



Macroinvertebrate Inventory of High Elevation Springs in Shenandoah National Park, 2022–2024



Spring-fed stream sampled for macroinvertebrates near the Pinefield Hut along the Appalachian National Scenic Trail within Shenandoah National Park, 25 September 2024.

NPS / H. GIRGENTE

Macroinvertebrate inventory of high elevation springs in Shenandoah National Park, 2022–2024

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Abstract

Virginia Tech partnered with the National Park Service Inventory and Monitoring program to sample macroinvertebrates and environmental measures related to water quality, habitat availability, and human use at 63 springs within Shenandoah National Park, of which 59 were assessed and analyzed. Thirty five of the 59 springs had been intentionally modified for human use via spring box or pipe installation and the remaining 25 had not been modified. The goals of this inventory were to assess the distributions of macroinvertebrates across springs in the park with an emphasis on identifying unique spring specialist taxa that might be considered rare. Additionally, we sought to determine whether macroinvertebrate diversity and the presence of rare taxa have been impacted by modifications made to springs and/or by visitor use (springs that are frequently visited/used by park goers).

We identified 205 unique taxa across the 59 springs. Of those taxa, we identified 17 rare taxa using NatureServe's Conservation Status Rank, where species with a status rank of at least vulnerable were labeled rare. Rare taxa were present in 32 springs, 19 of which were modified (having a spring box or pipe installed) and 13 were unmodified (no installations). There was no statistically significant difference in total taxa richness or abundance between the spring types. General linear models indicated decreased pH, flow (velocity), small woody debris, and increased proximity of springs to a spring signpost as factors associated with increased taxa richness in the macroinvertebrate community, while richness for rare taxa were most associated with increasing temperature, small woody debris, and increased distance (decreased proximity) of springs to a spring signpost. Furthermore, linear models showed that decreasing pH, flow, temperature, small woody debris, and increased proximity of springs to a spring signpost or Appalachian Trail shelters (referred to as AT huts) were associated with increased abundance of macroinvertebrate taxa, while decreases in temperature, increased cobble substrate, and increased proximity to spring signposts were associated with greater abundances of rare taxa. The diversity of spring types, locations and habitats appear to support a diversity of species that are not being negatively affected by visitor usage or spring box infrastructure. The springs provide a regional species pool at high density that are likely source populations for the headwater streams.

Acknowledgements

This project was made possible through the support from the National Park Service Inventory and Monitoring Division. Special thanks to Lisa Tabor for sample collection and field assistance, Sydney Haney for data collection and identification help, Marlaina Marvin for project coordination and data collection assistance, Hannah Swarm for site selection and data collection help, and Greg Pond for identification verification assistance. Finally, we thank Erin Borgman, Evan Childress, and Michelle Boone for providing crucial report revisions.

Introduction

Recent research suggests that there has been an 8.81% average decline in terrestrial insects per decade from 1925 to 2018, with the strongest evidence for decline coming from studies completed in North America (van Klink et al. 2020). Although the loss of biodiversity has emerged as a major issue worldwide, most scientific studies and general attention by the population have focused on vertebrate species, while insects and other invertebrates went generally unstudied in conservation research (Ceballos and Ehrlich 2002; Manne et al. 1999; Fox 2013; McKinney 1999; Thomas et al. 2004). Insect declines (percentage of species extinctions) are calculated to be larger than those of birds or plants over the same period of time (Thomas et al. 2004). As insects serve as a food source for other animals, their decline could create cascading effects within ecosystems across the world (Lister and Garcia 2018; Thomas et al. 2004).

Terrestrial and aquatic insect population declines are estimated at between 30–41%, nearly twice that of other animals such as mammals, birds, and amphibians, which are estimated at 22% of species decline (Dirzo et al. 2014). Based on a review of 73 studies involving insect decline and loss from a variety of regions across the globe, there may be an extinction of 40% of insect species around the world over the next several decades (Sánchez-Bayo and Wyckhuys 2019). As trends of decline develop, it leads us to further question what species are being lost and from what habitats. We are interested in how freshwater species are being impacted. Aquatic habitats and macroinvertebrates/organisms are vulnerable to changes in flow, habitat fragmentation, pollution, and invasive species introduction (Allan and Flecker 1993; Zwick 1992). Generally, aquatic species with one or more year life cycles are particularly sensitive to habitat changes, such as changes in temperature and dissolved oxygen (Long et al. 2016; Colom et al. 2022; Bhowmik and Schafer 2015).

Freshwater ecosystems support approximately 10% of all described animal species across all taxonomic groups, but they only account for less than 1% of the Earth's surface (Reid et al. 2019; Strayer and Dudgeon 2010). Therefore, freshwater ecosystems contain a disproportionately large number of species, designating these as some of the most productive ecosystems and also home to some of the most endangered species. Aquatic macroinvertebrates are small organisms with no internal skeletal system who have at least part, if not all, of their life cycle occurring in water (Orozco-González and Ocasio-Torres 2023). Macroinvertebrates that can be found in freshwater ecosystems originated from terrestrially derived insects and those of marine origin such as mollusks and crustaceans (Resh and Hauer 2007). Of these, aquatic insects are taxonomically diverse and functionally diverse. In North America, there are 13 insect orders with aquatic species but only five strictly aquatic orders: Ephemeroptera, Plecoptera, Odonata, Megaloptera and Trichoptera. The other orders contain both aquatic and terrestrial taxa with megadiverse orders (>20,000 species) of Trichoptera, Coleoptera and Diptera.

Despite several studies that have shown insect species decline, others have found increases in the abundance of more common taxa (Rumschlag et al. 2023). For example, a review of several studies showed an 11.33% increase in aquatic insect abundance (van Klink et al. 2020). Reasons for

increased abundance come from several lines of evidence: the spatial extent of the study, sampling approaches, land use and improved water quality of wadeable streams and river. While some species may be increasing in population, there may be entire species still being lost which cannot be expressed by the metric of abundance. When diversity is emphasized along with biomass, trends of species loss have been supported along with evidence of notable turnover in community composition (Outhwaite et al. 2020).

Project Goals

The purpose of this inventory was to identify the presence and distribution of rare spring macroinvertebrate species and their associated habitat characteristics in Shenandoah National Park in the northern Blue Ridge region of Virginia. Springs were sampled that had either been modified (a spring that was boxed and/or piped for water supply) or unmodified (a spring that was not boxed and/or piped) as well as springs that had variation in visitor usage, geology, and elevational gradients.

Methods

Of the 156 initial sites of interest identified by the park, 40 were wells, 115 were springs and spring boxes, and 1 was a surface spring (Figure 1). We conducted a site scouting trip in autumn 2022 to assess feasibility for site candidates. During this visit, wells were capped which impeded our ability to reach the water for sampling. Similarly, a surface spring could not be delineated from a stream and, therefore, could not be sampled for this project. As a result, springs that had varying levels of human modification became the focus of our sampling efforts. There were 52 spring sites where data were not taken for a variety of reasons: 19 mapped springs were not found; 1 spring was dry at the time of visitation; 3 springs were locked boxes with no output or water access; 3 sites were considered unsafe to visit due to danger of hunters/homes; and 26 springs had a round-trip hiking time that would have exceeded two hours (which would reduce the number of total sites that could realistically be sampled). This resulted in visiting 67 springs and spring boxes and collecting physiochemical variables (both physical and water chemistry measurements, such as substrate/vegetation composition, water flow, dissolved oxygen, pH, temperature, etc.) at each site.

In addition to the physiochemical variables measured, each site was characterized by its location, development type, geology, visitation level and other available information. The level of visitor traffic at a site was assessed using a combination of proximity to trails and roads, backcountry permits, presence of Appalachian Trail (AT) huts or spring signage (which denote a spring's location to visitors) to gauge the overall accessibility of the spring to the public. Sites were considered accessible to the public (or "close") if there was an AT hut present, the spring location was broadcast to visitors along the trail with signage, or the spring occurred right along a road or trail. Springs were considered inaccessible to the public (or "remote") if they did not occur at an AT hut, have spring signage, or occur right along a trail or road. Occasionally, a spring would be a short hike from a road or trail but be located in a steep ravine or involve heavy bushwhacking through dense vegetation to locate; these springs were also labeled as inaccessible to the public (or "remote") in this study because, despite their short Euclidean distance from high traffic areas, their existence would not be easy to discover or easy to navigate to by a visitor. Geological maps were used to identify each spring's underlying geology and spring development type was characterized as either modified, which refers to springs that have spring boxes or pipe installations for water supply, or unmodified, to refer to springs that had no human infrastructure built at the site location for water use (Figure 2). At each site a YSI handheld multiparameter meter was used to measure the following water quality variables: dissolved oxygen (in both percentage and mg/L); specific conductivity ($\mu\text{S}/\text{cm}$); pH; oxidation-reduction potential (mV); water temperature ($^{\circ}\text{C}$); and whether water was still or flowing. HOBO temperature loggers were also deployed at this time until autumn 2024 and programmed for bi-hourly measurements.

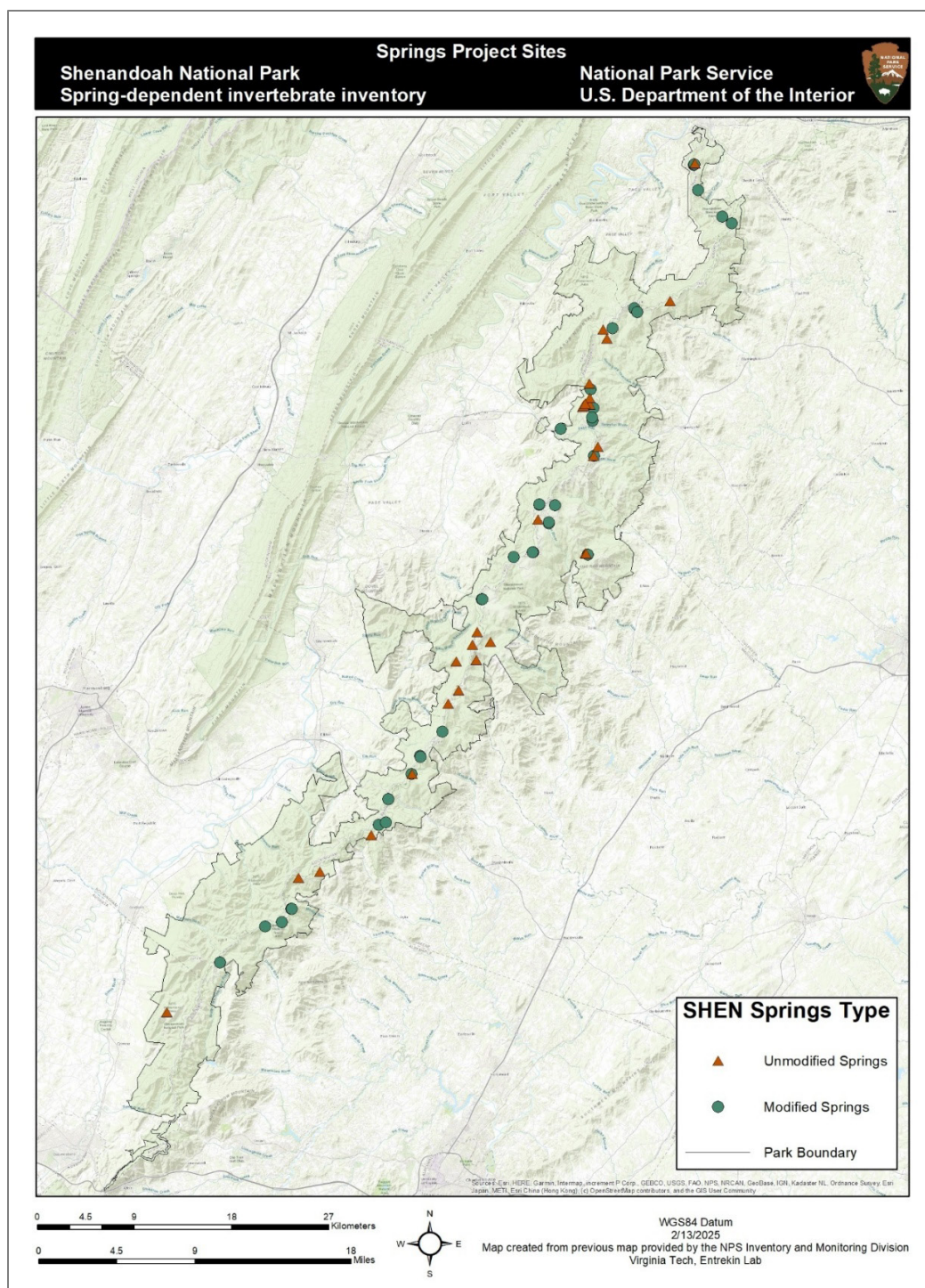


Figure 1. Study site map of the 67 original natural springs that were sampled across Shenandoah National Park between 2022 and 2023. Sites are color-coded by their spring modification type with springs modified for water use via spring box or pipe installation in pink and unmodified springs in blue. The number of sites later dropped to 63 and only 59 could be used in the final analysis.

