




Article

Biodiversity Monitoring of a Riparian Wetland in a Mixed-Use Watershed in the Central Appalachians, USA, before Restoration

Darien N. Becker^{1,2} , Jason A. Hubbart³  and James T. Anderson^{4,*} 

¹ School of Natural Resources, West Virginia University, 1145 Evansdale Drive, Morgantown, WV 26506, USA; dnl0009@mix.wvu.edu

² Welty Environmental Center, 1201 Big Hill Court, Beloit, WI 53511, USA

³ Division of Forestry and Natural Resources, West Virginia University, 322-B Percival Hall, Morgantown, WV 26506, USA; jason.hubbart@mail.wvu.edu

⁴ James C. Kennedy Waterfowl and Wetlands Conservation Center, Belle W. Baruch Institute of Coastal Ecology and Forest Science, Clemson University, P.O. Box 596, Georgetown, SC 29442, USA

* Correspondence: jta6@clemson.edu

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 are required to be collected 5–10 years after a project is complete; however, pre-restoration data that can inform the effectiveness of mitigation are often not collected. We conducted pre-restoration surveys on various taxa along or within Ruby Run, a tributary of Deckers Creek in north-central West Virginia, USA, from 2016 to 2020 to determine the baseline relative abundance and diversity within the stream and the associated riparian zone. In five years, we observed 237 species (154 plant, 58 bird, 13 fish, 6 small mammal, and 6 anuran) and 25 families of macroinvertebrates. 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



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1. Introduction

Wetlands are among the most valuable ecosystems in the world [1,2]. 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 (30%) development as well as agriculture (26%) and silviculture (23%) in the United States [3]. Since 1970, the greatest losses of wetlands have occurred in Louisiana, Mississippi, Arkansas, Florida, and the Carolinas [1]. In West Virginia, 24% of historical wetlands have been lost due to anthropogenic change [4]. Most wetland losses in West Virginia have been due, at least in part, to agricultural land use change, where wetlands were drained and filled to make the land more suitable for crop production [1]. 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 thereby altering wetland numbers and extent [5,6].

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 offspring [7–9]. 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 [3,10–12].

It is essential to periodically assess created and restored wetlands for their ecological diversity to ensure successful restoration practices. Vegetative community structures within created wetlands have been, on average, 26% lower than reference sites [2], and studies have showed that restored wetlands have significantly lower vegetative cover than natural wetlands [13,14]. Studies have alternatively found that mitigated wetlands have a higher diversity and abundance of wetland vertebrates (e.g., birds and anurans [15–17]) and provide similar or better wetland functions than reference wetlands [18–22]. Few studies have evaluated multiple taxa, and even fewer have evaluated faunal and floral response before, during, and after restoration, which could assist managers in identifying possible challenges and improvements to wetland mitigation.

In 2017, a headwater dam of Deckers Creek in Preston County, West Virginia, USA, was renovated to increase the water capacity of the impoundment. 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 pasture 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., twelve treatment areas) were proposed within the riparian zone of Ruby Run and used to delineate areas for faunal and floral sampling (Figure 1).



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.

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 will be used to quantify the ecological response to restoration activities conducted during summer 2021 and continuing into spring 2022 in Ruby Run, demonstrate how surveys for multiple taxa can be obtained in one area, and provide a characterization of the diversity and abundance of species occupying a typical central Appalachian mixed-use watershed. Specific monitoring objectives included: (1) quantifying the year-round relative

abundance and diversity of birds; (2) determining the annual relative abundance and diversity of wetland macroinvertebrates, small mammals, and anurans; (3) documenting annual plant diversity and relative abundance; and (4) identifying annual changes in 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, as we carefully placed survey points and monitoring plots of select taxa to facilitate monitoring after restoration.

2. Materials and 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 7778 ha in Preston County, West Virginia, USA, and encompasses 89.9 km of stream (Deckers Creek headwaters and its tributaries). Deciduous forests, palustrine wetlands (emergent, unconsolidated bottom, forested, and scrub–shrub), and rivers have been significantly fragmented and altered from agriculture. Developments of varying intensities also have impacted the watershed.

Elevation at the Ruby Run study site ranges from 517 to 524 m [23]. Soil is classified as silt loam, primarily Atkins silt loam, with lesser amounts of Ernest silt loam and Brinkerton Series [24]. The underlying geology is composed of sandstone, shale, clay, coal, and limestone [25].

