	4	0	30	0.067	0
	Vernon4	19	3	30	0.317	0.050
Summer 2020	Adams	0	0	30	0	0
	Cedar Mdw	0	0	30	0	0
	Hicks1	2	6	30	0.033	0.100
	Hicks2	0	2	30	0	0.033
	Hicks3	9	3	30	0.150	0.050
	Turnley	6	5	30	0.100	0.083
	Vernon1	7	6	30	0.117	0.100
	Vernon2	2	0	30	0.033	0

Appendix 6. Continued.

Season	Site	Captures		Trap nights	CPUE	
		<i>Chelydra</i>	<i>Chrysemys</i>		<i>Chelydra</i>	<i>Chrysemys</i>
Summer 2020	Vernon3	0	3	30	0	0.050
	Vernon4	1	1	30	0.017	0.017

Appendix 7. Number of sites visited by snapping turtles (*Chelydra serpentina*) from 2019 to 2020 within the Upper Deckers Creek watershed in Preston County, West Virginia, USA, according to their resident sites. Number of turtles tracked at each site ranged from 1–4 individuals (mean $\pm$ SE: 1.91 ± 0.21 individuals).

CHAPTER THREE: Environmental factors influencing bioaccumulation of Cd, Cr, Hg, Pb, Se, and Zn in freshwater turtles

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ABSTRACT

Land use practices such as underground and surface coal mining can result in FeS, Se, and Hg contamination in wetlands, and agricultural runoff also causes Zn, Cd, Cr, and Pb accumulation in wetland systems. These contaminants can bioaccumulate in water and food, posing a threat to human health if levels exceed U.S. Environmental Protection Agency limits. A total of 33 painted turtles (*Chrysemys picta*) and 24 snapping turtles (*Chelydra serpentina*) across 22 sites within a mixed-use watershed in north-central West Virginia, USA, were tested for Cd, Cr, Hg, Pb, Se, and Zn concentrations through non-destructive tissue sampling to identify land use effects on bioaccumulation. Concentrations were compared between species and tissue types, and concentrations in soil were also compared to turtle tissue concentrations. All metals were higher in nails than blood, but concentration differences between species varied across metals. Se levels in soil and turtle nails were positively correlated with proximity to mine land. Turtle nail Pb concentrations were positively correlated with wetland soil Pb concentrations. Percent agriculture within 30 m of wetlands was negatively correlated with Hg in blood but positively correlated in nails, and all samples analyzed had Hg levels that exceeded EPA consumption limits. Mining activity, whether current or historic, can have long-term effects on turtles. Turtle harvesting limits should be investigated further to ensure human health and safety for consumption.

Keywords: Acid mine drainage, Biomonitoring, Common snapping turtle (*Chelydra serpentina*), Eastern painted turtle (*Chrysemys picta*), Methylmercury, Mixed-use watershed, Wetland

Word count: 12,504

Environmental factors influencing bioaccumulation of Cd, Cr, Hg, Pb, Se, and Zn in freshwater turtles

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INTRODUCTION

Land use practices can introduce elements that may not naturally occur or result in higher concentrations than naturally found in a system. Acidic waters from underground and surface coal mining, for example, have been shown to contaminate nearby wetland systems with high concentrations of FeS and Se (Lemly 2008; Shapley 2011). Cadmium, Pb, and Zn are commonly associated with urbanized areas as they result from vehicle tire and brake lining wear as well as gas exhaust (Croteau et al. 2008). Other trace elements such as Cr, Cu, and Ti have been identified in higher concentrations in groundwater surrounded by agricultural fields compared to sites tested in bottomland hardwood forests (Kellner, Hubbard, and Ikem 2015). Increased wetland abundance and forest cover have similarly promoted high Hg concentrations in wetlands (Turnquist et al. 2011). Land use practices also mobilize metal concentrations found in soil (Xiao et al. 2020), which can indirectly impact organisms such as benthic macroinvertebrates (Dixit and Witcomb 1983), fishes (Scheuhammer et al. 2007), and reptiles (Bernanke and Köhler 2008).

Snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*) are two of the most abundant and widespread species of turtles in the United States (Ernst and Lovich 2009). Their high trophic position and diet preferences increase the likelihood of accumulating metals over time (Grillitsch and Schiesari 2010; Hopkins et al. 2013; Turnquist et al. 2011). Species of other taxa have exhibited this relationship as well. Small mammals found in grasslands had different concentrations of Cd and Cu in their tissues based on diet variation (insectivorous > herbivorous > frugivorous; Hunter and Johnson 1982).

Other characteristics such as body size (Bergeron et al. 2007; Kenyon, Landry Jr., and Gill 2001) and sex (Hopkins, Hepner, and Hopkins 2013) affect metal accumulation in turtles, but the literature is inconsistent regarding results comparing accumulation between sexes. Some studies have concluded that females transfer contaminants to their offspring and, therefore, adult male turtles have higher cumulative concentrations compared to females of the same species (Smith et al. 2016). Other studies have found that metal concentrations vary between sexes due to different feeding ecology (Hopkins et al. 2013). Alternatively, some studies concluded that there is no correlation between sex and metal bioaccumulation (Bergeron et al. 2007; Kenyon, Landry Jr., and Gill 2001; Schneider et al. 2009; Turnquist et al. 2011, Villa et al. 2017). This variation in patterns may be due to differences in studies such as focal species, wetland condition (e.g. disturbance, pH; Golet and Haines 2001, Schneider et al. 2009), and site-specific wetland factors such as food web complexity (Benjamin et al. 2018).

Bioaccumulation of metals does not go without consequence to turtles. Non-essential metals such as Hg can reduce fertility and increase probability of embryonic mortality (Thompson et al. 2017). Previous research found that snapping turtle hatchling survival is affected by nest substrate Hg contamination in addition to maternal transfer (Hopkins, Hepner,

and Hopkins 2013; Hopkins, Willson, and Hopkins 2013). Lead contamination negatively influences survival of young turtles including affecting righting ability (Burger 1998). Mercury bioaccumulation in freshwater turtles has implications for human health, particularly species that are commonly harvested for human consumption such as snapping turtles (Ernst and Lovich 2009; Colteaux and Johnson 2017; Sherwood 2017). For example, monitored total Hg levels accumulated in a population of snapping turtles in New York showed 61% of muscle samples and 100% of shell samples had Hg levels higher than the U.S. Environmental Protection Agency (USEPA) limits (Turnquist et al. 2011).