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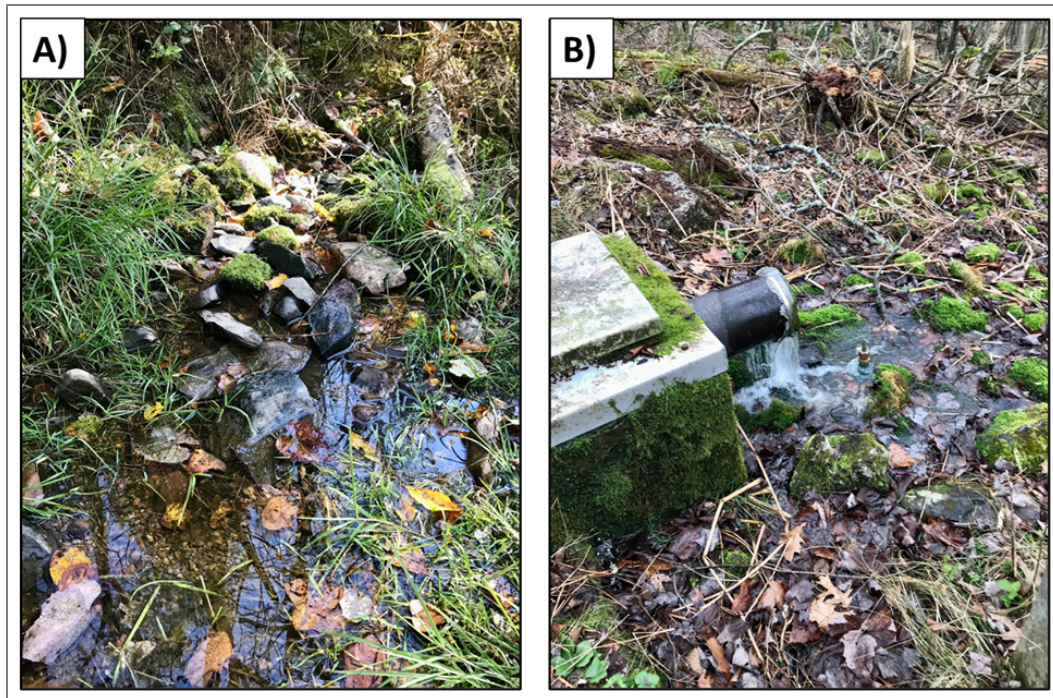


Figure 2. A) An example of an unmodified spring sampled for macroinvertebrates in Shenandoah National Park between 2022 and 2023 and B) an example of a modified spring that had both a pipe and spring box installed for water usage.

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In spring and summer 2023, all 67 sites were visited to assess water quality, benthic habitat (substrate and vegetation composition), and the associated macroinvertebrate community composition. However, data could only be collected at 63 sites due to logistical issues as well as two sites being dry at the time of collection. Substrate composition was assessed at both the spring outflow and at 10 meters from the spring following methods like those outlined in Scrimgeour and Winterbourn (1989) and Rosati et al. (2016). These methods used a visual assessment based on the Wentworth Scale to measure substrate size via sieving substrate from benthic macroinvertebrate core samples through coarse (>1 mm), fine (1 mm – 250 μ m), and very fine (250 μ m – 63 μ m) sieves. Substrate types included bedrock, boulders, cobble, gravel, sand, silt, clay, and moist soil (Appendix A). Vegetation cover was measured with similar proportional methods as substrate composition for each spring and included the following categories: grasses, bryophytes, undercut banks, leaf packs, submerged macrophytes, small woody debris, root mats, root wads, and riffles (Appendix A). Digital probes were also used during this trip to measure the same water quality parameters measured during the first trip, though the measurements weren't taken at every spring a second time. Based on the habitat assessment, all but eight springs were classified as hillslope; therefore, the variation in the geology, substrate composition, flow, and visitor usage variables were the most pertinent variables to assess habitat conditions related to the spring macroinvertebrate communities.

Macroinvertebrates were sampled at 63 of the original 67 spring sites. Two sites (FRNC2 and SIG1) were dry at the time of collection and one site (PMTH) was a late addition to the project and was not

revisited in time for sample collection. Finally, two sites (IVS1 and IVS2) were redefined as one site with two above-ground access points. To assess the community composition of benthic macroinvertebrates in each spring, three to five quantitative benthic samples were collected within a 10-meter reach using a mini-Surber sampler with a 500 μm mesh and a sampling area of 0.023 square meters. The number of benthic samples collected varied based on the length and size of the spring, though most were large enough to have five samples. To maximize the collection of rare taxa, one diversity sample per site was collected using a combination of dip-netting and hand picking. A dip-net fit with a 500- μm mesh was used to search for snails across five transects spaced equidistantly from the most upstream point to 10 meters downstream from the spring upwelling (Figure 3). Finally, two emergence traps were deployed at the upstream and mid-stream regions of each spring for larval to adult macroinvertebrate collection, which were left in place for 2 months and checked every two weeks. This resulted in each spring having 3 different types of macroinvertebrate samples: benthic, diversity, and emergence. The sampling protocols used in this study reflected those developed from Hauer and Lamberti (2017) and Rosati et al. (2016).

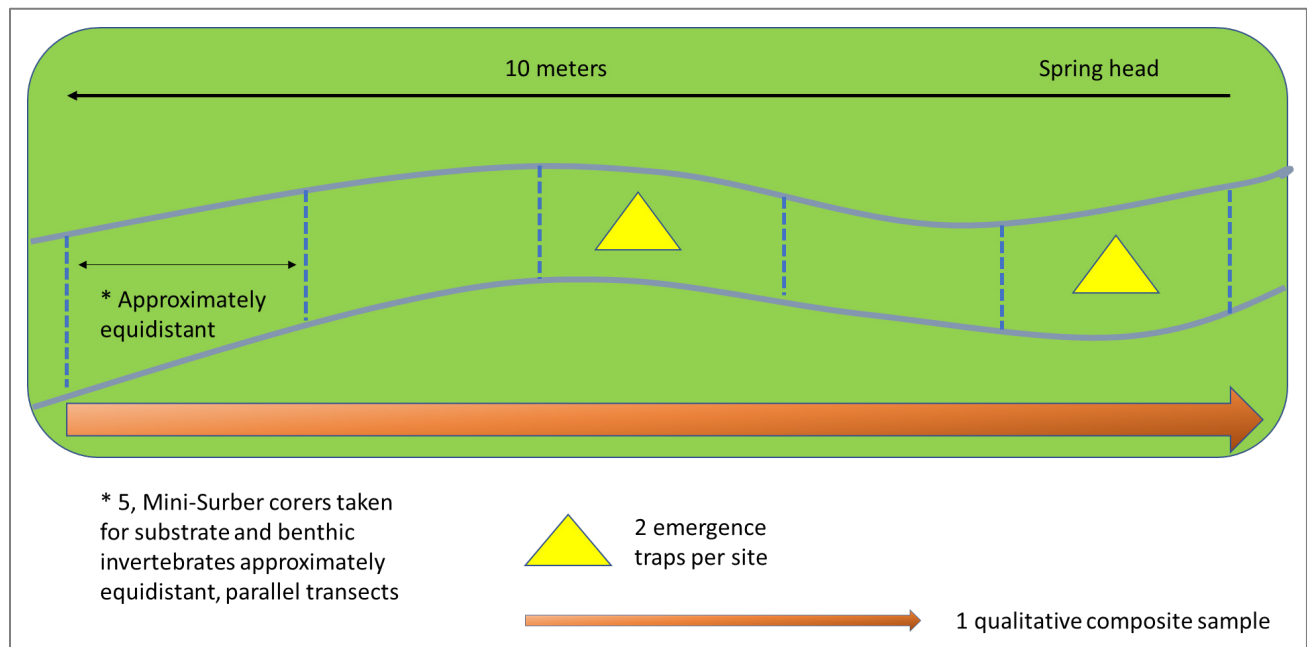


Figure 3. Study site sampling diagram that indicates reach length, quantitative and qualitative substrate and invertebrate sampling strategy.

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Each sample was processed following similar protocols from Hauer and Lamberti (2017) and Rosati et al. (2016) in which specimens were picked from the substrate of each sample and preserved in 95% ethanol for identification. All specimens were identified to the lowest taxonomic unit possible using the most current taxonomy keys available (Merritt et al. 2019, Tennessen 2019, Morse et al. 2017, Smith 2001, Holsinger 1978). Where necessary, especially for rare taxa, identifications were checked by secondary experts. Due to time constraints, only a subsample of the collected benthic samples were processed; benthic samples for identification were selected to strike a balance between

what would reflect the greatest spatial diversity of sites and the greatest community diversity. Identification of insects in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) were the focus of emergence sample processing because these orders are recognized to be more biologically sensitive than other emerging taxa. Rare taxa were defined using NatureServe's Conservation Status Rank where any taxa with a state or global listing of at least vulnerable was labeled as rare (NatureServe, 2025). When a specimen was identified to a genus that contains rare species (but species could not be determined), it was noted in the National Park Service (NPS) database but was not included in analyses as a rare taxon. The only exceptions were *Viehoplerla sp.*, *Zealuctra sp.*, and *Homoplectra sp.*, which were included in analyses as rare, because all species within these three genera meet the criterion for rarity. The resulting identification data were compiled into the NPS datasheet template and used for analysis. All samples are stored in the Virginia Tech Aquatic Entomology Lab in Blacksburg, Virginia. Identified specimens were sorted into vials according to their lowest taxonomic unit identified along with collection and location information. Rare species will be sent to the museum for verification.

Analytical Methods

Of the 63 springs sampled, 4 were excluded from analysis due to data issues, resulting in 59 sites used for analyses. Our first goal was to describe the springs by their location, visitor use (based on presence/absence of an AT hut or signpost and proximity of the spring to a road or trail) and physiochemical characteristics of each site (e.g., substrate composition, vegetation, pH, temperature, dissolved oxygen, flow, elevation, etc.). These characteristics were associated with diversity and abundance measures of each site's macroinvertebrate community. Diversity samples were processed for all springs and these data were used to compare richness and abundance between modified and unmodified springs using Kruskal Wallis or Analysis of Variance tests. Springs with rare taxa from diversity samples were investigated more closely by processing a subsample of quantitative samples and all emergence traps for Ephemeroptera, Plecoptera, Trichoptera and Odonata samples. All data were used in the Non-metric Multidimensional Scaling (NMDS) ordination techniques where compositional variables provided a visualization of differences across multiple related characteristics. Single, highly correlated variables or composite ordination axes were used in combination with general linear regression models related to spring taxa richness, relative abundance, and number of taxa with conservation status indicative of some level of rareness (methods similar to Horsák et al. 2018).

