APPENDIX A1: STANDARD OPERATING PROCEDURES FOR ADULT AND YOUNG-OF- YEAR FISH COLLECTION FROM HERRINGTON LAKE

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APPENDIX A1

SOP FOR PERFORMANCE MONITORING:

ADULT AND YOUNG-OF-THE-YEAR FISH, AND SURFACE WATER SAMPLING

E.W. BROWN STATION, HERRINGTON LAKE, MERCER COUNTY, KENTUCKY

RAMBOLL

DOCUMENT DEVELOPMENT AND APPROVAL

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CONTENTS

ATTACHMENTS

ACRONYMS AND ABBREVIATIONS

%	Percent
µg	Microgram
µm	Microns
aka	Also known as
bws	Below water surface
c	Celsius
CI	Curds Inlet
CAP	Corrective Action Plan
cm	Centimeter
COC	Chain of Custody
CPUE	Catch-per-unit-effort
DUP	Duplicate
EB	Equipment Blank
EPA	Environmental Protection Agency
e.g.	Example
FD	Field Duplicate
FO	Fish Ovaries
FR	Fish Remains (fish without ovaries)
FWB	Fish Whole-Body (whole-body fish sample)
ft	Feet
GPS	Global Positioning System
HI	Hardin Inlet
HNO3	Nitric Acid (Surface water sample preservative)
HQ	HQ Inlet
ID	Identifier
i.e.	Id est, meaning “that is”.
in	Inches
IRM	Interim Remedial Measure
ISARA	Investigation, Source Assessment, and Risk Assessment
KDOW	Kentucky Department for Environmental Protection, Division of Water
kg	Kilogram
KPDES	Kentucky Pollutant Discharge Elimination System
MCI	Middle Curds Inlet
mg	milligram
mg/kilogram	milligram per kilogram
MHL	Middle Herrington Lake
MS/MSD	Matrix spike/matrix spike duplicate
LCI	Lower Curds Inlet
LHL	Lower Herrington Lake
QA	Quality Assurance
QAPP	Quality Assurance Protection Plan
QC	Quality Control
RAO	Remedial Action Objective
Sample ID	Sample Identifier

SOP	Standard operating procedure
SRAA	Supplemental Remedial Alternatives Assessment
SW	Surface Water
The Cabinet	the Kentucky Energy and Environment Cabinet
UCI	Upper Curds Inlet
USEPA	United States Environmental Protection Agency
YOY	Young-of-the-Year
YSI	Yellow Springs Instruments
YYMMDD	Two-digit Year Month Day date format

1 OVERVIEW

This standard operating procedure (SOP) supplements the Kentucky Energy and Environment Cabinet (Cabinet)-approved SOPs that guided the Phase I and II field sampling program included in the E.W. Brown Corrective Action Investigation, Source Assessment, and Risk Assessment Report (Corrective Action ISARA), including:

- *SOP: Fish Sampling and Analysis* (Ramboll Environ 2017c) for adult fish sampling
- *SOP: Herrington Lake Young-of-the-Year (YOY) Bass Assessment and Phase II Fish Tissue Sampling* (Ramboll 2018) for YOY Bluegill sampling.
- *SOP: Aquatic Vegetation, Aquatic Invertebrate and Surface Water* (Ramboll Environ 2017d) for surface water sampling

The Supplemental Remedial Alternatives Assessment (SRAA) Report describes certain performance monitoring for three remedial action objectives (RAOs) defined for Herrington Lake (Ramboll 2021). This SOP addresses RAO 1 (adult fish monitoring) and RAO 2 (young-of-year [YOY] bluegill monitoring and co-located surface water sampling). Appendix A2 includes a separate SOP for the benthic community assessment to measure RAO 3 performance. In addition, Appendix A3 includes an SOP for sub-bottom profiling that will be conducted in support of the benthic community assessment.

The methods and descriptions in this SOP are consistent with the Kentucky Department for Environmental Protection Division of Water (KDOW) SOPs for fish sample collection and for fish sample preparation (KDOW 2016, 2017). The procedures outlined herein for the collection of fish and surface water are consistent with the United States Environmental Protection Agency (USEPA) sample collection guidance in *Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers (Flotemersch et al. 2006*) and *Guidance for Assessing Chemical Contamination Data for Use in Fish Consumption Guidelines* (USEPA 2000).

2 SAMPLE COLLECTION PROCEDURES

This section describes the adult fish, YOY bluegill, and collocated surface water sample collection procedures to monitor fish-tissue selenium concentrations and surface water quality at each YOY fish collection site.

The adult bluegill and bass, and YOY bluegill tissue sample collection described in this SOP is consistent with:

- The Kentucky Department for Environmental Protection Division of Water (KDOW) SOPs for fish sample collection and for fish sample preparation (KDOW 2016, 2017).
- United States Environmental Protection Agency (USEPA) *Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers* (Flotemersch et al. 2006).
- Downstream Strategies 2016 YOY sampling methods for bluegills collected in Curds Inlet.
- *SOP: Fish Sampling and Analysis* (Ramboll Environ 2017c) for Adult fish sampling
- *SOP: Herrington Lake Young-of-the-Year (YOY) Bass Assessment and Phase II Fish Tissue Sampling* (Ramboll 2018b) for YOY Bluegill sampling.

2.1 Adult Fish Collection for RAO 1

This section describes the adult bluegill and largemouth/spotted (Kentucky) bass sample collection and fish sample handling procedures prior to receipt by the laboratory for analysis for selenium in tissues.

Adult whole-body bluegill and largemouth bass will be collected from Curds Inlet and Middle Herrington Lake, with samples collected from locations shown on Figures 4-1A and 4-1B and summarized on Table 4-1 of the SRAA Report. Adult fish bluegill and largemouth bass samples will be collected consistent with the Quality Assurance Project Plan (Ramboll 2017b) and following the methodology provided in Appendix A-1 for the collection of adult fish tissues as part of the performance monitoring program in Herrington Lake. Consistent with the 2017 adult fish sampling, efforts will be made to collect bluegill and largemouth bass but if those species are unavailable in sufficient numbers at the time of sampling, other sunfish species and Kentucky bass will be considered reasonable substitutes, respectively.

Adult Fish Collection Seasonal Timing

The timing of bluegill spawning season in Herrington Lake can vary by year based on many factors including interannual and seasonal variability (e.g. a long cold winter, or early wet spring) but mid spring (late March to mid-May) is typically best for pre-spawn bluegill collection in Kentucky Lakes. Adult bluegill and largemouth bass collection will occur prior to spring spawning in March/ April to facilitate collection of gravid females, if available.

Adult Fish Collection Locations

The adult bluegill and largemouth bass collection will occur at each of the following areas as shown on Figures 4-1A and 4-1B and summarized in Table 4-1 of the SRAA Report:

1. Curds Inlet
2. Lower Herrington Lake outside Curds Inlet three locations LHL 1, LHL 2, and LHL 6
3. Middle Herrington Lake (near Gwinn Island Fish Camp located, near lake-mile 20)

Adult Fish Collection Procedures

- To the extent possible, adult bluegill collected for whole-body tissue sampling will consist of gravid females. If gravid bluegills are collected, ovaries will be removed, and the ovaries, including egg tissue, will be submitted for analysis separately from the remaining carcass. Ovary and carcass concentrations will be combined to provide whole-body concentrations, as done for the Corrective Action ISARA Report.
- Ovary/egg tissues will be collected for bluegill only. Adult largemouth bass ovary tissues are not planned for RAO 1 because ovary tissues were analyzed for largemouth bass and catfish in 2017 and the results did not exceed the USEPA ovary criterion (Ramboll 2019). In addition, studies indicate that bluegill are more sensitive to selenium exposure than largemouth bass (USEPA 2016c).
- The adult fish samples (whole-body and ovary/egg tissues) will be analyzed for selenium.
    - Individual adult fish will be grouped to form composited samples consisting of 2 to 5 bluegills or 2 to 3 largemouth bass.
    - Wherever possible, the individual adult fish within each composite sample should be of similar age-class, each measuring at least 75% in length, compare to the longest fish in the

sample. For the Phase I and II sampling, KDOW requested a 16-inch minimum bass length. This same practice will be followed for the performance monitoring. If a 16-inch bass is collected, the maximum individual length for that composite bass sample then becomes 21.5 inches. If two 16-inch or longer bass are not available, then the individual minimum length for that sample can be decreased to 12 inches. No collected bass should be shorter than 12 inches. For the adult bluegill composite samples, KDOW also requested the collection of reproductively mature individuals but noted that fish sampling is often constrained by the presence of fish at time-of-sampling, so the target lengths were intended as recommendations. Individual adult fish length will be recorded, along with other data including species, number of fish per sample, individual weight, and digital imagery of each sample.