Blood and nail concentrations are positively correlated with Hg and Pb concentrations in consumable parts of turtles (e.g. muscle and liver tissue; Hopkins et al. 2013; Powell 2014; Smith et al. 2016). Nail and muscle Hg in snapping turtles are positively correlated with muscle concentrations being 0.18 times higher than nails ($p < 0.001$; Hopkins, Hepner, and Hopkins 2013). Similarly, Powell (2014) found liver Hg concentrations were 0.47 times higher than nail concentrations in snapping turtles ($p < 0.02$). Blood and muscle Hg are positively correlated with muscle concentrations being 6.3 times higher than blood (Hopkins, Hepner, and Hopkins 2013).

Smith et al. (2016) found Cd and Pb concentrations in nail and liver tissues were not significantly different for painted turtles and snapping turtles. The same study found nail and muscle tissue concentrations of Cd, Cr, Fe, Mn, and Pb were not significantly different (Smith et al. 2016). Nail clipping and blood sampling are effective non-destructive alternatives to monitoring bioaccumulation of Hg and other metals as they eliminate negative impacts on individuals or populations (Benjamin et al. 2018; Smith et al. 2016); however, relationships between non-destructive tissues and more invasive tissues vary depending on the metal and turtle

species (Smith et al. 2016). Non-destructive sampling methods also have a strong positive correlation to environmental metals found in wetland sediment (Smith et al. 2016).

The primary purpose of this research was to collect data on concentrations of Cd, Cr, Hg, Pb, Se, and Zn accumulated in freshwater turtles at a series of wetlands and streams within a mixed-use (hardwood forest and agriculture) watershed in north-central West Virginia, USA, to identify effects of surrounding land use on turtle metal concentrations in wetlands.

Concentrations of metals were quantified among different size classes of snapping turtles and painted turtles, two common species found within the watershed. In addition, relationships between land use practices and metal bioaccumulation in turtles were investigated. The following hypotheses were tested to achieve these objectives: A) increased straight-line carapace length will result in higher concentrations of metals; B) snapping turtles will have significantly higher concentrations of analyzed metals than painted turtles; C) land use composition surrounding wetlands will affect metal concentrations in turtle tissues; D) metal concentrations in wetland soil and turtle tissues will be positively related; and E) turtle body condition will be negatively related to metal concentrations found in turtle tissue.

METHODS

Study sites

Twenty-six wetlands and three stream locations were surveyed in the Upper Deckers Creek watershed, a Hydrologic Unit Code 12 watershed that covers 7,778 ha in Preston County, West Virginia, USA, and encompasses 89.9 km of stream (Deckers Creek headwaters and its tributaries; Figure 1). Deciduous forests and wetlands (freshwater emergent wetlands [18%], freshwater ponds [42%], freshwater forested/shrub wetlands [25%], and riverine systems [15%]) have been significantly fragmented from a belt of agriculture. Development of varying intensities

have increasingly fragmented the watershed with northern (Masontown [Lat: 39.55056; Long: -79.79834], estimated population = 538 as of 2019 [USCB 2020]) and central (Reedsville [Lat: 39.51019; Long: -79.79799], estimated population = 604 as of 2019 [USCB 2020]) residential hubs within the watershed as well as barren and mine land.

Surveyed wetlands varied in size (0.04 to 7.45 ha) and had an even representation of wetlands surrounded by predominantly forested ($n = 13$; mean cover: 51.49 %, SE: 0.85 %) or agricultural ($n = 13$; mean cover: 51.20 %, SE: 0.69 %) land within 1 km, while 3 wetlands had an even combination of forested and agricultural land (mean forest cover: 41.19 %, SE: 1.91 %; mean agriculture cover: 46.30 %, SE: 1.10 %). Most sites ($n = 11$) sites were privately owned, 8 sites were associated with the West Virginia University J.W. Ruby Research Farm, and 10 sites were part of West Virginia Division of Natural Resources wildlife management area wetland systems. Direct and indirect wetland management practices varied by site (e.g. mowing edges, direct cattle access, chemical application, wetland plant removal).

The primary causes of water pollution in the Upper Deckers Creek watershed as assessed in 2014 by the watershed group Friends of Deckers Creek included acid mine drainage (AMD), fecal coliforms, sediment, and lead (Denicola et al. 2014). Since the turn of the century, with the earliest recording of mine activity in the watershed being the Richard Mine in 1903 (Irland 2016), the Upper Deckers Creek watershed had many active underground and surface mines and resultant AMD. Untreated water flowed into the creek prior to the Clean Water Act and Surface Mining Control and Reclamation Act (SMCRA) in 1977, causing significant repercussions to wildlife and water quality from reduced pH (USDA 2018). Projects funded by the American Recovery and Reinvestment Act of 2009 significantly decreased the impacts of AMD within the watershed. Additionally, fecal coliform levels have been difficult to address in the watershed due

to being introduced via both point sources (e.g. sewage treatment plants) and non-point sources (e.g. livestock field runoff; Denicola et al. 2014).

Increased alteration of the landscape from hardwood forest to agriculture has eliminated riparian vegetation that often can attenuate runoff to a given stream. With increased flow, quantity of runoff, and significant wetland loss in the region, sediment has accumulated within streams, causing stresses on aquatic biota according to the Total Maximum Daily Load (TMDL) established by the West Virginia Department of Environmental Protection (WVDEP; Denicola et al. 2014). Sources of Pb impacts on water quality in Deckers Creek are minimal compared to other metals; however, a foundry incident near the Fairfax Pond-Rehe Wildlife Management Area in 2006 caused Pb to be exposed to Deckers Creek and an adjoining 22.25 ha within the wildlife management area (Denicola et al. 2014).

Turtle capture and processing

At each site, ten aquatic hoop net traps of alternating sizes (0.91 m diameter and 0.76 m diameter; Flaherty, Anderson, and Michael 2008; Gulette, Anderson, and Brown 2019) were set at least 10 m apart for three trapping sessions in 2019, including: spring (18 April to 19 May), summer (1 July to 31 July), and fall (5 October to 1 November; Bowne, Bowers, and Hines 2006). Depending on the size of the wetland, traps were either set in a straight line along one side of the wetland (for larger wetland sites) or across the entire perimeter of the wetland when accessible (for smaller wetland sites).