Climate data were obtained from the National Oceanic and Atmospheric Association (NOAA) online weather repository from the Morgantown, West Virginia airport, located approximately 22.5 km from the study site [26]. The historic climate (1980 to 2020) of the local area is characterized by hot summers (i.e., hottest monthly mean temperature >22 °C), cold winters (i.e., coldest monthly mean temperature <0 °C), and no dry season (i.e., Dfa climate type; [27]). The long-term annual average precipitation is approximately 106 cm, and annual averages of daily maximum and minimum temperatures are approximately 17 °C and 6.3 °C, respectively [28]. July is the hottest and wettest month, with an average daily temperature of 23 °C and average monthly precipitation of 11.7 cm. January is the coldest month, with an average daily temperature of -0.4 °C, and February is the driest month with average monthly precipitation of 6.6 cm (28). During the period of study (2017 through 2020), the average air temperature (°C) ranged from 12.4 °C to 13.0 °C for 2018 and 2020, respectively (Figure 2). The minimum air temperature ranged from 0.2 °C to 2.1 °C in 2017 and 2020, respectively. The maximum air temperature ranged from 23.3 °C to 25.5 °C in 2017 and 2020, respectively. The total precipitation ranged from 1033 (mm) to 1389 mm in 2020 and 2018, respectively (Figure 3).

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 installed around 679 m (0.73 ha) of Ruby Run that protects approximately 2.22 ha of palustrine emergent wetlands and upland riparian areas from constant cow disturbance, ranging from 22 to 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 most large riffles are located with larger rocks and cobble substrate.

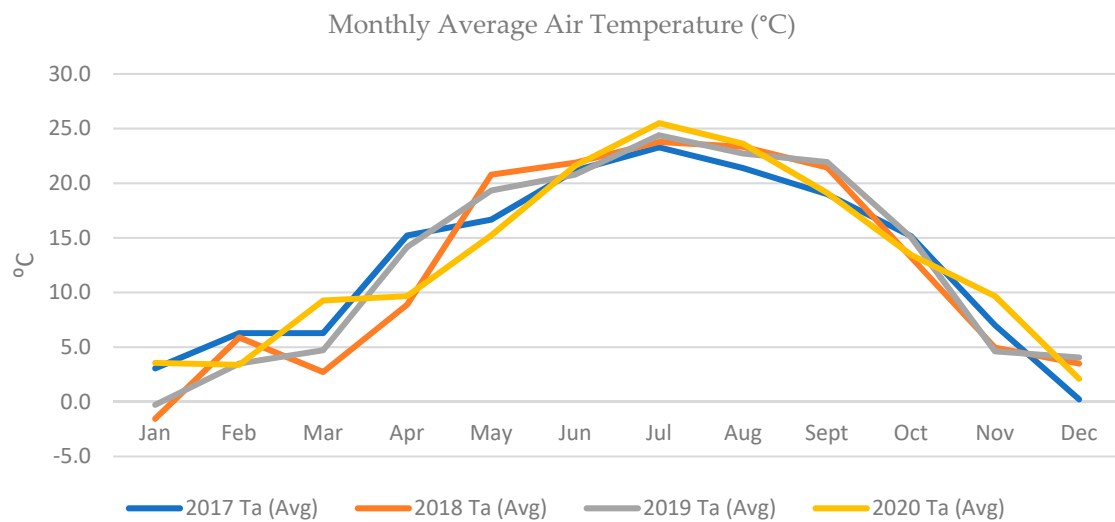


Figure 2. Monthly average temperature from 2017 to 2020 for Morgantown, West Virginia, USA, which is 22.5 km from the Ruby Run study site (NOAA 2022).