- For the collected gravid female bluegills (not required for bass), fish ovary tissues will also be removed and analyzed for selenium. For those samples, the separated carcass and ovary tissue analytical results will later be mathematically recombined to estimate whole-body concentrations for comparison to Phase I and II whole-body adult fish sample results.

2.1.1.1 Electrofishing

- Electrofishing is the preferred method for adult fish collection. It is efficient (high CPUE) and does not require equipment deployment. Compared to deployment-type collection methods (trotlines or gillnets), it also offers more precise collection location information and offers lower bycatch/mortality rates

Refer to *SOP: Fish Sampling and Analysis* Attachment C (Ramboll Environ 2017c) for electrofishing and seining procedures.

2.1.1.2 Trotlines

- Wherever adult bluegills or largemouth bass prove difficult to collect via electroshocking, collection efforts may require supplementation with duration-deployed (often overnight) multi-hooked fishing lines (aka trotlines) having 50 or less baited hooks. The 1.5-day on-effort sampling limit per region is a rough guideline that includes a portion of the overnight fishing gear deployment duration.

2.1.1.3 Gillnets

- Like trotlines, gillnets work well for nocturnal feeders but are less efficient for bass and bluegills. If duration-deployed (often overnight) gillnets are used, they will be clearly marked and labelled. Gillnet mesh-size for these target species typically ranges from 1 to 3 inches but will vary to optimize CPUE.

During Phase I and II sampling, most of the adult bluegill and bass samples were completed via electroshocking. In a few cases, additional collection methods including trotlines or gill netting were employed.

Adult Fish Collection Methods

- Water-filled containers (aka livewells) will temporarily hold collected adult fish until completion of sampling from a fishing region. Upon completion, any excluded live fish will be return to the collection location.
- Collected live non-target adult fish (e.g. small bass and any catfish or carp) will be immediately released.

- Due to many factors and influences, the lack of target species in sufficient numbers at time-of-sampling can constrain the ability to complete sampling within a practical timeframe. The 1.5-day limit per fishing region included multiple efforts with electroshocking equipment and one to two nights of gillnet/trotline deployment. Therefore, at the discretion of the field team leader and based on gauging level-of-effort, CPUE, and other environmental conditions, substitute species from a similar trophic level may replace the target species for certain samples not completed after 1.5-days on-effort.
- Upon completion of sampling in a fishing region, samples will be placed on ice until access to a freezer is available.

2.2 YOY Bluegill Collection for RAO 2

This section describes the YOY bluegill sample collection and sample handling procedures prior to receipt by the laboratory.

YOY whole-body sunfish will be collected from Curds Inlet and a reference area in Lower Herrington Lake as displayed in Figures 4-2A, 4-2B and summarized on Table 4-1 from the SRAA Report. At least two monitoring events are considered necessary to confirm tissue concentration trends. The results from the first YOY monitoring event will be used to inform the timing of the next YOY sampling event. The timing of the sampling will be determined based on consultation with the Cabinet.

YOY bluegill composite fish samples will be collected consistent with the Quality Assurance Project Plan (Ramboll 2017b) and following methodology provided in Appendix A-2 for the collection of YOY fish tissues as part of the performance monitoring program in Herrington Lake.

YOY Bluegill Collection Seasonal Timing

Related to Section 2.1.1 *Adult Fish Collection Seasonal Timing*, the timing of bluegill spawning season in Herrington Lake can vary by year based on many factors including interannual and seasonal variability (e.g., a long cold winter, or early wet spring) but bluegills in Kentucky lakes typically spawn in mid spring (early-April to mid-May). Collection timing for the YOY bluegills will occur in early-mid summer, one to two months after spawning season (early-June to late-July). During the 2018 YOY study sufficient YOY bluegills were available throughout June and July, but limited numbers were found in some smaller inlets (e.g. LHL3, HQ Inlet, and Hardin Inlet (Ramboll 2018). Along with the 2018 results, the results of the first performance monitoring sampling event, tentatively scheduled for summer 2022, will also help to guide the sampling for the second performance monitoring sampling event.

YOY Bluegill Collection Regions

For consistency and to allow direct comparison to the summer 2018 YOY study results reported in the Corrective Action ISARA Report, the proposed YOY bluegill monitoring locations include:

1. Curds Inlet (Upper, Middle, and Lower); three YOY regions located adjacent to E.W. Brown Station. If necessary, to collect sufficient samples, the lower Curds Inlet area can extend into HQ Inlet because these areas are very close and the 2018 data show that lower Curds Inlet and HQ Inlet YOY fish had similar selenium concentrations.
2. The target reference area will be Hardin Inlet (located approximately 3/4th of a mile upstream from Curds Inlet). Other reference areas, LHL 1 and LHL 6, will only be sampled if insufficient fish can be collected from Hardin Inlet at the time of sampling.

In addition to YOY fish collection, surface water samples will also be collected from each YOY fish collection regions. Selenium in surface water will be analyzed to provide supporting information for evaluating the selenium concentrations in YOY fish. Surface water selenium concentrations are not a direct measurement of performance, as the selenium concentrations in fish tissues supersede selenium concentrations in surface water according to the Kentucky water quality standards. The final determination of the locations for collection of the YOY fish and surface water will be determined at the time of sampling based on where the YOY fish are found.

- In the unlikely event that sufficient numbers of YOY fish are not available for each of the Curds Inlet regions (upper, middle, and outer Curds Inlet), a combination of the Curds Inlet regional samples may be considered.

YOY Bluegill Collection Procedures

The YOY-specific collection methods will target YOY bluegills in the 1–4-centimeter (cm) or 0.5–2-inch (in) length range.

- The number of individual fish within each YOY bluegill composite sample will be based on the number of individual YOY bluegills needed to meet the tissue mass requirement for performing the selenium tissue residue analyses (5 grams) and the number of YOY fish that can be readily obtained. For example, if collected YOY bluegills measure in the smaller range of 0.5−1 cm, achieving 5 grams for an analytical sample may require compositing up to 100 individual bluegills to be a reflective composite and have appropriate tissue mass. Where quality assurance samples are collected (e.g., duplicates and matrix spike/matrix spike duplicates), triple the number of fish are needed.
- YOY bluegill sample collection will occur in summer, when lake water temperatures are favorable and after bluegill spawning season is mostly complete. Efforts will be made to complete all the YOY bluegill sampling during one sampling event. If spawning results are found to be limited at the time of sampling, subsequent field efforts may be required.
- The primary YOY bluegill collection methods will include electroschocking, seining, dip-netting, and minnow trapping. Minnow traps tend to select for larger juvenile fish. The results of the Ramboll 2018 YOY study did not indicate any adverse physical effects from electrofishing YOY, and electroshocking will also not adversely affect the analytical results. Attachment A provides the YOY fish collection form for documenting collection date, method(s), and on-effort duration.

Additional details for collections/handling.

- Three separate YOY tissue samples composited from up to 100 individual fish will be collected from each YOY region.
- Collected YOY bluegills from one sampling region will be immediately placed in a small container (sanitized mason jar or similar) and placed on ice in a cooler until the minimum required number of individuals for that sample has been collected.
- When a sample is complete, record the number of individuals in the sample and the composited weight (individual YOY bluegill weight is very low).
- Seal each composite YOY bluegill sample in a Ziploc© bag and a capture digital image of the bagged sample that also includes the unique sample ID. Section 3 of this SOP describes the Sample ID nomenclature, adopted from the Phase I and II sampling events. Photographs of individual YOY fish will not be collected.
- Samples will be placed on ice until access to a freezer is available.

2.3 Collection of Surface Water Samples

This section describes the collection procedures for the surface water samples co-located with the YOY bluegill collection. With some notable exceptions, the surface water collection methods described herein are consistent with:

- *SOP: Aquatic Vegetation, Aquatic Invertebrate and Surface Water* (Ramboll Environ 2017) for surface water sampling

For the performance monitoring phase, each YOY Bluegill sampling location will include one mid-water-column depth surface water sample. Water depths at the 2018 YOY study sampling regions were less than 50ft. In any unexpected case that water depths (measured in ft. bws) at a YOY collection location should exceed 50, one surface water sample will be collected from 25 feet bws. Water temperature, depth, and dissolved oxygen will be recorded at time of sampling, using a water quality probe (e.g., YSI 650).

