Evaluation of physicochemical parameters in two different ecosystems

By

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ABSTRACT

Chapter 1:

Biological soil crusts are clumped together communities of organisms with soils that play an essential role in arid ecosystems. They have crucial roles in primary productivity, nitrogen and carbon cycling, mineralization, water retention, and soil stabilization. There is a gap in knowledge for desert crusts from the Cieneguilla Desert of Lima, Peru. This study measures metal concentrations and determines carbon/nitrogen associated with crust communities to characterize the BSC and establish potential biogeochemical relationships.

Chapter 2:

The importance of monitoring water quality is essential to maintain healthy aquatic environments for wildlife and human health. This study of Yellow, Creek in Poland, Ohio aims to identify spatial and temporal trends in physicochemical parameters, biological indicators, and benthic community structure. Water quality parameters (temperature, dissolved oxygen, conductivity, total suspended solids, and fecal coliform count) were measured using a YSI Pro 2030 or other standard methods. Nitrate, sulfate, and phosphate were measured using LaMotte nutrient kits. Macroinvertebrates were sampled using a 0.3 x 0.3 m Surber sampler. A two-way MANOVA of water quality parameters showed that season had significant influence on water quality. A one-way MANOVA showed that benthic macroinvertebrate community structure (density, diversity, and EPT richness) had a significant site*season interaction. Most physico-chemical and biological parameters were below maximum limits allowed by Ohio administrative code, but fecal coliform levels depended on season.

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Chapter I: Biological soil crust from Peruvian desert

Introduction

Biological Soil Crusts (BSC) are clumped layers of communities of organisms including cyanobacteria, algae, fungi, bryophytes, and lichens that have an intimate relationship with soil surfaces in arid ecosystems (Rozenstein & Karnieli 2015). Drylands makeup more than 40% of Earth's terrestrial ecosystems, and many of these environments face degradation due to human activity including population growth, energy and mineral extraction, and agriculture (Antoninka et al. 2020). BSC are labeled as "ecosystem engineers" in arid regions across the globe because of their crucial roles in primary productivity, nitrogen and carbon cycling, mineralization, water retention, and soil stabilization (Arana & Salinas 2016). BSC benefit humans through ecosystem services such as soil stabilization and erosion control (Belnap & Gillette 1998), improving soil fertility (Harper & Belnap 2001), and by fixing carbon through photosynthesis and creating bioavailable nitrogen for plants (Drahorad et al. 2013). Biological soil crusts are fragile and sensitive to destructive events like fires and trampling (Rajeev et al. 2013). If fires are not severe, surviving organisms of the crust can recolonize the area. In addition, invasive species like cheatgrass (*Bromus tectorum*) can increase the likelihood of fire due to the dense strands of grass that compete for light and moisture (Ponzetti et al. 2007). Livestock and vehicles can trample and destroy the cohesive characteristic of BSC which results in long recovery times of decades or even centuries (United States Department of Agriculture [USDA], n.d.)

Structure and Function

There are several different organisms that can be found in desert crusts. Cyanobacteria are typically the first organisms that provide the foundation of a BSC. These photoautotrophic prokaryotes make up the majority of the photosynthetic components of the crust, provide cohesiveness by secreting sticky substances, and create soil stability, an important process in erosion control (Warren et al. 2019). Photosynthetic blue-green algae within the BSC are adapted to living in dry environments despite their moisture requirements. When water is available, they help infiltrate water into the soil of the BSC reducing runoff and soil erosion (Warren et al. 2020). Within desert crust samples, fungi act as free-living organisms. They contribute to the nutrient cycle within the crust by acting as decomposers of organic materials (Bates & Garcia-Pichel 2009). Fungi in association with cyanobacteria assist in binding the particles together for the cohesive quality of the BSC layers and preventing desiccation of the other organisms within the BSC (Wang et al. 2019). Bryophytes are also common components of BSC (Warren et al. 2019). Short mosses like *Bryum argenteum*, *Didymodin vinealis*, and *Pterygoneurum ovatum* are the more common species found in BSC (Belnap & Eldridge 2003). Crusts that have a well-developed presence of moss are also shown to improve water holding capacity (Hu et al. 2019). Lichens, a symbiotic association between fungi and cyanobacteria, perform carbon and nitrogen fixation (United States Department of Agriculture [USDA], n.d.). Thus, nutrient fixation from lichen dominated desert crusts are important to these nutrient poor soils due to the arid ecosystem having no primary producers (Büdel et al. 2013).

Numerous studies of the Colorado Plateau, USA have shown that BSC are critical for the functioning of this ecosystem such as regulation of patterns of vascular plant establishment as well as fluxes of dust, carbon, nitrogen, and water (Steven et al. 2016). Similar studies in the United States, Venezuela, and South Africa have indicated that BSC can increase soil alkalinity from a pH of 8 to 10.5, which in turn increases availability of some plant nutrients (Belnap 2003). Similar ecosystems have also been identified in arid areas around the world including the Chihuahuan Desert in New Mexico, the Namib Desert of Africa, the Mojave Desert in California, and the Atacama Desert in Chile, all of which highly rely on the importance of BSC. In the Chihuahuan Desert of New Mexico, soil surface disturbances and climate change are affecting the rate of fixed carbon and nitrogen contributed to the environment by biological crusts by changing the successional stage of the crusts (Housman et al. 2006). In addition, fixed nitrogen, which typically would be assumed to be low in these arid ecosystems, like the Tengger Desert of Northern China (high temperatures and minimal precipitation), relies on the nitrogen fixed by soil crusts (Su et al. 2011). Carbon to nitrogen (C/N) ratio has an important role in the composition of the microbial community and mineralization rate, and the ratio is an overall indicator of soil quality (Sun et al. 2017).

Fog is the main source of moisture for the BSC in the Namib Desert of Africa which is dominated by terricolous lichens responsible for soil stabilization and primary productivity (Lalley & Viles 2005). Similarly, the moss-lichen surface features of desert crusts in the Mojave Desert in California, have different characteristics on the surface, like internal voids and vesicular pores, that allow for water to become easily trapped for crust organisms (Williams et al. 2012). Desert crusts found in the Atacama Desert in

Chile, one of the driest regions of the world, are shown to protect the soil from wind erosion and assist soil accretion (Wang et al. 2017).

The coastal Peruvian desert, also characterized by low precipitation and high UV radiation, has never been studied. There is a gap in knowledge of Peruvian BSC, which have a high conservation value and perhaps critical for sustaining life in such extreme environment.

Study Area

The study site is within the Cieneguilla Desert is a coastal fog desert located in the Cieneguilla Valley in the outskirts of Lima, Peru. The Cieneguilla Valley has not been urbanized as much as other valleys of Lima, so the desert countryside has been untouched. It is described as a fog desert with very high solar UV radiation, minimal rainfall levels (less than 5 millimeters annually) and minimal vegetation. These particular crusts form on top of sandy rocks scattered in the dry soil terrain.

Figure 1: Road to sampling location, Cieneguilla

Figure 2: Cieneguilla Valley, Lima, Peru

Goal

The goal of this study was to characterize the BSC and establish potential biogeochemical relationships.

Objectives

There is a larger study characterizing fungal and microbial communities, but this study only evaluates nonbiological data. The objectives of the study were to measure metal concentrations and determine C/N associated with biological crust communities.

Methods

Sample Collection

Samples were collected in January 2015 which is summer in Peru. Twenty-two BSC samples were collected in a transect with sterile tweezers and spatulas. Samples were collected along a transect of 1 kilometer using an existing trail that served as access to the hills surrounding the valley. Every 150 meters, 2, 3, or 4 samples (depending on accessibility) were taken either to the left or right of the main transect. Thus, samples were labeled as R if they were taken to the right, and L if they were taken to the left. Numbers 1 to 4 indicate sample taken. Samples labeled as S were crust samples only. Samples labeled as C were soil crust samples adjacent to cacti. Samples were stored at -20 °C (freezer) in Ziploc bags. They were then homogenized in texture using a mortar and pestle.

Inductive Coupled Plasma-Atomic Emission Spectrometry Methods of Biological Soil Crusts

Glassware

All glassware used in ICP-AES analysis was soaked in a 10% hydrochloric acid wash bath for 24 hours. Metal spatulas were soaked for 15 minutes prior to use in the acid bath. After acid bath, materials were rinsed with deionized water. Glassware was dried on drying paper and stored after cleaning in a covered container.

Samples for Metal Analyses

Acid digestion was performed in triplicate using the EPA method 3050 B using 0.5 grams of sample in triplicate instead of what the protocol called for which is 1 gram. NIST Reference Material 8704 Buffalo River Sediment (Gaithersburg, MD) was also used in triplicate at 0.5 grams to check recoveries. Procedural blanks (n=3) were also included to check for contamination. The Environmental Express Hotblock Pro Digestion system SC180 was used as the heating source for digestion (Charleston, SC). After digestion, soil crust samples were cooled down to room temperature and then diluted to 25 mL using Milli-Q deionized water.

ICP-AES Analysis

Soil crust samples, reference standards, and procedural blanks were analyzed with quality control checks in a Thermo Fisher iCAP 6500. Calibration standards (0.25, 0.5, 1.0, and 2.5 ppm) were made from a stock solution (Ricca Chemical, Arlington, Texas).

Total Carbon/Nitrogen Analysis

A sample of 300 mg was weighed and dried at 60 ℃ for 24 hours to a constant weight. Approximately 17 mg was weighed in triplicate using an ultra-micro balance. Samples were placed into a precombustion aluminum capsule ready for analysis on an

ECS 4010 Elemental Combustion System (N.C. Technologies, Bussero, Italy). The run time was 6 minutes per sample, and atropine in tin wrappers was used as a standard for calibration.

Results

Metal concentrations were highly variable for each metal (Table 1). As expected, Fe and Al were present in very high concentrations ranging from 4,200 to 40,600 ppm compared to As, Cr and Pb which were much lower. There was no particular trend between metal concentrations from crust samples (S) and soil crust associated with cacti vegetation (C). Similarly, analyses of carbon and nitrogen (Table 2) varied and did not show any particular trend as for type of sample (either crust samples or crust associated with cacti. In general, proportions of carbon were much higher than nitrogen (expressed in ratios).

Table 1. Average metal concentrations (parts per million) shown in the top row and standard deviation (bottom row) measured in 21 crust samples using ICP-AES.

Sample	Al	As	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
S1LA	5492.4	5.7	0.8	3.3	15.5	31.7	16856.3	5064.6	234.4	11.3	24.7	82.7
	282.5	0.3	0.2	0.7	2.0	8.7	1378.7	420.5	17.8	0.9	5.4	17.0
S1LB	5965.6	5.6	0.9	3.6	18.9	29.9	18308.4	4640.9	220.9	11.8	19.7	138.1
	323.3	0.8	0.2	0.8	4.9	8.6	806.9	329.9	16.4	1.6	4.7	122.4
S1LC	9729.3	13.4	2.3	6.9	28.5	74.7	30662.0	8425.4	402.1	18.6	78.9	263.0
	2020.5	4.3	0.2	0.6	6.2	7.6	8862.2	2358.4	101.3	0.3	8.4	24.8
S1RA	4236.3	3.0	0.6	2.9	9.7	25.9	12274.3	3342.5	156.8	9.8	16.3	72.7
	209.3	0.1	0.0	0.1	0.9	0.3	592.5	246.0	9.8	0.1	1.1	10.9
S1RB	9813.5	13.6	2.8	8.5	22.3	87.5	31114.7	7615.1	377.9	19.3	99.2	324.7
	686.3	1.8	0.4	1.1	1.1	8.2	5602.6	657.2	33.8	1.4	12.6	41.1
S2RA	6891.6	6.0	1.3	4.7	16.1	44.0	21191.6	4900.0	266.7	12.2	36.0	125.1
	245.2	0.6	0.3	1.0	0.6	10.2	675.1	286.4	15.0	1.3	8.8	35.9
S2RB	6899.6	5.9	1.0	4.2	20.3	36.4	21536.6	4420.5	224.4	12.4	21.9	76.5
	367.4	1.3	0.2	0.4	3.3	6.6	2623.6	330.9	13.6	1.0	2.6	8.5
S2RC	7882.3	8.6	1.6	4.7	21.2	52.4	23894.2	5621.1	277.2	13.4	49.3	132.6
	295.7	0.7	0.3	0.8	3.4	9.8	1671.2	305.4	10.5	1.7	10.8	28.4
S2RD	12023.6	18.7	3.8	9.8	32.9	119.4	40650.8	8849.2	467.9	23.3	114.4	427.9
	649.2	0.8	0.1	0.5	4.7	4.2	1955.1	698.0	34.0	1.7	4.1	7.2
S3RA	12270.7	19.3	3.3	8.9	60.9	107.8	35808.9	9030.1	401.3	40.6	139.1	369.3
	1088.2	1.6	0.3	0.2	28.3	8.4	6645.2	844.6	32.5	24.5	12.1	58.1
S3RB	9614.9	19.6	2.4	12.1	393.3	82.4	36003.2	9991.7	421.8	234.5	85.8	243.1
	388.8	2.3	0.7	10.6	622.5	17.6	7750.3	501.4	86.8	375.6	22.7	63.6
S3RC	11769.0	17.6	3.0	8.8	58.1	95.5	39763.7	10692.8	480.0	22.5	102.5	287.3
	1132.8	1.2	0.1	0.6	54.1	6.4	3842.7	2107.5	84.5	4.7	2.7	17.4
S3RD	10674.9	16.1	2.6	7.2	28.8	85.8	30844.7	7443.6	376.4	17.4	84.0	250.6
	748.5	1.4	0.5	1.2	8.5	8.3	5549.1	553.3	24.5	0.7	14.6	48.5
C1	6157.2	7.6	1.0	3.9	11.6	35.0	19492.6	5481.2	256.5	11.0	26.3	86.5
	951.9	0.2	0.3	1.3	1.4	11.9	2498.8	732.4	35.7	1.6	9.6	29.6
C1a	7563.6	9.3	1.7	5.0	16.4	55.5	23513.4	6527.1	328.6	13.2	55.8	165.6
	1436.8	2.5	0.2	0.7	3.6	5.0	4340.2	1275.8	62.0	0.9	6.1	20.0
C2	9362.2	15.4	2.3	6.4	45.6	78.0	30310.5	8242.6	385.3	22.8	72.4	293.2
	461.6	1.0	0.7	1.7	31.6	11.7	1371.5	579.6	26.7	10.1	20.7	103.0
C2a	12044.2	22.4	2.9	7.3	33.3	100.9	40271.4	10469.4	478.7	20.9	102.8	342.9
	614.6	2.0	0.9	2.1	5.5	15.7	2298.5	794.6	40.2	5.0	31.4	113.5
C3	4298.9	9.8	1.4	2.5	27.7	42.1	13638.5	6555.7	200.1	13.8	38.2	160.1
	965.1	2.0	0.1	0.5	20.3	4.1	3402.7	794.1	43.9	3.6	5.4	84.9
C3a	9605.6	16.0	1.4	4.7	31.1	53.0	30549.2	8273.8	373.7	14.9	49.2	219.4
	1640.6	3.6	0.3	1.0	8.3	8.9	6133.3	1564.7	72.5	2.2	9.6	119.1
C4	12322.3	23.4	2.2	6.1	30.1	78.7	39802.5	11089.3	495.5	16.4	80.5	234.4
	862.2	2.7	0.2	0.6	2.7	8.6	3638.4	1089.3	42.8	1.1	8.1	24.4
C5	11165.7	15.1	2.4	7.0	57.0	82.5	35373.9	9514.1	426.1	25.7	81.5	279.4
	222.8	1.2	0.4	1.2	53.2	10.9	1135.6	201.9	16.3	15.6	14.2	70.0