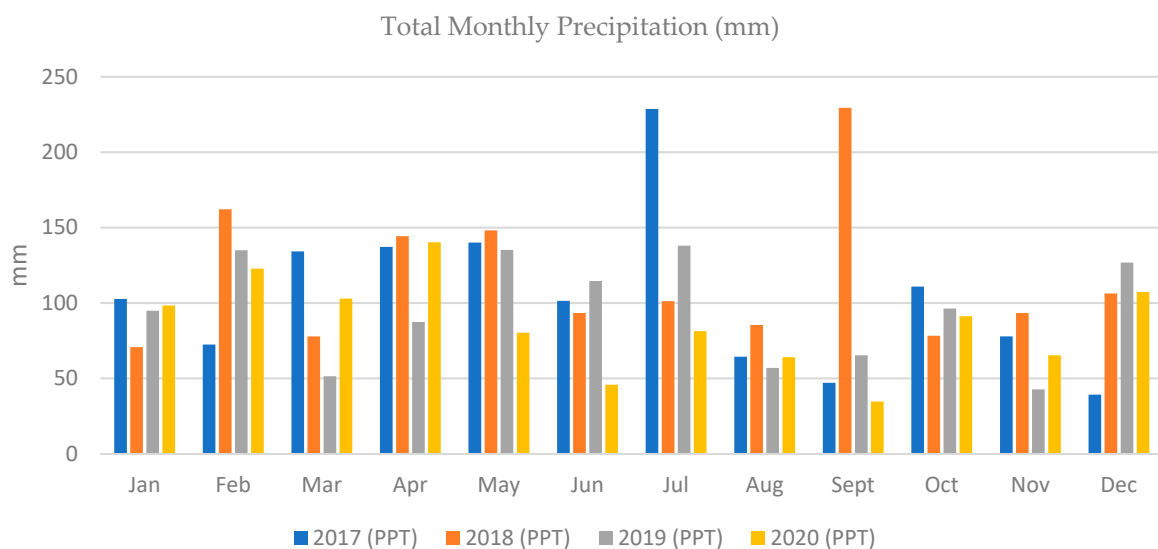


Figure 3. Monthly average precipitation from 2017 to 2020 for Morgantown, West Virginia, USA, which is 22.5 km from the Ruby Run study site (NOAA 2022).

This area is representative of similar mixed farm–woodland headwater streams and riparian habitats within the central and southern Appalachian regions. These regions are the focus of the Appalachian Landscape Conservation Cooperative, a regional effort with the goal of promoting larger contiguous deciduous forest landscape and improving stream water quality [29]. 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 [29,30].

Stream habitat degradation within the Upper Deckers Creek watershed primarily results from coal mining (i.e., acid mine drainage [31]). 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 [32], which directly affect organisms such as macroinvertebrates [33,34]. Treatments such as limestone channels and

settlement ponds were applied since 2002 and receiving waters have improved in terms of water quality and macroinvertebrate and fish diversity and abundance [35].

2.2. Bird Surveys

To determine bird use of wetlands, we conducted five ten-minute, 50 m radius point count surveys monthly from 2018 to 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 [36,37]. Survey locations were about 250 m apart along Ruby Run to prevent counting the same individuals between surveys [38]. 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/h to prevent decreased detection rates [39]. 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 the date, time, temperature (to the nearest degree Celsius), level of noise disturbance, wind, and sky condition [40]. We also recorded whether each bird was present within one of the twelve 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 to 2020 using a 5 cm diameter handheld PVC core sampler (with samples taken to a depth of 15 cm [41]). At each visit, we collected five randomly located benthic core samples at each of the five locations between the stream and fence, as determined using a random point generator in ArcGIS (v. 10.7, ESRI, Redlands, CA, USA). 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. Macroinvertebrates were preserved in 70% ethanol [41]. We identified and enumerated individuals to family (except earthworms [42]) for each soil core sample.

2.4. Small Mammal Surveys

We assessed temporal variation in small mammal relative abundance and diversity from 2017 to 2020 with Sherman live traps (5.1 cm × 6.4 cm × 16.5 cm, H.B. Sherman Traps, Tallahassee, FL, USA) placed 10 m apart along a 20 m × 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). Transect rows were located 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 [43] along with 20 dried mealworms to attract insectivores (e.g., short-tailed shrews (*Blarina brevicauda*)) and enhance the survival of captured individuals [44]. Every 24 h 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 [45] and equipped all other captured animals with #1005-1 Monel ear tags (National Band and Tag Company, Newport, KY, 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 [46].

With each capture, we took note of the 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 [47,48]. We took proper precautions against Hantavirus according to Mills et al. [49] and Kelt and Hafner [50].

2.5. Anuran Surveys

We evaluated anuran communities from 2017 to 2020 using nocturnal call count surveys. We followed standardized protocols developed by the U.S. Fish and Wildlife Service (outlined by Balcombe et al. [16]) 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/h [40,51]). 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 [52] to each species heard according to call intensity [16]: 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 [17,53]. We recorded call locations within one of the twelve proposed vegetative cover treatment areas, and we used maps to determine where they belong after the treatments have been implemented.