Surface water samples will be collected using grab sampling methodology (e.g., Beta bottle© or Kemmerer© sampler). The sampler will be lowered to mid-water depth (or other desired depth) and a messenger will then close the sampling container. The lowering process my occur multiple times until all the sample containers are filled.

The Surface water samples will be field-filtered for total and dissolved selenium only. Following collection of the total metals surface water samples the sample for filtered metals analysis will be collected using a 0.45-micron (µm) filter on the end of the tubing with the filtered water collected directly into the sample container.

Surface Water Collection Locations

Wherever possible and practical, the surface water samples will be collected in the same area and at the same time as YOY fish samples. The surface water samples should be collected from near the actual locale where the majority of the YOY bluegill sample was collected, within the YOY region.

3 SAMPLE IDENTIFICATION NOMENCLATURE

For the sample IDs, all sample media use the same Herrington Lake sample location acronyms developed in the CAP (Ramboll Environ 2017) as follows:

- **LHL** – Lower Herrington Lake
- **MHL** – Middle Herrington Lake
- **CI** – Curds Inlet
- **HQ** – HQ Inlet
- **HI** – Hardin Inlet

Quality assurance sample IDs will include the following acronyms suffixes:

- "**EB**" – Equipment blank;
- "**DUP**" or "**FD**"– Field duplicate
- "**MS**" or "**MS/MSD**" - Matrix spike/matrix spike duplicates.

Sample IDs for all sample media will include the sample collection date in the '**YYMMDD'** format as follows:

- "**210701**" represents July 1, 2021

Note: For fish samples, because one sample may require multiple days to complete (e.g., daytime electroshocking and then trotline overnight deployment to complete a composite sample for a particular adult fishing region), the sampling date recorded in the sample ID will reflect the day that the sample was completed.

3.1 Adult Bluegills and Bass

Each composited adult fish sample (consisting of 2–5 fish) will use the following naming convention:

- [sample matrix][discrete sampling number][(Species)]"- "[sampling region][sample date]

Composited adult fish tissue sample matrix will be indicated in the sample ID using the following prefixes:

- "**FWB**" – Fish Whole-Body, denotes a whole-body fish sample
- "**FO**" – Fish Ovary denotes an adult fish ovary sample (to be accompanied by an **FR** sample)
- "**FR**" – Fish Remains denotes an adult fish sample with ovaries extracted (accompanied by the **FO** sample from the same adult female)

For example:

- **FWB001(LMB)-UCI-210701** is whole-body adult fish **(FWB)** number **001**. It is a largemouth bass **(LMB)** collected from Upper Curds Inlet **(UCI)** on July 1, 2021 (**210701**).

3.2 YOY Bluegills

The following sample identification convention for the discrete YOY fish tissue composite samples will be followed using the prefix "**YOY**"

- "**YOY**" – YOY composite of 10 individuals

Each discrete sample will use the following general identification convention:

- [sample matrix code]**-**[discrete sampling number]**-**[YOY-specific sampling region]

The YOY bluegill sampling region acronyms include:

- **LHL** – Lower Herrington Lake YOY Sampling regions (**LHL1** and **LHL6** are include in the post-IRM monitoring)
- **CI** – Curds Inlet (divided into the three YOY sampling regions, **UCI** – Upper Curds Inlet, Middle Curds Inlet (**MCI**), Lower Curds Inlet (**LCI**)
- **HQ** – HQ Inlet
- **HI** – Hardin Inlet

The YOY bluegill sampling date recorded in the sample ID will reflect the day that the sample was completed. Because the YOY composite samples may consist of over 100 individuals, completion of a YOY sample may require multiple sampling days up to the limit of 1.5-days of on-effort sampling per YOY region. The YOY sampling date uses a two-digit year-month-day (**YYMMDD)** date format as follows:

"**210701**" is July 1, 2021

An example discrete YOY fish sample identification number is as follows:

- **YOY-001-LHL6-210701** indicates YOY bluegill composite (**YOY**) number 1 (**001**) collected from Lower Herrington Lake YOY sampling region LHL6 Cove located near Sunset Marina (**LHL6**), on July 1, 2021 (**210701**).

3.3 Surface Water

Each surface sample ID will use the following convention:

- "**SW**" – for surface water;
- [sample matrix code] [discrete sampling number] [sample depth, in feet] [sample date]

An example discrete sample identification number is as follows:

- **SW-001(2)-UCI-210701** – indicates a surface water sample (**SW**), number **001,** collected from Upper Curds Inlet (**UCI**) from 2-feet below water surface (bws) (**2**)**,** on July 1, 2021 (**210701**).
- The nomenclature for collected field duplicate surface water samples will be blind (i.e., the sample location will not be identified). The sample name will not include location, as follows: **SW-002-210701-DUP.** Location information for blind duplicates will be retained on the surface water field sampling forms, in field logbooks, and in linked records in a lookup table, for future reference and comparison to analytical results from the sister or parent sample(s).

4 HANDLING, PACKING, AND SHIPPING

The fish and surface water samples will be handled, packed, and shipped as follows:

4.1 Fish

- Samples will be labeled using the sample ID nomenclature described in Section 3.
- Samples will be wrapped in aluminum foil (dull side against the sample), double wrapped and bagged in Ziploc© bags and labeled with water-proof labels and permanent marker.
- Samples will be maintained via COC through shipment via overnight express and analytical laboratory reception as deemed appropriate to meet any laboratory-imposed sample-specific hold times.

Adult Fish

- Consistent with the Phase I and II adult fish sample documentation, documentation for the monitoring event will include completion of the forms previously provided in The *SOP: Fish Sampling and Analysis* and here as Attachment A (Part 1) and chain of custody (COC) forms as provided in Attachment A (Part 2) (Ramboll Environ 2017a). All field activities will be documented as detailed in the Herrington Lake Quality Assurance Project Plan (QAPP) (Ramboll Environ 2017b).
- Documentation of each collected individual adult fish will include length, weight, and fish number (within the sample) and each fish within a composite sample will be clearly and uniquely labeled (e.g., 1 of 5 bluegills, 2 of 5 bluegills, etc). A complete composited, whole-body adult fish sample should contain 2–5 same-species individuals having a minimum

length of at least 75% of the longest individual member (KDOW 2014). In the unlikely event that multiple fish meeting these criteria cannot be obtained, a sample of a single fish may be warranted.

- For collected gravid female adult bluegills, the ovaries will be extracted, weighed, aluminum foil wrapped (dull side against the sample), labeled, and Ziploc© bagged; then double Ziploc© bagged as a unique sample accompanying the remaining whole-body carcass, to ensure preservation of the ovary-carcass relationship for each analyzed individual adult female bluegill. Note that RAO1 and the post-IRM monitoring event does not focus on adult female bass ovary tissue collection but the pre-laboratory process for bass will be the same.
- The adult whole-body fish samples for bluegill and bass will be wrapped in aluminum foil (dull side against the fish) and Ziploc© bagged, and labelled with project name, sample identification, sample date and time, and the analyses requested. Each labelled sample will then be double-bagged to preserve the label(s) from water damage. Whole-body fish to be composited will be bagged together. The laboratory will composite the sample for analytical testing.
- The double-bagged samples will immediately be placed on ice (12-hour maximum hold time on ice) until transported to a freezer. All fish samples will be frozen for transport to the laboratory.

YOY Bluegills

- The YOY bluegills will be composited as described in Section 2 of this SOP. Multiple fish will be combined into a single sample. Based on the results of the 2018 YOY bluegill sample collection, the small individual weight of a single YOY bluegill may require the collection of up to 100 or more bluegills for each composite sample, to meet the minimum 5-gram weight for selenium analysis. The YOY bluegills will be frozen and wrapped in aluminum foil for shipment to the lab, as described in the SOP for Fish from the Phase I effort (Ramboll Environ 2017c). The double-bagged samples will be placed on ice (12-hour maximum hold time on ice) until transported to a freezer and all fish samples will be frozen before transport to the laboratory. Frozen fish samples will be maintained via COC through shipment via overnight courier to analytical laboratory reception.
- The YOY bluegill samples will be processed and analyzed in the lab within the hold time of 1 year for frozen fish samples.
- YOY bluegill composite sample preparation will be conducted in a laboratory environment, and processing (i.e., freeze-drying) will be conducted in accordance with the SOP for Preparation and Homogenization of Fish Tissue Samples (KDOW 2017[1]).