Traps were set in streams at least 25 m apart, but in areas where the channel was too narrow or water level was too shallow, the distance between traps varied as necessary to ensure each trap was properly set up. Each trap was baited with a half-can of sardines in a container so

the scent could escape and not be consumed. Bait was replaced every 24 hours for 3 consecutive days. Each trap also had a flotation device to allow captured individuals constant access to air.

Captured turtles were weighed to the nearest 5 g for individuals less than 600 g and to the nearest 100 g for individuals up to 10 kg with Pesola hanging scales (Baar, Switzerland), and to the nearest 500 g for individuals over 10 kg with a Grainger hanging scale (Lake Forest, Illinois, USA). Morphometric data including straight-line carapace length (SCL) and width, plastron length and width, and body depth were measured with calipers to the nearest 1 mm. Pre-cloacal tail and posterior plastral scute lengths were measured on snapping turtles to aid in determining sex with the pre-cloacal tail measuring less than the posterior plastral scute in females (Ernst and Lovich 2009; Mosimann and Bider 1960). Unique shell markings in the marginal scutes with a hand file or Dremel rotary tool (Racine, Wisconsin, USA) provided distinct identification, and every turtle was photographed for future reference (Cagle 1939; Ernst, Hershey, and Barbour 1974). After processing each turtle, they were released near their capture site.

Tissue and soil sample collection

One milliliter of blood from the dorsal or ventral caudal vein (Perpiñán 2015) and 1–2 mm trimmings from 3–4 hind toenails were collected from each captured individual for analysis (Hopkins et al. 2013; Powell 2014). Both blood and nail samples were collected in the field, where blood samples were collected using 3 mL non-heparinized syringes, and nail samples were taken with human or dog nail clippers, depending on the size of the turtle. All samples were stored in 1.5 mL Eppendorf tubes and tubes were stored in a cooler followed by transfer to a freezer at -20°C immediately upon return from the field until analysis.

Two surface soil samples at 0–10 cm depth were collected directly above the water line of each wetland using a 5 cm diameter handheld PVC core sampler to quantify a baseline of

stable, immobile environmental concentrations of metals (Hubbart 2002; Smith et al. 2016; Xiao et al. 2011). The PVC corer reduced potential soil sample contamination compared to a conventional steel soil sampler, which may slough off heavy metals (Hubbart 2002). The corer was rinsed after visiting each site to minimize soil sample cross-contamination.

Land use digitization

Wetland boundaries were digitized using ArcGIS (version 10.7, ESRI, Redlands, California) and agriculture within 30 m of the perimeter of each wetland was quantified based on most recently available orthophotos of the areas (Attum et al. 2008; Findlay and Houlihan 1997; Joyal, McCollough, and Hunter 2001; Marchand and Litvaitis 2004). Land use data were retrieved from the 2011 National Land Cover Data Set (Strager 2012). Because the wetlands varied in size, land use types were standardized by calculating the percent cover so they were comparable (Marchand and Litvaitis 2004). Distance to nearby mine land and roads (in meters) were also calculated using the Near function in ArcMap.

Hay, pasture, and cultivated crop land use types were combined and defined as ‘agriculture’. Similarly, current mining activity as of 2011 (NLCD ‘mine grass’ and ‘mine barren’) was combined with land that was mined prior to SMCRA (NLCD ‘Pre-SMCRA grass’ and ‘Pre-SMCRA barren’). Primary (e.g. state highway roads), secondary (e.g. driveways), and tertiary (e.g. graveled farm roads) roads were not analyzed separately but were combined and identified as ‘Census Roads (2011)’ (Strager 2012).

Sample analyses

Tissues from 61 painted turtles (range SCL = 51–180 mm, median SCL = 139 mm) and 33 snapping turtles (range SCL = 113–386 mm, median SCL = 257 mm) across 22 sites were chosen for analysis (Table 1). Some turtles (n = 34) had both tissue types analyzed, while others

had either blood (n = 35) or nail (n = 25) samples analyzed. All soil samples for each site and the subset of tissue samples were submitted to the West Virginia University National Research Center for Coal and Energy Analytical Lab.

Acid digestion using nitric acid and dilution to 100 mL was applied to tissue samples prior to analysis, and all samples were analyzed according to the USEPA method 3050B (1996) with a Perkin Elmer NexIon 2000 inductively coupled plasma mass spectrometer (Waltham, Massachusetts). For quality assurance, a linear calibration was completed using a blank (1 % HNO₃). A check verification standard and a blank were run after the calibration ($\pm$ 10 % recovery). Additional blanks were run after every 20 injections and at the end of each run with all blanks resulting in concentrations below the aqueous level of detection for each metal analyzed: 0.000025 mg/kg for Cd, 0.000018 mg/kg for Cr, 0.000003 mg/kg for Hg and Pb, 0.000185 mg/kg for Se, and 0.000031 mg/kg for Zn.

This suite of metals was chosen due in part to their status as metals and metalloids of primary interest according to USEPA (2007). The coal mining, agriculture, and development land use history of the Upper Deckers Creek watershed was also considered. Total Hg was measured instead of methylmercury (MeHg) because 70–100 % of Hg in turtle blood is methylated (Benjamin et al. 2018; Bergeron et al. 2007; Day et al. 2007), and keratinized portions of organisms (i.e. turtle nails) primarily contain MeHg (Hopkins et al. 2007; Hopkins et al. 2013; Thompson and Furness 1989).

Statistical analyses

All statistical analyses were conducted using the R statistical software (R Core Team 2020). To investigate relationships between land use practices, wetland soil metal concentrations, and turtle metal concentrations, redundancy analyses (RDA) were run with Cd, Cr, Hg, Pb, Se, and Zn

concentrations for each tissue or soil sample as response variables. Redundancy analyses identified strong effects in landscape predictors for concentrations of each metal while accounting for the explained variance between samples coming from different turtles at different sites (Legendre and Legendre 2012). Morphometric (SCL), landscape (percent agriculture and distance to nearest mines and roads), and environmental (soil metal concentrations) variables were used as predictors in the analyses (Table 2).