Table 2. Average (n=3) and standard deviation (SD) of carbon and nitrogen (by weight) of Peruvian BSC

	Carbon %		Nitrogen %		C : N
Sample	AV	SD	AV	SD	
S1LA	1.48	0.11	0.14	0.01	10 : 1
S1LB	3.71	0.63	0.36	0.02	10 : 1
S1LC	4.79	0.31	0.38	0.04	13 : 1
S1RA	1.47	0.16	0.17	0.01	9 : 1
S1RB	3.66	0.71	0.37	0.16	10 : 1
Base	4.03	0.18	0.39	0.03	10 : 1
S2RA	3.91	0.51	0.38	0.04	10 : 1
S2RB	1.93	0.21	0.22	0.13	9 : 1
S2RC	3.77	0.57	0.38	0.03	10 : 1
S2RD	8.91	0.81	1.04	0.06	9 : 1
S3RA	6.48	0.10	0.94	0.03	7 : 1
S3RB	5.41	0.07	0.43	0.07	13 : 1
S3RC	7.04	0.51	0.65	0.10	11 : 1
S3RD	9.23	0.17	1.05	0.07	9 : 1
C1	4.23	0.38	0.32	0.06	13 : 1
C1a	3.59	0.23	0.34	0.13	11 : 1
C2	4.44	0.51	0.38	0.06	12 : 1
C2a	3.60	0.20	0.29	0.03	12 : 1
C3	15.62	0.71	0.78	0.09	20 : 1
C3a	2.49	0.03	0.25	0.05	10 : 1
C4	6.16	0.29	0.64	0.15	10 : 1
C5	6.84	1.32	0.83	0.17	8 : 1

Discussion

Desert ecosystems are mainly characterized by low water content and nutrients, which in turn reduces rates of biogeochemical cycling. Thus, microbial metal metabolism becomes an important key factor for soil functioning (Liu et al. 2022). For instance, Mn can efficiently promote Cd stabilization by BSC (Peng et al. 2019). In this preliminary study of BSC from a Peruvian fog desert, high levels of important biological metals were found (Fe, Al, Zn and Mn) which is consistent with results from the Tengger desert of China (He et al. 2019).

Soil carbon to nitrogen (C/N) ratio is considered one of the most important variables to determine soil ecological function. However, there are very few studies on the role of BSC and C/N dynamics. In general, due to high variation in species composition and microenvironments within the crust, the role of BSC in C/N is not well understood. More recent research has revealed that BSC might be the key suppliers for nitrogen in dry ecosystems, driving community composition, diversity, assembly processes and biocrust succession (Xu et al. 2021). In this study, C/N ratios varied from 7 to 20, comparably with other systems where C/N ratios varied (ranging from 7 to 23) depending of the BSC community (Sun et al. 2017). Because microbial composition and biomass is directly dependent of nutrient and carbon availability, the rates of N fixation in BSC are expected to be highly variable. For instance, Xie and Steinberger (2004) reported C/N ratios of 0.5 in a sand dune desert ecosystem while Koyama et al. (2019) reported C/N ratios of 7 for soil crusts in the Mojave Desert.

Conclusion

Results presented in this chapter are preliminary and need to be further evaluated with microbial composition data. Yet, metal concentrations and C/N ratios fell into what other studies have reported in similar ecosystems around the world.

Chapter II: Water quality in Yellow Creek, OH: temporal and spatial variations

Introduction

The United States regulates water quality by setting standards in the Clean Water Act (1972) and Safe Drinking Act (1974) (Environmental Protection Agency [EPA] 2022b.). The importance of maintaining water quality is crucial to understanding health and environmental issues because freshwater is essential in all life processes on Earth (Akyildiz & Duran 2021). With the increase in human population and chemical use, it has become more difficult to evaluate and maintain healthy aquatic environments (Stucker & Lyons 2017). Healthy streams are important not only for the aquatic organisms that live within them, but also for the surrounding vegetation and terrestrial animals that rely on riparian zones for food, migration routes, and forest connectors to other habitats (Biggs et al. 2016).

Although recent research has measured the impacts of rapid urbanization on water scarcity, water quality assessment worldwide in a climate changing scenario has not been commonly addressed (Van Vliet et al. 2017). Urban stream characterization depends on the climatological region, physiographic region, and types of nearby urban development (Booth et al. 2015). In fact, urban streams face degradation mainly due to anthropogenic changes in infrastructure, land use, and development growth (Halstead et al. 2014). These urbanization changes affect the stream's morphology, aquatic species diversity, water chemistry, and overall habitat health (Blauch & Jefferson 2019). For example, a study of 9 metropolitan areas in the USA (Boston, MA; Raleigh, NC; Atlanta, GA; Birmingham, AL; Milwaukee Green Bay, WI; Denver, CO; Dallas-Fort Worth, TX; Salt Lake City, UT; and Portland, OR) showed that any level of urbanization had an effect on biological

stream's conditions by reducing threshold effects (Brown et. al 2009). In urban watersheds, pollutants, sediments, and nutrients quickly travel to streams by storm drains and pipes (McMillan & Noe 2017). Urban streams have point (i.e., discharge pipes from industry) and non-point (i.e., urban runoff) sources of pollution that greatly affect water quality during precipitation events, which are only exacerbated by the number of impervious surfaces in urban areas (Hasenmueller et al. 2017). For example, low water quality in urban streams near Cleveland, Ohio had a direct relationship with the intensity of urbanization and the low abundance and high mobility of large woody debris (Blauch & Jefferson 2019). Contamination of water is rarely the result of single pollutants, but more commonly a mixture of pathogens, plastics, and a variety of chemicals (Vermeulen et al. 2015). The Cuyahoga River, which runs through Cleveland and Akron, has many impervious surfaces that create excess non-point source runoff with pollutants such as polychlorobiphenyls (PCBs), metals, and polycyclic aromatic hydrocarbons (PAHs); these, in turn, tend to accumulate in sediments and fish tissues (Balanson et. al 2005).

With the increase in population and demand of agricultural practices (grazing, plowing, generation of animal wastes, application of pesticides and fertilizers) often results in an altered physical habitat that affects benthic communities that are also affected by other anthropogenic disturbances, metals, excess nutrients, and pollution runoff (Hall et al. 2018). Although in urban areas point source pollution has decreased in recent years due to regulations, non-point sources still pose a significant threat to stream health and aquatic communities by altering food sources and stream habitats (Lang 2004). A study of urban streams surrounding the cities of Akron, Cincinnati, Columbus, Cleveland, Dayton, and Toledo showed that high-magnitude annual peak discharge

events and hydrologic disturbances (such as out-of-season flood events) negatively affected fish and macroinvertebrate communities (Coleman et al. 2011). Similarly, urbanization in Anchorage, Alaska showed that impacted areas with higher human populations, road, and storm drain densities had a higher concentration of trace elements and salts which in turn decreased macroinvertebrate diversity (Ourso 2001). A study in St. Louis County, Missouri suggested that using chloride-based road salt on roads in municipal and non-municipal environments contributed towards loss of diversity and density of benthic macroinvertebrates in local urban streams (Haake et al. 2022).

Around the world, streams in urban settings face similar problems (Strokal et al. 2021). By 2050, more than two-thirds of the global population will live in areas where fast urbanization will increase not only the use of freshwater resources (Flörke & McDonald 2018) but also competition for water between cities and agriculture (Li et al. 2019). In Jakarta, Indonesia, a rapid increase in urbanization and population with a lack of wastewater treatment plants has heavily impaired the water quality where dissolved oxygen levels are below 5 mg/L (Luo et al. 2019). In Quito, Ecuador, urban streams degraded by land use (urban and agricultural) and sewage discharge negatively impacted diversity of aquatic insects and ecological quality especially in areas with poor sanitation (Ríos-Touma et al. 2022). Comparably, urban and peri-urban streams in the Brazilian Amazon with high levels of pesticides (e.g., atrazine) posed a high ecotoxicological risk to freshwater macroinvertebrates (Rico et al. 2022).

Physico-chemical parameters

Water quality measurements are useful tools to monitor the physical, chemical, and biological characteristics of the water. This type of information can be later used by

local, state, and federal agencies as well as private companies to make decisions to improve water quality (Environmental Protection Agency [EPA] 2012a). According to the U.S. EPA, there are many purposes to monitor water quality: (1) to evaluate if waters are meeting designated uses (e.g. recreational or drinking), (2) to identify specific pollutants and sources, (3) to determine trends (spatial or temporal), and (4) to screen for potential impairments. Measuring parameters like turbidity, temperature, pH, conductivity, dissolved oxygen, nutrients, and flow are key in understanding natural and anthropogenic factors over space and time in any given stream (Hamid et al. 2019). These physico-chemical parameters can typically explain changes in water quality by studying the trends temporally and spatially (Basto Salgado et al. 2005). Along with water quality monitoring, using biomonitoring in urban areas allows for an efficient way to understand the effect urbanization has on benthic communities; benthic macroinvertebrate indices like field biotic index and tolerance values give quantitative ratings that help determining the health status of a given stream (Cuffney et al. 2005).

Fecal Coliforms

Fecal coliforms are bacteria found in the intestines of warm-blooded mammals. The presence of these bacteria in streams are used as indicators for other potential pathogens that also live in the intestines of humans and animals (Environmental Protection Agency [EPA] 2012b). Pollution of fecal coliforms in urban areas can be from storm runoff, sewage overflows, septic systems, and upstream rural sources from manure (Zhang et al.2021). For example, in the Upper Green River Basin of Kentucky, straight pipe discharges and failed septic systems have impacted the water quality making the river unsafe for fishing, swimming, and body contact due to elevated fecal coliform

concentrations (Hannan & Anmala 2021). Fecal coliforms are considered the third largest impairment of rivers and streams in North Carolina, with undetermined biological sources (Vitro et al. 2017). In Arizona and much of the southwest of the United States, where recycled waters are needed to meet the general demand of a growing population, fecal coliforms levels were found to be higher than the established water quality standards for recreational use (Sanders et al. 2013).

Fecal coliforms are typically assumed to be free-floating bacteria, but a portion of them attaches to sediment particles; the levels of bacteria are then affected by sediment transport which increases their survival time (Bai & Lung 2005). In fact, levels of fecal coliforms are affected by sediment disturbance which occurs during precipitation events, recreational activities (boating or swimming), or engineering agitation due to levee failures (Saingam et al. 2020). As established, runoff from the surrounding watershed create an increase in fecal coliform numbers, but the resuspension of sediments from the disturbance also causes even more elevated fecal numbers (Pachepsky & Shelton 2011). Thus, fecal coliform that are stored as a reservoir in streambed sediments are a significant source of bacterial pollution under different flow conditions (Jamieson et al. 2003).