Fish Sample Volumes, Containers, and Hold Times

- Consistent with the Phase I and II samples, each composited adult fish sample will be comprised of 2 to 5 individuals. Unlike in Phase I, but consistent with Phase 2, the adult whole-body fish composite samples will be analyzed for selenium and percent moisture only.

[1] KDOW 2017 refers to a method of freeze dry for the whole fish, which is a time-consuming step in the sample preparation and analysis process. Sampling conducted in 2017 and 2017 required freeze drying to be done at a commercial freeze dry company because the analytical laboratories could not handle the volume of tissue in a reasonable timeframe. Ramboll will check to see if KDOW fish procedures are updated prior to sampling to see if freeze dry aliquots can be used rather than freeze dry of the entire fish (or if freeze drying is not required at all).

- Per Table 4-1 of the SRAA Report, adult fish collection from each sampling region will include two composited adult fish samples for each of two species, for a total of 6 adult bluegill composite samples and 4 adult bass composite samples. Section 2.1.2 describes the adult fish length-range recommendations and requirements.
- The adult fish tissue sample preparation and processing will occur in the laboratory environment in accordance with the standard operating procedures for preparation and homogenization of fish tissue samples (KDOW 2017) and as described in this SOP section. The adult fish samples, having a minimum weight of 5 grams, will be analyzed using USEPA method 7742 for selenium, with a fresh-frozen hold-time of 1 year. The 5-gram minimum does not include QC samples such as field duplicates or MS/MSDs, or any potential laboratory reruns.

4.2 Surface Water

Procedures for handling, packing, and shipping the collected surface water samples are as follows:

- The collected filtered and non-filtered surface water samples will be labeled accordingly using nomenclature that follows the guidelines described in Section 3 of this SOP.

Each submitted sample requires a note on each label and on each submitted SW COC indicating samples are filtered or non-filtered.

- The samples will be double wrapped with aluminum foil and labeled with water-proof labels.
- The samples will be placed immediately on ice and will be stored on ice or in a refrigerator until shipment to the laboratory.

The surface water samples will be maintained via chain of custody through shipment via overnight express to analytical laboratory reception, as deemed appropriate to meet the sample-specific laboratory hold times.

Surface Water Sample Volumes, Containers, and Hold Times

Chemicals of Interest	Analytical Methods	Minimum Volumes Required(a)	Container	Hold Times (b)
Selenium	USEPA 6010B/6020	50 ml	Glass or plastic HNO3 preservative	6 months

(a) The listed volumes are minimum requested.

(b) Assumes the SW sample is received by the laboratory on ice and below 4 Celsius (c)

5 PREPARATION AND ANALYSES AT THE LABORATORY

The physical compositing of the fish samples will occur at the laboratory. Adult fish laboratory analyses will include ovary tissue (wherever available for bluegills only) and whole-body tissue analysis for selenium. The laboratory analyses will be conducted on the composited samples of 2–5 individual fish per sample, and on the separated ovary and carcass samples (bluegills only). In order to limit the number of fish needed for analyses, ovary tissue will be extracted from selected individual gravid female bluegills where available, and the ovary tissue and corresponding carcass will be submitted separately for analysis, allowing for mathematical reconstruction estimation of the whole-body selenium tissue concentrations. This will satisfy sample volume requirements while minimizing the required number of adult bluegills collected.

- Adult bluegill and bass whole-body samples will be collected from Curds Inlet and Middle Herrington Lake. Each adult fish sample will be comprised of 2–5 individual fish. Field weights and lengths of each adult fish will be recorded.
- Adult female bluegills will also include analysis of ovary samples. Weight must be recorded for both the ovary tissue and remains, so that the whole-body fish estimate can be mathematically calculated.
- If confirmed with the Cabinet to be required, the handling of the fish will comply with "Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples, Commonwealth of Kentucky, Energy and Environment Cabinet, Department for Environmental Protection, Division of Water Effective Date: May 11, 2017" (Attachment A).
- Specifically, the Kentucky SOP includes a process call lyophilization (freeze drying). "In 2017, the Kentucky Division of Water elected to homogenize fish tissue samples by lyophilization, also known as freeze drying, instead of liquid nitrogen or dry ice homogenization. It was concluded that lyophilization would simplify and accelerate the sample homogenization process and limit chances of contamination. There were specific observations where the process of homogenizing samples that were frozen with liquid nitrogen/dry ice had damaged blender blades, introducing contaminants in the sample. Lyophilized samples can be homogenized easily in a blender with no risk of damaging the blender cups or blades".
- Results for fish tissue will be reported in wet and dry weight.

6 DATA RECORDING AND MANAGEMENT

Attachment A of this SOP includes the fish sample collection forms. At time-of-sampling, recorded information, at a minimum, will include:

- Names of field crew and oversight personnel
- GPS-Based Sample collection geographic location. For adult and YOY fish, this may consist of recording the centroid of the larger sampling region from which the sample was collected.
- Date, time, and sampling duration (in decimal days). Sample date(s) for some adult fish and YOY bluegills may include multiple dates to complete the YOY sampling for one sampling region. The sample date for the sample ID will be the sample completion date (i.e. the date when the collection of the required number of fish within a sample was completed)
- General weather conditions

- Substrate characterization – visual notes if available
- General water quality parameters
- Sample information (including matrix, sampling method, sample mass, sample ID, sample date and time)
- Habitat description at sampling location
- Digital image number (wherever images are required/captured)

Adult Bluegill Post-Laboratory Tissue Concentration Estimates

After the laboratory completes the adult ovary and carcass fish tissue analyses, the analytical results will be mathematically reconstructed to estimate whole-body selenium concentrations. Ovary and carcass concentrations will be recombined to provide whole-body concentrations, as done for the Corrective Action ISARA Report. The mathematical reconstruction calculates the proportional contribution of each analyzed fish part to then calculate the sum of the parts. The parts of the fish that will be analyzed are the ovaries and the remains (everything else after ovary extraction). A subset of the adult female bluegills will be analyzed for ovary samples for selenium and % moisture only.

The following equation estimates whole-body fish tissue selenium concentrations from composite samples (comprised of 2–5 adult female bluegills), where the ovary tissue and remains are analyzed separately for selenium:

$([W_R/W_{WB}] * C_R) + ([W_O/ W_{WB}] * C_O) = W_{BFC}$ (Selenium Only)

Where:

C_O = Chemical concentration in fish ovary sample (mg/kg)
C_R = Chemical concentration in remains composite sample (mg/kg)
W_O = Weight of fish ovary (mg/kg)
W_{WB} = Weight of fish whole-body fish (mg/kg)
W_R = Weight of fish remains (mg/kg)
W_{BFC} = Whole-body fish chemical concentration (mg/kg)

Fish concentration data will be reported in both dry and wet weight. Percent moisture will be calculated for each sample as follows:

Moisture Content = [(Wet weight – Weight after (Freeze) Drying)/Wet Weight] * 100

7 QUALITY ASSURANCE/QUALITY CONTROL

The quality assurance and quality control (QA/AC) will include the following:

- Quality assurance and quality control samples will be analyzed from a location to be determined in the field, based on sample availability for a duplicate and for MS/MSD analysis, consistent with the Phase I and II field efforts. One duplicate sample will be analyzed for every 10 samples. One MS/MSD tissue sample will be analyzed for 20 samples.
- A third-party data validation will be performed using USEPA Level II and Level IV validation procedures as described in the 2017 QAPP (Ramboll Environ 2017b).
- Kentucky Environmental Services Branch QA/QC split samples can be provided at Cabinet request.

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ADULT AND YOY FISH, AND SURFACE WATER SAMPLING - SOP ATTACHMENTS

Attachment A. Fish Data Collection Sheets

Attachment B. KDOW 2016 Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-Wadeable Streams and Rivers.

Attachment C. Standard Operating Procedures for Electrofishing and Seining

Attachment D. KDOW 2017 Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples.

Attachment E. Water Quality and Vegetation Field Data Sheet for Herrington Lake

Attachment F. Herrington Lake Surface Water Quality and Dissolved Oxygen Profile Data Collection Sheet.