Response values were standardized prior to analysis to give each metal equal weight in the analysis (i.e. concentrations between metals and tissue types) using the robustHD package (Alfons 2019). Predictors were also standardized because the scale of each predictor was inconsistent (i.e. percent, mg/kg, meters). Each set of RDA response variables were tested for linearity (gradient lengths ≤ 4) using a detrended correspondence analysis using the vegan package (Oksanen et al. 2019, version 2.5-6). The same package was used to conduct permutation tests to determine the significance of RDA axes (reported as p-values that determines the effect of the joint constraints in the RDA).

Eight RDAs were run to account for the significantly different concentrations of metals expected to be found between tissue types and turtle species. Landscape and soil metal concentrations were used in separate RDAs to minimize possible correlation between predictors. One additional RDA was run to identify soil metal relationships with land use practices, where percent agriculture and distance to nearby mines and roads were included as predictors like the turtle RDAs. Explanatory power of each RDA was determined using adjusted R^2 , which corrects for variance explained due to random chance. Permutation test p-values were considered of less importance to determine the top RDA due to the exploratory nature of the study and small sample sizes for each RDA.

Log-transformed SCL and weight were used to calculate body condition scores (BCI) for each turtle to determine the effects of metal bioaccumulation on body condition, where individuals with a positive BCI score have higher than average amounts of body fat and vice versa (Schulte-Hostedde et al. 2005). Body condition relates to different life history factors in turtles. Individuals with higher BCI, for instance, are more likely to reproduce or produce more offspring (Litzgus et al. 2008). Metal concentrations were standardized separately based on turtle species and tissue type to ensure equal weight of each metal in the analysis and combined forward stepwise and backward elimination model selection was conducted with the stats package for identifying the most parsimonious model (R Core Team 2020), which was identified as the model with the lowest Akaike's Information Criterion value that corrected for small sample size (AICc). Body condition values were standardized according to their species-tissue combination to assure they were comparable. Predictor strength in the top model was determined if the 85 % confidence interval did not include zero (Arnold 2010). Comparable models were considered if the model had a $\Delta AICc < 2$ (Burnham and Anderson 2002).

RESULTS

Land use composition across sites

Mean percent agriculture within 30 m of each of the 29 sites surveyed in 2019 varied between 0 and 79% (Table 3). Sites with forest as the predominant land use were primarily associated with wildlife management areas. Sites with agriculture as the predominant land use were primarily associated with the West Virginia University Research Farm or private farms where the land was used for hay and pasture. Distance from roads and mine land varied greatly between sites (range: 0–418.86 m; Table 3).

Comparing turtle species

All metals were detected in toenail tissue samples. Cadmium concentrations were 0 mg/kg in 29 painted turtle blood samples (54.7% of samples) and six snapping turtle samples (37.5% of samples). Blood Cr was not detected in 39 painted turtles (73.5% of samples) and the same six snapping turtle samples. Painted turtle nail concentrations were between 0.93 (e.g., Hg and Pb) and 3.88 times higher (i.e., Cr) than snapping turtle nail samples. In contrast, snapping turtle blood concentrations were as much as four times greater than painted turtle blood concentrations (Table 4). Order of mean concentrations for painted turtle blood was $Zn > Se > Hg \geq Pb > Cr \geq Cd$, while mean nail concentration order for painted turtles was $Zn > Se > Cr > Pb > Hg > Cd$. Snapping turtle order of mean concentrations in blood samples was $Zn > Se > Pb > Hg > Cr > Cd$, and nail sample mean concentration order was $Zn > Se > Cr > Pb \geq Hg > Cd$. Different relationships among metal concentrations in turtle species, tissue types, and predictor variables were observed (Appendices 1–6).

All samples exceeded the 0.0001 mg/kg limit of daily Hg for safe human consumption set by the USEPA (Farland 2000). Of the 128 samples that were analyzed for Hg, 34 nail samples (16 painted turtles and 18 snapping turtles) exceeded both the consumption limit as well as the 0.3 mg/kg fish tissue criterion that was established by the USEPA in the Clean Water Act (Section 304[a]; Borum et al. 2001; Driscoll et al. 2007). Lower threshold concentrations for MeHg were estimated to be at least 70 % of Hg detected (Bergeron et al. 2007), and 19 of the 34 nail samples (10 painted turtles and 9 snapping turtles) had MeHg levels exceeding both USEPA limits (Figure 2).

Painted turtle redundancy analyses

Blood concentrations of metals in painted turtles had a weak relationship with morphometric and landscape variables (adjusted $R^2 = 0.038$, $p = 0.096$; Figure 3A). There was a positive relationship between SCL and Pb, Se, Cd, and Zn concentrations in turtles. Selenium and Cr had a negative relationship with percent agriculture and distance from mine land. Turtles sampled from wetlands farther from roads had higher concentrations of Pb, Hg, and Zn.

Relating painted turtle blood concentrations to wetland soil concentrations was comparable to landscape (adjusted $R^2 = 0.037$); however, it failed the permutation test ($p = 0.173$; Figure 4A). Like the landscape RDA, there was a strong positive relationship between SCL and Pb and Cr concentrations in turtles. Concentrations of Hg and Pb in turtles were negatively related to Hg and Pb concentrations in wetland soil, respectively.

The RDA for painted turtle nail concentrations improved 10-fold compared to blood with an adjusted R^2 of 0.314 when relating to landscape predictors (Figure 3B), and the permutation test passed ($p = 0.002$). Carapace length had a strong negative relationship with Cd, Cr, Hg, and Pb. In contrast, the same metals had a weak positive relationship with distance from roads. Concentrations of Se in painted turtle nail samples were negatively correlated to percent agriculture, and Se and Zn showed a negative correlation with distance from mine land.

The painted turtle nail RDA with soil concentrations as predictors also passed its permutation test with a slightly higher adjusted R^2 (0.342; Figure 4B). Turtle size showed a similar negative relationship with Cd, Pb, Cr, and Hg. Turtle Se and Zn were positively related to soil Se and Zn, respectively, while Pb and Hg showed a negative relationship between turtle and soil concentrations.

Snapping turtle redundancy analyses

The RDA relating snapping turtle blood concentrations to landscape predictors had a low adjusted R^2 value (0.036) and the permutation test failed ($p = 0.352$; Figure 3C). There was a positive correlation with SCL and Hg, Se, and Zn concentrations in blood, while Cd showed a negative correlation. Blood Cd concentrations had a negative correlation with percent agriculture and distance from mine land. Lead levels in turtle blood were positively correlated with distance from mine land. Mercury and Zn concentrations were higher in turtles from wetlands farther from roads.