2.6. 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 [54]. 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 Daubermire [55] 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 the 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* spp.), alder (*Alnus* sp.), and multiflora rose (*Rosa multiflora*)), so we only used quadrats sized for herbaceous plants [56]. We used Radford et al. [57], Strausbaugh and Core [58], and Gleason and Cronquist [59] to identify plants to species.

2.7. In-stream Community Assessments

We surveyed fish communities in Ruby Run once annually between August and November from 2017 to 2020 using single-pass shocking methods by Huntsman and Petty [60] 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 the weight (to the nearest 0.1 g) and length (mm) according to standard length (caudal–peduncle). After processing, we returned each fish to the stream near their capture location.

We sampled stream macroinvertebrates once annually in May from 2017 to 2020 by obtaining kicknet samples (net dimensions 335 mm × 508 mm with 500 µm mesh) from four representative riffles [61]. We preserved all samples in 70% ethanol in the field, and we identified individuals to family for most individuals (except earthworms [42]).

2.8. Statistical Analysis

The mean annual diversity for each taxon with three or more species was calculated using R statistical software [62]. Because the primary objectives of this study were to estab-

lish 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' [63]) 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 the number of individuals per m^2 and H' of soil core samples for macroinvertebrates for each of the five wetland locations seasonally [64,65].

For small mammals captured at each trapline, we calculated the 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 the percentage of individuals captured divided by the number of trap nights for each survey period [66]. 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 [67].

We calculated species abundance and H' for anurans based on surveys in 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' for each $1 m^2$ quadrat and species richness and coverage based on plants identified to species during surveys. We calculated evenness (J') using Pielou's evenness index [68]:

$$J' = H' / H'_{\max}, \quad (1)$$

where H' = Shannon–Wiener diversity index and H'_{\max} = the maximum Shannon–Wiener diversity index. We present standard errors with the means because we are most interested in the precision of the sample means.

3. Results

3.1. Birds

Fifty-eight bird species were observed between 2018 and 2020 [40]. Out of the 2880 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).

Table 1. Bird species with an average of >0.1 individuals per 50 m radius plot per survey 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	Standard Error
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

The highest Shannon diversity was observed in the summer months (May–July) and attributable to the presence of less common migratory birds such as the Nashville warbler

(*Leiothlypis ruficapilla*) observed in 2018 and even less common out-of-range observations such as the western meadowlark (*Sturnella neglecta*) found in May 2020 (Figure 4 [40]). 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.

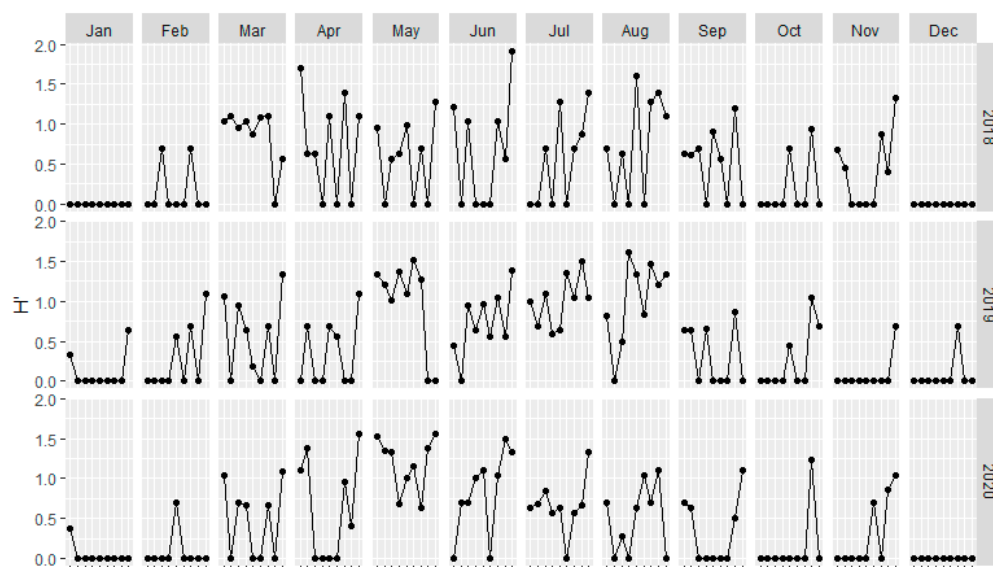


Figure 4. Monthly Shannon diversity of birds within the riparian area of Ruby Run at the JW Ruby Research Farm, Reedsville, West Virginia, USA, from 2018 to 2020. Birds were recorded in five equidistant 50 m radius point counts along the stream as well as along transects between each point count area. Lines are plotted to show trends in diversity from downstream to upstream.