Because time constraints did not allow all collected samples to be processed, the number of available samples for each site varied based on which samples were selected for identification. Only diversity samples were used to characterize the rare taxa detected and the macroinvertebrate communities in each spring and by spring type. Taxa were grouped by spring type (modified or unmodified) and eight different metrics (total richness and abundance for total taxa, Ephemeroptera, Plecoptera, and Trichoptera [EPT], Plecoptera only, and rare taxa) were used to characterize macroinvertebrate communities. Richness for each site was calculated by counting the number of unique taxa per site using all sample types available. Relative abundance was calculated by summing the number of individuals identified to a unique taxon across all available samples for each site. Because excluding benthic samples had no influence on the difference in taxa abundance across sites, we used all

available sample data to look at possible environmental conditions influencing taxa relative abundance using general linear models. Environmental and usage data taken at each site were averaged across sampling dates and standardized and split into three groups: water variables (temperature, pH, flow, specific conductance, dissolved oxygen, and oxygen reduction potential), habitat variables (elevation and percent composition of small woody debris, leaf packs, moss, cobble, gravel, sand, and silt), and usage variables (presence of an AT hut or spring signpost and distance to a road or trail). A Pearson correlation matrix was constructed and variables that were highly correlated were removed ($r > 0.8$). Eighteen generalized linear models with a Poisson distribution were used to relate six of the eight response metrics (excluding EPT and Plecoptera richness) with the three environmental gradients to account for response variables being count data and non-parametric. After each model was constructed, variance inflation factor (VIF) tests were run on each model to assess the influence of multicollinearity. Variables in models that had VIF scores over five were removed and rerun. See Table 9 in Appendix B for all macroinvertebrate samples processed for each spring.

Results

Of the 59 springs included in analyses, 44 were underlain by basaltic, 6 siliciclastic, and 9 granitic geology (Table 1). Most of the basaltic springs (57%) were modified via spring box or pipe installation. Because most of the springs were basaltic, we did not run further analysis on spring-geology relationships. Spring water chemistry varied but not by boxing/piping or by geology, exclusively; pH ranged from 4.88 to 7.26, temperatures spanned 5.3 °C to 13.1 °C, and specific conductivity and oxygen reduction potential (ORP) varied vastly between 11.3 $\mu\text{S}/\text{cm}$ to 127.3 $\mu\text{S}/\text{cm}$ and 63.0 mV to 370.0 mV (Table 2). Spring benthic habitat was mostly gravel, followed by cobble and sand. Some springs also had a large (>50% cover) proportion of silt. Because springs are often situated at the toe of rockslides, boulders are a common substrate (Appendix A).

Table 1. Summary of springs and spring types across the three underlying geologic types that were sampled in fall 2023 across Shenandoah National Park.

Spring Type	Modified	Unmodified	All
Basaltic	25	19	44
Siliciclastic	4	2	6
Granitic	5	4	9
Total	34	25	59

Table 2. Ranges of physical and chemical parameters at each spring in Shenandoah National Park.

Environmental Parameter	Spring Type		
	Modified	Unmodified	All
pH	5.10–7.26	4.88–7.15	4.88–7.26
Temp (°C)	5.4–12.7	5.3–13.1	5.3–13.1
Specific Conductance ($\mu\text{S}/\text{cm}$)	14.7–82.5	11.3–127.3	11.3–127.3
DO (mg/L)	2.65–11.70	1.00–12.34	1.00–12.34
DO (%)	23.40–101.0	10.0–102.4	10.0–102.4
ORP (mV)	64.0–347.0	118.0–370.0	64.0–370.0
Elevation (m)	520–1043	399–1048	399–1048
Road Distance (m)	2.76–1596.84	0.78–1740.43	0.78–1740.43
Trail Distance (m)	1.78–1491.29	0.65–486.77	0.65–1491.29

A total of 205 unique taxa were identified across the 59 springs. More taxa were documented at unmodified springs (155 taxa) than modified springs (141 taxa). Out of 205, only 17 of the identified taxa were categorized as rare according to their conservation status (Table 3). Rare taxa were primarily composed of insects in the order Trichoptera (7) followed by Plecoptera and Odonata (3),

Ephemeroptera (2), and two non-insect organisms, a spring-associated amphipod and an aquatic snail (Table 3).

Table 3. Rare and potentially rare taxa found during sampling in 2022 and 2023 in Shenandoah National Park.

Taxon ID	Scientific Name (Source)	Conservation Status	Present in # Sites
ADIC	<i>Adicropheps hitchcocki</i> (Flint, 1965)	Imperiled in VA (S2 status from NatureServe)	1
AMEL2	<i>Ameletus cryptostimulus</i> (Carle, 1978)	Vulnerable in VA (S3 status from NatureServe)	2
AMEL3	<i>Ameletus tarteri</i> (Burrows, 1987)	Nationally vulnerable (G3 status from NatureServe)	1
CORD	<i>Cordulegaster erronea</i> (Hagen in Selys, 1878)	Vulnerable in VA (S3 status from NatureServe)	1
FONT	<i>Fontigens orolibas</i> (Hubricht, 1957)	Vulnerable in VA (S3 status from NatureServe)	24
HOMO	<i>Homoplectra</i> sp. (Ross, 1938)	Both species are rare in VA: <i>doringa</i> (vulnerable, S3 status from NatureServe) and <i>monticola</i> (imperiled, S2 status from NatureServe)	1
LANT	<i>Lanthus vernalis</i> (Carle, 1980)	Vulnerable in VA (S3 status from NatureServe)	1
NEOP3	<i>Neophylax mitchelli</i> (Carpenter, 1933)	Not documented in VA, but found in TN (globally vulnerable, G3 status from NatureServe).	2
OPHI	<i>Ophiogomphus howei</i> (Bromley, 1924)	Critically imperiled in VA (S1 status from NatureServe)	1
PSEU3	<i>Pseudostenophylax sparsus</i> (Banks, 1908)	Vulnerable in VA (S3 status from NatureServe)	1
PSIL2	<i>Psilotreta rossi</i> (Wallace, 1970)	Imperiled in VA (S2 status from NatureServe)	1
PYCN2	<i>Pycnopsyche flavata</i> (Banks, 1914)	Vulnerable species (S3 status from NatureServe)	1
RHYA3	<i>Rhyacophila invaria</i> (Walker, 1852)	Vulnerable in VA (S3 status from NatureServe)	1
STYG	<i>Stygobromus spinosus</i> (Hubricht and Mackin, 1940)	Vulnerable in VA (S3 status from NatureServe)	4
VIEH	<i>Viehoplerla</i> sp. (Ricker, 1952)	Not documented in VA, but is in TN and NC (vulnerable, G3 status from NatureServe). Only one species is described, so this is likely <i>Viehoplerla ada</i> .	1
ZAPA	<i>Zapada fumosa</i> (Baumann and Grubbs, 20215)	Critically imperiled in VA (S1 status from NatureServe).	4
ZEAL	<i>Zealeuctra</i> sp. (Ricker, 1952)	No species documented in VA, but one in NC (<i>uwharrie</i>) - imperiled in VA (S2 status from NatureServe)	1

Kruskal-Wallis tests showed no statistically significant differences in observed taxa richness ($p = 0.58$) or total abundance ($p = 0.73$) between the spring types (Figure 4). On average, taxa richness and abundance in modified springs ($\bar{x} = 17.1$ taxa and 195 individuals, respectively) did not differ from unmodified springs ($\bar{x} = 15.1$ taxa and 134 individuals, respectively) (Figure 4). Similarly, rare taxa richness (p -value 0.39) and abundance (p -value 0.21) in modified springs ($\bar{x} = 61.7$ individuals and 0.82 taxa, respectively) did not differ from unmodified springs ($\bar{x} = 11$ individuals and 0.64 taxa, respectively). These trends also held true for EPT and Plecoptera-only taxa richness and abundance, known sensitive taxa to oxygen and temperature (Figure 5). Rare taxa were found in both spring types in relatively equal amounts ($n_{\text{total}} = 32$, $n_{\text{modified}} = 19$, $n_{\text{unmodified}} = 13$).

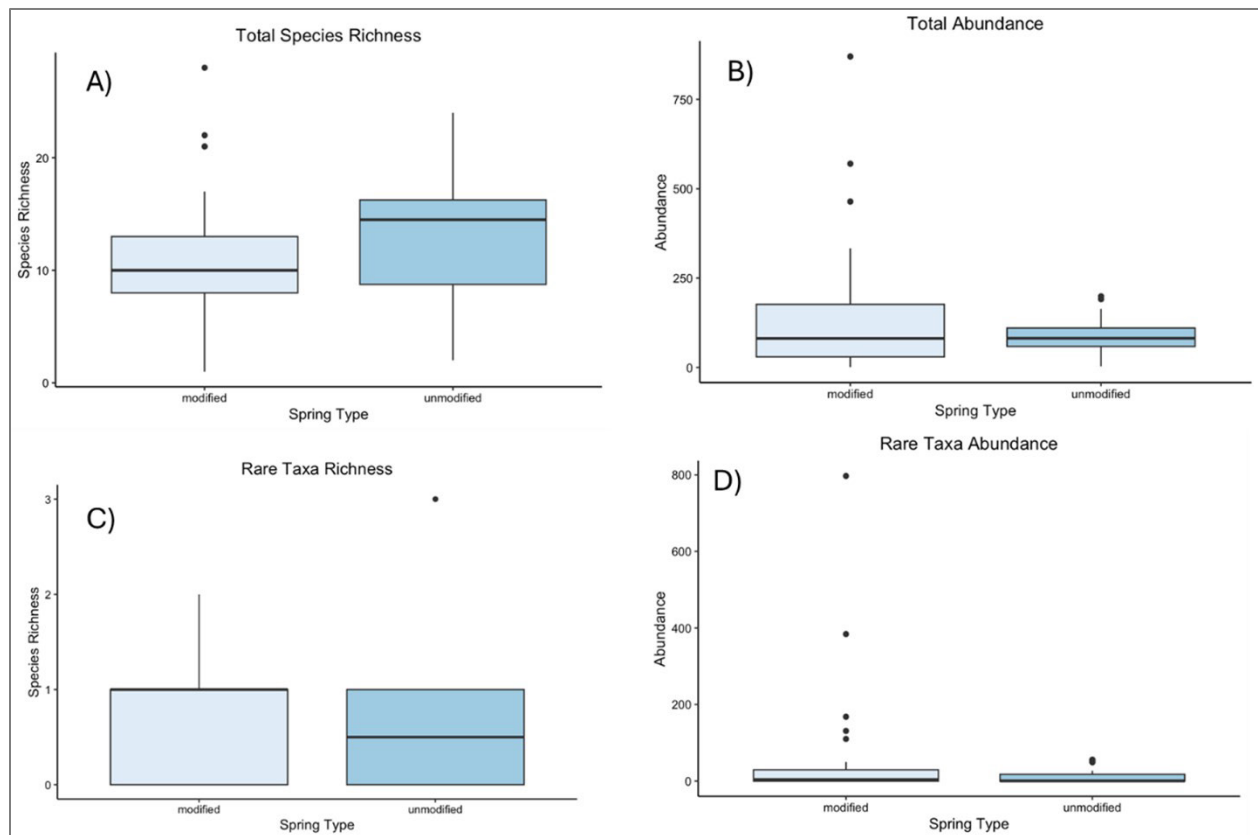


Figure 4. Macroinvertebrate metrics observed between modified ($n = 34$) and unmodified ($n = 25$) natural springs along a 10 m reach within Shenandoah National Park between 2022 and 2023. A) Total observed taxa richness between modified and unmodified springs, B) Total number of all macroinvertebrate individuals (abundance) collected in modified and unmodified springs, C) Taxa richness for taxa that were designated rare as defined by their conservation status in modified and unmodified springs, and D) Abundance of individuals that were identified as rare according to their conservation status for modified and unmodified springs.