Despite the small sample size of snapping turtle blood, relating snapping turtle blood concentrations to soil concentrations improved the adjusted R^2 five-fold compared to landscape predictors (0.185; Figure 4C); however, the permutation test still did not pass ($p > 0.1$). Lead and Hg concentrations in turtles were negatively related to Pb and Hg levels found in wetland soil, respectively. Like the landscape RDA, larger snapping turtles had higher concentrations of Se in their blood compared to smaller conspecifics.

Snapping turtle nail concentrations were best described with soil concentrations (adjusted $R^2 = 0.142$; Figure 4D) compared to landscape predictors (adjusted $R^2 = 0.009$; Figure 3D), but permutation tests for both RDAs failed ($p > 0.1$). There was a negative relationship between Pb in turtle nails and Pb in wetland soil. Alternatively, there was a positive correlation between soil Se and Se levels in turtle nails. Distance from mine land was negatively correlated to turtle Se, and distance from roads was negatively correlated to Cr, Pb, and Hg. Chromium and Hg had a positive correlation with percent agriculture, and SCL was negatively correlated to Zn but had a positive correlation with Cd.

Soil redundancy analyses

All metals were detected in soil samples of the 29 sites surveyed. The RDA that described the relationship between landscape parameters and wetland soil concentrations was weak ($p = 0.258$, adjusted $R^2 = 0.022$); however, one site was considered an outlier due to the amount of Pb and Zn (457.26 and 904.98 mg/kg, respectively), possibly due to measurement error or sample contamination, and was removed from further analysis to avoid skewing due to the regression-based nature of the RDA (Zuur, Ieno, and Smith 2007). The RDA improved slightly with an adjusted R^2 of 0.037 ($p = 0.186$; Figure 5). Distance from roads had a strong positive relationship with Zn and Se in soil. There was a strong positive relationship with distance from mine land and Cd, Pb, and Hg in soil, while wetland soil Cr concentrations were lower in wetlands farther from mine land. Most metals found in soil were negatively related to percent agriculture within 30 m (e.g. Hg, Se, and Zn).

Body condition

There were no strong effects of metal concentrations on turtle body condition across species, tissue types, and metals. The most parsimonious model for the relationship between turtle BCI and metal concentrations was the null model ($AIC_c = 363.28$, $AIC_{wt} = 0.62$). A Zn-only model had some support with $\Delta AIC_c = 1.02$ ($AIC_{wt} = 0.38$), where BCI decreased from 0.02 to 0.002 with an increase in Zn concentrations from 50 to 100 mg/kg, respectively. The 85 % confidence interval, however, overlapped zero (-0.0007–0.0001).

DISCUSSION

Difference between turtle species and tissue types

Study results indicated all metals were higher in nails than blood as expected, but concentrations were different between species and varied across metals. Observed differences between turtle

species and tissue types were likely attributable to diet (Gall, Boyd, and Rajakaruna 2015; Haskins et al. 2017; Wittman and Hu 2002) and the temporal inference of each tissue type—nails reflecting long-term accumulation (Hopkins et al. 2013; Slimani et al. 2017) and blood reflecting recent accumulation (Burger et al. 2005; Evers et al. 2005). Although both painted turtles and snapping turtles are omnivorous generalists (Ernst and Lovich 2009), snapping turtles are able to bioaccumulate higher short-term levels of metals due to their larger prey items including fish, other turtles, mallard (*Anas platyrhynchos*) ducklings and mute swan (*Cygnus olor*) cygnets (Ernst and Lovich 2009). Specifically, nail Hg can be significantly higher than blood concentrations due to the high affinity for Hg that is found in keratinized tissue such as nails (Hopkins et al. 2013). The same study found turtle blood Hg concentrations were most related to dietary Hg uptake (Hopkins et al. 2013); however, other studies showed that blood Hg is affected by additional factors such as body mass (Day et al. 2005; Hopkins, Hepner, and Hopkins 2013). Benjamin et al. (2018) found Blanding's turtle (*Emydoidea blandingii*) nail Hg concentrations also reflected dietary Hg concentrations.

Carapace length was related to increased blood Hg levels in both species, which is similar to what has been found previously (Schneider et al. 2009; Turnquist et al. 2011); however, there was not a relationship exhibited between SCL and Hg in snapping turtle nail concentrations, which could be because larger snapping turtles have exhibited the ability to reach an equilibrium of uptake and excretion of metals (Golet and Haines 2001). This study also showed larger painted turtles had lower nail concentrations of metals such as Hg and Pb compared to smaller conspecifics (Figure 2B), which could be due to the shift from being more carnivorous to more herbivorous over time (Ernst and Lovich 2009). Schneider et al. (2011) had mixed results depending on the species in question, where the red-headed Amazon river turtle (*Podocnemis*

erythrocephala) exhibited a strong positive relationship between SCL and Hg in scute samples, while the six-tubercled Amazon River turtle (*Podocnemis sextuberculata*) did not exhibit a relationship. The observed difference was likely attributed to the different feeding habits of the turtle species (Schneider et al. 2011).

Selenium concentrations were 1.7 times greater in painted turtle nails relative to snapping turtle nails unlike similar studies in which relative trophic level effects on Se concentrations were conducted. In Tennessee, there were no significant differences in Se bioaccumulation found between snapping turtles, map turtles (*Graptemys* spp.), musk turtles (*Sternotherus odoratus*), and red-eared sliders (*Trachemys scripta elegans*; Van Dyke, Hopkins and Jackson 2013). A similar study in Virginia found that feeding ecology was related to MeHg and Se bioaccumulation (snapping turtles $\geq$ musk turtles > painted turtles > northern red-bellied cooter [*Pseudemys rubriventris*]), where more carnivorous omnivores like snapping turtles accumulated higher concentrations than other omnivores within the same system (Bergeron et al. 2007).

