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SOP: AQUATIC VEGETATION AND AQUATIC INVERTEBRATE SAMPLING AND ANALYSIS

HERRINGTON LAKE, KENTUCKY



Protocol for Sampling Aquatic
Vegetation for Tissue Analysis

Standard Operating Procedures (SOP) Document Control: Standard Operating Procedure for Collection of Aquatic Vegetation and Aquatic Invertebrates for Tissue Residue Analyses		
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ATTACHMENT A

Water Quality and Vegetation Data Collection Sheets for Herrington Lake
Aquatic Invertebrate Data Collection Sheet for Herrington Lake

ACRONYMS AND ABBREVIATIONS

CAP	Corrective Action Plan
GPS	Global position system
MS/MSD	matrix spike/matrix spike duplicate
QAPP	Quality Assurance Project Plan
SOP	standard operating procedure
USEPA	U.S. Environmental Protection Agency

1 OVERVIEW

This standard operating procedure (SOP) describes the field methods for the collection of aquatic vegetation and aquatic invertebrates for the analysis of chemical concentrations in tissue as described in the E.W. Brown Corrective Action Plan (CAP) (Ramboll Environ 2017a). The approaches outlined herein for the collection of aquatic vegetation and invertebrates from Herrington Lake is consistent with the collection of representative samples as described by the United States Environmental Protection Agency (USEPA) *Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers* (Flotemersch et al. 2006), and procedures for the collection environmental media (e.g., Alaska Department of Environmental Conservation Appendix B Standard Operating Procedures [2002], British Columbia Field Sampling Manual [2013] and the U.S. Geological Survey procedures for collecting and processing aquatic invertebrates and fish [Scudder 2008]). The sampling SOPs are generally consistent with the *Kentucky Methods for Assessing Biological Integrity of Surface Waters* (2002), with the difference being that the Kentucky guidance for algae sampling is intended to evaluate algal and sediment dwelling organism community composition and the vegetation and invertebrate sampling specified in the Herrington Lake CAP is intended for chemical analyses. As such, methods of sampling, handling, and preservation for taxonomic identification do not apply for Herrington Lake CAP sampling program.

2 PROCEDURES FOR SAMPLE COLLECTION

Aquatic vegetation sampling will consist of macrophytes, periphyton, and phytoplankton. Aquatic invertebrates will be sediment dwelling or pelagic macroinvertebrates to the extent available. As stated in the CAP, the collection of biological organisms is dependent upon the presence of those organisms and they are not always evenly distributed in the natural environment. As such, aquatic vegetation and aquatic invertebrates will be collected opportunistically throughout each target sampling location and/or area. The CAP states that vegetation and invertebrates will be collected as they are available within the littoral zone approximately 100 meters of the transects identified in the CAP (i.e., 50 meters in each direction, parallel to the transect affording the most coverage of the proximate area to allow flexibility for the field team based on field conditions at the time of sampling). Samples will be collected on the shoreline closest to the E.W. Brown Station on the side of the Auxiliary Pond, when sufficient biotic material is available because this will allow an understanding of potential influences, if any.

Water quality sampling specified in the CAP will be collected prior to any vegetation or invertebrate material collection to avoid the possibility of sediment disturbance in the water that may affect water quality measures. As stated in the CAP, the field collection will be opportunistic and will be based on field conditions at the time of sampling. The remainder of this SOP describes collection, storage, and shipment procedures. All field activities will be documented as detailed in the Herrington Lake Quality Assurance Project Plan (Ramboll Environ 2017b).

2.1 Aquatic Vegetation

Aquatic vegetation tissues will be collected for analyses according to the following procedures:

- Global position system (GPS) coordinates will be recorded for each location plant materials are collected, along with additional information provided in Section 6 (*Data Recording and Management*).

