LARGE RIVER EMERGING CONTAMINANTS SAMPLING AND ANALYSIS PROJECT, 2023 AND 2024

Water/ Biological Tissue/ Macroinvertebrate Community

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Authorization for Release

The analyses, opinions, and conclusions in this document are based entirely on EnviroScience's unbiased, professional judgment. EnviroScience's compensation is not in any way contingent on any action or event resulting from this study.

To the best of their knowledge, the undersigned attest that this document, and the information contained herein, is accurate and conforms to EnviroScience's internal Quality Assurance standards.

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EXECUTIVE SUMMARY

EnviroScience, Inc. (EnviroScience) was hired by the Ohio EPA and Brownfield Restoration Group to provide technical assistance with sampling Ohio large river sites in 2023 and 2024 for emerging contaminants. The contract proposed that 151 large river sites would be sampled for surface water, fish tissue, macroinvertebrate tissue, macroinvertebrate communities, and mussel communities. All sample collections were conducted by EnviroScience staff and chemical lab testing was completed by Eurofins Cleveland. All fish sampling, water sampling, and macroinvertebrate taxonomic identifications were conducted by Level 3 Qualified Data Collectors per the Ohio EPA Credible Data Certification Program. Macroinvertebrate sampling was conducted by Level 2 and Level 3 Qualified Data Collectors.

A total of 149 of the 151 sites were sampled during 2023 and 2024. Two sites were not sampled due to denied permission and had no comparable replacement. Two of the 149 sites sampled were denied access permission but were replaced with other Ohio EPA large river sites. One of the 149 sites was moved downstream due to denied access permission. All 149 sites were sampled within the dates of September 6th to October 13th, 2023, and May 13th to September 19th, 2024. According to the study plan, the goal for collection rates for all sites were 100% for water and macroinvertebrates, and 80% for each target fish species. Target fish species included spotfin shiner (*Cyprinella spiloptera*), bluegill (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*). The overall sample collection rates of the 149 sites were as follows: surface water (100%), macroinvertebrate community (100%), macroinvertebrate tissue (99.3%), spotfin shiner tissue (93.3%), bluegill tissue (83.2%), and channel catfish tissue (78.4%).

Standard sampling guidance and protocols for the targeted emerging contaminants, which were identified in the workplan, were used throughout the entirety of the project. Field collected data and the macroinvertebrate community data can be found attached to this report. All analytical chemical data, mussel data, and macroinvertebrate voucher specimens will be submitted to Ohio EPA and Brownfield Restoration Group separately from this report. Per the workplan, data analyses and evaluations were not conducted by EnviroScience as part of this project.

1.0 INTRODUCTION

EnviroScience, Inc. (EnviroScience) was hired by the Ohio EPA and Brownfield Restoration Group to provide technical assistance with sampling Ohio EPA large river sites for Emerging Contaminants (PFAS: per- and polyfluoroalkyl substances). Data collected will be used in support of Ohio EPA's evaluation of the potential to exceed the draft aquatic life criteria regarding emerging contaminants using Method 1633, which covers 40 specific parameters. The work performed will assist the Ohio EPA in its efforts to evaluate the levels and prevalence of those parameters within surface waters and biological tissue in large rivers of Ohio.

Surface water and biological sampling was conducted at 149 of the 151 proposed sites on large rivers throughout the state of Ohio as identified by the Ohio EPA. Sampling occurred in 2023 and 2024. In addition to water and tissue sampling, a macroinvertebrate community assessment using the U.S. EPA's Rapid Bioassessment Protocol (RBP) was completed at each site. The overall sample collection rates of the 149 sites are as follows: surface water (100%), macroinvertebrate community (100%), macroinvertebrate tissue (99.3%), spotfin shiner tissue (93.3%), bluegill tissue (83.2%), and channel catfish tissue (78.4%). A separate report will be submitted which details mussel sampling and assessment protocols used to estimate community composition, distribution, and abundance at all sites.

2.0 METHODS

EnviroScience collected water samples, fish and macroinvertebrate tissue samples, and composite benthic macroinvertebrate community samples at each site. Collection methods followed standardized Ohio EPA methods for water and tissue sampling and US EPA (Barbour et al., 1999; RBP) methods for macroinvertebrate community assessments. Sampling methods included grab water samples, boat electrofishing to collect fish tissue samples, and d-frame kick nets for macroinvertebrate samples for tissue analysis and community assessment. All field collections and laboratory identifications were conducted and directly overseen by staff who have received the appropriate Ohio EPA Qualified Data Collector (QDC) status for the various disciplines.