Attachment A: Part 1

Example Fish Data Collection Sheets for Herrington Lake from KDOW 2017 for Preparation and Homogenization of Fish Tissue Samples

LARGE RIVER BIOLOGICAL SAMPLE – Fish p1 of 2 Reviewed by: _____

STREAM NAME:	LOCATION(Lat/Long):
STATION ID:	DATE:

FISH COLLECTION			
Collectors:		River Type:	☐ Large Streams ☐ Small River ☐ Run of River ☐ Regulated Flow
Time of Electrofishing	☐ Day ☐ Night	Type of Electrofishing Unit:	☐ BPEF_____# of Units ☐ 2.5 GPP ☐ 5.0 GPP
LEFT BANK		RIGHT BANK	
Method(s):	☐ Boat ☐ BPEF ☐ Barge ☐ Seine	Method(s):	☐ Boat ☐ BPEF ☐ Barge ☐ Seine
Total Time:	Start: Finish:	Total Time:	Start: Finish:
Voltage Applied:		Voltage Applied:	
Amp output:		Amp output:	
Percent Applied:		Percent Applied:	
Boat Shock Time:		Boat Shock Time:	
Alternate Shock Time:		Alternate Shock Time:	
Total Shock Time:		Total Shock Time:	
Electrofishing Total # of Voucher Jars:			
Left Bank:		Right Bank:	
Seine Time:	# of Efforts:	Seining Total # of Voucher Jars:	

Comments:

Attachment A: Part 2

Fish Data Collection Forms

Herrington Lake Monitoring Phase
Surface Water Collection and Profiling Data Form

Sampling Region (e.g. Curds Inlet):		
GPS Coordinates (or where they can be found if collected electronically):		
Investigators:		**Date:**
Water Quality Probe (e.g. YSI 650 MDS):		**Start Time:**
Probe Calibration Date:	**Weather at start:**	
Secchi Disk Depth (in feet):	**Water Depth (in ft surface to bottom):**	
Notes / Observations:		

SW Sample Depth (feet bws)	Dissolved Oxygen (DO in mg/L)	Water Temperature (°C or °F)	Surface Water Sample? (Y/N)	SW Sample ID

Notes:

°C	Degrees Celsius
°F	Degrees Fahrenheit
bws	below water surface
mg/L	milligrams per liter
mS/cm	milliSiemens per centimeter
pH	Potential of Hydrogen
Y/N	Yes or No

Herrington Lake Monitoring Phase
Adult Fish Tissue Collection
and Chain-Of-Custudy Form

Sampling Location ID (e.g. LHL-2):	**Fish Sampling Location Description:**

KDFWR Wildlife Collection Permit#:	**Notes / Observations:**
Date:	
Start Time:	
GPS Coordinates or Geographic Location:	
Investigators:	**Weather at Start:**

Flow status (circle one): runoff event high flow low flow normal other

Sample #	Fish #	Genus	Species	Length (mm)	Weight (grams)	Comments
	001					
	002					
	003					
	004					
	005					
	006					
	007					
	008					
	009					
	010					
	011					
	012					
	013					
	014					
	015					

Length (mm) of 75%tile of Longest Fish:	**Total # Fish Collected in Sample:**	
Collected by:	**Date:**	**Time:**
Relinquished by:	**Date:**	**Time:**
Received by:	**Date:**	**Time:**

Notes:

Herrington Lake Monitoring Phase
Young-Of-The-Year Fish Tissue Collection and Chain-Of-Custudy Form

Primary Fish Collector(s):	**Notes:**
Sampling Date(s):	
Weather Forecast:	
Air Temp: **Water Temp:**	

YOY Sampling Region (circle one):

Curds Inlet **HQ InletLHL1(Rocky Arm)** **LHL2(Dix Dam)** **LHL3 Cove** **LHL 6 Cove**

Sample ID: e.g. (YOYBASS-001-LHL6), or (YOYBASS - 001TS - LHL6)	**Sampling Location Description** e.g. 50' north of CI2, west shore)	**Sampling Method** (Seine, Electrofish, Minnow Trap etc.)	**Start Time** (24hr clock)	**Sampling Duration** (in hrs)	**Sample Size** (N=)	**No. in Photo**[a] (N=)

Notes:

a) Image containing groups of the collected YOY bluegills (typically accompanied by the Sample ID label and/or at time of placing the sample on the weighing scale) to mesaure field weights before frozen for shipment to the laboratory for whole-body analysis for Selenium.

Attachment B

Kentucky Department for Environmental Protection Division of Water (KDOW) 2016 Standard Operating Procedure (SOP) for Collection of Fish in Large Wadeable and Non-Wadeable Streams and Rivers

Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-wadeable Streams and Rivers

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

Effective Date: September 1, 2016

Revision Date: September 1, 2016

Revision No: 1.0

Document Control No: DOWSOP03041

Action By:	Signature	Date
Rodney N. Pierce, Prepared, SOP Author	Rod N Pe	8/30/2016
Jacob Culp Reviewed, Environmental Biologist Consultant	Jack Culp	9-1-2016
Melanie Arnold Reviewed, Monitoring Section Supervisor	Melanie Arnold	8/30/16
Michelle Cook Approved, Water Quality Br Quality Assurance Coordinator	Michelle Cook	8/30/16
Andrea Keatley Approved, Water Quality Br. Manager	Andrea Keatley	8/31/16
Lisa Hicks Approved, Division Quality Assurance Officer	Lisa Hicks	9/7/16

Revision History

Date of Revision	Page(s) Revised	Revision Explanation
9/1/2016	ALL	New Document

Suggested Citation: Kentucky Division of Water (KDOW). 2016. Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-wadeable Streams and Rivers, Version 1.0. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Table of Contents

Scope and Applicability

This manual has been developed by the Kentucky Division of Water (KDOW) as guidance for the uniform and accurate collection, field processing, field handling and quality assurance/quality control (QA/QC) of fish samples collected from the large wadeable and non-wadeable waters of Kentucky. The methods defined herein are required for all fish collection, field processing, field handling and QA/QC activities resulting in information that could be used for water quality assessments. Advantages of using fish as biological indicators include their 1) widespread distribution from small streams to all but the most polluted waters; 2) utilization of a variety of trophic levels; 3) stable populations during summer months; and 4) the availability of extensive life history information (Karr et al. 1986). The methods used for collecting fish community structure data for use in the large river biotic index development are outlined in this manual.

Any data submitted to KDOW for review will undergo QA/QC and those identified as not following the methods set forth in this document will be flagged as not suitable for the Integrated Report to Congress on Water Quality in Kentucky (305[b] and 303[d] Reports). These data may be retained in KDOW files for other data purposes.

Definitions

Anode – the positive electrode.

Backpack Electrofisher– unit designed for electrofishing.

Backpack Electrofishing (BPEF) – electrofishing with a backpack electrofisher.

Barge Electrofishing – use of a small boat to carry a generator and pulsator.

Cathode – The negative electrode.

DC – Direct current

Dip Net – A net (of appropriate size for size fish being collected) with 3/16 inch mesh affixed to a fiberglass handle.

Electrofishing – The use of electricity to provide a sufficient electrical stimulus in fish to permit easy capture by netting.

GPP – Generator powered pulsator electrofisher

KDOW – Kentucky Division of Water

Large Streams = free-flowing streams with catchment areas greater than 150-200 mi^2 , with most of the channel accessible for sampling and with most of the stream depth less than 1 meter. All of sampling reach is wadeable.

Netter – The individual who nets the captured fish during electrofishing operations.

Non-wadeable – stream sections that cannot be traversed by foot and sampling cannot be performed without the aid of a boat.

Probe – Pole fitted with a metal ring or dropper array.

PPE – Personal Protective Equipment

Rat Tail – cable that is dragged behind a backpack electrofisher and serves the cathode.

Restricted flow Non-wadeable river = a flowing river with a catchment area greater than 200mi^2 and the presence of low-head dams in the system, most areas around the bank may not be wadeable for sampling and with a mean average thalwag depth greater than 4 meters. Most of sampling reach is non-wadeable with generally no areas that are wadeable.

Run-of-river non-wadeable river = a free-flowing stream with a catchment area greater than 150-200 mi^2 , with most areas around the bank that may or may not be wadeable for sampling and with a mean average thalwag depth greater than 1 meter and less than 4 meter. Most of sampling reach is non-wadeable with small areas that are wadeable.

Sample Point – Latitude and longitude that identifies sampling location.

SDS – safety data sheet

Seine – A 10 or 15 foot length by 6 foot width net with 3/16 in mesh affixed to two brails.

Seine effort – One seine effort equal approximately seining 9.2 m^2 (100ft^2) area.

Shocking seconds – time (in seconds) recorded on the electrofisher that the unit is actively electrofishing.

Small River = a free-flowing stream with a catchment area greater than 150-200 mi^2 , with most areas around the bank that are accessible for sampling and with a mean average thalwag depth less than 1 meter. Most of sampling reach is wadeable with small areas that are non-wadeable.