Quality Habitat Evaluation Index

The Quality Habitat Evaluation Index (QHEI) was developed by the Ohio EPA to rapidly assess the macro-habitat quality using quantitative measurements that properly represent variables that affect aquatic vertebrates and invertebrates (Rankin 1989). The QHEI is used to provide a quantified evaluation, of the physical macrohabitat characteristics that affect an aquatic ecosystem (Taft & Koncelik 2006). Modifications of the stream environment, such as human activities like channel dredging and agricultural

changes in the watershed, create habitat disturbances which then affect nutrient cycles and aquatic community structure (Rankin 1989

Benthic macroinvertebrates

Benthic macroinvertebrates (benthos) are bottom dwelling aquatic organisms found in bodies of waters among rocks, vegetation, and woody debris. Benthos are useful indicators of water quality because they respond to disturbances in predictable ways, they are easy to collect and identify, and they have limited mobility (Environmental Protection Agency [EPA] 2022a). In fact, benthic macroinvertebrates are common biological indicators of stream health because their diversity depends on their sensitivity to environmental stressors (Medupin 2020). In addition, biological communities of benthic macroinvertebrates can reflect the past and current status of the stream. Some benthos species are more tolerant whereas other species require environments with optimal physico-chemical conditions (Akyildiz & Duran 2021). Indeed, the Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa (mayflies, stoneflies, and caddisflies) are the more intolerant taxa of pollution within the benthic macroinvertebrates, while Diptera and Oligochaeta are indicators of polluted areas because they are more tolerant organisms (Akamagwuna et al. 2019). Waterbodies that yield samples with a high diversity and abundance of low pollution tolerance species show better water quality. In contrast, water bodies that show samples with low diversity and pollution tolerant species have less healthy biological conditions.

Goal

The goal of this study was to assess the relationship between river water quality and the distribution and composition of benthic macroinvertebrate communities in Yellow Creek, Poland, Ohio.

Objectives

1. Monitor and relate relevant physico-chemical and biological parameters

2. Catalogue benthic macroinvertebrate communities and stream habitat quality

Study Area

Yellow Creek, a stream located in Northeastern, Ohio, is part of the Mahoning River watershed. The Mahoning River watershed extends into five counties Portage, Stark, Mahoning, Trumbull, and Columbiana in Ohio and Lawrence County in Pennsylvania (Yellow Creek WAP 2015). Yellow Creek, a small tributary (as shown in Figure 1) begins in Northeast Columbiana County and expands north into eastern Mahoning County. Yellow Creek sub-watershed has an area of 63.62 square kilometers (Yellow Creek WAP 2015). Yellow Creek flows through the Village of Poland which is located just southeast of the city of Youngstown in Northeastern, Ohio. That majority of land use in the study area of Poland, OH consists of residential-single family (Yellow Creek WAP 2015).

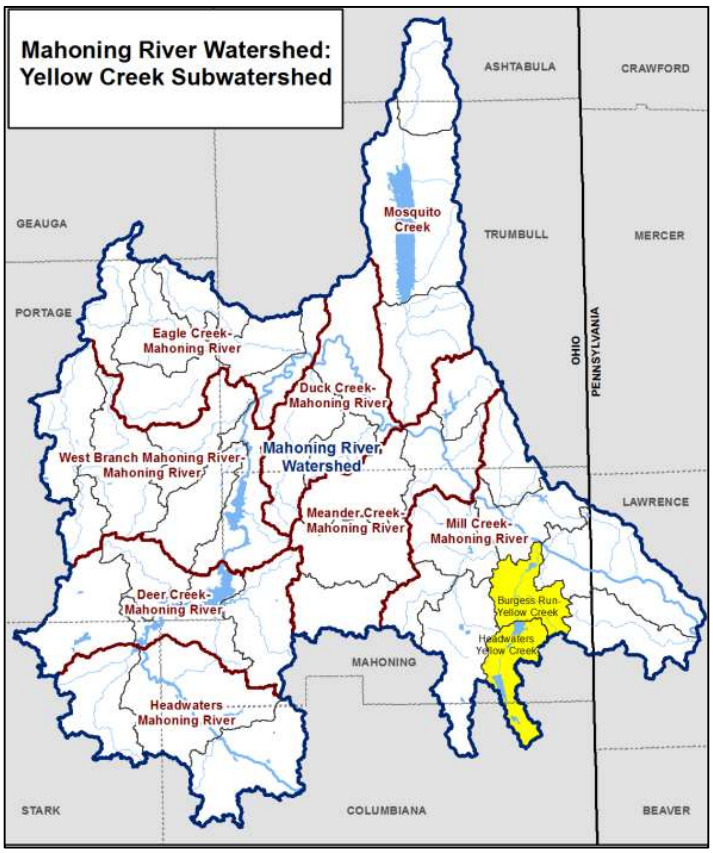

Figure 1. Yellow Creek Watershed (Natural Resources Conservation Service, United States Geological Survey, Mahoning County GIS, Trumbull County GIS, United States Census Bureau, 2013)

Sampling Sites

In this study, 3 sites were chosen along Yellow Creek within Poland, Ohio (Figure 2 -5). At each site, 4 sampling points were taken starting down stream and moving up stream in a transect of approximately 20 meters (Table 1). Site 1 Cemetery, located between a road and cemetery which had the potential for nutrient run off, had the widest stream channel and the highest current, and the riparian zone was well covered with vegetation. Site 2 Library, located behind The Municipal Poland Library in Poland, adjacent to a large parking lot and under a road bridge (Route 224). Site 2 had a storm drain flowing directly into the sampling reach, and had the lowest flow. Site 3 Woods,

located within the Poland Municipal Forest which serves as a nature sanctuary and recreational area. Site 3 had visually the best riparian zone with abundant vegetation, a very shallow stream with a normal current (Poland Municipal Forest Restoration Assessment 2019).

Table 1: Experimental design for water quality monitoring in Yellow Creek, Poland, Ohio

Season	Site	Parameters measured
Summer 21 n=8	Cemetery Library Woods	Temperature, dissolved oxygen, conductivity Flow Turbidity Depth pH Fecal coliforms QHEI Sulfate, phosphate and nitrate
Fall 21 n=6	Cemetery Library Woods	Temperature, dissolved oxygen, conductivity Flow Turbidity Depth pH Fecal coliforms QHEI Sulfate, phosphate and nitrate Benthic macroinvertebrates
Winter 22 n=5	Cemetery Library Woods	Temperature, dissolved oxygen, conductivity Flow Turbidity Depth pH Fecal coliforms QHEI Sulfate, phosphate and nitrate Benthic macroinvertebrates
Spring 22 n=5	Cemetery Library Woods	Temperature, dissolved oxygen, conductivity Flow Turbidity Depth pH Fecal coliforms QHEI Sulfate, phosphate and nitrate Benthic macroinvertebrates

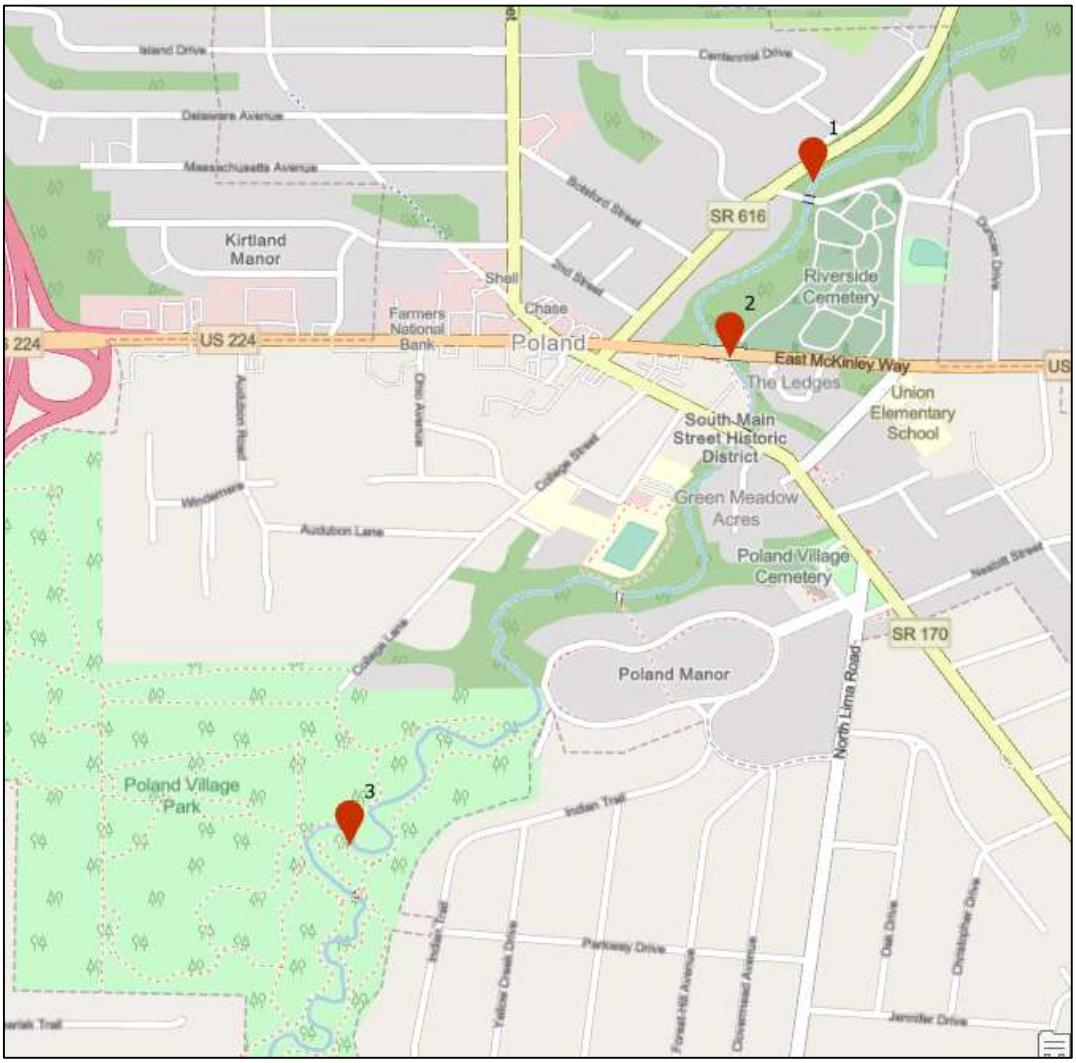

Figure 2. Sampling sites, Cemetery (1), Library (2), and Woods (3) displayed.

Figure 3. Site 1 Riverside Cemetery

Figure 4. Site 2 Poland Library

Figure 5. Site 3 Poland Woods

Methods

Physico-chemical Parameters

Samples were collected in a 125 mL Nalgene bottle that had been acid washed before use. Water was collected from mid-stream channel just below the surface of the water, transported on ice to the laboratory, and analyzed within 48 hours.

- Nitrate (measured as nitrate-nitrogen), phosphate (measured as low range phosphate), and sulfate were all measured in parts per million using nutrient kits (LaMotte, Chestertown, Maryland). Five or ten mL of water was mixed with the assigned reagents for a colorimetric assay following manufacturers' instructions.
- Velocity. A FP111-FP211 Global Flow Probe (Global Water, Gold River, California) was used to measure average velocity of the stream (km/hr).
- Temperature (°C), dissolved oxygen (DO mg/L), and conductivity (mS/cm) were measured in the field using a multiparameter probe (Model 2030 Pro, YSI, Yellow Springs, Ohio).
- Depth (cm) was measured using a meter stick from mid-stream channel.
- Turbidity was determined by a Secchi tube in cm (Thomas Scientific, Swedesboro, NJ). Water was collected from mid-stream channel just below the surface following manufacturers' instructions.

Total Suspended Solids

The Standard Method 2540 D Total Suspended Solids (APHA, 1999) was followed (Appendix A). Samples were collected in 500 mL or 1000 mL Nalgene bottles depending on the season and water turbidity. Before collecting samples, bottle was rinsed 3 times with sample

water. Samples were immediately placed on ice in a cooler, transferred to the laboratory at YSU and stored at 4 °C until further analyses. Glass microfiber filters -47mm- (Whatman, Marlborough, Massachusetts) were prepared for filtration by rinsing them with 20 mL of reagent water 3 times. They were then dried in an oven at 103-105 °C for 1 hour and cooled in a desiccator. A heating and cooling cycle continued until there was a balanced weight change of less than 4%. On the day of the analysis, a dried filter was placed on a funnel apparatus and rinsed with a small amount of milli-Q water. Water samples collected in Nalgene bottles were allowed to equilibrate to room temperature, mixed for 10 seconds, and then transferred to the funnel. The sample bottle was rinsed twice with 10 mL of milli-Q water, and the funnel was rinsed once with the same milli-Q water. Filters were then placed in a drying oven for 103-105 °C for 1 hour in a heating and cooling cycle until there was a less than 4% weight change. Determination of suspended solids were calculated using the following formula:

mg total suspended solids/L = [(A-B) *1000]/ (sample volume in mL)

where:

A: weight of the filter + dried residue, mg

B: weight of filter, mg

Fecal Coliforms

The 9222 B Standard Total Coliform Membrane Filter Procedure (APHA 1999) was followed (Appendix B). All supplies and glassware were autoclaved before analyses. Samples were collected using a 125 mL Nalgene bottle. After collection, water samples were immediately placed on ice in a cooler, transferred to the laboratory and kept at 4 °C. Samples were analyzed within 24 hours of the collection time. Membrane Filters (Millipore, Tullagreen, Carrigtwohill, County Cork, Ireland.), 47-mm, 0.45-µm (gridded) were placed on the porous part of the filter

funnel with the grid portion facing upwards. The chosen sample amount (either 100 ml or 10 ml) was measured in a graduated cylinder then transferred to the filter funnel. The funnel sides were rinsed 3 times with 10 mL of autoclaved water. After completed filtration, filters were removed and rolled onto a mFC plate of containing a rosolic acid medium which inhibits bacteria growth except for fecal coliforms (Aulenbach 2009). Samples were then incubated for 24 ± 2 hours at 44.5 ± 0.2°C. After incubation, colonies (blue) were counted using a microscope at 10 to 15 magnifications and white fluorescent light source.