2.1 SAMPLING LOCATIONS

A total of 149 of the 151 sites in Ohio were sampled for water quality, fish tissue, macroinvertebrate tissue, and macroinvertebrate community composition. The 149 sites were previously assessed by Ohio EPA in 2020 and 2021 as part of their biological and water quality assessment program.

One site on the Scioto River (600940) and one site on Paint Creek (304031) were dropped due to access permission being denied. Replacement sites were not recommended by the Ohio EPA and none were identified in the workplan for these two locations. One site on the Mahoning River (N03K31) and another site on the Stillwater River (H06P07) were dropped and replaced with other sites given by the Ohio EPA due to access restrictions. The replacement for the Mahoning River was site code N03W13. The replacement for the Stillwater River was site code H06G04. Both replacement sites were within 1.9 miles of the original sites. One site on the Olentangy River (V04S16) at RM 2.7 had restricted access due to construction activities. With permission from Ohio EPA, the site was moved downstream to Olentangy River RM 0.9, which corresponded with a large river site in Ohio EPA's 2024 biological survey.

The 149 sites were located in 11 major Ohio drainages and included the following rivers and streams:

- Auglaize River
- Big Darby Creek
- Blanchard River
- Cuyahoga River
- Grand River
- Great Miami River

- Hocking River
- Killbuck Creek
- Licking River
- Little Miami River
- Mad River
- Maumee River
- Mahoning River
- Mohican River
- Muskingum River
- Olentangy River
- Paint Creek
- Raccoon Creek
- Salt Creek
- Sandusky River
- Sandy Creek
- Scioto River
- Stillwater River
- St. Joseph River
- Tiffin River
- Tuscarawas River
- Walhonding River
- Whitewater River
- Wills Creek

All 149 sites were sampled within the dates of September 6th to October 13th, 2023, and May 13th to September 19th, 2024, with 86% of the sites sampled in 2024. The sampling period included in the workplan was June to September in both 2023 and 2024. However, the sampling period was extended to October in 2023 and started in May in 2024. Both time extensions are outside the standard Ohio EPA biological stream sampling period however Ohio EPA gave preapproval to both extensions.

2.2 CONTAMINANT SAMPLING GUIDANCE

Field sampling hygiene protocols are critical to ensuring that testing results reflect actual contaminant levels in the analyzed media. All sampling methods adhered to the protocols described within the *Sampling, Analysis and Assessment of Per- and Polyfluoroalkyl Substances (PFAS) Under NYSDEC's Part 375 Remedial Programs* (NYSDEC, 2023), and the *General PFAS Sampling Guidance* (MDEQ, 2018).

In summary, the following protocols were used during sampling and processing to minimize sample bias:

- Powder-free nitrile gloves were used when collecting and handling water samples and processing tissue samples. New gloves were used with each sample.
- Any approved sunscreen products were applied away from the sample processing and staging areas.
- Laboratory contaminant-free jars and caps were used for all water samples. Clean aluminum foil was used for fish tissue processing and wrapping fish samples. Macroinvertebrate tissue samples were placed in lab supplied contaminant-free jars.
- Fish live-well was constructed of polypropylene.
- The fish processing area was covered in high density polyethylene (HDPE) sheeting, with a HDPE fillet board and a stainless-steel fish measuring stick attached. These items were cleaned with Alconox and contaminant free water between each fish sample.
- Food and drinks were consumed away from the sample processing area.
- Polypropylene clipboards, non-waterproof paper, and pencils and fine point Sharpie markers were used to record field data.
- Each channel catfish sample was filleted with a properly decontaminated stainless steel filet knife.
- Field staff were instructed to wear well-laundered synthetic or 100% cotton clothing, with most recent launderings not using fabric softeners.
- Sampling waders were constructed of PVC coated denier nylon.
- All sample containers and foil wrapped samples were placed in lab supplied Ziploc style plastic bags and transported in wet ice coolers (water samples) or dry ice coolers (tissue samples). Each sample was sealed in their own bag.