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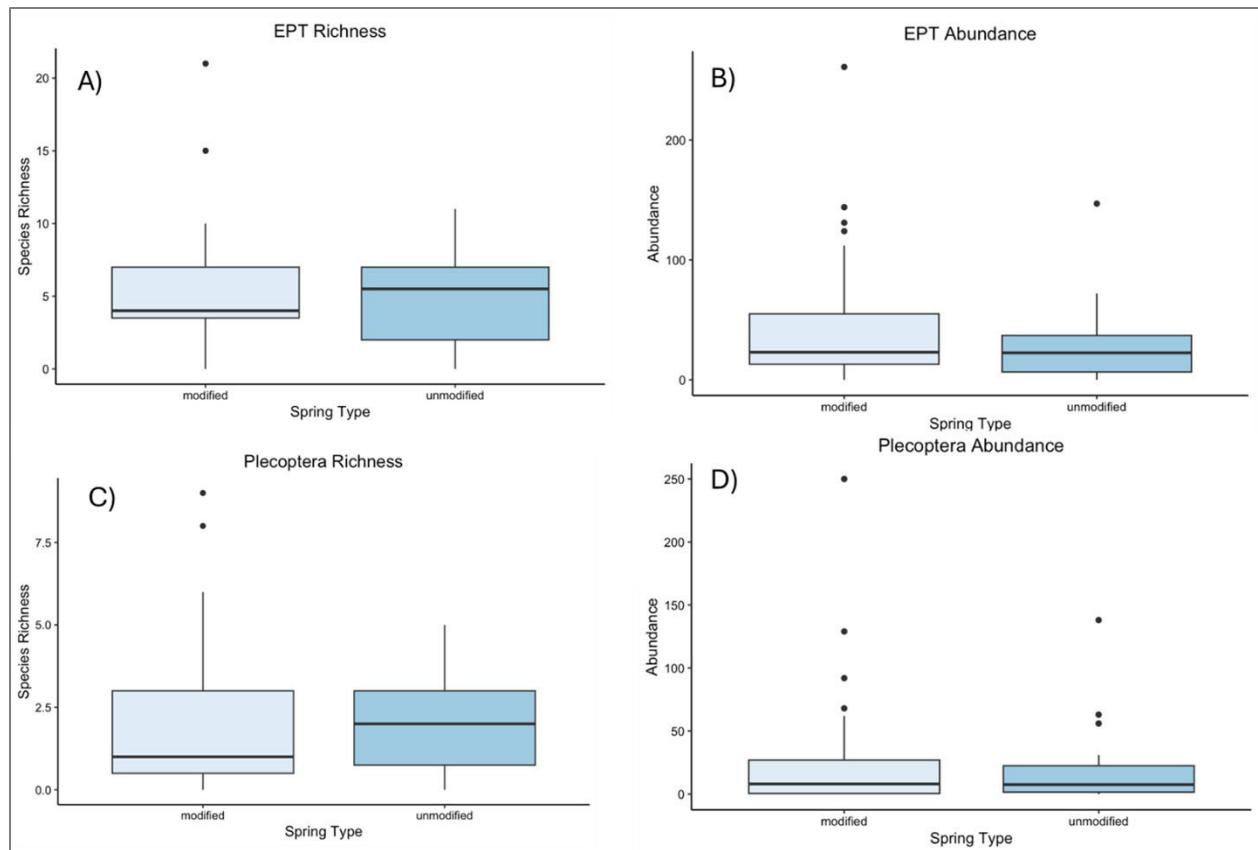


Figure 5. Ephemeroptera, Plecoptera and Trichoptera (EPT) and Plecoptera-specific metrics observed between modified ($n = 34$) and unmodified ($n = 25$) natural springs within Shenandoah National Park between 2022 and 2023. A) EPT taxa richness between modified and unmodified springs, B) Total number of all EPT individuals (abundance) collected in modified and unmodified springs, C) Taxa richness for all members of the order Plecoptera in modified and unmodified springs, and D) Abundance of individuals in the order Plecoptera for modified and unmodified springs.

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We predicted that differences in macroinvertebrate communities between modified and unmodified springs would be correlated with lower average oxygen levels and warmer average temperatures in the modified springs, specifically those having spring box installations. However, we did not identify significant environmental differences, nor did we observe differences in the taxa richness of the community as visualized by the lack of clustering between sites based on spring type in the NMDS ordination between spring types (Figure 6).

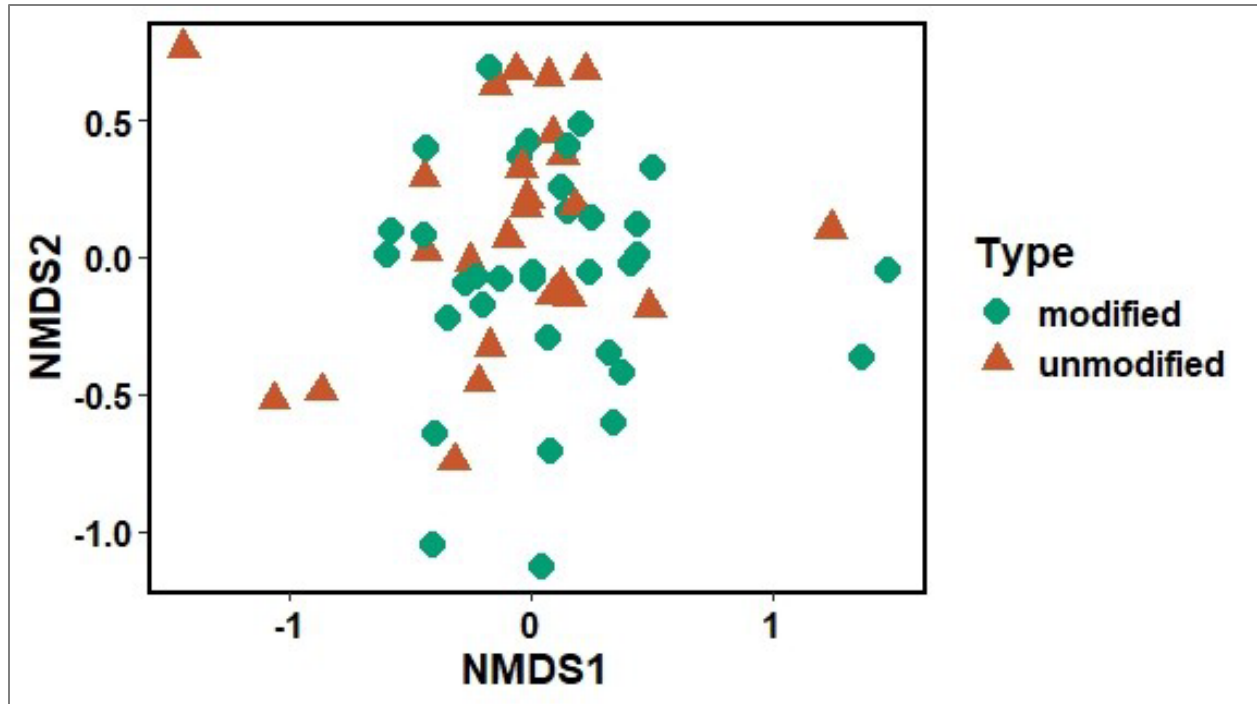


Figure 6. Non-metric multidimensional scaling analysis with each taxon's presence or non-detection across all sampled springs. Ordination used selected Surber samples and all diversity samples and EPT (Ephemeroptera, Plecoptera, and Trichoptera) from emergence samples. The lack of clustering between sites suggests no significant difference in macroinvertebrate communities between spring types.

NPS

Although not statistically significant, there was more separation between spring types based on taxa abundance at the genus or species level (Figure 7). When we added an environmental matrix to the abundance ordination to explain variation in the community abundance, ORP was the variable that explained the most variation across sites (Figure 7). Trail and road distance and water temperature were other variables weakly related to NMDS axis 1. Elevation was most related to variation across NMDS axis 2 (Table 4).

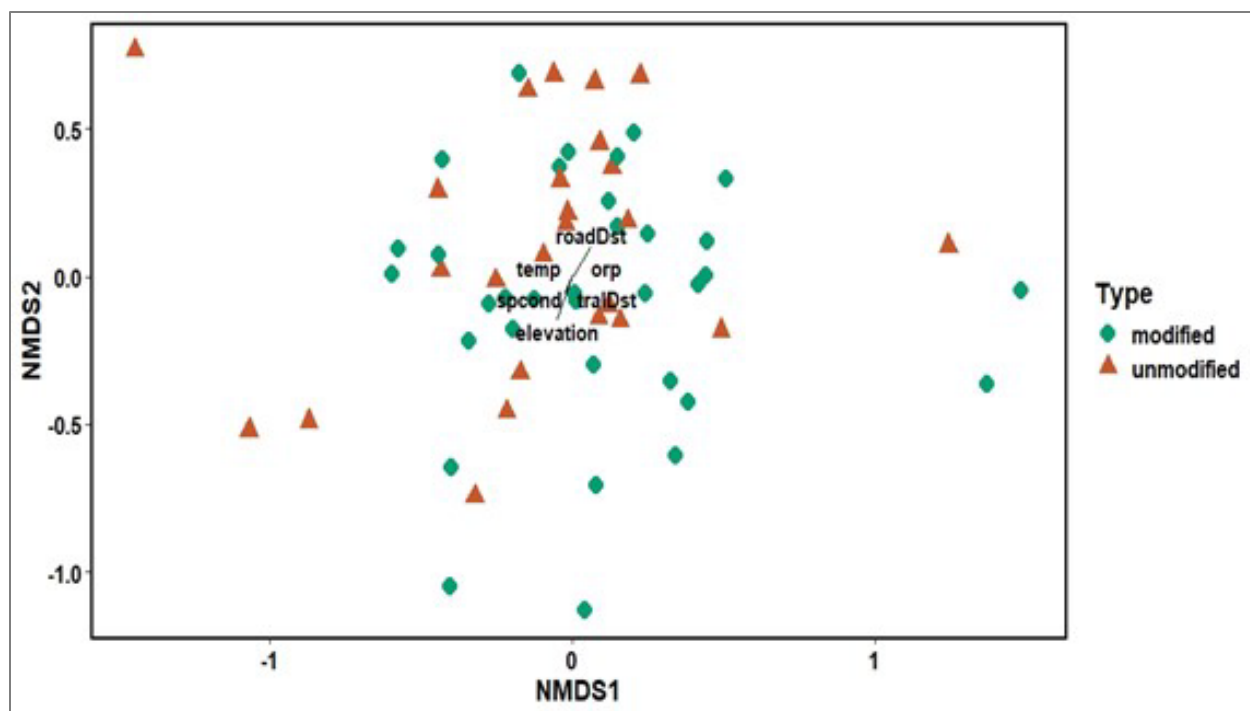


Figure 7. Site by species NMDS ordination identifying environmental variables associated with patterns in modified and unmodified spring macroinvertebrate communities. Vectors have been scaled with a multiplier so that length is related to the strength of correlation between the variables and patterns in the data. See Table 4 for environmental scores. Although not statistically significant, there was more separation between spring types based on taxa abundance at the genus or species level.

NPS

Table 4. Environmental variables related to NMDS abundance across all springs. Although not statistically significant, ORP (**bolded**) was the variable that explained the most differences across the community composition followed by water temperature.