Zinc concentrations in the present study were as high as 400-fold greater in nails than blood and 40-fold greater than any other metal tested, similar to what was found in a population of Mediterranean pond turtles (*Mauremys leprosa*) in a contaminated mining district in Spain (Zn > Cu > Pb > Hg > Cd; Martínez-López et al. 2017). A population of Kemp's Ridley sea turtles (*Lepidochelys kempii*) off the coasts of Texas and Louisiana, USA, had a similar order of metal concentrations in blood samples collected from 106 individuals (Zn > Cu > Hg > Pb > Ag; Orvik 1997).

High concentrations of Zn across species is likely due to the important role Zn has in turtle development; it is found in all tissue types due to its affinity toward blood plasma (Ehsanpour et al. 2014). Despite the potential toxic effects that could occur in turtles with high

levels of Zn (Ehsanpour et al. 2014), it has been reported to not be of consequence to turtles due to an equilibrium of Zn uptake and excretion being reached (Maffucci et al. 2005). Similarly, homeostatic regulation of Zn has been observed in Hawksbill sea turtles (*Eretmochelys imbricata*; Ehsanpour et al. 2014), green turtles (*Chelonia mydas*; Storelli et al. 2008), and Caspian pond turtles (*Mauremys caspica*; Yadollahvand et al. 2014) to prevent toxic effects. Other metals such as Cu, Cd, and Hg reach an equilibrium (Schneider et al. 2011) or undergo detoxification processes involving metallothionein (Roesijadi 1996). These equilibria being reached could explain why there was not a significant relationship between body condition and accumulation of metals in this study.

Land use effects on turtle heavy metal concentrations

Cadmium, Se, and Zn were negatively related to distance from mine land across species and tissue types, which was expected due to acid mine drainage runoff and other contaminants associated with mining activities (Lemly 2008; Locke et al. 2020). Similar Se patterns were found in mud turtle (*Kinosternon subrubrum*) blood at the Savannah River Site in Georgia, where heavy impacts from coal combustion residue were present in the system (Cochran et al. 2018). Interestingly, Se was the only metal in this present study that showed a linear relationship where distance from mining was positively related to Se levels in soil, and soil Se concentrations further showed a positive relationship with concentrations found in turtle nails. It is likely there are missing links in this linear relationship with Se in the environment such as the long-term uptake of metals by plants that are food sources of snapping turtles and painted turtles in various life stages (Bischoff 1999). Another missing link that was not investigated in this study is the maternal transfer of Se that has been observed in snapping turtles (Hopkins, Hepner, and

Hopkins 2013), leatherback turtles (*Dermochelys coriacea*; Guirlet, Das, and Girondot 2008) and red-eared sliders (Nagle, Rowe, and Congdon 2001).

This study found turtles had higher concentrations of Pb in wetlands further from roads which is opposite from the relationship between Pb and roads that has been reported in diamondback terrapins (*Malaclemys terrapin*) and plants (Burger 2002; Croteau et al. 2008; Ferretti et al. 1995). The lower concentrations of Pb in turtles from wetlands directly adjacent to roads (0 m away) compared to wetlands farther from roads (> 400 m away) could be due to low vehicle traffic, as most contaminants affiliated with roads are from vehicle wear (Croteau et al. 2008; Forman et al. 2003; Kobringer 1984). Other metals associated with roads such as Cd and Cr in turtles also showed positive or weak negative relationships with proximity to roads, which could be because trace amounts were detected in all sample types across species.

Direct land use impacts on metal bioaccumulation in turtles have not been thoroughly studied; however, studies on other bioindicators such as macroinvertebrates have shown that individuals from wetlands surrounded by organic cultivated lands (e.g. barley [*Hordeum vulgare*], pea [*Pisum sativum*] and white clover [*Melilotus* spp.]) had higher concentrations of MeHg compared to individuals from wetlands surrounded by cultivated crops or grasslands (Bates and Hall 2012). This could explain why turtle Hg concentrations were higher in turtles from wetlands that had increased forest cover as forest was the most natural land use type. Wetland soil RDAs had higher adjusted R^2 values compared to landscape RDAs, which could be due to the uptake of metals from nesting substrate; however, Nagle, Rowe, and Congdon (2001) found that nesting substrate had no effect on red-eared slider hatchling physiology that came from artificial nests with contaminated substrate. Since the majority of turtles sampled were older, the contamination could have resulted from ingesting plants that had accumulated metals

from the soil such as cattail (*Typha* spp.) and duckweed (*Lemna minor*; Ernst and Lovich 2009; Parra et al. 2012).

Some historic problem mining areas within the watershed have since been reclaimed (34.78 ha), but about 75 % of mines that were permitted in the 1990s (278.74 ha) have been completely released (WVDEP 2020), meaning the area is not actively mined and defined as ‘released acres’ (Nazarro 2009). Whether or not this released land has been reclaimed is uncertain. Although reclaimed mine land is no longer active, environmental contamination can remain in the system. Yu et al. (2011) found that proximity to a gaseous diffusion plant did not significantly affect accumulation of Cd, Cr, Cu, Hg, and Pb; however, Nagle, Rowe, and Congdon (2001) found significantly higher concentrations of As, Cd, Cr, and Se in wetlands affected by coal ash compared to reference sites. A larger sample size of wetlands with control sites that are not impacted by mining activities would be recommended, and long-term studies are recommended to further investigate the effects of different mining land use history (i.e. remediated lands, abandoned mines).

The creation of wetlands increases bioavailable Hg in the system that can convert to MeHg (Wolfe et al. 1998). Sinclair et al. (2012) investigated the differences in MeHg concentrations in natural and created wetlands in Saskatchewan and found created wetland sediment had significantly higher concentrations compared to natural wetlands. Although Hg is not one of the primary contaminants affiliated with roads, light switches in vehicles with the model year 2002 or older are made with Hg (ELVS 2012). Other parts such as automatic brake system switches have historically been made with Hg (Dormody et al. 2005); however, turtles from wetlands further from roads had higher concentrations of Hg which is opposite of what would be expected if contamination was coming primarily from vehicles.