Qualitative Habitat Evaluation Index

QHEI was determined by visual observations and pictures, then catalogued according to the QHEI field sheet (Appendix C). Different metrics involving substrate, coverage, channel morphology, erosion, riffle/pool quality, and gradient, were computed and reported with a score out of 100. The Yellow Creek Watershed Action Plan (2015) states that the watershed is approximately 63.62 square kilometers. Ranking of water habitat health was determined as follows in table:

Table 2. QHEI narrative ranges from the Ohio EPA for larger streams (≥ 20 sq. mi).

Rating	QHEI Range for Larger Streams
Excellent	≥75
Good	60 to 74
Fair	45 to 59
Poor	30 to 44
Very Poor	< 30

Macroinvertebrate Benthic Sampling

Benthic macroinvertebrates were collected using a 0.3 x 0.3 m Surber net sampler with a 500 µm net was used to collect organisms from stream bottom. Sediment, rocks, and leaves were dislodged by hand for 30 seconds to disturb organisms into Surber that was sitting perpendicular to flow. After collection, samples were placed into a Ziploc bag on ice in a cooler. In the laboratory, organisms were sorted and stored in 70% ethanol until they were identified to the family level using taxonomic keys Peckarsky et al. (1990), Merritt and Cummings (1996). The total number of benthic samples taken at each site during the extent of the study is displayed in Table 3.

Table 3. Sampling design for benthic macroinvertebrates

Site	Water depth (cm)	Sampling method	Fall 2021	Winter 2022	Spring 2022	Total
Cemetery	13-56	Surber	16	16	16	48
Library	12-50	Surber	16	16	16	48
Woods	9-47	Surber	16	16	16	48

Data Analysis

Physico-chemical and biological parameters were analyzed using three approaches:

1. For water quality parameters, a two-way Multiple ANOVA (Analysis of Varianc test (Type III sum of squares and a significance level of 0.05) and Pearson correlation were performed.
2. For benthic community structure, one-way Multiple ANOVA (Analysis of Variance) test (Type III sum of squares and a significance level of 0.05), Pearson correlation, and non-metric multidimensional scaling (NMDS) coordination plot analysis were performed.

3. Different indexes were used to evaluate the benthic community structure
    a. Total invertebrate density expressed as number of organisms per m^2
    b. EPT richness (Ephemeroptera [mayfly], Plecoptera [stonefly], Trichoptera [caddisfly]) expressed as number of these three families per sample
    c. Community diversity index (Shannon-Weiner H') determined by the following formula:

$$H' = -\sum_{i=1}^{s}[(p_i)\ (log_2 p_i)$$

Where:

H = Index of species diversity

s = Number of species

p_i = Proportion of total sample belonging to the i^{th} species

    d. Hilsenhoff Field Biotic Index (shown in Table 4) was used to measure macroinvertebrates' responses to changes in dissolved oxygen levels.

Table 4. Ranking of the field biotic index for water quality and degree of organic pollution (Hilsenhoff 1987).

FBI	Water Quality	Degree of Organic Pollution
0.00 – 3.50	Excellent	No apparent organic pollution
3.51 – 4.50	Very Good	Possible slight organic pollution
4.51 – 5.50	Good	Some organic pollution
5.51 – 6.50	Fair	Fairly significant organic pollution
6.51 – 7.50	Fairly Poor	Significant organic pollution
7.51 – 8.50	Poor	Very significant organic pollution
8.51 – 10.00	Very Poor	Severe organic pollution

The field biotic index was calculated using the formula:

$$FBI = \frac{\sum[(TV_i)(n_i)]}{N}$$

Where:

TV_i = Tolerance value for each family (Soil & Water Conservation Society of Metro Halifax 2004)

n_i = The number of individuals in the family

N = The total number of individuals in the collection

Results

Physico-Chemical Parameters

Several physico-chemical parameters showed notable variation seasonally and modest variation spatially (Table 5) showing a dynamic system over time while other parameters remained more stable.

Lower water temperatures were measured during the winter season with the lowest temperatures recorded as 3 ℃ while higher water temperatures were recorded during the summer season with highest temperatures of 21.5 ℃.

Measured pH values did not vary much seasonally with values ranging from 7.87 to 8.11. Changes in dissolved oxygen (DO) showed an inverse relationship with temperature. The variability of dissolved oxygen between sites may suggested that site 3-Woods had some oxygen demand, but not enough to be a stressor. Concentrations were found to be the lowest in the summer ranging from 6.4 to 8.2 mg/L, and they were found to be the highest during the winter ranging from 13.6 to 13.8 mg/L.

Conductivity values were reported higher during summer with a value of 0.8 to 1.0 mS/cm, and lower during fall with a value of 0.5 mS/cm. The higher conductivity during the summer suggested dissolved solids from run off or biological activity. (Hanafiah et al. 2018)

Total Suspended Solids varied depending on precipitation events, but the summer had the highest values between 9.7 to 11.3 mg/L. The winter season had the lowest values ranging from 4.1 to 4.3 mg/L.

Fecal coliform numbers per 10 mL were the highest during fall ranging from 17 to 19 colonies, and lowest during the winter ranging from 3 to 4 colonies. Fecal coliforms were also affected after precipitation events showing an increase in the number of colonies.

Nitrate was characterized to be highest in the summer with value of 0.7 ppm, and lowest in the winter ranging from 0.3 to 0.4 ppm. Sulfate concentrations ranged from 168 to 173ppm in the spring but were much lower during the winter ranging from 8.5 to 14.3 ppm. Phosphate had the higher values in summer ranging from 0.3 to 0.4 ppm while fall, winter, and spring had similar values ranging from 0.1 to 0.2 ppm.

Table 5. Physico-chemical and biological parameters of Yellow Creek, OH (sampling period 2021-2022) Mean in left-hand column and standard deviation in right-hand

Season	Site	Temperature °C		pH		Dissolved O2 mg/L		Conductivity mS/cm		Suspended Solids mg/L		Fecal Coliforms per 10 mL	
Summer 21	Cemetery	21.6	1.8	8.03	0.08	8.2	0.4	1.0	0.6	9.7	8.5	11.4	2.9
	Library	21.3	1.4	7.95	0.09	7.5	0.6	0.8	0.2	11.3	8.7	10.6	3.7
	Woods	21.4	1.5	7.87	0.11	6.4	0.8	0.8	0.2	10.0	7.6	11.5	3.4
Fall 21	Cemetery	9.2	5.5	8.03	0.06	13.1	2.9	0.5	0.1	4.4	2.4	19.0	4.8
	Library	9.4	5.6	8.03	0.05	12.9	3.0	0.5	0.1	4.9	2.9	18.1	5.5
	Woods	9.2	5.6	8.00	0.06	11.8	2.6	0.5	0.1	4.4	3.0	17.1	6.3
Winter 22	Cemetery	2.9	3.1	8.04	0.05	13.8	1.2	0.7	0.2	4.1	2.2	3.2	2.9
	Library	3.1	3.2	8.03	0.05	13.6	1.0	0.6	0.1	4.2	2.1	3.8	3.7
	Woods	3.3	3.2	8.02	0.04	13.6	1.1	0.6	0.1	4.3	2.3	3.7	3.6
Spring 22	Cemetery	15.6	5.4	8.11	0.13	11.6	1.9	0.8	0.1	7.4	2.9	12.9	1.9
	Library	16.2	5.4	8.08	0.09	11.5	2.1	0.8	0.1	7.6	3.1	12.1	2.7
	Woods	16.1	4.8	8.06	0.05	10.2	2.2	0.8	0.2	8.0	3.1	12.2	2.6

Season	Site	Nitrate (NO_3-N) ppm		Sulfate (SO_4^{2-}) ppm		Phosphate (PO_4^{3-}) ppm	
Summer 21	Cemetery	0.7	0.1	45.0	8.3	0.3	0.1
	Library	0.7	0.1	52.9	9.6	0.4	0.1
	Woods	0.7	0.1	47.9	6.5	0.4	0.1
Fall 21	Cemetery	0.5	0.1	8.5	6.7	0.2	0.1
	Library	0.4	0.2	14.3	6.6	0.1	0.1
	Woods	0.4	0.2	13.4	6.8	0.1	0.0
Winter 22	Cemetery	0.3	0.1	28.0	22.9	0.2	0.1
	Library	0.3	0.1	33.0	20.4	0.2	0.1
	Woods	0.4	0.2	37.0	30.8	0.2	0.1
Spring 22	Cemetery	0.6	0.1	170.0	38.1	0.2	0.1
	Library	0.5	0.1	173.0	37.1	0.1	0.1
	Woods	0.6	0.1	168.0	47.2	0.2	0.1

Physico-chemical and biological parameters seemed to follow the same pattern among the three sites, Cemetery, Library, and Woods (Figure 6 and 7). However, a two-way Multiple ANOVA (Table 6), showed that season had a significant effect on physico-chemical and biological parameters, but site had borderline significance ($p=0.054$). Univariate ANOVA of the individual parameters (within the Tests of Between-Subjects Effects) showed statistical significance only for season (Table 7). Thus, Post hoc test (Table 8) revealed that higher significance was found for dissolved oxygen, temperature, nitrate, sulfate, phosphate, and fecal coliforms while pH and suspended solids had the lowest significant differences among the parameters. In contrast, site did not have an effect on water quality parameters ($p>0.05$).

A correlation matrix was performed to establish correlation between the water quality parameters measured (Table 9). Temperature was inversely correlated with dissolved oxygen ($r=0.912$, $p<0.001$) and directly with nitrate ($r=0.785$, $p<0.001$). Dissolved oxygen was inversely correlated with nitrate ($r=-0.733$, $p<0.001$).

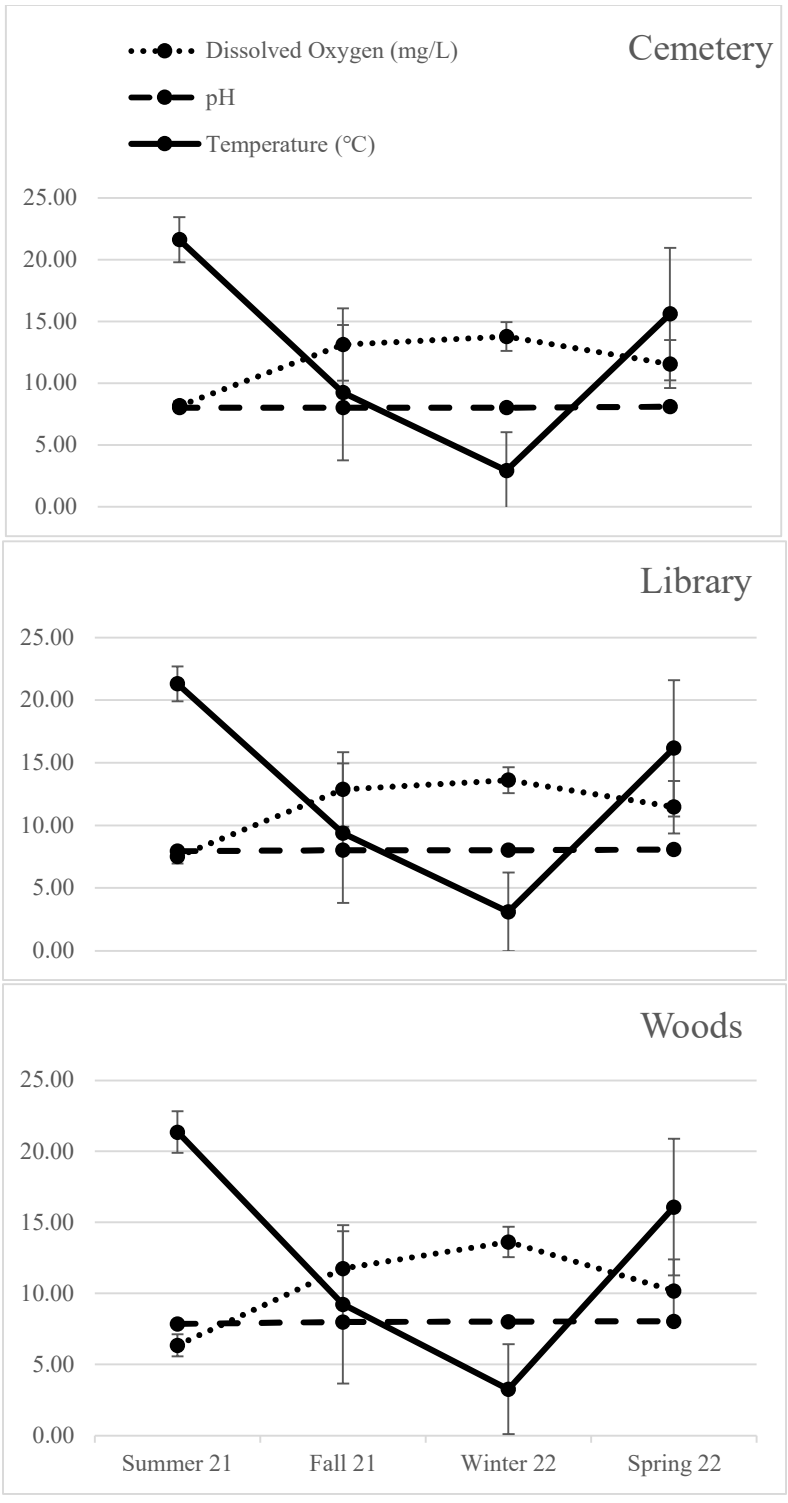

Figure 6. Mean values and standard deviation of dissolved oxygen, pH, and temperature at each sampling site.