- Dedicated sampling equipment and supplies were used when samples to minimize the potential for cross-contamination.

2.3 FIELD OBSERVATIONS

For all sampling activities, stream flow conditions were assessed prior to sampling to ensure that elevated river flows that would hinder the collection of valid samples were not present. This evaluation included examination of National Weather Service data and forecasts and USGS streamflow data from applicable gaging stations.

A field water chemistry assessment was conducted at all 149 sites at the time of each biological sampling event. Water chemistry was assessed using guidance in the *Ohio EPA Surface Water Field Sampling Manual* (Ohio EPA, 2021b) and the *EnviroScience Quality Assurance Program Plan (QAPP) for Aquatic Survey* (EnviroScience, 2023). Measurements were taken using a portable YSI Pro DSS Multi-Parameter Water Quality Meter, or equivalent. Measured parameters included water temperature, pH, specific conductance, dissolved oxygen, time of measurement, and depth in water column of measurement. All water quality meters were calibrated in accordance with the manufacturer's specifications at the beginning of each sampling day. In addition, water clarity was measured using a transparency tube. Water clarity was used to estimate water turbidity at the time of each fish sample collection.

At each site before collection of macroinvertebrate samples, a modified habitat assessment was conducted to give additional information for the macroinvertebrate community assessments. The habitat assessment was modeled after the Ohio EPA Field Collection Data Sheets for Macroinvertebrate Sampling. Habitat assessments were not required as part of the workplan. All field observations and habitat assessment were recorded in a tablet containing a custom digital field form created by EnviroScience using ArcGIS's Survey123.

2.4 WATER QUALITY SAMPLING

EnviroScience biologists conducted surface water quality sampling at all 149 sampled sites. Water sample collection followed standard procedures in the *Surface Water Field Sampling Manual for Water Quality Parameters and Flows* (Ohio EPA, 2021b), the *Surface Water PFAS Sampling Guidance* (EGLE, 2022), and the *Quality Assurance Program Plan (QAPP) for Aquatic Survey* (EnviroScience, 2023). The sampling for this task was completed by an individual(s) certified as a Level 3 QDC for Chemical Water Quality Assessment per the Ohio EPA Credible Data Certification Program.

In summary, water samples were collected while wading in the water body (or from the boat) by inverting the container (opening down) and then immersing directly upstream of the collector. The sampling container was then righted (turned so that opening was pointed upwards) to collect the sample. Samples were collected at a depth of 15-30 cm below the water's surface. At all sites, a field blank was collected by pouring lab grade, contaminant free water from its original container into a prelabeled, lab-issued sample container. Duplicates of both water samples and field blanks were collected at 15 sites as noted in the work plan. Aqueous equipment blanks were collected from two fish sampling nets and two macroinvertebrate sampling nets. Equipment blanks were collected by pouring lab grade contaminant free water from its original container through a decontaminated sampling net and into a prelabeled, lab-issued bottle. Two equipment blanks for each sampling net were collected randomly within the sampling period.

All water samples, field blanks, and equipment blanks were collected by staff after prewashing hands and while wearing appropriate PPE following the guidelines above. All samples were immediately placed in a cooler with wet ice after collection. A total of 307 aqueous samples were collected and analyzed by Eurofins Cleveland lab using Method 1633.

2.5 FISH TISSUE SAMPLING

A total of 147 of 149 sites were sampled for fish tissue. Target fish species were not collected from the Grand River (G02S13) due to no target species being observed after significant effort nor from the Stillwater River (H06G04) due to equipment failure. Fish tissue collection followed standard procedures in the *Biological Criteria for the Protection of Aquatic Life: Volume III, standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities* (Ohio EPA, 2015), the *Fish Tissue Field Collection Manual* (Ohio EPA, 2021a), the *Quality Assurance Program Plan (QAPP) for Aquatic Survey* (EnviroScience, 2023), the *USEPA's Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (Barbour et al.,1999), and the *Fish Tissue PFAS Sampling Guidance* (MDEQ, 2019).