Turtle movement between wetlands could be confounding the site relationships found in this study (Ehsanpour et al. 2014; Turnquist et al. 2011; Yu et al. 2011). In a concurrent study, 66 % (n = 27) of telemetered snapping turtles relocated to at least one other wetland within the Upper Deckers Creek watershed, and 30 (1 telemetered snapping turtle and 29 marked painted turtles; 4.44 %) turtles were recaptured at different sites between 2019 and 2020 (Chapter 2, this document). Turnquist et al. (2011) considered snapping turtles with outlier concentrations of metals as potential immigrants from nearby watersheds due to their possible > 1 km overland movement (Ernst and Lovich 2009). Due to the size of the Upper Deckers Creek watershed, movement among wetlands is increasingly possible, and further investigation is necessary to determine how movement affects metal bioaccumulation in turtles. Because of the short-term nature of blood metal levels, localized bioaccumulation could be elucidated.

Land use effects on soil concentrations

Contrary to expectations, agricultural land use was negatively correlated to soil metals (i.e. Hg, Pb, and Zn; Lowrance et al. 1997, Lowrance 1998). A study in China found that physical characteristics such as topography and upland land use significantly correlated with metals such as Co, Cu, and Zn (Liu et al. 2016). Opposite trends were found in New York and Connecticut where pastures had higher concentrations of Hg in the A soil horizon (Richardson and Moore 2020). The same study found that adjacent land use types (e.g. single- and multi-family homes, varying degrees of development and industry) affected the concentrations of Hg found in urban forests with industrial land use associated with higher soil Hg concentrations (Richardson and Moore 2020).

Results in this study regarding agricultural effects on metals in soil are opposite of what Patra and Sharma (2000) found, where higher Hg concentrations in cultivated crop land because

of Hg coat dressings on crop seeds. Similarly, a study on the Beiyun River in China found there were higher concentrations of Cr in farmland than forestland, and forestland was moderately polluted with Cd, Cr, and Zn, which was related to concentrations found in river sediment (Liu et al. 2019). The negative relationship found in this study between agricultural land and metals in the soil could be due to uptake into plants and further plant removal from the system (i.e. phytoremediation; Chaney et al. 2005; Zhang et al. 2018). Separating hay and pasture from cultivated crops rather than analyzing all as ‘agriculture’ could be further investigated to determine differences in metal contamination between land use practices (i.e. livestock introduction compared to seasonal removal of crops).

Effects of metal bioaccumulation on body condition

This study showed there was no relationship between Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations and BCI across turtle species or tissue types. This is consistent with what Overmann and Krajicek (1995) found when they investigated the effects of Pb concentrations on snapping turtle BCI in three wetlands of varying levels of Pb contamination (i.e. control, moderate, and heavy contamination) in Missouri. This lack of effect, however, could be due to small sample sizes at each of the wetlands (15, 14, and 8 individuals, respectively; Overmann and Krajicek 1995).

Physiological effects resulting from bioaccumulation of metals has been thoroughly studied *in situ* and in laboratory studies (reviewed by Grillitsch and Schiesari 2010); however, effects of increased metal burdens on turtle BCI has not been studied as thoroughly. Hopkins et al. (2005) found that when western fence lizards (*Sceloporus occidentalis*) were fed Se-contaminated prey items, BCI was affected differently between sexes. Selenium did not affect BCI in juvenile brown house snakes (*Lamprophis fuliginosus*) that were exposed to

contaminated prey over 10 months (Hopkins et al. 2004). This observation was attributed to individual variability that could have been masking potential effects of Se bioaccumulation (Hopkins et al. 2004). Guirlet and Das (2012) reported no effect of growth (i.e. length and weight) in red-eared sliders that were given Cd-contaminated diets of varied concentrations. They also identified higher concentrations of Cd in feces than blood, implying there is effective elimination (Guirlet and Das 2012). There have been reports of no relationships between metal concentrations and BCI in birds as well. Feral pigeons (*Columba livia*) were investigated in Slovakia to determine if dead bird body size was affected by bone Pb concentrations, and there was no relationship (Janiga and Žemberyová 1998). In contrast, American alligators (*Alligator mississippiensis*) experienced lower BCI values when fed mice with higher concentrations of selenomethionine, which significantly increased liver and kidney Se concentrations (Finger Jr. et al. 2017).

CONCLUSIONS

This study indicates that Cd, Cr, Pb, Hg, Pb, Se, and Zn are present within the Upper Deckers Creek watershed. Mining activity, whether current or historic, could have an impact on the environment that could be seen for decades in freshwater turtles and should be considered when implementing and managing wetland buffer areas. Identifying human perceptions and behaviors regarding turtle harvest and consumption would be complementary to this study. Further studies should also be conducted to investigate environmental concentrations of metals in locations where turtles are commonly collected for human consumption, specifically snapping turtles (Sherwood 2017; WVDNR 2014). This current study showed Hg concentrations in 57% of nail samples analyzed exceeded both EPA consumption (0.0001 mg/kg) and fish tissue limits (0.3 mg/kg). Over half of the nail samples exceeding EPA limits in Hg also had estimated MeHg

concentrations exceeding both EPA limits, which is concerning since MeHg is the most toxic form of Hg (Slimani et al. 2017). Although nails are not a consumable part of the turtle, Hg concentrations in parts of the turtle that are consumable (i.e., muscle and liver tissue) can be up to 0.47 times higher than keratinized tissue (i.e., nails; Hopkins, Hepner, and Hopkins 2013; Powell 2014). Estimated muscle Hg concentrations based on the nail Hg concentrations also exceeded the consumption limit. Turtle consumption advisories should be considered to ensure the safety of residents who choose to consume turtles within the watershed.

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The authors have no conflict of interest in publishing this work.

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TABLES

Table 1. Number of tissue samples analyzed from snapping and painted turtles captured during 2019 within the Upper Deckers Creek watershed in Preston County, West Virginia, USA.

Random individuals were chosen based on the quality of the tissue sample (i.e. large enough blood samples without lymph) and dominant land use surrounding the wetland they were originally captured in. The number of turtles varied by how many individuals were captured and had tissues collected. Of the 128 tissue samples collected, 22 wetlands were represented: 8 wetlands had forest, 11 wetlands had agriculture, and 3 wetlands had both forest and agriculture as the dominant land use types within 1 km of the wetland.

	Nail samples			Blood samples			Total
	Ag	Forest	Both	Ag	Forest	Both	
Snapping turtle	13	10	7	9	6	1	46
Painted turtle	12	9	8	27	19	7	82
Total	25	19	15	36	25	8	128

Table 2. A summary of the predictor variables used to investigate the effects of land use types on freshwater turtle Cd, Cr, Pb, Hg, Pb, Se, and Zn bioaccumulation within the Upper Deckers Creek watershed in Preston County, West Virginia, USA. The four main categories of predictors are land use composition factors, soil metal concentrations, turtle morphometric factors, and tissue type. Expected relationships between each predictor and metal bioaccumulation are reported. Note: Wetland soil metal concentrations were additionally used as response variables for the soil metal redundancy analyses where only land use composition predictors were used.