Most Related Environmental Parameter	NMDS 1	NMDS 2	R ²	P-value
Temperature (°C)	-0.95825	-0.28593	0.0452	0.266
Specific Conductance (μS/cm)	-0.63237	-0.77467	0.0213	0.550
ORP (mV)	0.98291	0.18409	0.1035	0.055
Elevation (m)	-0.45301	-0.89150	0.0065	0.833
Road Distance (m)	-0.66684	0.74520	0.0077	0.789
Trail Distance (m)	0.99855	0.05382	0.0014	0.964

Next, we identified taxa that were most responsible for differences across the communities (Figure 8, Appendix B). From left to right across NMDS axis 1, ORP, temperature, specific conductance, and trail and road distances tended to decline and weakly correspond to more Ephemeroptera, Plecoptera and Trichoptera taxa. Rare taxa were not necessarily related to these same patterns. Therefore, we also conducted general linear models to identify the suite of environmental conditions predictive of

our macroinvertebrate metrics. Based on a combination of environmental conditions and taxon associations, NMDS 1 represents a gradient from left to right as an increase in flowing water, where all the net-spinning caddisflies were more abundant in springs on the right side of the axis, also represented by more unmodified springs, but not exclusively. The left side of the axis shows more taxa adapted to still water and lower dissolved oxygen.

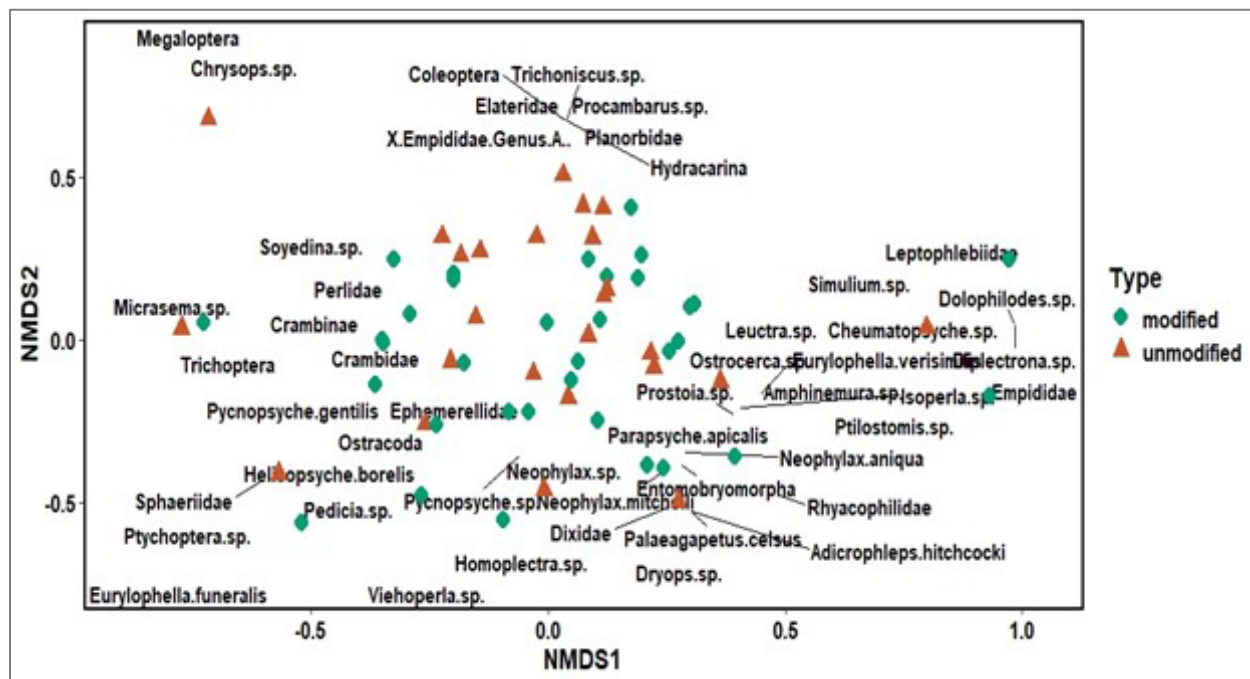


Figure 8. NMDS ordination for taxa associations in ordination space. See Appendix B, Table 8 for all species scores.

NPS

General linear models used to associate macroinvertebrate metrics with measures of water quality, habitat, and visitor usage revealed that total richness was associated with decreased flow and pH, more small woody debris, and the presence of spring signposts (Table 5). All environmental variables measured had a statistically significant association with both total and rare taxa abundance (Table 5). In contrast, none of the measured environmental variables had a statistically significant association with rare taxa richness (Table 5). See Appendix C for environmental variables correlated with each other and with trail use and road access information.

Table 5. The p-values extracted from the summary results for a generalized linear model using a Poisson distribution. Only p-values for statistically significant relationships are recorded and non-significant relationships are denoted with a “–”. The direction of significant relationships is denoted next to each p-value in parentheses with a “+” for positive or “-” for negative.

Environmental Parameter	Rare Richness	Total Richness	Total Abundance	EPT Abundance	Plecoptera Abundance	Rare Abundance
pH	–	2.31e-05 (-) ^A	< 2e-16 (-)	< 2e-16 (-) ^A	< 2e-16 (-) ^A	< 2e-16 (-) ^A
Temperature (°C)	–	0.03557 (+)	< 2e-16 (+)	< 2e-16 (+) ^A	6.56e-16 (+) ^A	< 2e-16 (+)
Specific Conductance (µS/cm)	–	0.00181 (+)	< 2e-16 (+)	< 2e-16 (+)	< 2e-16 (+)	< 2e-16 (+)
Dissolved Oxygen (%)	–	–	0.0364 (-)	0.012 (-)	–	Removed
Oxygen-reduction Potential (mV)	–	1.24e-05 (+)	< 2e-16 (+)	< 2e-16 (+)	< 2e-16 (+)	< 2e-16 (+)
Flow presence	–	0.04873 (-) ^A	7.33e-09 (-) ^A	–	–	< 2e-16 (+) ^A
Elevation (m)	–	–	< 2e-16 (+)	5.21e-12 (-)	1.71e-10 (-)	< 2e-16 (+)
Small woody debris (%)	–	0.00037 (-) ^A	< 2e-16 (-) ^A	< 2e-16 (-) ^A	< 2e-16 (-) ^A	< 2e-16 (-) ^A
Leaf packs (%)	–	1.62e-05 (+)	–	8.04e-15 (+)	4.99e-13 (+)	< 2e-16 (-)
Moss (%)	–	0.02958 (+)	3.25e-06 (+)	–	< 2e-16 (+)	–
Cobble (%)	–	0.00124 (-)	< 2e-16 (+)	< 2e-16 (-)	< 2e-16 (-)	< 2e-16 (+) ^A
Gravel (%)	–	–	< 2e-16 (-)	–	–	< 2e-16 (-)
Sand (%)	–	–	1.05e-07 (+)	0.00708 (+)	7.76e-09 (+)	< 2e-16 (+)
Silt (%)	–	0.02317 (-)	< 2e-16 (-)	< 2e-16 (-)	0.00623 (-)	–
Presence of an AT hut	–	–	3.29e-14 (+)	5.65e-16 (+)	4.56e-13 (+)	< 2e-16 (+)
Presence of a spring signpost	–	0.00311 (-) ^A	< 2e-16 (+) ^A	< 2e-16 (+) ^A	< 2e-16 (+) ^A	< 2e-16 (+) ^A
Distance to road (m)	–	–	< 2e-16 (+)	< 2e-16 (+)	< 2e-16 (+)	4.7e-14 (-)
Distance to trail (m)	–	–	–	0.00166 (+)	–	2.2e-08 (-)

^A The strongest relationships (also in bold font).

Discussion

Our project evaluated macroinvertebrate presence and abundance in springs throughout Shenandoah, with special consideration of rare taxa, and identified spring characteristics related to taxa abundance, such as spring modifications, which can guide rehabilitation and conservation plans to protect these springs for the future. The results of our study contribute to the small pool of research conducted on springs, as they are less studied than other aquatic ecosystems, though they contribute significantly to local and regional diversity that benefits higher ecosystem functionality (Cantonati et al. 2012).

The sampled springs support even greater diversity than the streams they flow into (Jastram et al. 2013) and have a seemingly neutral response to the presence of spring infrastructure and a complex response to visitor usage. It is important to note that springs with over 2 hours round-trip travel time were specifically avoided to increase the number of sites able to be sampled. Consequently, all springs visited were relatively accessible and springs that were categorized as inaccessible in this study were not defined based on Euclidean distance from visitors but on decreased probability of visitors going out of their way to locate and hike off trail to find them. Therefore, all interpretations related to visitor usage are relative and in need of further investigation.

We hypothesized that the environmental conditions would be distinct among modified and unmodified streams and result in distinct communities; however, this difference was not observed in the environment or within the macroinvertebrate communities. This finding seems to coincide with Ilmonen and Paasivirta's (2005) study that suggested some distinction in the community composition for specialized taxa yet more common taxa were found to occur in all spring/habitat types. Other studies suggested more community distinction based on habitat specialization and dispersal mode (Horsák et al. 2015).

The lack of differentiation between communities of modified and unmodified springs could be explained by the age of the infrastructure in place at modified springs and the spatial proximity of unmodified springs to modified springs. Many of the spring boxes and pipes in place at modified springs were built in the mid to late 19th century in the early days of Shenandoah's designation as a national park. At the time of their inception, they may have had a much larger impact on the diversity and abundance of macroinvertebrates. However, if so, their installation appears to have been a pulse disturbance, and these communities may have rebounded in the decades since so that the infrastructure is no longer interfering with their ability to persist on the landscape.

Total abundance of macroinvertebrates was also related to water conditions, habitat, and usage. Spring communities, however, showed some different patterns compared to overall richness. Taken together, abundances seem to be greater in springs at higher elevation, with less wood, gravel, and silt but greater cobble, sand, and moss cover. These characteristics correspond with cooler water and more oxygen. Perhaps counterintuitively, the abundances were also greater at springs closer to roads, trails and signposts. Although speculative, this relationship could be related to more retentive springs having greater fine organic matter as food. Rare taxa and total taxa abundance aligned with lower pH, greater temperature, specific conductance, ORP, and elevation (Table 5). EPT and Plecopteran

abundance were also explained by greater temperature, specific conductance, ORP, and lower elevation. That in combination with lower elevation suggests more retentive springs are suitable habitat for certain EPT taxa. This contrasts with rare abundance, that includes EPT, but also crustaceans and gastropods that have different environmental requirements that correspond with the higher elevation sites.

Fitting the NMDS ordination with environmental vectors showed Oxidation Reduction Potential (ORP) to explain the most variation in the macroinvertebrate community (Figure 7). ORP impacts oxygen availability in the water column for aquatic organisms and generalized linear models further supported that increasing oxygen availability was significantly associated with increases in all assessed macroinvertebrate metrics, apart from total richness, which was positively associated but not significant (Table 5). The influence of increased ORP in shaping abundance and diversity patterns of spring macroinvertebrate communities found here are coherent with the finding of others in both riverine and lacustrine systems (Tatenda et al. 2025; Tatenda et al. 2022; Shiyun et al. 2018; Horsák et al. 2015) and should be explored further in future studies.

Though we found no significant difference in the total or rare taxa abundance between spring types, rare taxa abundance increased in springs further from areas of frequent human disturbance, suggesting disturbance from visitor use may affect rare taxa abundance more than disturbance from past spring modifications. Lack of differentiation between invertebrate communities in the springs indicates that using park resources to remove decommissioned spring infrastructure may be unnecessary. Instead, working to keep remote springs inaccessible to the public and implementing efforts to minimize visitor impacts at accessible springs could prove more beneficial to supporting at-risk invertebrate populations in the future.

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Appendix A: Physical and anthropogenic spring attributes

Table 6 and Table 7 describe spring attributes.