Per the work plan, target species included spotfin shiner (*Cyprinella spiloptera*), bluegill (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*). A minimum of an 80% success rate per fish species was expected for the entire project. Based on the 147 sites sampled for fish tissue, the tissue collection rate for each fish species was as follows: spotfin shiner (93.3%), bluegill (83.2%), and channel catfish (78.4%). The field sampling for fish tissue collection was completed by individual(s) certified as a Level 3 QDC for fish community biology using all methods per the Ohio EPA Credible Data Certification Program. Additionally fish tissue duplicate samples were collected from 17 sites (spotfin shiner, 8; bluegill, 6; channel catfish, 3). These samples were not required as part of the work plan. All collected fish tissue samples were analyzed by Eurofins Cleveland lab using Method 1633.

Fish sampling was conducted using a boat mounted pulsed DC current single anode array electrofishing system (Smith-Root Apex). Dedicated sampling equipment and supplies were used when collecting fish tissue samples to minimize the potential for cross-contamination. Sampling consisted of electrofishing habitat types typically frequented by the three fish species of interest. Fishing efforts generally occurred within a 400–700-meter sampling zone near the designated Ohio EPA large river site. Sampling times varied depending on sampling success, with times ranging between 60 and 120 minutes. Once fish were collected, they were retained in a high-density polyethylene live-well for tissue processing.

The number of fish collected for each fish species varied depending on their goal individual count and target sample weights for laboratory analysis. Whole-body composite spotfin shiner samples targeted 5-10 individuals, whole-body composite bluegill samples targeted 3-5 individuals, and channel catfish samples consisted of skin-on fillet composites with a target of 3-5 individuals of consumable size. All tissue samples needed a minimum weight of 20 grams. Given the spotfin shiner species is smaller, an exception of samples as low as 5 grams were accepted for spotfin shiner tissue samples. Required in the workplan, fish composite samples met the 25% difference between the largest and smallest individual within a site's specific fish tissue sample. Most fish tissue samples hit their target individual count and weight; however, single samples of species were collected due to low catch or fish not meeting the 25% difference between individuals.

After collection, fish were processed on shore on a flat surface covered in contaminate free plastic cover. All fish were worked up on a polyethylene fish processing board covered in clean aluminum foil. Fish were removed from the live-well filled with ambient water and euthanized. Fish were then enumerated, measured for total length, and batched weighed per composite sample or individual channel catfish. Spotfin shiner whole-body composite samples were wrapped together in clean aluminum foil and placed in a plastic bag. That bag was then placed in another plastic bag containing the appropriate label to prevent label contamination. Bluegill whole-body composite samples were wrapped individually in clean aluminum foil and placed in separate plastic bags. All composite bags were then placed in a larger plastic bag with the appropriate label. Channel catfish were filleted using decontaminated stainless steel fillet knives.

Knives were individually wrapped in clean aluminum foil and a dedicated knife was used for each channel catfish sample. Fillet samples were wrapped in clean aluminum foil and placed in separate plastic bags. All composite bags were then placed in a larger plastic bag with the appropriate label. All bagged samples were immediately put in a cooler with dry ice.

The polyethylene fish processing board was rinsed with contaminant free water and relined with clean aluminum foil prior to each fish species processed. Individuals processing fish tissue samples wore powderless nitrile gloves which were replaced with new nitrile gloves for each fish species handled. All fish processing equipment was decontaminated using Alconox, followed by a rinse with deionized, contaminant free water between sites. All field fish data was recorded in a tablet containing a custom digital field form created by EnviroScience.

2.6 MACROINVERTEBRATE TISSUE SAMPLING

EnviroScience biologists collected 148 composite macroinvertebrate samples for tissue analysis from the 149 sites sampled. A macroinvertebrate tissue sample was not analyzed for one site on the Little Miami River (600580) because not enough mass was found after collection efforts. Macroinvertebrate tissue was collected by using a d-frame kick net as well as hand picking in order to obtain sufficient mass for analytical processing. Macroinvertebrate tissue duplicate samples were collected from 7 sites. These samples were not required as part of the work plan. All collected macroinvertebrate tissue samples were analyzed by Eurofins Cleveland lab using Method 1633.