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 [69].

3.2. Wetland Macroinvertebrates

All macroinvertebrates sampled were in various larval stages, except for earthworms, which consisted of both adults and juveniles. Wetland soil core samples revealed 11 families across seven orders between 2018 and 2020 ($n = 275$ soil samples across five wetlands; Table 2 [40]), where earthworms (Oligochaeta) were the most abundant taxon out of the 89 individuals collected across years (47.0%), followed by mosquito larvae (Chironomidae; 15.7%) and biting midge larvae (Ceratopogonidae; 13.5%). The mean annual H' ranged from 0.27 to 0.34 (Figure 5).

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 to 2020. Relative abundance was calculated as number of individuals per square meter. All taxa were larval forms aside from Oligochaeta, which consisted of both adults and juveniles.

Class	Order	Family	Individuals per m ²	
			Mean	SE
Oligochaeta			3565.07	640.85
Insecta	Coleoptera	Hydrophilidae	169.77	169.77
Insecta	Coleoptera	Ptilodactylidae	339.53	224.58
Insecta	Coleoptera	Staphylinidae	84.88	84.88
Insecta	Diptera	Ceratopogonidae	1018.59	640.85
Insecta	Diptera	Chironomidae	1188.36	662.95
Insecta	Diptera	Empididae	84.88	84.88
Insecta	Diptera	Psychoidae	169.77	169.77
Insecta	Diptera	Tabanidae	339.53	224.58
Insecta	Diptera	Tipulidae	84.88	84.88
Collembola	Poduromorpha	Neanuridae	84.88	84.88
Insecta	Trichoptera	Rhyacophilidae	84.88	84.88
Arachnid	Trombidiformes		84.88	84.88

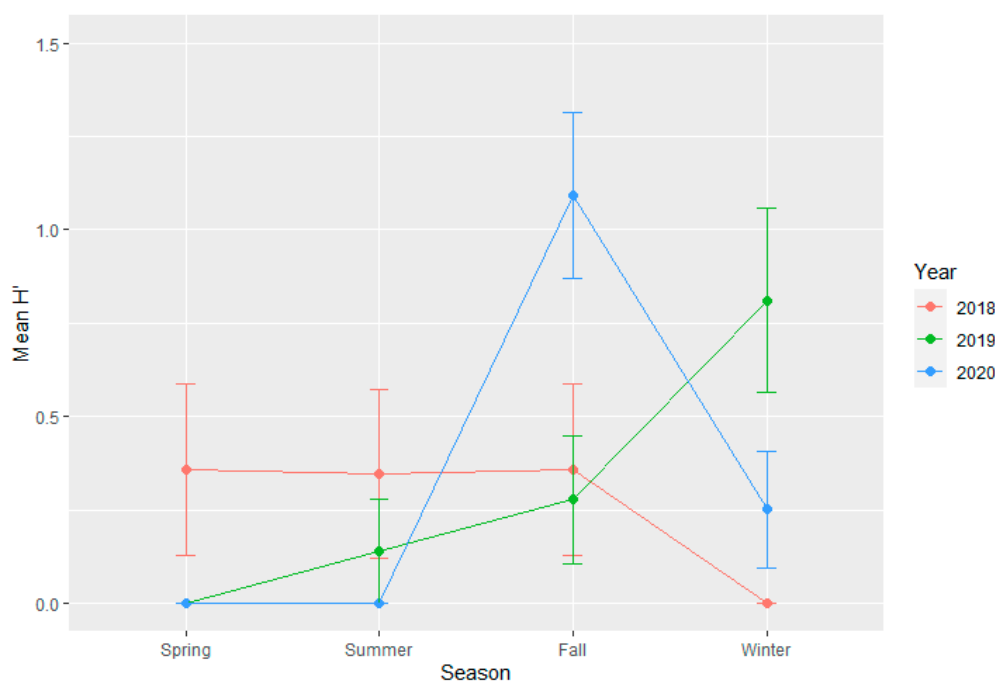


Figure 5. Seasonal variation in benthic macroinvertebrate Shannon diversity (H') across five wetlands within the Ruby Run riparian zone at the J.W. Ruby Research Farm in Preston County, West Virginia, USA, during 2018–2020. Error bars indicate standard error for each mean value.