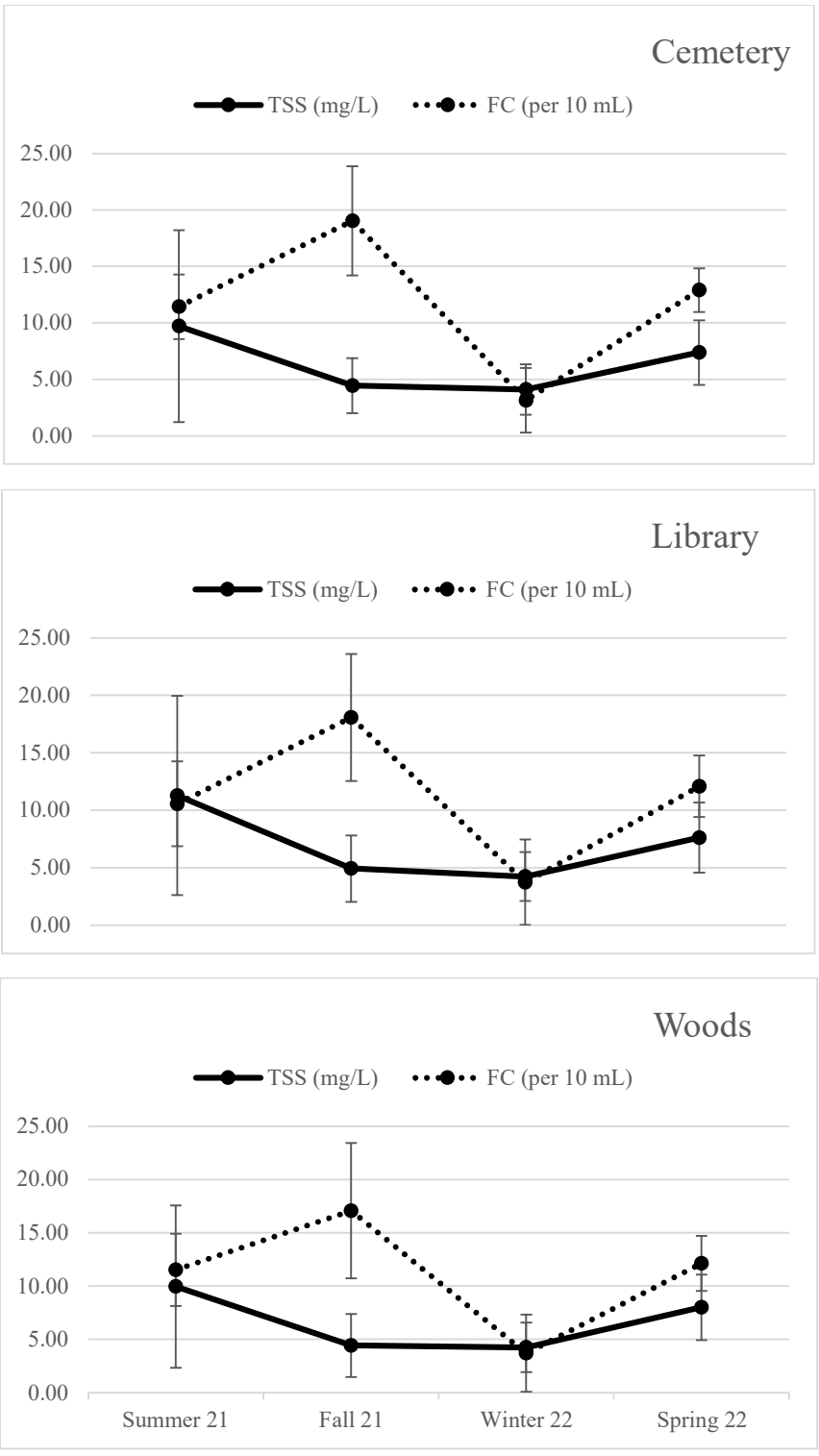

Figure 7. Mean and standard deviation values of total suspended solids (TSS) and fecal coliforms (FC) measured at each sampling site.

Table 6. Two-way MANOVA considering the site and season as factors for physico-chemical and biological parameters in Yellow Creek, OH showing the F test statistic and degrees of freedom.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1	86559.463b	9	49	<.001
	Wilks' Lambda	0	86559.463b	9	49	<.001
	Hotelling's Trace	15898.677	86559.463b	9	49	<.001
	Roy's Largest Root	15898.677	86559.463b	9	49	<.001
site	Pillai's Trace	0.439	1.56	18	100	0.086
	Wilks' Lambda	0.582	1.690b	18	98	0.054
	Hotelling's Trace	0.681	1.816	18	96	0.034
	Roy's Largest Root	0.623	3.462c	9	50	0.002
season	Pillai's Trace	2.547	31.892	27	153	<.001
	Wilks' Lambda	0.003	35.412	27	143.748	<.001
	Hotelling's Trace	20.523	36.232	27	143	<.001
	Roy's Largest Root	9.947	56.366c	9	51	<.001
site * season	Pillai's Trace	0.383	0.409	54	324	1
	Wilks' Lambda	0.662	0.397	54	254.446	1
	Hotelling's Trace	0.445	0.39	54	284	1
	Roy's Largest Root	0.229	1.377c	9	54	0.222

Table 7. Tests of Between-Subjects Effects for physicochemical parameters with site and season of Yellow Creek, OH showing the F test statistic and degrees of freedom.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	DO	442.331a	11	40.212	9.745	<.001
	cond	2.083b	11	0.189	2.645	0.008
	temp	3236.417c	11	294.22	14.726	<.001
	pH	.217d	11	0.02	2.696	0.007
	TSS	426.045e	11	38.731	1.179	0.322
	colonies	1760.676f	11	160.061	8.544	<.001
	Nitrate	1.664g	11	0.151	5.48	<.001
	Sulfate	238393.189h	11	21672.108	32.668	<.001
	Phosphate	.747i	11	0.068	6.229	<.001
Intercept	DO	8477.016	1	8477.016	2054.397	<.001
	cond	33.435	1	33.435	467.132	<.001
	temp	10347.896	1	10347.896	517.911	<.001
	pH	4354.517	1	4354.517	596340.17	<.001
	TSS	2974.229	1	2974.229	90.566	<.001
	colonies	8647.612	1	8647.612	461.6	<.001
	Nitrate	16.779	1	16.779	607.683	<.001
	Sulfate	292345.69	1	292345.69	440.678	<.001
	Phosphate	3.077	1	3.077	282.32	<.001
site	DO	16.004	2	8.002	1.939	0.153
	cond	0.069	2	0.035	0.484	0.619
	temp	0.682	2	0.341	0.017	0.983
	pH	0.042	2	0.021	2.886	0.064
	TSS	0.079	2	0.04	0.001	0.999
	colonies	4.377	2	2.189	0.117	0.890
	Nitrate	0.028	2	0.014	0.514	0.601
	Sulfate	218.079	2	109.04	0.164	0.849
	Phosphate	0.005	2	0.003	0.25	0.780
season	DO	419.956	3	139.985	33.925	<.001
	cond	1.797	3	0.599	8.371	<.001
	temp	3235.146	3	1078.382	53.973	<.001
	pH	0.134	3	0.045	6.136	0.001
	TSS	421.174	3	140.391	4.275	0.009
	colonies	1741.386	3	580.462	30.984	<.001
	Nitrate	1.579	3	0.526	19.068	<.001
	Sulfate	237982.36	3	79327.454	119.577	<.001
	Phosphate	0.699	3	0.233	21.385	<.001

Table 7. Continued

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
site * season	DO	4.504	6	0.751	0.182	0.981
	cond	0.189	6	0.032	0.441	0.848
	temp	0.722	6	0.12	0.006	1.000
	pH	0.031	6	0.005	0.7	0.651
	TSS	4.707	6	0.785	0.024	1.000
	colonies	14.18	6	2.363	0.126	0.993
	Nitrate	0.057	6	0.01	0.346	0.909
	Sulfate	195.326	6	32.554	0.049	0.999
	Phosphate	0.043	6	0.007	0.663	0.680
Error	DO	235.198	57	4.126		
	cond	4.08	57	0.072		
	temp	1138.864	57	19.98		
	pH	0.416	57	0.007		
	TSS	1871.898	57	32.84		
	colonies	1067.837	57	18.734		
	Nitrate	1.574	57	0.028		
	Sulfate	37813.796	57	663.4		
	Phosphate	0.621	57	0.011		
Total	DO	8917.328	69			
	cond	41.033	69			
	temp	16028.483	69			
	pH	4435.854	69			
	TSS	5525.534	69			
	colonies	12103.875	69			
	Nitrate	21.4	69			
	Sulfate	540161.14	69			
	Phosphate	4.827	69			
Corrected Total	DO	677.529	68			
	cond	6.163	68			
	temp	4375.281	68			
	pH	0.633	68			
	TSS	2297.943	68			
	colonies	2828.513	68			
	Nitrate	3.238	68			
	Sulfate	276206.99	68			
	Phosphate	1.368	68			

Table 8. Post Hoc test showing the significant values (less than 0.05) among the physico-chemical parameters for each season (summer 1, fall 2, winter 3, and spring 4).

Dependent	(I) season	(J) season	Mean Difference (I-J)	Std. Error	Sig.
DO	1	2	-5.15726*	0.63941	<.001
		3	-6.24460*	0.67296	<.001
		4	-3.63643*	0.67296	<.001
	2	1	5.15726*	0.63941	<.001
	3	1	6.24460*	0.67296	<.001
		4	2.60817*	0.72688	0.004
	4	1	3.63643*	0.67296	<.001
		3	-2.60817*	0.72688	0.004
COND	1	2	.40107*	0.08324	<.001
		3	.27674*	0.08761	0.014
	2	1	-.40107*	0.08324	<.001
		4	-.30257*	0.0906	0.008
	3	1	-.27674*	0.08761	0.014
	4	2	.30257*	0.0906	0.008
TEMP	1	2	11.83968*	1.34527	<.001
		3	18.02524*	1.41585	<.001
		4	5.17857*	1.41585	0.003
	2	1	-11.83968*	1.34527	<.001
		3	6.18556*	1.46419	<.001
		4	-6.66111*	1.46419	<.001
	3	1	-18.02524*	1.41585	<.001
		2	-6.18556*	1.46419	<.001
		4	-12.84667*	1.52929	<.001
	4	1	-5.17857*	1.41585	0.003
		2	6.66111*	1.46419	<.001
		3	12.84667*	1.52929	<.001
DEPTH	1	3	-11.19833*	1.97046	<.001
		4	-9.70000*	1.97046	<.001
	2	3	-6.47333*	2.03774	0.014
	3	1	11.19833*	1.97046	<.001
		2	6.47333*	2.03774	0.014
	4	1	9.70000*	1.97046	<.001
PH	1	4	-.12276*	0.0296	<.001
	4	1	.12276*	0.0296	<.001
TSS	1	2	5.44901*	1.72598	0.014
		3	5.84429*	1.81654	0.012
	2	1	-5.44901*	1.72598	0.014
	3	1	-5.84429*	1.81654	0.012

Table 8. continued

Dependent Variable	(I) season	(J) season	Mean Difference (I-J)	Std. Error	Sig.
FC	1	2	-6.83135*	1.31362	<.001
		3	7.70476*	1.38255	<.001
	2	1	6.83135*	1.31362	<.001
		3	14.53611*	1.42974	<.001
		4	5.68611*	1.42974	0.001
	3	1	-7.70476*	1.38255	<.001
		2	-14.53611*	1.42974	<.001
		4	-8.85000*	1.49332	<.001
	4	2	-5.68611*	1.42974	0.001
		3	8.85000*	1.49332	<.001
NITRATE	1	2	.25317*	0.05131	<.001
		3	.40762*	0.05401	<.001
	2	1	-.25317*	0.05131	<.001
		3	.15444*	0.05585	0.044
	3	1	-.40762*	0.05401	<.001
		2	-.15444*	0.05585	0.044
		4	-.27000*	0.05833	<.001
		3	.27000*	0.05833	<.001
SULFATE	1	2	35.76548*	7.78935	<.001
		4	-122.47619*	8.19806	<.001
	2	1	-35.76548*	7.78935	<.001
		4	-158.24167*	8.47793	<.001
	3	4	-137.66667*	8.85491	<.001
	4	1	122.47619*	8.19806	<.001
		2	158.24167*	8.47793	<.001
		3	137.66667*	8.85491	<.001
PHOSPHATE	1	2	.22976*	0.03258	<.001
		3	.16810*	0.03429	<.001
		4	.23476*	0.03429	<.001
	2	1	-.22976*	0.03258	<.001
	3	1	-.16810*	0.03429	<.001
	4	1	-.23476*	0.03429	<.001

Table 9. Correlation matrix for physico-chemical parameters (dissolved oxygen, conductivity, temperature, pH, total suspended solids, nitrate, sulfate, and phosphate) and microbial indicator (fecal coliforms) measure in Yellow Creek, OH.