Table 6. Overview of spring attributes in relation to geology, spring type, and potential for visitor use.

Object ID	Latitude	Longitude	Site ID	Geology	Spring Type	Open or Closed Box	Hut Nearby	Spring Sign Post	Road Distance (m)	Trail Distance (m)
151	38.6972	-78.3224	BMSG	basaltic	unmodified	no box	no	yes	197.65	35.32
132	38.6365	-78.3201	BNS2	basaltic	unmodified	no box	no	no	46.76	13.31
32	38.6364	-78.3201	BNS3	basaltic	modified	closed	no	yes	35.24	2.77
134	38.4439	-78.4704	BRFH1	basaltic	unmodified	no box	yes	yes	20.89	0.65
127	38.1808	-78.7885	CSH1	basaltic	unmodified	no box	no	no	1740.43	7.17
149	38.4922	-78.4489	CVSW	basaltic	unmodified	no box	no	no	131.41	486.77
131	38.8334	-78.1758	DKRG	basaltic	modified	closed	no	no	539.50	145.35
113	38.3892	-78.5132	DMPH	granitic	modified	closed	yes	no	32.31	23.12
37	38.3899	-78.5140	DMSR	siliciclastic	modified	closed	yes	no	117.11	394.65
142	38.3891	-78.5136	DMTG	basaltic	modified	closed	yes	no	22.04	306.88
145	38.2507	-78.6828	DOR1	basaltic	modified	closed	no	yes	321.40	2.65
39	38.2218	-78.7316	DUS1	siliciclastic	modified	closed	no	no	70.60	185.94
40	38.7343	-78.3023	EKW8	siliciclastic	unmodified	no box ^A	no	no	416.44	188.91
109	38.7420	-78.3061	EKWW	granitic	unmodified	no box	no	no	87.78	188.13
143	38.8774	-78.2045	FHC1	basaltic	modified	open	no	no	308.21	11.23
72	38.8787	-78.2029	FHC2	basaltic	unmodified	no box	no	no	461.29	23.23
76	38.8776	-78.2038	FHC3	basaltic	modified	closed	no	no	416.62	6.99
95	38.5970	-78.379	FRNC2	granitic	modified	no box	yes	yes	4.22	5.20
153	38.5973	-78.3795	FRNC3	basaltic	unmodified	closed	yes	yes	22.56	1.96
97	38.3246	-78.5675	FSEE	granitic	unmodified	no box	no	no	202.24	204.52

^A No box seen where sampled; did not sample at start of spring.

Table 6 (continued). Overview of spring attributes in relation to geology, spring type, and potential for visitor use.

Object ID	Latitude	Longitude	Site ID	Geology	Spring Type	Open or Closed Box	Hut Nearby	Spring Sign Post	Road Distance (m)	Trail Distance (m)
144	38.7641	-78.2334	GSS1	granitic	modified	no box	yes	no	4.79	3.13
152	38.3333	-78.5594	HHR1	basaltic	modified	no box	yes	yes	70.42	5.47
43	38.5963	-78.3628	HMLK	siliciclastic	modified	closed	no	no	9.74	542.99
110	38.7588	-78.2726	HSS1	granitic	modified	open	no	yes	20.65	26.94
135	38.7589	-78.2723	HSS2	basaltic	unmodified	open	no	yes	43.99	7.57
42	38.4817	-78.4544	HTRO	siliciclastic	modified	no box	no	no	0.78	359.54
5	38.3350	-78.5521	HTS1	basaltic	modified	open	no	yes	581.65	4.05
105	38.5576	-78.3877	HWBL0	granitic	modified	no box	no	yes	160.09	94.73
82	38.5574	-78.3880	HWBL1	basaltic	unmodified	no box	no	yes	164.63	86.58
71	38.4688	-78.4511	HWCW	basaltic	modified	no box	no	no	67.73	69.86
7	38.6601	-78.3544	HWS1	basaltic	unmodified	closed	no	no	31.86	1491.29
140	38.6442	-78.3150	HZLW	basaltic	unmodified	no box	no	no	363.74	403.64
79	38.4681	-78.4720	IMAP	basaltic	modified	no box	no	no	1159.13	2.88
130	38.8275	-78.1657	IRS1	basaltic	modified	no box	no	yes	100.32	13.25
147	38.2649	-78.6548	IVS1	basaltic	unmodified	closed	yes	yes	65.16	10.34
49	38.2648	-78.6543	IVS2	basaltic	unmodified	closed	yes	no	61.53	0.85
89	38.6803	-78.3272	JCN1	basaltic	unmodified	no box	no	no	80.55	3.83
86	38.6796	-78.3223	JCN2	basaltic	unmodified	no box	no	no	411.34	69.99
87	38.6853	-78.3221	JNAH	basaltic	modified	no box	no	no	158.44	151.70
85	38.6778	-78.3301	JNHS	basaltic	modified	no box	no	no	43.71	111.46
133	38.7558	-78.2693	LHW1	basaltic	unmodified	open	no	no	85.69	308.38
53	38.2542	-78.6650	LMC1	basaltic	modified	closed	no	no	2.76	51.67
116	38.4835	-78.4354	LRLP	granitic	modified	no box	no	no	1150.22	44.11
148	38.4331	-78.4823	LWSM	basaltic	unmodified	no box	no	no	128.84	253.69

^A No box seen where sampled; did not sample at start of spring.

Table 6 (continued). Overview of spring attributes in relation to geology, spring type, and potential for visitor use.

Object ID	Latitude	Longitude	Site ID	Geology	Spring Type	Open or Closed Box	Hut Nearby	Spring Sign Post	Road Distance (m)	Trail Distance (m)
51	38.5194	-78.4435	LWSS1	basaltic	unmodified	closed	yes	yes	9.93	8.64
102	38.5195	-78.4433	LWSS2	granitic	modified	closed	yes	no	3.46	2.12
137	38.5556	-78.3314	OLD1	basaltic	modified	no box	no	yes	1381.12	51.25
129	38.5552	-78.3310	OLD2	granitic	unmodified	no box	no	no	47.67	10.29
83	38.5544	-78.3295	OLD3	basaltic	modified	no box	yes	yes	1596.84	56.13
99	38.4096	-78.4892	PCSC1	granitic	modified	open	no	yes	34.06	2.75
124	38.2908	-78.6457	PFH1	basaltic	unmodified	no box ^A	yes	yes	22.12	6.36
4	38.6768	-78.3190	PMTH	basaltic	modified	no box	yes	yes	26.49	1.85
6	38.6659	-78.3202	PNRM1	basaltic	modified	closed	no	no	68.36	54.41
58	38.6687	-78.3206	PNRM2	granitic	modified	closed	no	no	167.51	1.18
150	38.5849	-78.3812	PSEW	basaltic	unmodified	no box	no	no	170.52	27.61
136	38.5541	-78.4086	RKSH1	basaltic	unmodified	no box	yes	yes	276.28	6.69
56	38.7430	-78.2964	RVC1	basaltic	unmodified	closed	yes	no	28.20	2.21
92	38.3751	-78.5232	SBMT1	basaltic	modified	open	no	yes	31.64	1.63
91	38.3751	-78.5225	SBMT2	basaltic	modified	no box	no	yes	15.79	2.75
62	38.2952	-78.6229	SIG1	basaltic	modified	no box	no	no	121.87	122.95
90	38.6790	-78.3289	SLS1	basaltic	modified	no box	no	no	40.09	36.34
88	38.6805	-78.3272	SLS2	basaltic	modified	no box	no	no	70.47	17.00
33	38.6922	-78.3214	SLS3	siliciclastic	boxed	closed	no	no	132.82	161.89
138	38.3544	-78.5489	SRG1	basaltic	boxed	closed	no	no	193.41	41.75
64	38.8562	-78.2005	SSB1	basaltic	boxed	open	no	no	134.97	135.01
67	38.5815	-78.3702	WHOC	basaltic	boxed	closed	no	no	278.00	40.15
119	38.5820	-78.3699	WHOK	basaltic	unboxed	no box	no	no	806.28	1.54

^A No box seen where sampled; did not sample at start of spring.

Table 7. Overview of substrate type and percent composition within a 50-m reach of each spring.

Object ID	Site ID	Substrate Type (% in 50-Meter Reach)							
		Bedrock	Boulder	Cobble	Gravel	Sand	Silt	Clay	Moist Soil
151	BMSG	0	25	35	10	15	15	0	0
132	BNS2	0	0	10	55	15	20	0	0
32	BNS3	0	0	55	30	5	10	0	0
134	BRFH1	0	10	30	35	10	15	0	0
127	CSH1	0	0	20	5	70	5	0	0
149	CVSW	0	10	20	0	0	70	0	0
131	DKRG	0	10	25	15	35	15	0	0
113	DMPH ^A	–	–	–	–	–	–	–	–
37	DMSR	0	35	35	20	5	5	0	0
142	DMTG	0	10	25	20	10	35	0	0
145	DOR1	0	30	30	20	5	15	0	0
39	DUS1 ^A	–	–	–	–	–	–	–	–
40	EKW8	0	50	20	15	5	10	0	0
109	EKWW	0	25	15	20	0	40	0	0
143	FHC1	0	0	70	15	5	10	0	0
72	FHC2	0	0	0	10	0	0	0	90
76	FHC3	0	0	70	17.5	0	12.5	0	0
95	FRNC2 ^A	–	0	–	–	–	–	–	–
153	FRNC3	0	10	30	45	5	10	0	0
97	FSEE	0	0	10	0	0	90	0	0
144	GSS1	0	30	15	40	5	10	0	0
152	HHR1	0	15	30	25	10	20	0	0
43	HMLK ^A	–	–	–	–	–	–	–	–
110	HSS1	0	20	50	5	20	5	0	0

^A Data not taken.

Table 7 (continued). Overview of substrate type and the percentage for which it composes the reach of each spring.

Object ID	Site ID	Substrate Type (% in 50-Meter Reach)							
		Bedrock	Boulder	Cobble	Gravel	Sand	Silt	Clay	Moist Soil
135	HSS2	0	15	20	35	25	5	0	0
42	HTRO	0	60	10	10	0	20	0	0
5	HTS1	0	0	50	10	20	20	0	0
105	HWBL0 ^A	–	–	–	–	–	–	–	–
82	HWBL1	0	10	25	25	20	20	0	0
71	HWCW	0	50	20	15	10	5	0	0
7	HWS1	0	0	10	30	35	25	0	0
140	HZLW	0	75	0	5	10	10	0	0
79	IMAP	0	10	60	15	5	10	0	0
130	IRS1	0	5	25	35	20	15	0	0
147	IVS1	0	25	20	30	5	20	0	0
49	IVS2	0	30	20	30	5	15	0	0
89	JCN1	0	0	35	50	5	10	0	0
86	JCN2	0	0	25	45	15	15	0	0
87	JNAH	0	25	25	15	0	35	0	0
85	JNHS	0	5	10	0	0	85	0	0
133	LHW1	0	0	0	0	100	0	0	0
53	LMC1	0	5	30	35	5	25	0	0
116	LRLP	0	5	25	20	0	50	0	0
148	LWSM	0	5	20	35	40	0	0	0
51	LWSS1	0	5	40	30	15	10	0	0
102	LWSS2	0	5	10	35	25	25	0	0
137	OLD1	0	5	70	10	10	5	0	0
129	OLD2	0	10	10	10	25	45	0	0

^A Data not taken.

Table 7 (continued). Overview of substrate type and the percentage for which it composes the reach of each spring.