3.3. Small Mammals

Six species of small mammals were captured from 2017 to 2020 (Table 3 [40]), 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 [40]. All observed small mammals were native species to West Virginia [70].

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 to 2020. Numbers 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 (CPUE) with standard error (SE) were calculated.

Species	Scientific Name	Number of Individuals	CPUE	
			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

The number of trap nights in a session for a trapline varied from 27.5 to 54 per month and was primarily affected by bait loss (presumably raccoons (*Procyon lotor*)). Damage to and the 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. Ants or slugs also consumed some bait, thereby potentially affecting the trapping success. The highest CPUE reported was 49.2 captures per 100 trap nights in September 2020 (Figure 6). Recapture rates were 10.87% in 2017, 10.67% in 2018, 2.80% in 2019, and 15.15% in 2020.

3.4. 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 4 [40]). 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 score 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 [71].

3.5. Vegetation

The number of surveyed quadrats varied from 63 in 2016 to 82 in 2020 ($n = 445$ plots across six visits in 4 years; mean \pm SE: 74.17 ± 2.91 quadrats) with 154 total plant species observed, where 67% of the plants observed were native [40]. Plant diversity per 1 m² quadrat among years ranged from 0.22 to 0.26 (Table 5). 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-leaved goldenrod (*Solidago rugosa*) in 2018 and 2020 ($14.59 \pm 2.32\%$ and $15.27 \pm 2.50\%$, respectively; Table 6).

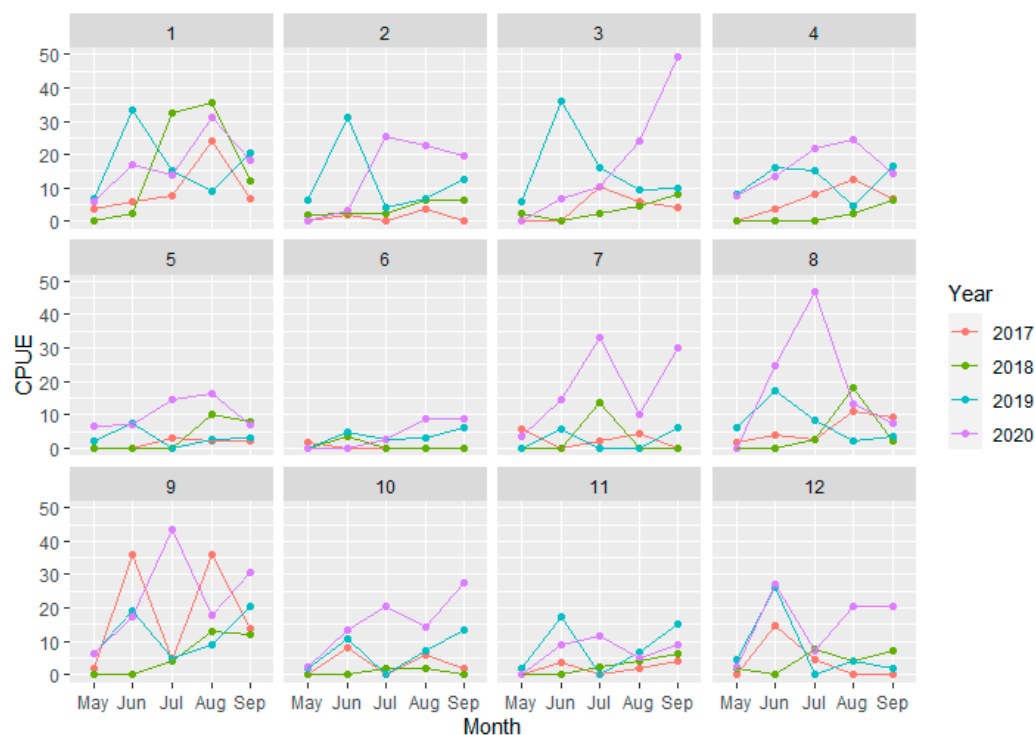


Figure 6. Variation of small mammal catch-per-unit-effort (CPUE; calculated as the number of captures per 100 trap nights) among the 12 traplines surveyed along Ruby Run at the JW Ruby Research Farm, Reedsville, West Virginia, USA, across years (2017–2020). Six unique species were captured across the 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*).