		DO	COND	TEMP	pH	TSS	FC	Nitrate	Sulfate	Phosphate
DO	Pearson Correlation	1	-.358**	-.912**	.296*	-.380**	-.339**	-.733**	-0.17	-.532**
	Sig. (2-tailed)		0.003	<.001	0.013	0.001	0.004	<.001	0.162	<.001
	N	69	69	69	69	69	69	69	69	69
COND	Pearson Correlation	-.358**	1	.412**	0	0.218	-0.145	0.215	.262*	0.15
	Sig. (2-tailed)	0.003		<.001	0.999	0.071	0.233	0.076	0.03	0.217
	N	69	69	69	69	69	69	69	69	69
TEMP	Pearson Correlation	-.912**	.412**	1	-0.106	.439**	.407**	.785**	.388**	.430**
	Sig. (2-tailed)	<.001	<.001		0.387	<.001	<.001	<.001	<.001	<.001
	N	69	69	69	69	69	69	69	69	69
pH	Pearson Correlation	.296*	0	-0.106	1	-.274*	-0.116	-0.219	.300*	-.310**
	Sig. (2-tailed)	0.013	0.999	0.387		0.023	0.341	0.07	0.012	0.01
	N	69	69	69	69	69	69	69	69	69
TSS	Pearson Correlation	-.380**	0.218	.439**	-.274*	1	0.051	.488**	0.127	.417**
	Sig. (2-tailed)	0.001	0.071	<.001	0.023		0.679	<.001	0.3	<.001
	N	69	69	69	69	69	69	69	69	69
FC	Pearson Correlation	-.339**	-0.145	.407**	-0.116	0.051	1	.425**	0.018	-0.128
	Sig. (2-tailed)	0.004	0.233	<.001	0.341	0.679		<.001	0.882	0.295
	N	69	69	69	69	69	69	69	69	69
Nitrate	Pearson Correlation	-.733**	0.215	.785**	-0.219	.488**	.425**	1	.251*	.480**
	Sig. (2-tailed)	<.001	0.076	<.001	0.07	<.001	<.001		0.037	<.001
	N	69	69	69	69	69	69	69	69	69
Sulfate	Pearson Correlation	-0.17	.262*	.388**	.300*	0.127	0.018	.251*	1	-0.192
	Sig. (2-tailed)	0.162	0.03	<.001	0.012	0.3	0.882	0.037		0.113
	N	69	69	69	69	69	69	69	69	69
Phosphate	Pearson Correlation	-.532**	0.15	.430**	-.310**	.417**	-0.128	.480**	-0.192	1
	Sig. (2-tailed)	<.001	0.217	<.001	0.01	<.001	0.295	<.001	0.113	
	N	69	69	69	69	69	69	69	69	69

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Quantitative Habitat Evaluation Index

QHEI was monitored at each site during the 4 seasons as shown in Table 10. The results for each site are very consistent through all 4 sampling seasons, which was to be expected. The Woods had the highest QHEI score of 61.5 during fall 2021 sampling, and Library had the lowest score of 41.6 during winter 2022 sampling. The Cemetery had a stable score with a range between 53.1 to 53.4 throughout the sampling period.

Table 10. QHEI scores based on site and season with the mean in the left-hand column and the standard deviation in the right-hand.

Season	Site	QHEI	
Summer 21	Cemetery	53.3	0.4
	Library	42.0	0.0
	Woods	61.0	0.0
Fall 21	Cemetery	53.4	0.2
	Library	41.8	0.4
	Woods	61.5	0.5
Winter 22	Cemetery	53.1	0.5
	Library	41.6	0.5
	Woods	60.8	0.8
Spring 22	Cemetery	53.4	0.2
	Library	42.0	0.0
	Woods	61.2	0.4

Benthic Community Structure

During the length of the study 8 benthic macroinvertebrate orders and 10 families were identified and catalogued in the 3 sites along Yellow Creek during the seasons of fall 2021, winter 2022, and spring 2022 (Table 11). The most abundant family collected was Chironomidae, followed by Heptageniidae (mayflies) and Hydropsychidae (caddisflies). Oligochaetes were only recorded once throughout the sampling period at the Cemetery during fall 2021. Also, Decapoda was rarely catalogued only during the Fall 2021 at the Cemetery and Library and during spring 2022 at the Library site.

Density (number of organisms per m^2), Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, Shannon Weiner Index (H'), and the Field Biotic Index (FBI) were calculated as shown in Figure 8. A one-way multiple ANOVA test, showed a significant site*season interaction for EPT, density, and H' ($p = < 0.001$) for each index (Table 12, 13). FBI was not included in this test because it did not show significance for the site*season interaction. Similarly, a correlation

matrix was performed to view the relatedness of each index (Table 14). EPT richness highly correlated with H' diversity ($r=0.876$, $p=0.002$) and density ($r=0.784$, $p=0.012$).

NMDS ordination analyses for the benthic macroinvertebrate abundance revealed some clustering according to season (Figure 9). Fall-Library (F2) and Spring-Library (S2) communities clustered for the winter due to their lower abundance compared to the other 2 sites during those same seasons. Overall, Fall-Cemetery and Fall-Woods benthic communities showed the highest abundance of macroinvertebrates while Winter-Library had the lowest.

Table 11. Abundance of family of macroinvertebrates catalogued per site during the sampling period of the study in Yellow Creek. Samples were collected 4 times each season for fall 2021, winter 2022, and spring 2022.

		Order	Coleoptera	Diptera		Ephemeroptera				Plecoptera	Tricoptera	Oligochaeta	Veneroida	Decapoda
		Family	Elmidae	Chironomidae	Simuliidae	Ephemerellidae	Heptageniidae	Baetidae	Caenidae	Perlidae	Hydropsychidae		Corbiculidae	
Season	Site													
Fall 21	Cemetery		24	237	7	10	65	7	1	7	48	1	44	2
Fall 21	Library		1	55	3	0	1	1	0	0	7	0	33	1
Fall 21	Woods		58	247	1	21	68	17	11	3	53	0	56	0
Winter 22	Cemetery		23	28	1	0	16	2	3	0	47	0	8	0
Winter 22	Library		1	0	0	0	0	0	0	0	0	0	3	0
Winter 22	Woods		15	23	0	2	10	9	6	0	37	0	8	0
Spring 22	Cemetery		87	110	2	15	53	11	14	5	33	0	7	0
Spring 22	Library		14	36	3	6	25	9	10	0	0	0	9	2
Spring 22	Woods		58	114	1	26	45	23	9	5	31	0	6	0

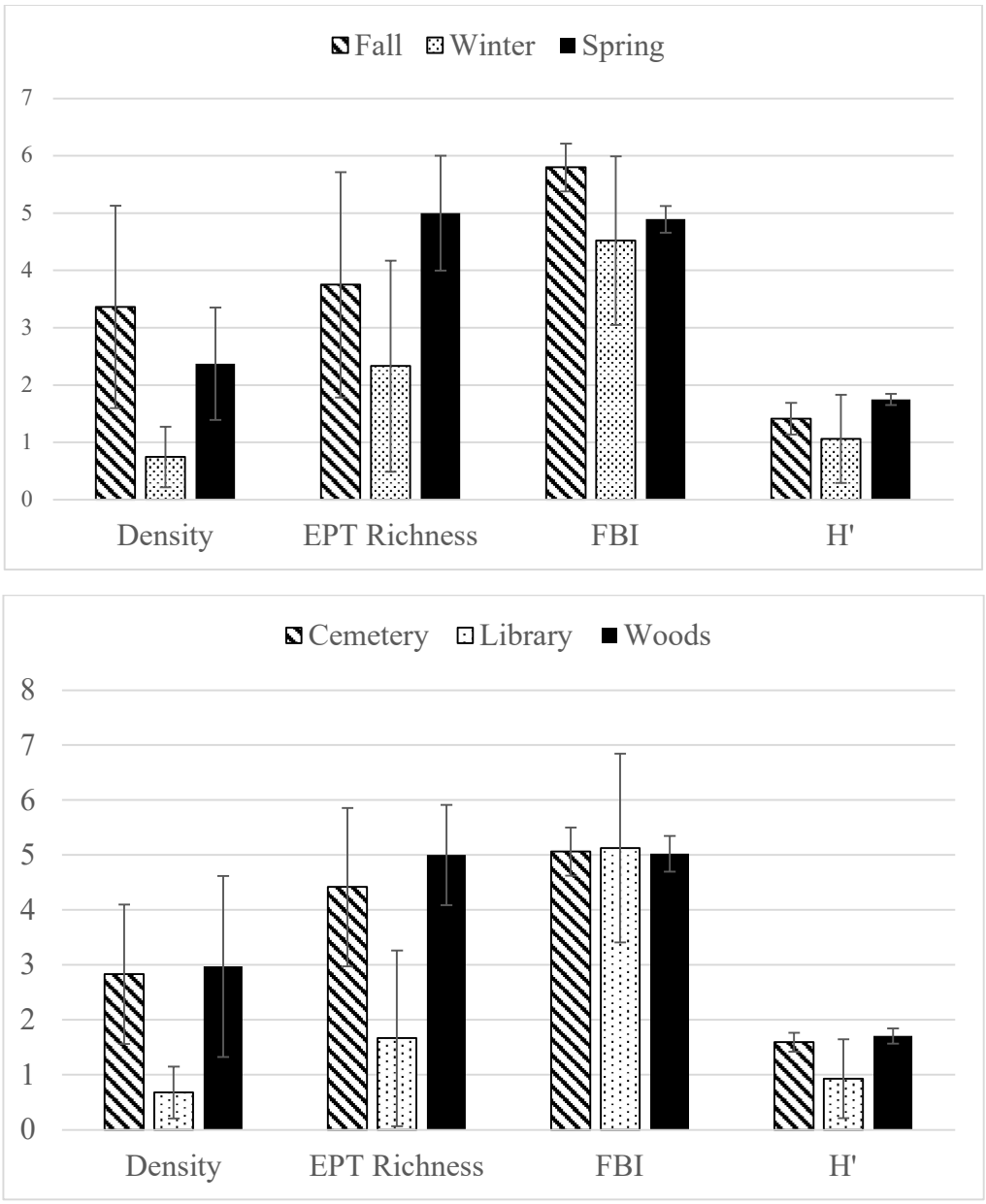

Figure 8. Benthic macroinvertebrate community structure shown as organism density (per 0.01 m^2), EPT family richness (Ephemeroptera, Plecoptera, Trichoptera), family diversity (Shannon-Weiner H'), and FBI Hilsenhoff Field Biotic Index. Mean and standard deviation values are display

Table 12. Two-way MANOVA considering the site and season as factors for benthic macroinvertebrate indices in Yellow Creek, OH showing the F test statistic and degrees of freedom.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	0.998	2831.910b	4	24	<.001
	Wilks' Lambda	0.002	2831.910b	4	24	<.001
	Hotelling's Trace	471.985	2831.910b	4	24	<.001
	Roy's Largest Root	471.985	2831.910b	4	24	<.001
season	Pillai's Trace	1.828	66.562	8	50	<.001
	Wilks' Lambda	0.004	91.076b	8	48	<.001
	Hotelling's Trace	42.94	123.452	8	46	<.001
	Roy's Largest Root	37.063	231.641c	4	25	<.001
site	Pillai's Trace	1.073	7.237	8	50	<.001
	Wilks' Lambda	0.03	28.600b	8	48	<.001
	Hotelling's Trace	28.822	82.863	8	46	<.001
	Roy's Largest Root	28.702	179.390c	4	25	<.001
season * site	Pillai's Trace	2.011	6.821	16	108	<.001
	Wilks' Lambda	0.004	22.524	16	73.959	<.001
	Hotelling's Trace	42.417	59.649	16	90	<.001
	Roy's Largest Root	39.037	263.499c	4	27	<.001

Table 13. One-way Multiple ANOVA considering the site*season interaction for Benthic Macroinvertebrate Indices of Yellow Creek, OH. FBI is not listed because it was not significant for the interaction.

	Parameter	Sum of Squares	df	Mean Square	F	Sig.
EPT	Between Groups	128.056	8	16.007	45.493	<.001
	Within Groups	9.5	27	0.352		
	Total	137.556	35			
Diversity	Between Groups	10.785	8	1.348	84.542	<.001
	Within Groups	0.431	27	0.016		
	Total	11.215	35			
Density	Between Groups	66714.22	8	8339.278	217.757	<.001
	Within Groups	1034	27	38.296		
	Total	67748.22	35			

Table 14. Correlation Matrix between Benthic Macroinvertebrate Indices for Yellow Creek, OH.