Object ID	Site ID	Substrate Type (% in 50-Meter Reach)							
		Bedrock	Boulder	Cobble	Gravel	Sand	Silt	Clay	Moist Soil
83	OLD3	0	10	5	15	25	45	0	0
99	PCSC1	0	30	10	15	25	20	0	0
124	PFH1	0	10	35	40	5	10	0	0
4	PMTH	0	25	10	55	5	5	0	0
6	PNRM1	0	40	30	10	5	15	0	0
58	PNRM2	0	0	50	15	5	30	0	0
150	PSEW ^A	–	–	–	–	–	–	–	–
136	RKSH1 ^A	–	–	–	–	–	–	–	–
56	RVC1	0	15	40	40	2.5	2.5	0	0
92	SBMT1	0	5	60	10	20	5	0	0
91	SBMT2	0	60	5	30	5	0	0	0
62	SIG1 ^A	–	–	–	–	–	–	–	–
90	SLS1	0	0	0	0	0	0	0	100
88	SLS2	0	0	35	50	5	10	0	0
33	SLS3	0	0	25	60	5	10	0	0
138	SRG1	0	10	30	15	15	30	0	0
64	SSB1	0	0	100	0	0	0	0	0
67	WHOC	0	5	15	15	10	55	0	0
119	WHOK	0	10	15	25	15	35	0	0

^A Data not taken.

Appendix B: Species scores for NMDS ordination

Table 8 shows species scores for non-metric multidimensional scaling ordination and Table 9 outlines which samples were processed.

Table 8. Species scores for non-metric multidimensional scaling ordination assessing species richness grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
Acari	-0.079033958	0.10031939	0.092825528
<i>Adicrophleps hitchcocki</i>	0.299014107	-0.527104621^A	-0.343108653
<i>Alloperla</i> sp.	-0.008028517	0.346200273	-0.236164598
<i>Ameletus</i> sp.	-0.062672118	-0.159279169	-0.22336186
<i>Ameletus cryptostimulus</i>	-0.017683299	0.220085789	-0.32967544
<i>Ameletus tarteri</i>	-0.246945924	0.27017057	-0.245304103
<i>Amphinemura</i> sp.	0.501306802	-0.171818629	-0.154506991
<i>Amphinemura wui</i>	0.124335491	0.124651691	-0.220285388
<i>Anabolia</i> sp.	0.103491119	0.016428807	-0.266737033
<i>Anthopotamus</i> sp.	0.103491119	0.016428807	-0.266737033
Apataniidae	-0.189159983	0.089116945	0.504408824
Aphididae	0.077541945	0.393735411	-0.036869265
Arachnida	0.063366286	0.610927564^A	0.042465171
<i>Atherix</i> sp.	0.105736391	-0.241152596	0.166288217
<i>Atrichopogon</i> sp.	-0.178426752	0.361441039	-0.552265879
Baetidae	-0.06735838	-0.091481846	-0.189095435
Bivalvia	0.076886591	0.543797206^A	-0.043934679
<i>Bezzia</i> sp. <i>Palpomyia</i> sp. <i>Probezzia</i> sp.	-0.034417494	0.200075726	0.156071356
Brachycentridae	0.143935583	0.179814949	0.23009863
<i>Caenis</i> sp.	0.10560569	0.32544597	-0.25200053
Cambaridae	-0.081504513	0.368696629	-0.052844232
<i>Cambarus</i> sp.	0.114465803	0.417145055	-0.158351899
<i>Ceratopogon</i> sp. <i>Culicoides</i> sp. <i>Stilobezzia</i> sp.	-0.051526944	0.084668681	0.136111579
Ceratopogonidae	0.141542274	0.446828814	-0.106521451
Ceratopogoninae	0.218363958	0.46915897	0.470537284
<i>Cernotina</i> sp.	-0.00310487	0.069936633	0.432961715
<i>Cheumatopsyche</i> sp.	0.984755275^A	0.047676922	-0.275688307
Chironomidae	-0.017791515	-0.159388789	0.08807462

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
Chironomini	0.168119153	0.471952464	-0.134999546
Chloroperlidae	-0.035638121	0.189531541	-0.246075078
<i>Chrysops</i> sp.	-0.770766473	0.804641056 ^A	0.349242248
Cicadellidae	0.12525286	0.465117686	-0.204818723
<i>Clinocera</i> sp.	0.201818456	0.188038591	-0.19918909
Coleoptera	0.037981555	0.674917744 ^A	0.074519268
Collembola	0.142297343	0.316153875	0.004681298
Cordulegaster erronea	0.266033454	-0.05524807	0.141899867
<i>Corynoneura</i> sp.	0.114137949	0.093525139	-0.120749543
Crambidae	-0.255488095	-0.089386554	-0.168173515
Crambinae	-0.433358128	0.00013643	0.455147197
<i>Cryptolabis</i> sp.	0.114465803	0.417145055	-0.158351899
Cyclopoida	0.114429789	-0.139521152	-0.134826497
<i>Cymbiodyta</i> sp.	0.06500063	-0.046639521	0.256990757
Decapoda	0.349644351	0.09287045	0.059942631
<i>Dicranomyia</i> sp.	0.003412824	-0.216817668	0.089628134
<i>Dicranota</i> sp.	-0.013151463	0.078474781	0.149690838
<i>Diplectrona</i> sp.	0.984755275 ^A	0.047676922	-0.275688307
<i>Diplectrona modesta</i>	-0.150652217	0.026688756	-0.295155388
Diplopoda	0.242729733	0.348589589	-0.20253792
Diptera	-0.074356898	0.240716458	0.109337382
<i>Dixa</i> sp.	0.082455012	0.049047338	0.209760683
Dixidae	0.257962849	-0.514080936 ^A	0.260701552
<i>Dolophilodes</i> sp.	0.984755275 ^A	0.047676922	-0.275688307
<i>Dryops</i> sp.	0.338797499	-0.660799202 ^A	0.807541459
Elateridae	0.037981555	0.674917744 ^A	0.074519268
Elmidae	-0.124407755	0.338421921	-0.045667448
<i>Eloeophila</i> sp.	0.191522469	0.375959231	-0.184897378
Empididae Genus A	0.037981555	0.674917744 ^A	0.074519268
Empididae	1.031664665	-0.208338361	0.047945779
Entomobryomorpha	0.278387209	-0.384815207	0.162608471
<i>Epeorus</i> sp.	0.120377652	0.412619188	-0.261494592
<i>Epeorus vitreus</i>	0.141572155	0.537693511 ^A	-0.275116476
<i>Ephemerella</i> sp.	-0.359858604	0.10565638	-0.068428306

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness for each grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
<i>Ephemerella catawba</i>	0.242729733	0.348589589	-0.20253792
Ephemerellidae	-0.219295894	-0.162006307	-0.618591543
Ephemeroptera	0.249858644	0.069069206	0.0562228
<i>Eristalis</i> sp.	0.266033454	-0.05524807	0.141899867
<i>Eurycea</i> sp.	0.141572155	0.537693511^A	-0.275116476
<i>Eurylophella</i> sp.	-0.328226688	0.054578296	-0.546367651
<i>Eurylophella funeralis</i>	-0.640827609	-0.747581611^A	-0.405805843
<i>Eurylophella verisimilis</i>	0.447671741	-0.170579649	-0.12717838
<i>Eusphalerum</i> sp.	0.141572155	0.537693511^A	-0.275116476
<i>Fattigia pele</i>	-0.246945924	0.27017057	-0.245304103
<i>Fontigens orolibas</i>	-0.209864228	-0.278337887	0.182872739
Gammaridae	0.13174184	0.125874276	-0.165327675
Gastropoda	0.108473161	0.37606711	-0.125647282
Gomphidae	0.134831304	0.083305161	-0.523237849
<i>Habrophlebia vibrans</i>	0.141572155	0.537693511^A	-0.275116476
<i>Helicopsyche borealis</i>	-0.291038031	-0.348806118	0.153955086
<i>Hemerodromia</i> sp.	0.266033454	-0.05524807	0.141899867
Hemiptera	-0.178426752	0.361441039	-0.552265879
Heptageniidae	0.039974754	-0.019294652	-0.101485358
<i>Hexatoma</i> sp.	-0.168055645	0.288847433	0.129528659
<i>Homoplectra</i> sp.	-0.116386583	-0.737687099^A	-0.146832573
Hyalidae	0.338138624	-0.006648642	-0.124362591
<i>Hybomitra</i> sp.	-0.228566427	0.342462173	0.324261766
Hydracarina	0.037981555	0.674917744^A	0.074519268
<i>Hydraena</i> sp.	0.089202926	0.545798203^A	0.009840434
Hydrophilidae	0.153000283	0.206193493	0.328580911
Hydropsychidae	-0.219243634	-0.093255176	-0.299858957
<i>Isonychia</i> sp.	0.10560569	0.32544597	-0.25200053
<i>Isoperla</i> sp.	0.401830039	-0.210032419	-0.276624959
Ixodida	0.089202926	0.545798203	0.009840434
<i>Lanthus vernalis</i>	0.242729733	0.348589589	-0.20253792
<i>Lepidostoma</i> sp.	-0.127695485	-0.25058146	0.078326516
Lepidostomatidae	-0.074058957	-0.268740558	0.102324824
Leptoceridae	0.010186109	0.178307237	0.236884517

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness for each grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
Leptophlebiidae	0.793852241	0.220984873	-0.016749349
<i>Leuctra</i> sp.	0.583885525	0.065792143	0.004167288
<i>Leuctra truncata</i>	0.141572155	0.537693511	-0.275116476
Leuctridae	-0.088805291	0.06240061	-0.172658864
<i>Limnephilidae</i>	-0.261182206	-0.216907198	-0.147239152
<i>Limnophila</i> sp.	0.09875858	0.497135298	-0.053778122
Limoniidae	0.073867214	0.242334798	0.075733197
Lymnaeidae	-0.228566427	0.342462173	0.324261766
Megaloptera	-0.884471533	0.901564836^A	0.354480918
<i>Micrasema</i> sp.	-0.733084823^A	0.157630863	-0.020412157
<i>Microvelia</i> sp.	-0.089117886	0.378426321	-0.432153537
<i>Molanna</i> sp.	0.114465803	0.417145055	-0.158351899
<i>Molanna blenda</i>	-0.268644962	0.156253705	0.087258376
<i>Molophilus</i> sp.	0.078327991	0.09894853	0.089963213
<i>Myrmeleontidae</i>	0.141572155	0.537693511^A	-0.275116476
Nematoda	-0.303382562	0.159959303	0.347162612
Nemouridae	0.051298529	0.046475709	0.080115613
<i>Neophylax</i> sp.	0.153624736	-0.343316771	-0.002358966
<i>Neophylax aniqua</i>	0.284090319	-0.348720242	0.135217867
<i>Neophylax mitchelli</i>	0.245553397	-0.401941862	0.203573203
<i>Nigronia fasciata</i>	-0.216801347	0.10095767	-0.249835625
<i>Nigronia</i> sp.	0.141572155	0.537693511^A	-0.275116476
<i>Oemopteryx contorta</i>	-0.281941184	-0.001241421	-0.097772548
Oligochaeta	-0.014233662	-0.039340545	0.027060839
<i>Ophiogomphus howei</i>	-0.246945924	0.27017057	-0.245304103
<i>Optioservus</i> sp.	-0.089117886	0.378426321	-0.432153537
Orthocladinae	0.079907447	0.315915631	-0.122050789
Ostracoda	-0.291521973	-0.259899697	0.339933758
<i>Ostrocerca</i> sp.	0.408852318	-0.112481173	-0.126180441
<i>Palaeagapetus celsus</i>	0.299014107	-0.527104621	-0.343108653
<i>Paraleptophlebia</i> sp.	0.439147626	0.088287504	-0.101747953
<i>Paranemoura perfecta</i>	0.134831304	0.083305161	-0.523237849
<i>Parapsyche</i> sp.	-0.240399251	0.11337061	0.005825954
<i>Parapsyche apicalis</i>	0.237338453	-0.355652762	-0.319018035