- Submerged aquatic vegetation (i.e., macrophytes, periphyton, and algae) material will be collected from natural substrata by hand or using pre-cleaned (i.e., decontaminated) nets or scraping tools (e.g., trowel). Plant material will be collected by field personnel from boats, from the shoreline, from wadeable areas of the lake, or from swimmable/snorkeling depths consistent with the health and safety protocols. Vegetation samples may also include collection using plankton tow nets. The field team will select the optimal sample collection method to efficiently gather sufficient plant volume needed, to collect a variety of species when a variety of species are available, to adequately handle the materials in accordance with this SOP, and to ensure safety of the field personnel.
 - Effort must be made to minimize disturbance to the aquatic habitat while sampling. Plant material will be rinsed thoroughly at the time of collection in the lake water where collected to remove excess sediment.
 - Plant samples will be kept submerged in water from the associated transect and will be covered until they plant material is processed for shipment (vegetation will not be allowed to desiccate).
 - The abundance of the vegetation material available for collection at any transect in Herrington Lake is dependent upon the presence of the vegetation in specified areas. Because vegetation material may not be present in all areas or the amount of effort to obtain plant material may be prohibitive, a time limit for each transect is estimated at 4 hours for vegetation collection.
- Macrophytes (i.e., larger plant material) may not be available at all locations, and as such, algae or phytoplankton may be collected, as follows:
 - Algae may be scraped from rocks in the littoral zone along the shoreline. Algae will be rinsed with lake water to remove debris and sediment particles.
 - Phytoplankton may be collected using a 0.5 micrometer mesh using a plankton net or a dip net with appropriate mesh size. If plankton are collected, the material will be sorted to ensure that the sample contains plant material only.
- The composite sample will include sampling of multiple species roughly proportional to the presence of the species in the sample area and the reasonableness of collecting the vegetation material (e.g., submerged aquatic vegetation in the upper few feet of the water column of the lake will be preferentially collected versus vegetation at depths of three feet or greater).
- Plant material will be transferred to a holding container (e.g., a clean bucket or Ziploc bags) until the appropriate sample volume is achieved for each location. Each vegetation sample for the Herrington Lake CAP sampling will include composite aquatic vegetation that reflects the types of vegetation readily available at any given transect.
- Samples will be photographed upon collection.
- Plant species will be identified to the extent possible or practical. The types of vegetation included in each sample will be generally described but note that the purpose of the sampling is chemical analysis, taxonomic identification will not be performed. When sampling is completed, individual vegetation will be identified to the lowest taxonomic level practical.

2.2 Aquatic Invertebrates

Aquatic invertebrates in the Herrington Lake Study Area are most likely to be present along the rocky edges of the lake. There may be challenges to collection of aquatic invertebrates given the depth of water, water level fluctuations, and the lack of fine sediment substrates in the littoral zone of the lake, therefore samples of invertebrates will be collected opportunistically near each Herrington Lake transect. Aquatic invertebrate tissues will be collected for analyses as indicated in the CAP. The following procedures are provided:

- Aquatic invertebrates will be collected using pre-cleaned sediment grab samplers (e.g., petite ponar dredge), dip nets or scraping tools (e.g., trowel) depending on substrate conditions. Aquatic invertebrate samples may also include collection using plankton tow nets. The field team will select the optimal sample collection method to efficiently gather sufficient aquatic invertebrate volume from each transect. Aquatic invertebrates are often associated with aquatic vegetation. As such, net sweeps within vegetation may be a beneficial way to collect aquatic organisms.
- Aquatic invertebrates may be collected by field personnel from boats, from the shoreline, from wadeable areas of the lake, or from swimmable/snorkeling depths consistent with the health and safety protocols.
- If substrates collected along with aquatic invertebrates include significant amounts of coarse material or organic debris, on-site sieving may be necessary. In this case, samples may be sieved in the field at the time of collection with coarse material or organic material returned to the portion of the lake where collected.
- The abundance of the aquatic invertebrates available for collection at any transect is dependent upon the presence of the vegetation in specified areas. Because vegetation material may not be present in all areas or the amount of effort to obtain plant material may be prohibitive, a time limit for each transect is estimated at 4 hours for aquatic invertebrate collection.
- Aquatic invertebrates will be depurated prior to sample packaging and shipment. Failing to depurate or correct for gut contents increases the probability of either false positives or false negatives. The [ASTM \(2003\)](#) protocol E1688 for bioaccumulation studies with invertebrates calls for gut contents to be purged to avoid overestimation of bioavailability when metals are the contaminant of concern.
- The organisms may be held in lake water from the collection site in a cooler at approximately 10°C for a 24-hour depuration period (ASTM 2003). Water from melted ice will be contained so that it does not contaminate the holding water. Water in which the organisms are held will be returned to the specific transects where they were collected or will be discharged to a sanitary sewer system.

3 SAMPLE VOLUMES, CONTAINERS, AND HOLD TIMES

The sample volume required for each analysis is a minimum of 2 grams of vegetation and 2 grams of aquatic invertebrates for each of the tissue tests described in detail in the CAP (listed below). When abundant vegetation and aquatic invertebrates are available, 5 grams of tissue per method is preferred. Collectively, this is 8 to 20 grams of vegetation per CAP transect and 8 to 20 grams of aquatic invertebrates per CAP transect. Where quality control samples are collected, sample volume requirements will be proportionally greater (e.g., approximately double volume for sample duplicates).