Table 4. 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 to 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 [17]).

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 5. Mean (\pm SE) annual variation of Shannon diversity (H') and Pielou’s evenness (J) of plant species per 1 m² quadrat in 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 6. 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>Symphotrichum 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

3.6. In-stream Communities

Between the seven reaches across four years, 13 species of fish were captured (Table 7 [40]). All fish species sampled were native to West Virginia [72]. The 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 [40]. Shannon diversity ranged from 0.69 to 1.11 (mean ± SD: 0.87 ± 0.03; Table 8).

Table 7. 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 to 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 8. 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

From the four stream riffles assessed from 2018 to 2019 (no macroinvertebrates were found in 2020), 16 invertebrate families across eight orders were represented (Table 9), 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 (Perlodidae), which accounted for 50.7% of all samples. Ceratopogonids were the most common taxon represented in 2019 (56%; Table 9).

Table 9. Macroinvertebrate assemblages within stream riffles surveyed in Ruby Run at JW Ruby Research Farm in Preston County, West Virginia, USA, from 2018 to 2019. Relative abundance was determined as number of individuals per square meter. All taxa were larval forms aside from Oligochaeta, which consisted of both adults and juveniles.

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

4. Discussion

4.1. Bird Diversity

We found over 50% of the birds consisted of song sparrows and red-winged blackbirds consistently across the study period. 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 [73], and most observations of red-winged blackbirds were singing males during the breeding season 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 meadows 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* [73]), and were regular but not abundant in Ruby Run. 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 [73], which could be due to increased edge effects from the small size of the wetland area. The intersection of the adjacent woodlands with the riparian area along Ruby Run likely created an edge effect that attracted generalist bird species and specialists of open grassland and forested areas [74]. It is also possible that Ruby Run serves as a keystone structure for increasing habitat heterogeneity and thus attracting diverse avian species [75]. Increased wetland size and vegetative diversity and heterogeneity are associated with riparian wetland bird richness and abundance in general [76,77]. Moreno-Mateos et al. [78] found opposite relationships between bird species abundance and wetland size; however, they attributed this to inconsistent surveying effort to account for larger wetlands. Seasonal patterns in avian abundance and diversity also were evident, with the lowest numbers occurring during the winter, as the pool of potential species is diminished, and the lack of winter cover reduced bird use. Although, changes in water availability in relation to phenological patterns also potentially influenced some species [79], such as Canada Geese.

4.2. Macroinvertebrate Assemblages

The current study showed that 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 [80], 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, indicating that while 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 [81]. Ephemeropterans, plecopterans, and trichopterans (EPT) were present within stream samples, thus indicating Ruby Run has improved stream water quality since an upstream segment was identified as low priority (iron load: 0.59 kg/day as of 2014) and management was implemented to address acid mine drainage in the Deckers Creek watershed [31]. EPT richness is negatively related to percent sand in streams [82], 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 [83]. Despite using similar trapping methods, Osbourne and Anderson [83] had higher CPUE for taxa such as *Peromyscus* spp., which may have been due to trapping near field–forest transition zones that were not abundant in our study. The low CPUE in the current investigation may be due to ants and slugs that consumed bait 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, at least 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 destroyed 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 ran 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 [84]. Alternatively, Bissonette and Rosa [85] showed that road distance did not affect the 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. 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 [86,87]. Babbitt et al. [88] 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 was evident as per tadpole bycatch during macroinvertebrate surveys as well as leopard frog and green frog bycatch during small mammal surveys. Amphibian diversity declined over the four years of the study. Continued monitoring is necessary to determine if the observed declines in anuran diversity are attributable to long-term trends on the study site, representative of the general decline in amphibians around the world, or the result of short-term land use and precipitation patterns [89,90].

4.5. Wetland Vegetation Diversity

Wetland vegetation diversity and evenness did not deviate significantly among years despite the inconsistent sampling effort. The 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 [40,91,92]. 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 [93]. Other plant species such as glossy-leaved aster (*Symphotrichum puniceum*) and Allegheny blackberry (*Rubus allegheniensis*) were not detected by Balcombe et al. [93], which could be because both species are facultative upland species.