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness for each grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
<i>Pedicia</i> sp.	-0.320231268	-0.469422682	0.267427195
<i>Peltoperla</i> sp.	0.132308054	-0.227522931	-0.135272479
Peltoperlidae	0.100551246	-0.153244297	-0.111044132
Pericomaini	-0.001954792	0.187879635	0.138012934
Perlidae	-0.359858604	0.10565638	-0.068428306
Perlodidae	0.073634737	0.254058858	-0.067379135
Perloidea	0.266033454	-0.05524807	0.141899867
Philopotamidae	0.141572155	0.537693511 ^A	-0.275116476
<i>Phylocentropus</i> sp.	-0.246945924	0.27017057	-0.245304103
Planorbidae	0.037981555	0.674917744 ^A	0.074519268
Platyhelminthes	-0.189159983	0.089116945	0.504408824
Plecoptera	0.22388239	0.149615993	-0.364144045
Podocopida	0.114202943	0.267672604	-0.131543469
Poduridae	0.128934489	0.481490773	-0.220677878
<i>Polycentropodidae</i>	-0.217242094	-0.06849042	-0.082368156
<i>Procambarus</i> sp.	0.037981555	0.674917744 ^A	0.074519268
<i>Prodiamesa</i> sp.	0.114465803	0.417145055	-0.158351899
<i>Prostoia</i> sp.	0.39204193	-0.229100347	-0.433129183
<i>Pseudolimnophila</i> sp.	-0.027104865	0.30043132	0.109571149
<i>Pseudostenophylax</i> sp.	-0.219030105	-0.297714121	0.411507008
<i>Pseudostenophylax sparsus</i>	-0.433358128	0.00013643	0.455147197
<i>Psilotreta</i> sp.	-0.228559369	0.229301293	0.109266436
<i>Psilotreta rossi</i>	0.134831304	0.083305161	-0.523237849
Psychodidae	0.338138624	-0.006648642	-0.124362591
<i>Psychomyia</i> sp.	-0.031131136	0.420198354	0.284620318
Psychomyiidae	-0.114709209	-0.221859174	-0.302490036
<i>Ptilostomis</i> sp.	0.673818571 ^A	-0.212947184	-0.306258821
<i>Ptychoptera</i> sp.	-0.700392738 ^A	-0.54778244	0.603080934
<i>Pycnopsyche</i> sp.	-0.061296404	-0.354859913	-0.234655653
<i>Pycnopsyche flavata</i>	0.141572155	0.537693511 ^A	-0.275116476
<i>Pycnopsyche gentilis</i>	-0.415896574	-0.271732774	-0.099867154
<i>Remenus bilobatus</i>	0.103491119	0.016428807	-0.266737033
<i>Rhyacophila</i> sp.	-0.13566069	-0.176824205	-0.202182686
<i>Rhyacophila carolina</i>	0.040843298	0.300218336	-0.369668614

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness for each grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
<i>Rhyacophila invaria</i>	0.089202926	0.545798203^A	0.009840434
<i>Rhyacophila nigrita</i>	-0.092899276	0.06460914	0.045969913
<i>Rhyacophilidae</i>	0.485671685	-0.47788227	-0.399994377
<i>Sacodes pulchella</i>	0.10560569	0.32544597	-0.25200053
Saldidae	-0.178426752	0.361441039	-0.552265879
Sericostomatidae	-0.088385888	-0.048782126	-0.286429166
<i>Sialis</i> sp.	-0.178426752	0.361441039	-0.552265879
Simuliidae	0.136959933	0.226792091	-0.444885985
<i>Simulium</i> sp.	0.596545139^A	0.125443674	-0.317353183
<i>Sminthuridae</i>	0.141572155	0.537693511	-0.275116476
<i>Soyedina</i> sp.	-0.560875776^A	0.239079545	0.066085146
Sphaeriidae	-0.600649862^A	-0.429670352	0.509877639
Staphylinidae	0.201687629	0.543406658	0.529493767
<i>Stenonema</i> sp.	0.300480965	0.025179272	-0.506939238
<i>Stygobromus spinosus</i>	-0.086527109	-0.059773485	0.121321324
<i>Suwallia marginata</i>	0.134831304	0.083305161	-0.523237849
<i>Sweltsa</i> sp.	0.037852772	-0.286172284	-0.211190201
Symphyleona	0.338138624	-0.006648642	-0.124362591
<i>Symplecta pilipes</i>	0.177855819	0.24563797	0.181458167
Tabanidae	-0.253858858	0.383465246	0.106463933
<i>Tallaperla</i> sp.	0.017589434	-3.02E-05	-0.125565303
Tanypodinae	-0.021636083	-0.014611384	0.084114232
Tanytarsini	0.143381183	0.256623984	-0.185315558
Thaumaleidae	0.186372902	0.221738147	-0.0121967
<i>Thienemanniella</i> sp.	0.163692022	0.390299882	-0.214860695
Thysanoptera	0.121057601	0.431129979	-0.150730071
<i>Tipula</i> sp.	0.091965324	0.174789908	0.275314697
<i>Tipula Nippotipula</i> sp.	0.313741821	-0.049082305	-0.576835962
<i>Tipula Platytipula</i> sp.	0.158265929	0.083788708	-0.00509033
<i>Tipula Sinotipula</i> sp.	0.233972485	0.266361016	0.071102823
Tipulidae	-0.010342955	0.496814571	0.221424706
Tipuloidea	-0.067590034	0.187078963	0.24757017
<i>Trichoniscus</i> sp.	0.037981555	0.674917744^A	0.074519268
Trichoptera	-0.615668736^A	-0.018278805	-0.376538802

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 8 (continued). Species scores for non-metric multidimensional scaling ordination assessing species richness for each grouped by unboxed and boxed. Greater value indicates taxa driving differences in variation across the spring communities.

Species	NMDS1	NMDS2	NMDS3
Tricladida	-0.052155577	-0.298816092	-0.304064479
<i>Ulomorpha</i> sp.	0.10560569	0.32544597	-0.25200053
<i>Viehoplerla</i> sp.	-0.116386583	-0.737687099^A	-0.146832573
<i>Wormaldia</i> sp.	0.123599069	-0.081893347	-0.011514359
<i>Wormaldia modesta</i>	-0.030927119	0.356940137	-0.42978232
<i>Zapada fumosa</i>	0.264310846	0.044024711	-0.373624068
<i>Zealeuctra</i> sp.	0.134831304	0.083305161	-0.523237849

^A Values are used to guide interpretation of NMDS in Figure 8.

Table 9. Number of samples processed and sample type for each spring. An – indicates samples were not processed.

Sites	Spring Type	Processed Sample Types			
		Benthic Samples	Diversity Samples	Emergence Samples with EPTO	Total Emergence Samples
BMSG	unmodified	–	1	2	3
BNS2	unmodified	–	1	3	6
BNS3	modified	–	1	3	3
BRFH1	unmodified	2	1	1	2
CSH1	unmodified	–	1	–	2
CVSW	unmodified	–	1	1	2
DKRG	modified	–	1	–	2
DMPH	modified	–	1	1	1
DMSR	modified	1	1	2	2
DMTG	modified	–	1	2	2
DOR1	modified	1	1	1	2
DUS1	modified	–	1	2	2
EKWW	unmodified	–	–	–	–
EKW8	unmodified	–	1	–	2
FHC1	modified	–	1	–	1
FHC2	unmodified	–	–	–	–
FHC3	modified	–	–	2	2
FRNC3	modified	–	1	1	2
FSEE	unmodified	–	1	–	1
GSS1	unmodified	–	1	2	2

^A 1 site had 2 samples.

Table 9 (continued). Number of samples processed and sample type for each spring. An – indicates samples were not processed.

Sites	Spring Type	Processed Sample Types			
		Benthic Samples	Diversity Samples	Emergence Samples with EPTO	Total Emergence Samples
HHR1	modified	–	–	–	2
HMLK	modified	2	1	–	–
HSS1	modified	–	1	2	2
HSS2	modified	–	1	2	2
HTRO	unmodified	3	1	–	2
HTS1	modified	–	1	1	2
HWBL0	modified	–	–	–	–
HWBL1	modified	–	1	1	2
HWCW	unmodified	–	1	2	2
HWS1	modified	–	1	–	2
HZLW	unmodified	–	1	2	2
IMAP	unmodified	5	1	–	2
IRS1	modified	–	1	1	3
IVS1	modified	–	1	2	2
JCN1	unmodified	–	–	4	4
JCN2	unmodified	–	1	–	2
JNAH	unmodified	–	1	–	–
JNHS	unmodified	–	1	–	2
LHW1	modified	–	1	2	4
LMC1	modified	–	1	1	2
LRLP	unmodified	–	1	2	2
LWSM	unmodified	–	1	–	2
LWSS1	modified	–	1	–	2
LWSS2	modified	–	1	1	1
OLD1	unmodified	–	1	1	1
OLD2	unmodified	–	1	1	1
OLD3	modified	–	1	2	2
PCSC1	modified	–	1	2	2
PFH1	unmodified	3	1	1	1
PNRM1	modified	–	–	2	2
PNRM2	modified	–	1	1	2
PSEW	unmodified	–	1	2	2
RKSH1	modified	–	1	–	–

^A 1 site had 2 samples.

Table 9 (continued). Number of samples processed and sample type for each spring. An – indicates samples were not processed.

Sites	Spring Type	Processed Sample Types			
		Benthic Samples	Diversity Samples	Emergence Samples with EPTO	Total Emergence Samples
RVC1	modified	–	1	–	2
SBMT1	modified	2	2	1	1
SBMT2	unmodified	–	1	2	2
SLS1	unmodified	–	1	–	3
SLS2	unmodified	–	1	1	2
SLS3	modified	–	1	1	2
SRG1	modified	–	1	–	2
SSB1	modified	–	1	1	1
WHOC	modified	3	–	1	1
WHOK	modified	2	1	–	2
Totals	–	24	56	65	116
Total Sites	–	10	55 ^A	40	57

^A 1 site had 2 samples.

Appendix C: Correlations

Tables 10 and 11 show the correlations between covariates.

Table 10. Shows the correlation between environmental variables displayed in graphic format in Figure 9 above.

Environmental Parameter	pH	Temperature	Specific Conductance	Dissolved Oxygen (mg/L)	Dissolved Oxygen (%)	Oxygen Reduction Potential	Flow	Elevation
pH	–	–	–	–	–	–	–	–
Temperature	0.76	–	–	–	–	–	–	–
Specific Conductance	0.48	0.60	–	–	–	–	–	–
Dissolved Oxygen (mg/L)	0.79	0.45	0.26	–	–	–	–	–
Dissolved Oxygen (%)	0.79	0.51	0.30	0.99	–	–	–	–
Oxygen Reduction Potential	0.57	0.27	0.07	0.69	0.66	–	–	–
Flow	0.48	0.18	0.25	0.62	0.60	0.39	–	–
Elevation	-0.09	0.16	-0.03	-0.18	-0.15	-0.17	0.02	–

Table 11. Shows the correlation matrix across all environmental variables and all springs in tabular format displayed in graphic format in Figure 10 above.

Environmental Parameter	Temperature	Specific Conductance	Oxygen Reduction Potential	Elevation	Distance to Road	Distance to Trail
Temperature	–	0.60	0.27	0.16	-0.41	-0.22
Specific Conductance	0.60	–	0.07	-0.03	-0.30	0.09
Oxygen Reduction Potential	0.27	0.07	–	-0.17	-0.34	-0.09
Elevation	0.16	-0.03	-0.17	–	-0.61	-0.40
Distance to Road	-0.41	-0.30	-0.34	-0.61	–	-0.18
Distance to Trail	-0.22	0.09	-0.09	-0.40	-0.18	–

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