Wadeable – stream locations that can easily be traversed on foot and efficient sampling can be performed.

Health & Safety Policy/Section

Supervisors must make employees aware of proper safety procedures before the employee is engaged in electrofishing. Prior to field work, new crew members should receive orientation on equipment, procedure and risks involved. This orientation should include: explain equipment components and function, demonstration of equipment and hazards associated with electrofishing.

For general safety purposes, field crews should consist of more than one field person. At least two, and preferably all, crew members must have CPR and first aid training.

Members of a field crew should familiarize themselves with the nearest hospital, doctor's office or instant medical care provider.

Each field crew should use the following personal protective equipment (PPEs) (as deemed necessary) for each sampling trip: waders, boots, long pants, hearing protection, eye protection, bug repellent, sunscreen and hand sanitizer. If additional PPE is deemed necessary and not available the site must not be sampled.

Each field crew shall take an inventory/checklist of PPEs before each sampling trip making sure that all equipment is working properly. If any PPE is found to be inadequately working, such as leaking, ripped, etc., it should be repaired or replaced before leaving for the sampling trip.

Field crew allergies, such as bee stings, should be identified before the sampling trip.

Field crews should be properly dressed for the weather conditions. Coats, gloves and head coverings should be used during the late fall, winter and early spring to reduce the threat of hypothermia. Shorts can be worn under waders during the summer to reduce the threat of heat exposure (as deemed necessary).

Drinking water and other liquids should be available to field crews during sampling trips. Water coolers with ice can assist in reducing dehydration and heat exposure illnesses.

When transporting a formaldehyde container inside a vehicle, it must be transported in a secondary leak proof container of sufficient volume to hold the amount in the storage container. When pouring formaldehyde into collection jars, gloves should be worn to prevent skin exposure.

Unless placing a specimen into a collection jar, the lid shall remain closed to prevent the splashing of formalin out of the jar. Jars should be kept away from the facial area to reduce splashing and inhalation exposure. Collection jars should be inspected before use to check for damage. If damage is found, the jar is discarded. Plastic collection jars should be utilized.

Gasoline cans should have tight seals to eliminate the escape of fumes. Electrofisher should be refueled in an open area. Care should be taken when pouring gasoline into the electrofisher so that spillage and inhalation and skin exposure can be reduced.

Field crews should ensure containers are properly sealed before transport to prevent spill and release of fumes.

Personnel Qualifications / Responsibilities

All field crew members will meet at least the minimum qualifications for their job classification. Fish sample collection will be done by Division of Water or partner agency biologists with specialized expertise in fisheries management, fisheries biology, fisheries science or related field. The nature of the sampling protocols for this group requires specialized knowledge of habitats and taxonomy. The fisheries biologist should have knowledge of taxonomy and be familiar with the taxonomic references listed in Appendix A. Fisheries biologist are considered to be qualified if they have specific advanced academic training and/or several years professional experience in field collection of fish assemblages. Division of Water personnel with the required expertise usually holds the title Environmental Biologist Specialist or Environmental Biologist Consultant. Individuals assisting with sampling will be under the direct supervision of a fisheries biologist.

Equipment and Supplies

- Field Datasheet or Waterproof Notebook
- Dipnets
- Electrofisher (DC backpack shocker or GPP)
- Probes
- Anode rings
- Spare probe and rings
- Rat tail
- Boat
- Electrofishing barge
- Fuel: gasoline or batteries
- Field guide (e.g. Peterson's Field Guide Freshwater Fishes)
- Seine (15 foot)
- Formalin and SDS sheet
- Voucher jars (Various sizes)
- 5 gal bucket
- Live well
- Waterproof paper for sample labels

- Lineman's gloves if using non-insulated probes or nets
- Waders and boots
- Polarized sunglasses
- Copies of field protocols
- Pencils
- Clipboard
- First aid kit
- Global Positioning System (GPS) Unit

Methods

Cautions

While following these sampling techniques, it is important to keep the sampling reach intact and undisturbed. Field personnel should not disturb the reach until sampling has occurred. Doing so could result in degradation of the sample. If the sampling reach has been disturbed by other activities, sufficient time should be allowed for the water to clear and fish to settle back into normal habitats. Electrofishing in turbid water can result in underestimates of the fish community. The experience of the crew and their ability to see and net the fish improves the effectiveness of sampling the reach. Polarized sunglasses are recommended when electrofishing, since they will cut down on the glare of the water. In addition, features such as water clarity, flow, depth and time of day need to be considered to obtain optimal success in sampling.

The sampling reach must not be associated within the immediate area (<100 meters) of major tributary confluences or human structural influences, such as bridges, road crossings (fords), low head dams or any other similar structure, unless the purpose of obtaining the fish community data is related to these influences. If these conditions are not adequate or practical, sampling needs to be postponed until an efficient sampling effort can be obtained.

Instrument Calibration

Select the electrofisher settings based on the conductivity of the water. To minimize stress and mortality, it is important to use the minimum amount of electrical energy to stun fish. Select initial voltage setting 150-400 V for high conductivity (>300 µS/cm), 500-800 V for medium conductivity (100 to 300 µS/cm) and 900-1100 V for low conductivity (<100 µS/cm) waters) pulse width (2-6 ms) and pulse frequency (30-120 Hz). Adjust the voltage, pulse width and pulse frequency to efficiently capture fish without inducing excessive stress and mortality.

Type of Collections

To ensure collection of standardized fish community data, stream size (i.e., drainage area) and depth (i.e. wadeable and non-wadeable) have been used to designate streams into four classes: Large Streams, Small River, Run-of-River Non-wadeable Rivers and Restricted Flow Non-wadeable Rivers.

Sampling Periods

The sampling index period is June through October. In some cases, sampling outside of these index periods is necessary to assess immediate impacts (e.g., chemical spills) or to adhere to specific guidelines set forth by the U.S. Fish and Wildlife Service or KDOW for trend monitoring and bioassessment in streams containing federally listed threatened or endangered species. For routine bioassessment or baseline data collection, samples collected outside of these index periods will be considered unacceptable. Also, fish samples should not be collected during periods of excessively high or low flows or within 14 days of a known scouring flow event. Scour events occur when excessive rain fall occurs and river substrates have been altered. In addition, excessive turbid waters should not be sampled.

Sample Reach

Wadeable Large Streams and Small Rivers

A. At each site, a sampling reach of a 300 m length will be established.
B. Latitude and longitude will be determined for each site at the downstream location.

Run-of-River and Restricted Flow Non-wadeable Rivers

A. Sample reaches will be determined by methods presented in Flotemersch et al. (2006).
B. At each site, a sampling reach of a 500 m length will be established.
C. Latitude and longitude will be determined for each site at the downstream location.

Sampling Methods

Wadeable Large Streams and Small Rivers

A. The sampling crew will consist of a minimum of at least three.
B. A combination of electrofishing and seining techniques will be utilized at all wadeable sites. Dip nets and seines shall have $3/16^{th}$ inch mesh. Electrofishing and seining collections will be kept separate.

<u>Barge Electrofishing Method</u>

A. A barge electrofisher is the preferred electrofishing gear in wadeable large streams and small rivers

B. A tote barge or similar electrofisher capable of producing at least 2,500 watts should be used with a single anode.
C. One crew member will navigate the barge and operate the electrofishing unit.
D. The other crew members will work the anode and dip stunned fish.
E. Stunned fish are placed in a live well carried in the barge.
F. Anode operator should also carry a dip net (Barbour et al. 1999).
G. One pass on each bank reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled (Barbour et al. 1999). One pass of the stream channel is allowed if stream width is small enough to allow one zig zag pattern and all habitats to be sampled efficiently.
H. The sampling zone on each bank extends from the edge of the water to the center of the river or to depth of 3 ft.
I. Some circumstances (e.g. swift water) may require the use of a seine (rather than a dip net) and electrofishing.
    1. The seine may be set perpendicular to the current (to act as a block net) by two crew members.
    2. The anode operator(s) applies current upstream to downstream to the seine.
    3. Stunned fish are carried by current into the seine where they are captured.
    4. The electrofishing operator may need to dislodge specimens caught in the substrate.
J. Collected fish should be frequently transferred from dip nets and seines to the live well to lessen stress and mortality.
K. In addition, water in the live well should be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality of fish.
L. At the conclusion of each sampling run, record the time spent electrofishing (in seconds) (Appendix B).