		EPT	DENSITY	FBI	DIVERSITY
EPT	Pearson Correlation	1	.784*	0.111	.876**
	Sig. (2-tailed)		0.012	0.777	0.002
	N	9	9	9	9
DENSITY	Pearson Correlation	.784*	1	0.345	0.546
	Sig. (2-tailed)	0.012		0.363	0.128
	N	9	9	9	9
FBI	Pearson Correlation	0.111	0.345	1	0.322
	Sig. (2-tailed)	0.777	0.363		0.398
	N	9	9	9	9
DIVERSITY	Pearson Correlation	.876**	0.546	0.322	1
	Sig. (2-tailed)	0.002	0.128	0.398	
	N	9	9	9	9

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

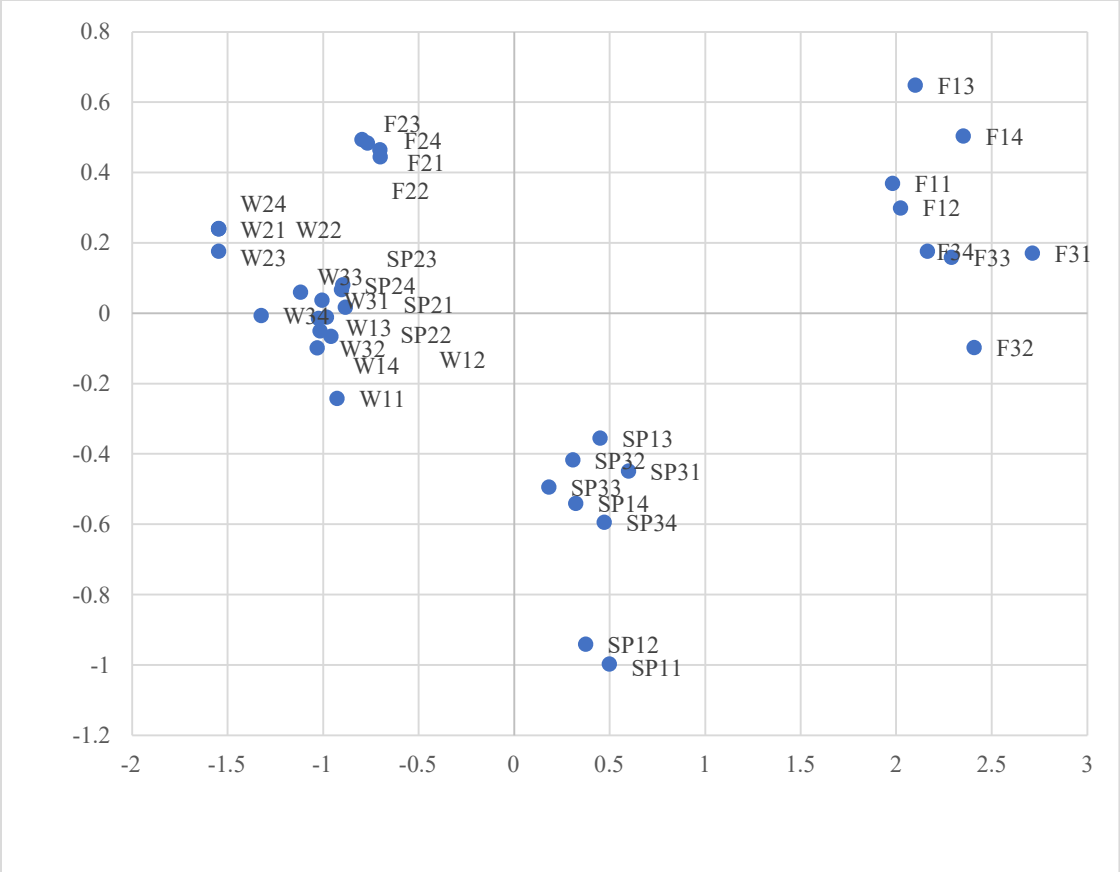

Figure 9. NMDS ordination plots of the mean of benthic macroinvertebrates shown per date of Fall 21 (F), Winter 22 (W), and Spring 22 (S). Site is also shown as Cemetery (1), Library (2), and Woods (3). Sampling date is represented as 1 through 4, indicating 4 benthic sampling events per season.

Discussion

This study examined the distribution of benthic macroinvertebrate communities and assessed water quality in Yellow Creek, Poland, Ohio with the aim to evaluate the environmental health of the stream to better understand water management issues in this urban stream.

Relationships between environmental variables and ecological indicators such as benthic macroinvertebrates in urban rivers could reveal not only stream health, but also might point to stressors affecting water quality.

Water quality and stream health

Study sites were selected based on proximity to potential pollution points to see if there was a difference in water quality and stream health based on a spatial and temporal scale for an urban stream.

The sites were easily accessible which allowed for frequent sampling during all 4 seasons. This study showed that there was no significance between sites for physico-chemical parameters, but there was a marked effect based on seasonality. In general, water temperature, pH, dissolved oxygen, total suspended solids, phosphate, and fecal coliform from the 3 sites followed a common pattern displaying a temporal variability of the parameters marked by seasonality. Temperature and dissolved oxygen had an inverse relationship as expected, pH and phosphate remained relatively stable throughout the 4 sampling seasons, and fecal coliforms and total suspended solids did fluctuate with precipitation events as predicted. Nitrate had higher measurements (0.7 ppm) during summer 2021 that could be due to runoff from upstream pollutants like fertilizers.

However, sulfate had the highest readings during spring 2022 (168.0 to 173.0 ppm), and this increase may be due to sulfate entering the stream during snow melt events (Shanley et al. 2008).

Water quality parameters were compared to standards and metrics to determine status of the water in Yellow Creek. According to the Ohio EPA statewide water quality criteria 3745-1-35, pH measured at the three sites at Yellow Creek fell within the range (6.5 to 9.0). As for water temperature, Yellow Creek had very different averages values compared to other streams in Ohio. Average values for Ohio streams are as follows: winter temperatures between 8.3 and 10℃, spring temperatures from 12.2 to 23.3 ℃, summer temperatures around 28 ℃, and fall ranging from 22.8 to 8.3 ℃ (Ohio Administrative Code 3745-1-35). In this study, averages water temperature measured at Yellow Creek in the winter (2.9 to 3.3℃), in the spring (15.6 to 16.2℃), and in the summer (21.3 to 21.6 ℃), were much lower. Site 3 has significant tree canopy cover which may contribute to the lower temperature. It was only the fall (9.2 to 9.4 ℃) that had relatively similar values to Ohio's average. In contrast, all recorded dissolved oxygen in Yellow Creek was higher than the average criteria (5.0 mg/L), with the lowest during summer 2021 at the Woods (6.4 mg/L) and the highest at the Cemetery during winter 2022 (13.8 mg/L). It is not clear why such pattern in both temperature and dissolved oxygen were observed. A possible explanation is perhaps the occurrence of mild summers and colder winters, a trend somehow contradictory to what has been observed in other aquatic systems.

According to the Ohio Administrative code 3745-1-37, recreational use criteria limits a maximum of 126 cfu/100 mL to be considered acceptable. The highest fecal coliform levels occurred during fall 2021 with an average of ~190 colonies/100 mL.

QHEI scores were very consistent throughout the seasons at each site averaging 53 for Cemetery, 42 for Library, and 61 for Woods. Comparing those scores to the QHEI narrative ranges from the Ohio EPA, the Cemetery site was rated as "fair", the Library site fell into "poor" category, and Woods site was ranked as "good". A study looking at small urban streams' tributaries to the Cuyahoga River (20–52 km^2 drainage area) in Northeastern Ohio showed a similar QHEI scored of 62 (Walton et al. 2007). It is important to note that Cemetery and Library were considered and characterized as more "stressed" because of the proximity to roads, bridges, and traffic. Specifically, the Library site where sampling was conducted in a stretch of the river under a bridge that supports heavy traffic at all times. The Woods site had more vegetation surrounded by a well conserved riparian area, with less influence of anthropogenic disturbances (e.g., a bridge). Thus, QHEI scores somehow did reflect initial observations.

Benthic macroinvertebrate communities

Abundance and different indexes of benthic macroinvertebrate communities at each study location were measured to get information about the status of benthic communities in response to stream quality. In fact, this study found a significant site and season interaction for benthic macroinvertebrates.

Benthic communities were limited to the families of Elmidae, Chironomidae, Simuliidae, Ephemerellidae, Heptageniidae, Baetidae, Caenidae, Perlidae, Hydropsychidae, and Corbiculidae as well as Oligochaeta and Decapoda in much less

proportion. Chironomidae was by far the most abundant family throughout the seasons. Chironomidae, a pollution tolerant family, is used as an indicator family for biomonitoring because they respond rapidly and sensitively to disturbances in the environment (Beneberu et al. 2014). Generally, a site with abundance of organisms belonging to Chironomidae is considered of "poor water quality" depending on which midges were found (Molineri et al. 2019). However, in this study high amounts of organisms in more sensitive families such as Heptageniidae and Hydropsychidae were also found. Comparably, benthic communities from an urban stream in Greater Manchester, UK affected by sewage overflow had also abundance organisms of environmentally tolerant taxa like Oligochaeta, Baetidae, and Chironomidae while sites without the discharge had more pollution sensitive species like Heptageniidae (Medupin 2020).

Benthic macroinvertebrate communities are found to be affected by heavy rain fall due to change in stream substrate resulting in a loss of habitat within Korean peninsula streams (Bae & Park 2019). During fall, Yellow Creek had higher amounts of woody debris and leaves that provided a habitat for benthic macroinvertebrates; however, spring had a higher rainfall and snowmelt which is suspected to have created a higher velocity pushing the debris downstream, which could affect the overall abundance of organisms. In addition, local scale factors, such as velocity and depth, affect benthic communities due to the physical force placed on the organisms, while velocity also affects other variables like substratum composition, food delivery, and dissolved oxygen (Sandin & Johnson 2004). In the present study, spring 2022 had fewer individual organisms found in the major families (Table 10) than fall 2021 as well as a lower

dissolved oxygen and higher water velocity which could explain the smaller number of organisms collected. Non-metric multidimensional scaling of the family-level dataset showed that differences of the interaction of sampling location and seasonality had an effect on abundance of benthic communities. Roy et al. 2003 found a positive relationship between tolerant benthic communities and conductivity perhaps due to contamination sources such as urban runoff and effluent from sewage. Kim et al. (2019) found that land-use coverage is the primary factor affecting benthic communities with water quality being the second factor. However, in this study because water quality parameters were influenced only by seasonality no further evidence of an effect on benthic communities can be withdrawn from the dataset collected.

Forested areas tend to reduce runoff of sediment and nutrients which maintains a more stable flow, temperature, and channel morphology allowing for food sources and habitats for benthic macroinvertebrates; agricultural disturbances of the riparian vegetation cause destabilization of environmental variables which negatively affect benthic communities and decreases FBI scores (Kasangaki et al. 2006). This supports the suggestion that benthic macroinvertebrates in Yellow Creek were affected by the nutrient run off and disturbances in the riparian zones. The Library site had a storm runoff drain, a road bridge, as well as a frequented parking lot that acted as sources for anthropogenic pollution. Nevertheless, the FBI was found to be similar among the three sites, with scores of 5.06 at Cemetery, 5.13 at Library at 5.13, and 5.00 at Woods. Consequently, the FBI did not show a significant site/season interaction statistically compared to EPT Richness, density, and H' diversity, which indeed were statistically significant. Overall,

Yellow Creek sites fell within the ranking "good" which indicates some degree of organic pollution.

Study limitations

River sampling is very dependent on the current weather conditions. During spring, water velocity was too high to safely sample from the midstream channel. Also, Yellow Creek was often covered with ice during the winter months, which limited accessibility and benthic macroinvertebrate collection. The flow meter, Secchi tube, and YSI would freeze during winter sampling generating no data and/or meaningless data for those sampling dates. Collectively, these factors might have contributed to the lack of a clear effect on site and season interaction for water quality. More sampling dates and/or sites would be necessary to establish a more robust dataset. More studies on the distribution of benthic macroinvertebrates are needed to establish correlations with environmental parameters.

Conclusions

The results of this study revealed that water quality parameters in Yellow Creek, Poland Ohio followed a clear pattern marked by seasonality. Site differences were not found. Most physico-chemical and biological parameters were below maximum limits allowed, but fecal coliform levels depended on season (Ohio Administrative Code 3745-1-35). Benthic communities were influenced by the interaction of season and sampling site. Benthic communities and QHEI evaluation together showed that Yellow Creek might be impacted by organic pollution. This study as well as other future studies could contribute to a better understanding of the distribution of freshwater macroinvertebrates in urban streams impacted by sewer overflows, salt runoff and urbanization. Stream

health assessment could be used to address and focus on areas in need of effective management of urban streams.