The macroinvertebrate tissue sample target weight was 20 grams. Although, a minimum of 5 grams was acceptable for the laboratory analysis given macroinvertebrates are small. Macroinvertebrates were collected and placed in a jar of ambient river water until sufficient mass had been collected. Macroinvertebrates were then passed through a decontaminated sieve and moved using an appropriate gloved hand or clean stainless-steel forceps to the lab-provided, prelabeled and pre-weighed plastic bottle. The bottle was then weighed for total invertebrate mass. The bottle was then placed into a plastic bag and immediately placed in a cooler with dry ice. A majority of the macroinvertebrate tissue samples were comprised of crayfish, gastropods, or corbicula. All data pertaining to macroinvertebrate tissue sampling was recorded in a tablet containing a custom digital field form created by EnviroScience. All sample collections for macroinvertebrates were conducted or overseen by an individual with Level 2 QDC per the Ohio EPA Credible Data Certification Program. All equipment used for macroinvertebrate tissue collection was rinsed with ambient water between sites.

2.7 MACROINVERTEBRATE COMMUNITY SAMPLING AND LABORATORY METHODS

EnviroScience biologists conducted representative composite sampling of the macroinvertebrate community using methods outlined in USEPA's Rapid Bioassessment Protocol (Barbour et al., 1999) at all 149 sampled sites. Duplicate samples for taxonomic analysis were also collected at eight sites for a total of 157 samples that were sorted, enumerated, and identified. The field sampling (Level 2) and taxonomic identifications (Level 3) for this task were completed by individuals with the appropriate QDC level per the Ohio EPA Credible Data Certification Program.

The macroinvertebrate community sampling utilized the multihabitat sampling technique described in US EPA's *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (Barbour et al., 1999). Benthic macroinvertebrates were collected systematically from all available instream habitats by kicking the substrate or jabbing with a D-frame dip net. A total of 20 jabs (or kicks) were taken from all major habitat types in the reach, resulting in sampling of approximately 3.1 square meters of habitat. Sample debris and invertebrates were transferred to 1-liter jars with 95% ethanol. Field observations regarding the types of macroinvertebrates, habitat, and water

quality observations used as additional info for community identification were recorded in a tablet containing a custom digital field form created by EnviroScience. All samples after collection in the field were retained for taxonomic analysis in the EnviroScience Aquatic Biology Laboratory.

The 157 samples retained for taxonomic analysis were preserved and identified to the standard identification levels called for in Level 3 Credible Data surveys as described in Ohio EPA's *Biological Criteria for the Protection of Aquatic Life: Volume III*, 2015. Macroinvertebrate sample sorting was conducted by lab technicians that were trained and over seen by a Level 3 QDC staff. A 200-organism subsample and a large and rare pick was conducted following the US EPA's Rapid Bioassessment Protocol (Barbour et al., 1999). Each sample was spread across a gridded white tray. A random square was selected using a random number generator. Contents of the square were transferred to a petri dish, viewed under the microscope, and invertebrates were removed and counted. Additional squares were selected one at a time until the 200-organism target was met. Once started, a square was always picked to completion even if it exceeded the organism target. After subsampling, the remaining debris were picked through, and a handful of large and rare organisms were added to the sample. The large and rare search targeted material to supplement the subsample such as more mature specimens and organisms not represented in the subsample. After sorting, samples were labeled with collection information and stored in vials of 80% ethanol, except for midges, which were cleared in hot KOH, and slide mounted using CMC-1019.

Samples were identified to the taxonomic level of resolution as outlined in Ohio EPA's *Macroinvertebrate Taxonomic Level* document (Ohio EPA, 2019) and *Master Taxa List* (Ohio EPA, 2023). A voucher collection was built including at least one representative of each taxa found project wide and stored in individual labeled vials or marked on the slide. All macroinvertebrate community data was entered into an Excel document.

3.0 RESULTS

All field data collected in the digital field forms and macroinvertebrate community results for each site are attached to this report in Excel formats (Appendix A; Appendix B). Water and tissue chemical results for contaminants were provided to Ohio EPA as a raw data file and cohesive visual aid using R and a Shiny Application (Chang et al., 2004; R Core Team, 2025). Macroinvertebrate voucher specimens will be physically provided to the Ohio EPA upon completion. The mussel community results were provided in their own separate report. Per the workplan, data analyses and evaluations were not conducted by EnviroScience as part of this project, and thus, not included in this report.

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Appendix A

Ohio EPA Large River Contaminants Sampling Field Data

Appendix B

Ohio EPA Large River Contaminants Sampling Macroinvertebrate Community Data