<u>Backpack Electrofishing Methods</u>

A. Note: At large streams and small river sites a single backpack electrofishing unit may not provide sufficient power to collect fish. However, some sampling site may prevent tote barge access. In these situations a backpack electrofisher may be used. Depending on sampling condition, a second backpack unit may be needed in order to provide a sufficient electrical field to collect fish. If two backpack units are used, one unit is designated as the primary unit. The primary unit will be the unit that electrofishing time is recorded from. The secondary unit will provide support to the primary unit by cutting escape routes off from fish fleeing the sampling area.
B. One member of the field crew operates each backpack electrofishing unit.
C. The other field crew members dip stunned fish and carry the bucket used to transport captured fish.
D. The anode operators will also carry a dip net (Barbour et al. 1999).
M. One pass on each bank reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled (Barbour et al. 1999). One pass

of the stream channel is allowed if stream width is small enough to allow one zig zag pattern and all habitats to be sampled efficiently.

E. The sampling zone on each bank extends from the edge of the water to the center of the river or to depth of 3 ft.
F. Crew members with dip nets walk alongside and behind the anode operator(s) to collect stunned fish.
G. Some circumstances (e.g. swift water) may require the use of a seine (rather than a dip net) and electrofishing.
    1. The seine may be set perpendicular to the current (to act as a block net) by two crew members.
    2. The anode operator(s) applies current upstream to downstream to the seine.
    3. Stunned fish are carried by current into the seine where they are captured.
    4. The electrofishing operator may need to dislodge specimens caught in the substrate.
H. Collected fish should be frequently transferred from dip nets and seines to a bucket of water to lessen stress and mortality.
I. In addition, water in the bucket should be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality offish.
J. At the conclusion of sampling, record the time spent electrofishing (in seconds) (Appendix B).

<u>Seining</u>

A. Habitats not effectively sampled by electrofishing are sampled by seining once electrofishing activities have concluded.
B. Seining is a better technique for collecting some minnow species that are not as affected by the electric current.
C. Use a seine that is at least 15 feet long, 6 feet tall and with a mesh size of $3/16^{th}$ inch. The brails must be sturdy to be used in swift runs.
D. There are 3 seining techniques that may be utilized at all stations where fish collections are conducted: seine hauls, kick seining and specific habitat seining
    1. Seine Hauls
        i. Seine hauls are used in shallow areas near the shore with very little structure or in swift runs.
        ii. Seine hauls are generally performed in a downstream direction (Etnier and Starnes 1993, Jenkins and Burkhead 1993 and Hendricks et al. 1980).
        iii. Seining with the current is more efficient because there is less drag on the net and takes advantage of a fish's tendency to escape upstream. Seine operators can also move more quickly to trap fish, and there is no pressure wave in front of the seine, which can cause fish to move away from the net.
        iv. Two members of the field crew will each take a brail and begin moving with the current through the targeted habitat.

v. Make sure that the lead line is down on the bottom, there is an adequate bag in the seine and that the floats at the top of the seine are floating on the surface.
vi. When the seine haul is finished, the seine is beached by dragging it onto the shore.
vii. When there is only a small shoreline area to beach the seine, the brails are brought close together at the shoreline and the lead line slowly pulled into shore by hand.
viii. If the seine cannot be beached, then in one motion, the seine is quickly lifted out of the water and carried onto shore.

2. Kick Seining
   i. Kick seining will be conducted in riffle and run areas of the stream.
   ii. Kick seining involves two crew members holding the seine in a position downstream of the area to be sampled.
   iii. The brails are slightly angled downstream so that the flow forms a bag or pocket in the seine.
   iv. A third crew member disturbs (or kicks) the substrate while moving toward the seine.
   v. After reaching the seine, crew members lift the seine out of the water.
3. Specific Habitat Seining
   i. Sometimes specific habitat seining might be utilized, if specific habitats within the sampling reach could not be adequately electrofished.
   ii. Specific habitat seining involves encircling specific habitat (i.e. woody debris pile) with a seine and thrusting the brails into the habitat (or crew member disturbs the habitat) to force fish out.
   iii. After disturbing the habitat the seine is lifted out of the water.

E. After each seine effort, fish are briefly examined by the fish biologist for the species present and then placed in a bucket of water.
F. Large fish are identified, recorded and released immediately after each seine haul.
G. Smaller fish are identified and released or retained as a voucher after all seining has been completed.
H. A minimum of five seine efforts will be used and will continue until no new species are collected in three consecutive efforts or until a maximum of 90 minutes of effort is reached.
I. One seine effort equal approximately seining 9.2 m^2 (100ft^2) area.
J. If five seine efforts have been expended and no new species were encountered in the last three efforts, seining may cease if all appropriate habitats in the reach have been sampled.
K. Minimum and maximum times are defined as the start to finish of the seining effort.
L. Record the time spent seining (in minutes start to finish) and the number of efforts (Appendix B).

Run-of-River and Restricted Flow Non-wadeable Rivers

- The sampling crew will consist of a minimum of two or three. Crew size will depend on the electrofishing boat.
- Electrofishing will be utilized at all non-wadeable sites. Dip nets shall have 3/16th inch mesh. Left and right bank electrofishing collections will be kept separate.

<u>Electrofishing</u>

A. Collection of fish will follow methods described in Flotemersch et al. (2006) and Flotemersch and Blocksom (2005).
B. The LR-BP method specifies that a single bank is electrofished for 1000 m or a pair of 500 m banks is sampled. Preferred method is the paired 500 m bank.
C. This method is appropriate in Run-of-River and Restricted Flow sites.
D. Daytime electrofishing is conducted at Run-of-River sites
E. Nighttime electrofishing is conducted at Restricted Flow sites.
F. Run-of-River and Restricted Flow sites, each bank (500 m left and right) along the riparian habitat in the main channel will be electrofished with boat mounted electrofishing gear.
G. The electrofishing crew should consist of one boat operator (who maneuvers the boat and controls the electrofishing unit) and one dip-netter (who collects stunned fish and places them in the livewell). Under some circumstances it may be necessary to increase the number of dip-netters (e.g. fast water, low visibility or size of boat).
H. For each bank, electrofishing will start in the upstream portion of the reach and proceed downstream to the end of the reach.
I. During electrofishing, the boat should be operated at a speed near, or if velocities are low just above the current of the river and maneuvered in and out of shoreline habitat.
J. Fish should be dipped from the water and placed into a livewell for processing. In the cases where large quantities of fish are present at once (i.e. when large schools of gizzard shad are encountered), only a representative sample of these fish should be collected (i.e. one or two scoops of the dip net).
K. If large quantities of fish are collected during the sample run and the live well is at capacity, the sampling run should be suspended. The boat should be maneuvered to a location that will not influence additional sampling. Fish should be processed and sampling should continue after fish have been processed. This will help reduce mortality of released fish.
L. At the conclusion of each sampling run the electrofishing settings (i.e. pulse width, percent applied and shocking seconds) for each run should be recorded (Appendix B).
M. Some shallow portions of reaches (e.g. shoals/riffles) may require that the boat be beached and alternate electrofishing techniques employed. Alternate methods include the use of a handheld anode attached to the boat electrofishing unit, hand maneuvering the boat into shallow portions or the use of a backpack electrofisher. In these cases, one crew member operates the anode/boat/backpack electrofisher while the other dips stunned fish with a dip net. The shallow portion of the reach is sampled in this fashion and the electrofishing time is added (if gear other than the electrofishing boat is used)

to the boat electrofishing time to calculate total electrofishing effort. After the shallow portion is electrofished with the alternate methods, the boat is then maneuvered over the shallow portion and boat electrofishing is then continued.

N. Any deviation from boat electrofishing is recorded on the biological verification form (Appendix B).

Sample Processing and Preservation

A. Young of the year fish should not be retained as voucher or included in field counts.
B. Wadeable seining and electrofishing fish collections should be kept separate. Wadeable sites will result in one jar of voucher specimens for electrofishing, one jar for seining and a list of released species.
C. Non-wadeable paired 500m left and right bank samples should be kept separate. Non-wadeable sites will result in two voucher jars (one for left and right banks) and a list of released species.
D. Vouchers specimens.
    1. A minimum of two specimens of all species will be kept as vouchers from the sample event as either retained specimens or photographs. Voucher specimens will be of at least 2 different age/size classes.
    2. Retained specimens are preserved in the field with a 10%-15% buffered formalin solution.
    3. Field containers should be large enough to accommodate the largest specimen without distorting it.
    4. If at all possible, large specimens will be identified in the field, photographed, recorded and released. Retained large specimens vouchers should have a slit made in the abdomen to permit entrance of preservative into the body cavity. This is particularly important in warm weather to prevent partial decomposition of internal organs.
    5. If a specimen represents a significant ichthyological find (e.g., state or drainage record) or the specimen is hard to identify, then they are to be preserved and retained.
    6. If a species or genus is viewed but not collected and if positively identified, these records should be noted (i.e., Hypentelium nigricans, Micropterus spp. or Lepomis spp.).
    7. Federally protected species must be identified, photographed and released immediately.
E. While at the sampling location, all fish samples will receive a label.
    1. The label is placed in the sample jar (labels placed in the jar will be written in No. 2 pencil on waterproof paper).
    2. The label will consist of the following information:
        a. station ID,
        b. stream name,
        c. county,
        d. date sampled,

e. collectors' initials and
f. collection method.