Chemicals of Interest	Analytical Methods	Hold Times (a)
Metals (selenium, arsenic, cadmium, lead, and zinc)	USEPA 6010/6020	6 months
Mercury	USEPA 7470 and EPA 7471	6 months
Methylmercury	USEPA 1630 and USEPA 1631E	6 months
Percent Moisture	Lab SOP	6 months

a) Assumes samples received on ice and frozen upon receipt by laboratory

- Vegetation and aquatic invertebrate samples will be collected in pre-cleaned jars, bottles, plastic bags, or aluminum foil. If aluminum foil is used, the samples will be wrapped in aluminum foil with the dull side against the sample. Vegetation and aquatic invertebrates will be packaged separately. A single sample container with sufficient volume for all analyses for vegetation and a single sample container with sufficient volume for all analyses for aquatic invertebrates will be submitted to the laboratory for each transect.
- Samples will be held on ice¹ until final packaging, labeling, and shipment.

4 SAMPLE NOMENCLATURE

The CAP identifies the transect-numbering protocols planned for the Herrington Lake sample locations, as follows:

- LHL – lower Herrington Lake
- UHL – upper Herrington Lake
- MHL – mid-Herrington Lake
- DR – Dix River
- CI – Curds Inlet

¹ Samples will be placed on wet ice. Dry ice may be used in place of wet ice if necessary.

- HQ – HQ Inlet
- HI – Hardin Inlet

Within the CAP, the LHL, UHL, and MHL transects are further numbered as Transect 1, 2, 3, for each portion of the lake (e.g., LHL-1, LHL-2). The transect nomenclature from the CAP will be used along with nomenclature for vegetation and invertebrates described in Sections 4.1 and 4.2, respectively.

Quality assurance samples for both vegetation and invertebrates will be labeled, as follows:

- “EB” – for equipment blanks;
- “DUP” – for field duplicate samples; and
- “MS/MSD” or “M” – for matrix spike/matrix spike duplicates.

4.1 Aquatic Vegetation

The aquatic vegetation material matrix codes for samples that will be collected include the following:

- “AV” – for aquatic vegetation;

The following sample identification convention for the discrete sediment samples will be followed using the prefix “AV.” Each discrete sample will use the following general identification convention:

- [sample matrix code][discrete sampling number][sample depth, in feet][sample date]

An example discrete plant material sample identification number is as follows:

- **AV-001(0.0-2) - LHL1-170912** – indicates the plant sample number 1 (AVG001) collected from the 0.0- to 2-foot water depth interval (0.0-2) on September 12, 2017 (170912).
- The nomenclature for duplicate samples will include the matrix (AV), the depth (0.0-2), the transect number (LHL1) and the date but not the exact sample location within that transect (blind duplicate) such as: **AV (0.0-2) - LHL1-170912-DUP**.

4.2 Aquatic Invertebrates

The aquatic invertebrate material matrix codes for samples that will be collected include the following:

- “AI” – for aquatic invertebrate;

The following sample identification convention for the discrete samples will be followed using the prefix “AI.” Each discrete sediment sample will use the following general identification convention:

- [sample matrix code][discrete sampling number][sample depth, in feet][sample date]

An example discrete invertebrate sample identification number is as follows:

- **AI-001(0.0-2) - LHL1-170912** – indicates the invertebrate sample number 1 (AI001) collected from the 0.0- to 2-foot depth interval (0.0-2) on September 12, 2017 (170912).
- The nomenclature for duplicate samples will include the matrix (AI), the depth (0.0-2), the transect number (LHL1) and the date but not the exact sample location within that transect (blind duplicates) such as: **AI (0.0-2) - LHL1-170912-DUP**

5 HANDLING, PACKING, AND SHIPPING

The following identifies the procedures that will be used to handle, pack, and ship the aquatic vegetation and aquatic invertebrate samples:

- Samples will be labeled using nomenclature that follows typical nomenclature guidelines described in Section 4.
- Samples will be double wrapped with and labeled with water-proof labels.
- Samples will be placed immediately on ice and will be stored on ice or in a refrigerator until shipment to the laboratory.
- Samples will be maintained via chain of custody until shipment via overnight express to the analytical laboratory as deemed appropriate to meet hold times described in Section 3.