Parameter	Value	Expected relationship
Roads	Minimum road distance from wetland (m)	Negative
Mine land	Minimum mine ^a land distance from wetland (m)	Negative
Agriculture	% cultivated crops, agriculture, hay, and pasture within 30 m	Positive
Soil concentrations	Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations (mg/kg)	Positive
Species	Snapping turtle or Painted turtle	Snapping turtle > Painted turtle
SCL	Straight-line carapace length (mm)	Positive
Tissue type	Blood or nail tissue	Nail > blood

^a Mine land includes grass and barren land affiliated with mining (“mine grass” and “mine barren” [Strager 2012]) and land that was mined prior to 1977 when the Surface Mining Control and Reclamation Act was enacted (“Pre-SMCRA grass” and “Pre-SMCRA barren” [Strager 2012]).

Table 3. Descriptive statistics for percent agriculture within 30 m of each wetland and distance to nearest mine land and roads for 29 wetlands in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, that were sampled for soil analysis in 2019. Tissues from turtles surveyed in 22 of the sites were used for turtle tissue analysis. Values reported are distances in meters for all parameters but percent agriculture. Agriculture values were calculated using the 2011 National Land Use/Land Cover Dataset provided by the West Virginia GIS Technical Center (Strager 2012).

Type	Min	Max	Mean	SE
<i>Soil analysis</i>				
Ag within 30 m	0.00	79.95	23.41	4.93
Mine land	0.00	934.63	331.74	60.89
Roads	0.00	418.86	78.53	17.38
<i>Turtle analysis</i>				
Ag within 30 m	0.00	79.10	26.54	5.72
Mine land	0.00	934.63	363.09	72.77
Roads	0.00	252.41	77.17	15.47

Table 4. Mean ($\pm$ SE) concentrations for each metal tested in nail and blood samples from snapping and painted turtles across three trapping sessions during 2019 in the Upper Deckers Creek watershed in Preston County, West Virginia, USA. Twenty-six wetlands and 3 stream locations were surveyed from 18 April to 19 May (spring), 1 July to 31 July (summer), and 5 October to 1 November (fall).

		Cd	Cr	Hg	Pb	Se	Zn
Snapping turtle	Nail	0.02 (0.004)	0.99 (0.14)	0.42 (0.06)	0.44 (0.08)	1.04 (0.19)	203.72 (9.30)
	Blood	0.0001 (0.00006)	0.0003 (0.0002)	0.005 (0.001)	0.02 (0.01)	0.42 (0.10)	3.94 (0.75)
Painted turtle	Nail	0.07 (0.19)	1.63 (0.35)	0.39 (0.075)	0.41 (0.08)	1.77 (0.31)	254.79 (17.08)
	Blood	0.0001 (0.00003)	0.0002 (0.00005)	0.007 (0.0001)	0.005 (0.001)	0.37 (0.04)	1.47 (0.15)

FIGURES

Figure 1. Mosaic of land use within the Upper Deckers Creek watershed in Preston County, West Virginia, USA (inset). Twenty-six wetlands and 3 stream locations were surveyed during 2019 for freshwater turtles from 18 April to 19 May (spring), 1 July to 31 July (summer), and 5 October to 1 November (fall). Wetlands were either predominantly surrounded by forested ($n = 13$) or agricultural ($n = 13$) land within 1 km, while three sites had an even combination of both. Eight sites were associated with West Virginia University, 11 sites were privately owned with various management practices applied (e.g. mowing, cattle access, chemical application), and 10 wetlands were in wildlife management areas.

Figure 2. Concentrations of total mercury (Hg) found in nail and blood tissue samples from snapping turtles and painted turtles in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, during 2019. Lower bars for each point indicate the lower threshold for the estimated MeHg concentration defined by Bergeron et al. (2007) as 70–100% of Hg being methylated. The zoomed subsection emphasizes samples between the 0.0001 mg/kg recommended daily consumption level (dashed line; Borum et al. 2001; Farland 2000) and the 0.3 mg/kg fish tissue criterion (dotted line; Driscoll et al. 2007).

Figure 3. Redundancy analysis (RDA) biplots relating land use to painted turtle (A) blood and (B) nail, and snapping turtle (C) blood and (D) nail Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations from individuals captured in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, during 2019. Response variables for each RDA are the six metals analyzed in turtles, and predictors included are straight-line carapace length (SCL), percent agriculture within 30 m, and distance from nearest mine land and roads. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

Figure 4. Redundancy analysis (RDA) biplots relating soil metal concentrations to painted turtle (A) blood and (B) nail and snapping turtle (C) blood and (D) nail Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations from individuals captured in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, during 2019. Response variables for each RDA are the six metals analyzed in turtles, and predictors included are straight-line carapace length (SCL) and the six metals analyzed in soil. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

Figure 5. Soil redundancy analysis (RDA) biplot to relate soil Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations with surrounding land use practices. Soil samples were analyzed from 28 wetland sites (one outlier site omitted) in the Upper Deckers Creek watershed in Preston County, West Virginia, USA, during 2019. The multivariate response variable was the six metals analyzed in wetland soil. Land use variables including distance to nearby roads and mine land were used as predictors. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

APPENDICES

Appendix 1. Relationships between Cd concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data.

Appendix 2. Relationships between Cr concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data..

Appendix 3. Relationships between Hg concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data.

Appendix 4. Relationships between Pb concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data.

Appendix 5. Relationships between Se concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data.

Appendix 6. Relationships between Zn concentrations found in snapping turtle and painted turtle tissue types in Preston County, West Virginia, USA during 2019 (i.e. blood and nails) and the following predictors: (A) Percent agriculture within 30 m of each wetland; (B) minimum wetland distance from mine land and (C) roads; (D) straight-line carapace length (SCL; g); and concentrations of Cd, Cr, Hg, Pb, Se, and Zn in wetland soil (E–J). Loess lines are included to elucidate the trends in the data.