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Appendix

Appendix A: Total Suspended Solids

1. Samples were collected in 500 mL or 1000 mL Nalgene bottles depending on the season and water turbidity.
2. Before collecting samples, bottle was rinsed 3 times with sample water.
3. Samples were immediately placed on ice in a cooler, transferred to the laboratory at YSU and stored at 4 °C until further analyses.
4. Glass microfiber filters were prepared for filtration by rinsing them with 20 mL of reagent water 3 times.
5. They were then dried in an oven at 103-105 °C for 1 hour and cooled in a desiccator.
6. The filters were heated and cooled using the oven/dessicator until there was a weight change of less than 4%.
7. On the day of the analysis, a dried filter was placed on a funnel apparatus and rinsed with a small amount of milli-Q water.
8. Water samples collected in Nalgene bottles were allowed to equilibrate to room temperature, mixed for 10 seconds, and then transferred to the funnel.
9. The sample bottle was rinsed twice with 10 mL of milli-Q water, and the funnel was rinsed once with the same milli-Q water.
10. The filters were heated and cooled using the oven/dessicator until there was a weight change of less than 4%.
11. Determination of suspended solids were calculated using the following formula:

mg total suspended solids/L = (A-B) *1000 sample volume in mL

where:

A: weight of the filter + dried residue, mg

B: weight of filter, mg

Appendix B: Fecal Coliforms

Sample collection:

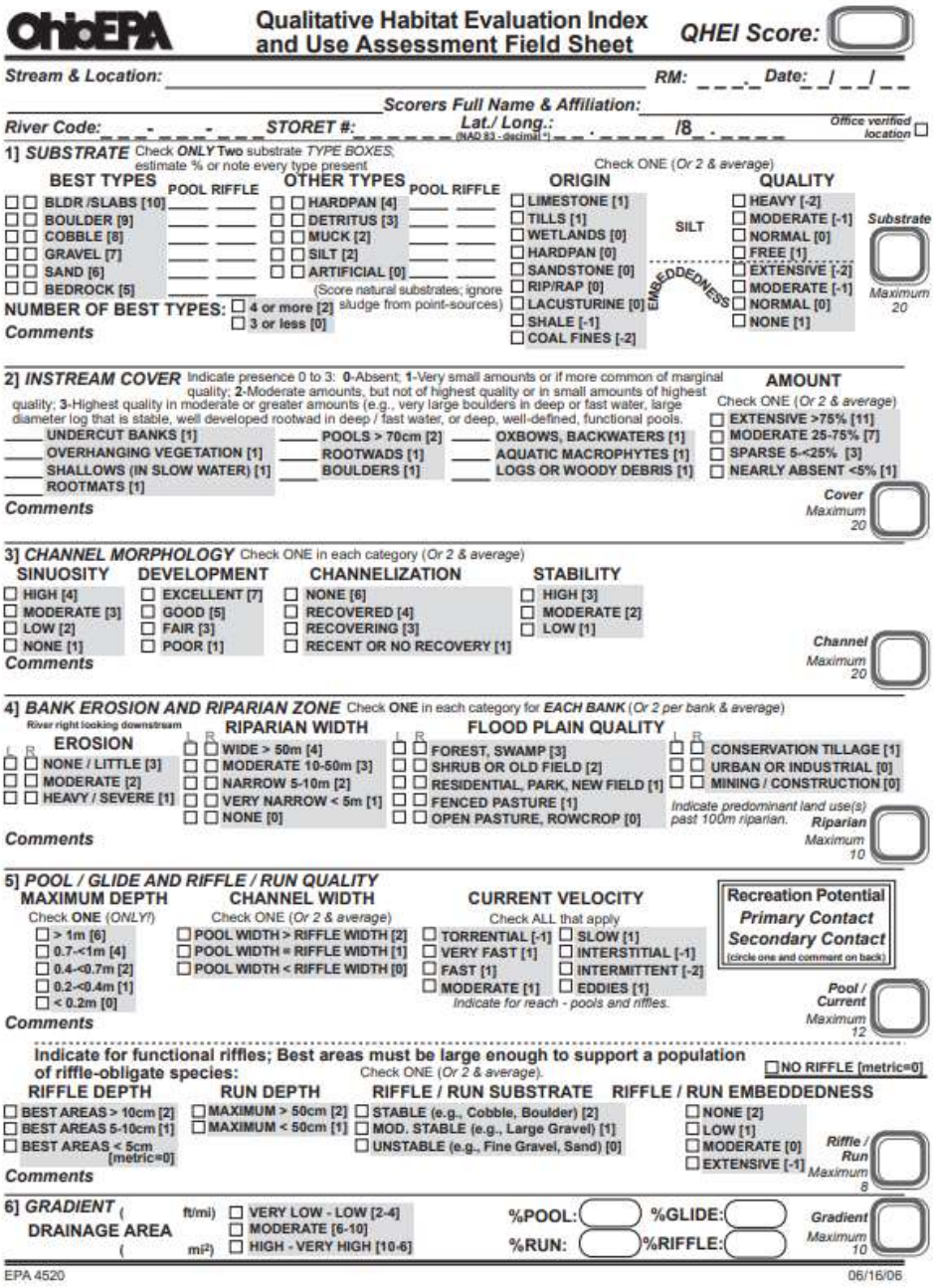
1. Sample water was collected using autoclaved 125 mL Nalgene bottles to prevent contamination.
2. All glassware and supplies were autoclaved and sterilized before analyses.
    a. Funnel
    b. Tweezers
    c. Bottle of approximately 300 mL of water for rinse
    d. Glass graduated cylinder
3. After collection, water was placed in a cooler on ice and transported to the laboratory for analyses
4. At the lab, water samples were placed at 4 ℃ in a fridge before analyses.
5. Water samples were analyzed within 24 hours of the collection date.

Method

1. Using sterile tweezers, a sterile membrane filter was placed on the porous part of the funnel with the grid portion face upwards.
2. 100 mL of sample water was transferred from the collection bottle to funnel.

3. Turn on the vacuum and slowly pour the measured sample water into the funnel.
4. With the filter still in place and vacuum on, the interior sides of the funnel were rinsed 3 successional times with 20 mL of autoclaved water.
5. Once the filtration and rinses were completed, the vacuum was turned off.
6. The funnel was removed and the filter paper was removed with sterile tweezers.
7. The filter paper was placed on a prepared mFC agar plate in a rolling motion to prevent bubbles
8. Plates were then incubated for 24 ± 2 hours at 44.5 ± 0.2°C.

Appendix C. QHEI Field Sheet



OhioEPA Qualitative Habitat Evaluation Index and Use Assessment Field Sheet — QHEI Score: ☐

Stream & Location: _____ RM: _ _ _._ Date: _ _/_ _/_ _

Scorers Full Name & Affiliation: _____

River Code: _ _ - _ _ - _ _ STORET #: _ _ _ _ _ _ Lat./ Long.: (NAD 83 - decimal °) _ _ . _ _ _ _ /8_ . _ _ _ _ Office verified location ☐

1] SUBSTRATE Check **ONLY Two** substrate *TYPE BOXES*, estimate % or note every type present

BEST TYPES	POOL	RIFFLE	OTHER TYPES	POOL	RIFFLE
☐☐ BLDR /SLABS [10]	____	____	☐☐ HARDPAN [4]	____	____
☐☐ BOULDER [9]	____	____	☐☐ DETRITUS [3]	____	____
☐☐ COBBLE [8]	____	____	☐☐ MUCK [2]	____	____
☐☐ GRAVEL [7]	____	____	☐☐ SILT [2]	____	____
☐☐ SAND [6]	____	____	☐☐ ARTIFICIAL [0]	____	____
☐☐ BEDROCK [5]	____	____	(Score natural substrates; ignore sludge from point-sources)		

NUMBER OF BEST TYPES: ☐ 4 or more [2] ☐ 3 or less [0]

Check ONE (*Or 2 & average*)

ORIGIN		QUALITY	
☐ LIMESTONE [1]	SILT	☐ HEAVY [-2]	Substrate
☐ TILLS [1]		☐ MODERATE [-1]	
☐ WETLANDS [0]		☐ NORMAL [0]	
☐ HARDPAN [0]		☐ FREE [1]	
☐ SANDSTONE [0]	EMBEDDEDNESS	☐ EXTENSIVE [-2]	
☐ RIP/RAP [0]		☐ MODERATE [-1]	
☐ LACUSTURINE [0]		☐ NORMAL [0]	Maximum 20
☐ SHALE [-1]		☐ NONE [1]	
☐ COAL FINES [-2]			

Comments

2] INSTREAM COVER Indicate presence 0 to 3: **0**-Absent; **1**-Very small amounts or if more common of marginal quality; **2**-Moderate amounts, but not of highest quality or in small amounts of highest quality; **3**-Highest quality in moderate or greater amounts (e.g., very large boulders in deep or fast water, large diameter log that is stable, well developed rootwad in deep / fast water, or deep, well-defined, functional pools.

			AMOUNT
____ UNDERCUT BANKS [1]	____ POOLS > 70cm [2]	____ OXBOWS, BACKWATERS [1]	Check ONE (*Or 2 & average*)
____ OVERHANGING VEGETATION [1]	____ ROOTWADS [1]	____ AQUATIC MACROPHYTES [1]	☐ EXTENSIVE >75% [11]
____ SHALLOWS (IN SLOW WATER) [1]	____ BOULDERS [1]	____ LOGS OR WOODY DEBRIS [1]	☐ MODERATE 25-75% [7]
____ ROOTMATS [1]			☐ SPARSE 5-<25% [3]
			☐ NEARLY ABSENT <5% [1]

Comments Cover Maximum 20

3] CHANNEL MORPHOLOGY Check ONE in each category (*Or 2 & average*)

SINUOSITY	DEVELOPMENT	CHANNELIZATION	STABILITY
☐ HIGH [4]	☐ EXCELLENT [7]	☐ NONE [6]	☐ HIGH [3]
☐ MODERATE [3]	☐ GOOD [5]	☐ RECOVERED [4]	☐ MODERATE [2]
☐ LOW [2]	☐ FAIR [3]	☐ RECOVERING [3]	☐ LOW [1]
☐ NONE [1]	☐ POOR [1]	☐ RECENT OR NO RECOVERY [1]	

Comments Channel Maximum 20

4] BANK EROSION AND RIPARIAN ZONE Check **ONE** in each category for ***EACH BANK*** (*Or 2 per bank & average*)

River right looking downstream

EROSION (L R)	RIPARIAN WIDTH (L R)	FLOOD PLAIN QUALITY (L R)	(L R)
☐☐ NONE / LITTLE [3]	☐☐ WIDE > 50m [4]	☐☐ FOREST, SWAMP [3]	☐☐ CONSERVATION TILLAGE [1]
☐☐ MODERATE [2]	☐☐ MODERATE 10-50m [3]	☐☐ SHRUB OR OLD FIELD [2]	☐☐ URBAN OR INDUSTRIAL [0]
☐☐ HEAVY / SEVERE [1]	☐☐ NARROW 5-10m [2]	☐☐ RESIDENTIAL, PARK, NEW FIELD [1]	☐☐ MINING / CONSTRUCTION [0]
	☐☐ VERY NARROW < 5m [1]	☐☐ FENCED PASTURE [1]	*Indicate predominant land use(s) past 100m riparian.*
	☐☐ NONE [0]	☐☐ OPEN PASTURE, ROWCROP [0]	

Comments Riparian Maximum 10

5] POOL / GLIDE AND RIFFLE / RUN QUALITY

MAXIMUM DEPTH	CHANNEL WIDTH	CURRENT VELOCITY	
Check **ONE** (*ONLY!*)	Check ONE (*Or 2 & average*)	Check ALL that apply	
☐ > 1m [6]	☐ POOL WIDTH > RIFFLE WIDTH [2]	☐ TORRENTIAL [-1]	☐ SLOW [1]
☐ 0.7-<1m [4]	☐ POOL WIDTH = RIFFLE WIDTH [1]	☐ VERY FAST [1]	☐ INTERSTITIAL [-1]
☐ 0.4-<0.7m [2]	☐ POOL WIDTH < RIFFLE WIDTH [0]	☐ FAST [1]	☐ INTERMITTENT [-2]
☐ 0.2-<0.4m [1]		☐ MODERATE [1]	☐ EDDIES [1]
☐ < 0.2m [0]		*Indicate for reach - pools and riffles.*	

Recreation Potential
Primary Contact
Secondary Contact
(circle one and comment on back)

Comments Pool / Current Maximum 12

Indicate for functional riffles; Best areas must be large enough to support a population of riffle-obligate species: Check ONE (*Or 2 & average*). ☐ NO RIFFLE [metric=0]

RIFFLE DEPTH	RUN DEPTH	RIFFLE / RUN SUBSTRATE	RIFFLE / RUN EMBEDDEDNESS
☐ BEST AREAS > 10cm [2]	☐ MAXIMUM > 50cm [2]	☐ STABLE (e.g., Cobble, Boulder) [2]	☐ NONE [2]
☐ BEST AREAS 5-10cm [1]	☐ MAXIMUM < 50cm [1]	☐ MOD. STABLE (e.g., Large Gravel) [1]	☐ LOW [1]
☐ BEST AREAS < 5cm [metric=0]		☐ UNSTABLE (e.g., Fine Gravel, Sand) [0]	☐ MODERATE [0]
			☐ EXTENSIVE [-1]

Comments Riffle / Run Maximum 8

6] GRADIENT (ft/mi) ☐ VERY LOW - LOW [2-4] ☐ MODERATE [6-10] ☐ HIGH - VERY HIGH [10-6]

DRAINAGE AREA (mi²)

%POOL: ☐ %GLIDE: ☐ %RUN: ☐ %RIFFLE: ☐

Gradient Maximum 10

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Appendix D. Sampling dates

Summer 21	Fall 21	Winter 22	Spring 22
6/28	10/10	1/22	4/10
7/5	10/23	2/6	4/24
7/12	11/1	2/27	5/9
7/19	11/13	3/10	5/25
7/26	12/4	3/20	6/13
8/2	12/22		
8/9			
8/23			