6 DATA RECORDING AND MANAGEMENT

Field notes will be recorded during sampling activities and, at a minimum, will include the following:

- Names of field crew and oversight personnel
- Sample location (GPS of the CAP transect)
- Date, time, and duration of sampling
- General weather conditions
- Substrate characterization
- General water quality parameters
- Sample information (including matrix, sampling method, sample mass, sample ID, sample date and time)
- Habitat description where collected
- Photograph number when pictures are taken (if necessary)

7 QUALITY ASSURANCE

One quality assurance sample will be collected (from a location to be determined in the field based on sample availability) for a duplicate and for matrix spike/matrix spike duplicate (MS/MSD) analysis. If this is not feasible, the laboratory will analyze a lab spiked blank and spiked duplicate. Data validation will be performed in accordance with Section 2.4.1 of the CAP.

8 REFERENCES

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- Ramboll Environ 2017a. Herrington Lake E.W. Brown Corrective Action Plan. Submitted to the Kentucky Division of Water Agreed Order No. DOW – 17001.
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- Scudder, B.C., Chasar, L.C., DeWeese, L.R., Brigham, M.E., Wentz, D.A., and Brumbaugh, W.G., 2008, Procedures for collecting and processing aquatic invertebrates and fish for analysis of mercury as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 2008–1208, 34 p.

Attachment A

Water Quality and Vegetation Data Collection Sheets for Herrington Lake

**Aquatic Invertebrate Qualitative Data Collection Sheet
for Herrington Lake**

WATER QUALITY AND VEGETATION FIELD DATA SHEET

WATERSHED FEATURES	Predominant Surrounding Landuse <input type="checkbox"/> Forest <input type="checkbox"/> Commercial <input type="checkbox"/> Field/Pasture <input type="checkbox"/> Industrial <input type="checkbox"/> Agricultural <input type="checkbox"/> Other _____ <input type="checkbox"/> Residential	Local Watershed NPS Pollution <input type="checkbox"/> No evidence <input type="checkbox"/> Some potential sources <input type="checkbox"/> Obvious sources Local Watershed Erosion <input type="checkbox"/> None <input type="checkbox"/> Moderate <input type="checkbox"/> Heavy
RIPARIAN VEGETATION (18 meter buffer)	Indicate the dominant type and record the dominant species present <input type="checkbox"/> Trees <input type="checkbox"/> Shrubs <input type="checkbox"/> Grasses <input type="checkbox"/> Herbaceous dominant species present _____	
INSTREAM FEATURES	Estimated Reach Length _____m Estimated Stream Width _____m Sampling Reach Area _____m ² Area in km ² (m ² x1000) _____km ² Estimated Stream Depth _____m Surface Velocity _____m/sec (at thalweg)	Canopy Cover <input type="checkbox"/> Partly open <input type="checkbox"/> Partly shaded <input type="checkbox"/> Shaded High Water Mark _____m Proportion of Reach Represented by Stream Morphology Types <input type="checkbox"/> Riffle _____% <input type="checkbox"/> Run _____% <input type="checkbox"/> Pool _____% Channelized <input type="checkbox"/> Yes <input type="checkbox"/> No Dam Present <input type="checkbox"/> Yes <input type="checkbox"/> No
LARGE WOODY DEBRIS	LWD _____m ² Density of LWD _____m ² /km ² (LWD/ reach area)	
AQUATIC VEGETATION	Indicate the dominant type and record the dominant species present <input type="checkbox"/> Rooted emergent <input type="checkbox"/> Rooted submergent <input type="checkbox"/> Rooted floating <input type="checkbox"/> Free floating <input type="checkbox"/> Floating Algae <input type="checkbox"/> Attached Algae dominant species present _____ Portion of the reach with aquatic vegetation _____%	
WATER QUALITY	Temperature _____° C Specific Conductance _____ Dissolved Oxygen _____ pH _____ Turbidity _____ WQ Instrument Used _____	Water Odors <input type="checkbox"/> Normal/None <input type="checkbox"/> Sewage <input type="checkbox"/> Petroleum <input type="checkbox"/> Chemical <input type="checkbox"/> Fishy <input type="checkbox"/> Other _____ Water Surface Oils <input type="checkbox"/> Slick <input type="checkbox"/> Sheen <input type="checkbox"/> Globs <input type="checkbox"/> Flecks <input type="checkbox"/> None <input type="checkbox"/> Other _____ Turbidity (if not measured) <input type="checkbox"/> Clear <input type="checkbox"/> Slightly turbid <input type="checkbox"/> Turbid <input type="checkbox"/> Opaque <input type="checkbox"/> Stained <input type="checkbox"/> Other _____
SEDIMENT/SUBSTRATE	Odors <input type="checkbox"/> Normal <input type="checkbox"/> Sewage <input type="checkbox"/> Petroleum <input type="checkbox"/> Chemical <input type="checkbox"/> Anaerobic <input type="checkbox"/> None <input type="checkbox"/> Other _____	Deposits <input type="checkbox"/> Sludge <input type="checkbox"/> Sawdust <input type="checkbox"/> Paper fiber <input type="checkbox"/> Sand <input type="checkbox"/> Relict shells <input type="checkbox"/> Other _____ Looking at stones which are not deeply embedded, are the undersides black in color? <input type="checkbox"/> Yes <input type="checkbox"/> No