Temporal variation in the riparian wetland vegetation between 2016 and 2020 indicated consistent percent covers of facultative upland species relative to obligate or facultative wetland species [40], and the probability of facultative upland species occurrence in wetlands is relatively low (1–33% [94]). Ho and Richardson [95] showed that wetland-dependent species (obligate or facultative wetland species) increased in richness over time with higher water inundation. Additionally, because riparian wetlands are occasionally inundated for short periods of time (e.g., flooding from snowmelt or significant rain events), upland species can establish within the riparian area during times of flood recession.

The presence of cattle or heavy mowing equipment can drastically alter the microtopography in wetlands, which can alter natural pooling within a wetland [96]; however, the effects of microtopographic change in wetland plant diversity is inconsistent. Hong et al. [97] found that nutrient runoff has a stronger effect on plant diversity than microtopography, and the effects that cattle have on the microtopography is not certain [98]. Alternatively, wetlands managed via disking, which is considered among the most intense form of wetland management [99], had microtopographies comparable to natural wetlands and exhibited the highest plant diversity compared to natural and non-disked wetlands [96].

4.6. Fish Assemblages

The only fish species sampled in 2017 were largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*) and creek chub (*Semotilus atromaculatus* [40]), which are considered species tolerant of disturbance in streams [100]. Leonard and Orth [101] found that green sunfish and creek chub were comparable when 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 the increased presence of disturbance-intolerant species such as spottail shiner (*Notropis hudsonius*) and central stoneroller (*Campeostoma 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 [102] and should be compared with more frequent sampling to elucidate possible seasonal habitat use of Ruby Run throughout the year as either a core habitat or a corridor.

5. Conclusions

We documented 235 species (6 anurans, 6 small mammals, 13 fish, 58 birds, and 154 plants) of which 78% were native and 25 families of macroinvertebrates in this mixed-use watershed, which is typical of the mid-Appalachians (Table 10). Ruby Run had a good representation of anurans and to a lesser degree small mammals and turtles, but lacked many wetland-dependent birds, likely due to a lack of open water and the small size of the wetland. However, this lack of species richness indicates that Ruby Run is a prime candidate for restoration. Wetland mitigation efforts should continue to be monitored for success using multiple taxa given that land use change can affect taxa in many ways. This approach would result in a well-rounded assessment rather than focusing on a single taxon [103]. The multiple taxa approach used here can serve as an example of monitoring for other systems and localities globally. 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. However, lacking documented species richness, it is unlikely that land managers would be aware that a decline is occurring. Hence, this underscores the importance of the approach illustrated in the current investigation.

Table 10. Number of species by taxa within West Virginia, number of species by taxa that are wetland/aquatic dependent, number of species documented in Ruby Run (this study), and the percent of species we documented compared to the total number in West Virginia and the percent of species we documented compared to the total number that are wetland-dependent [16,69–72].

Taxon	West Virginia	Wetlands		Ruby Run	
	No. Species	No. Species	No. Species	% of West Virginia Species	% Wetland Species
Plants	2344	1500	154	6.57%	4.20%
Fish	181	181	13	7.18%	7.18%
Anurans	15	15	6	40.00%	40.00%
Birds	266	103	58	21.80%	7.77%
Small mammals	36	9	6	16.67%	22.22%

The continued monitoring of plant and animal abundance and diversity post-restoration is important to determine restoration success [104]. Efforts from government organizations such as the U.S. Army Corps of Engineers and the U. S. 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 reverse wetland losses 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 and the incorporation of additional adaptive restoration and management activities [105], wetlands can become habitat sinks, especially for organisms such as macroinvertebrates and amphibians that depend on water quality, nutrient cycling, and hydrologic cycles for the majority of their life history [106]. Wetland size reduction up to 50% has been predicted to reduce richness by 10–16% in any taxa [84]. The hydroperiod of mitigated wetlands is also important to consider as some amphibians depend on semipermanent wetlands to breed and safely undergo metamorphosis [107].

Once mitigation has 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 additional information regarding time requirements for riparian wetland succession. Commonly, wetland monitoring continues 3–5 years post-restoration to determine restoration success; however, the 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 [108]). 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 the improvement of wetland conditions.

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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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