INORGANIC SUBSTRATE COMPONENTS (should add up to 100%)			ORGANIC SUBSTRATE COMPONENTS (does not necessarily add up to 100%)		
Substrate Type	Diameter	% Composition in Sampling Reach	Substrate Type	Characteristic	% Composition in Sampling Area
Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"-10")		Muck-Mud	black, very fine organic (FPOM)	
Gravel	2-64 mm (0.1"-2.5")				
Sand	0.06-2mm (gritty)		Marl	grey, shell fragments	
Silt	0.004-0.06 mm				
Clay	< 0.004 mm (slick)				

HERRINGTON LAKE MACROINVERTEBRATE SAMPLING FORM

SITE	HERRINGTON LAKE TRANSECT ID		
	DATE	TIME	<input type="radio"/> Grab sample <input type="radio"/> Hester Dendy
	FORM COMPLETED BY:		Sample Volume:
	OTHER:		Depuration Time
WEATHER	Present conditions (check all that apply)		
	<input type="radio"/> Heavy Rain <input type="radio"/> Overcast <input type="radio"/> Steady Rain <input type="radio"/> Partly Cloudy <input type="radio"/> Intermittent Rain <input type="radio"/> Clear/Sunny	Inches of rain in last 24 Hours Other:	
OBSERVATIONS	Check all that apply		
	FLOW	WATER CLARITY	WATER COLOR
	<input type="radio"/> Dry <input type="radio"/> Stagnant/Still <input type="radio"/> Low <input type="radio"/> Normal <input type="radio"/> High <input type="radio"/> Flood over banks	<input type="radio"/> Clear/Transparent <input type="radio"/> Cloudy/Slightly Turbid <input type="radio"/> Opaque/Very Turbid <input type="radio"/> Other:	<input type="radio"/> None <input type="radio"/> Brown/Muddy <input type="radio"/> Green <input type="radio"/> Milky/White <input type="radio"/> Tannic/Black <input type="radio"/> Other:
	WATER SURFACE		
	<input type="radio"/> Clear/Sunny <input type="radio"/> Oily sheen that breaks when disturbed <input type="radio"/> Oily sheen that does not break when disturbed		
	<input type="radio"/> Some foam <input type="radio"/> More than 3" foam	Color Color	
	WATER ODOR		
<input type="radio"/> Natural/None <input type="radio"/> Fishy <input type="radio"/> Sewage	<input type="radio"/> Gasoline <input type="radio"/> Chlorine <input type="radio"/> Sulfur	<input type="radio"/> Other	
TAXA GROUPS	MARK THESE TAXA AS X, R, C, or D		
	X = not found, R (rare)=1-9, C (common)=10-99 and D (dominant)=100 individuals or greater		
	<input type="checkbox"/> Stonefly Nymphs	<input type="checkbox"/> Net Spinning Caddisflies	<input type="checkbox"/> Midge Fly Larvae
	<input type="checkbox"/> Mayfly Nymphs	<input type="checkbox"/> Dobsonfly/Helgrammite	<input type="checkbox"/> Black Fly Larvae
	<input type="checkbox"/> Water Penny Larvae	<input type="checkbox"/> Dragonfly & Damselfly	<input type="checkbox"/> Lunged Snails
	<input type="checkbox"/> Riffle Beetles	<input type="checkbox"/> Crayfish	<input type="checkbox"/> Aquatic Worms
	<input type="checkbox"/> Aquatic Snipe Flies	<input type="checkbox"/> Crane Flies	<input type="checkbox"/> Leeches
	<input type="checkbox"/> Caddisflies	<input type="checkbox"/> Aquatic Sow Bugs	
	<input type="checkbox"/> Gilled Snails	<input type="checkbox"/> Scud	
		<input type="checkbox"/> Clams & Mussels	