Data and Records Management

Released fish are counted and recorded in the fisheries biologist's field notebook or on the field datasheet (Appendix B). Photographed fish are recorded with the file number from the camera.

Record the time spent electrofishing (in seconds).

Record the time spent seining (in minutes) and the number of efforts.

Completed Chain-of-Custody (KDOW 2009b) if fish samples will not be retained by fish crew leader.

All records are to be stored in project files.

Quality Control and Quality Assurance

A field crew will consist of at least one trained fisheries biologist who is knowledgeable of the identification and nomenclature of Kentucky fishes. This fisheries biologist is to ensure that voucher collections of all fish are taken, specimens are preserved correctly for laboratory examination and sample jars are labeled correctly. All released specimens will be noted in field notebooks or datasheets. After any sampling has been completed, all sampling gear will be thoroughly cleaned to remove all fish so that no fish are carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no fish are present.

Five percent of samples taken in a season will be duplicated by a field crew. The samples will be selected randomly by numbering each collected site as 1-X. Sites will be chosen for replication using a random numbers table or other random numbering method. Replicates will be collected by a different fisheries biologist (if possible) within the same index period.

Field data must be complete and legible and entered on field data sheet (Appendix B) or field notebook. While in the field, the field team should possess sufficient copies of standardized field data forms and chains-of-custody for all anticipated sampling sites, as well as copies of all applicable Standard Operating Procedures.

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Appendix B. Fish Verification and Field Datasheet

LARGE RIVER BIOLOGICAL SAMPLE – Fish p1 of 2 Reviewed by: _____

STREAM NAME:	LOCATION(Lat/Long):
STATION ID:	DATE:

FISH COLLECTION			
Collectors:		River Type:	☐ Large Streams ☐ Small River ☐ Run of River ☐ Regulated Flow
Time of Electrofishing	☐ Day ☐ Night	Type of Electrofishing Unit:	☐ BPEF_____# of Units ☐ 2.5 GPP ☐ 5.0 GPP
LEFT BANK		RIGHT BANK	
Method(s):	☐ Boat ☐ BPEF ☐ Barge ☐ Seine	Method(s):	☐ Boat ☐ BPEF ☐ Barge ☐ Seine
Total Time:	Start: Finish:	Total Time:	Start: Finish:
Voltage Applied:		Voltage Applied:	
Amp output:		Amp output:	
Percent Applied:		Percent Applied:	
Boat Shock Time:		Boat Shock Time:	
Alternate Shock Time:		Alternate Shock Time:	
Total Shock Time:		Total Shock Time:	
Electrofishing Total # of Voucher Jars:			
Left Bank:		Right Bank:	
Seine Time:	# of Efforts:	Seining Total # of Voucher Jars:	

Comments:

LARGE RIVER BIOLOGICAL SAMPLE – Fish p2 of 2

Reviewed by: _____

Stream Name:	Location:
Station Id:	Date:

	Species	Left Bank Released	Left Bank DELT	Right Bank Released	Right Bank DELT
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
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24					
25					
26					

Attachment C

Standard Operating Procedures
Electrofishing and Seining

Occupational Safety & Health Data Sheet

Electrofishing

ELECTROFISHING EQUIPMENT (Figure 1) is used by fisheries' biologists to shock fish in order to survey fish populations. Sending an electric current (either AC or DC) into the water momentarily stuns fish that are within the effective range of the unit, so people can dip them from the water with nets.

2. Electrofishing is the most effective means of finding out the year/class ratio of fish in any body of water. Netted fish are identified, counted, measured, weighed, and examined for identifying marks, such as tags, clipped fins, or brands. The fish are then returned unharmed to the water from which they were netted.

Accident Potential

3. The most obvious accident potential in electrofishing is from electrical shock. Because most boats have aluminum hulls and are floating in water that is being subjected to electrical discharge great enough to stun fish, electrofishing can be a dangerous operation. In addition, an electrical short in the equipment or its wiring can electrify the entire boat.

4. As with any operation in or near water, there is also a hazard of drowning. The people netting fish from the water stand in the bow of a boat. Even on a boat with a waist-high railing, a person could fall overboard while reaching too far out for a fish. And because the tank for holding captured fish is located amidship, the wet fish in the net often must be passed over the deck, creating a slipping hazard.

5. Because the electric generator is usually located near the boat operator, its exhaust pipe and cylinder head present a burn hazard, especially when the water is rough.

Types of Equipment Required

6. Electrofishing is conducted in a variety of environments, each with distinct problems. Projects are conducted both during daylight hours and in darkness with the use of lights.

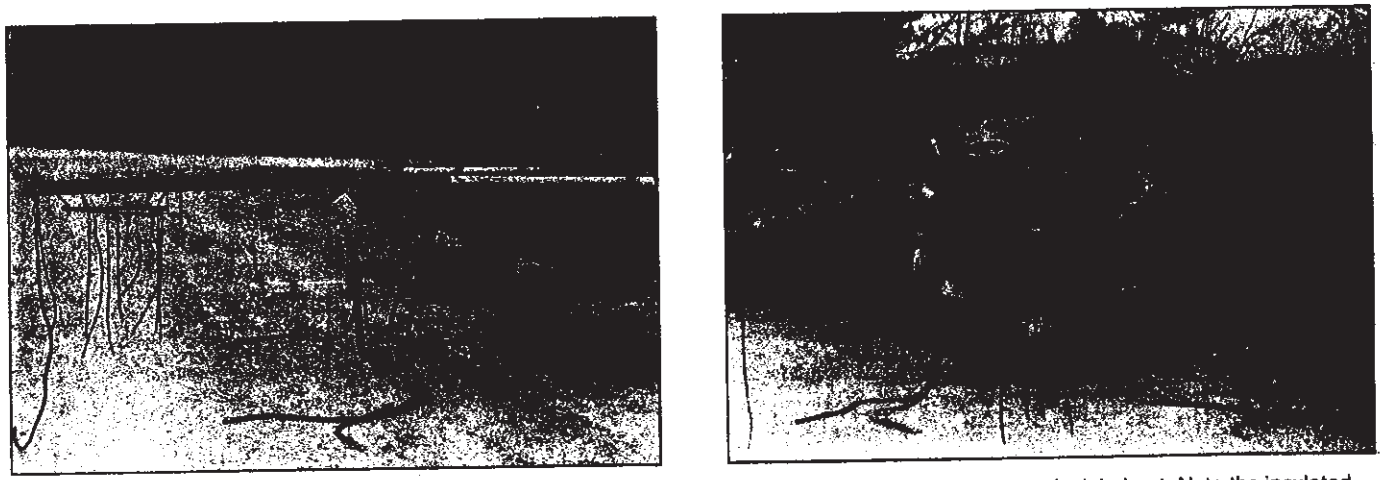

Figure 1. The side view *(left)* and front view *(right)* are of an electrical shocker device mounted on the bow of a johnboat. Note the insulated waist-high safety rails located around the dipping area. (The boat shown here is mounted on a trailer for overland towing to location.)

Projects involve surveys on major rivers, streams, reservoirs, large and small lakes, and in ponds. The types of electrical needs are dictated by the water chemistry and the species of fish involved. The electrical needs include DC (pulsed and nonpulsed) and both two- and three-phase AC.

7. Each electrofishing unit requires the same basic equipment. The primary boat used is the flat-bottomed johnboat. Generators can vary but should be capable of producing AC or DC. (The maximum electrical output capacity is determined by specific needs.) Choice of outboard engines to power the boat also can vary, depending on the need. Boats require a fish-holding tank complete with aerator (Figure 2). Because of the noise created by the outboard motor and the generator, each electrofishing unit should be equipped with an intercommunication system providing a minimum of four headphone jacks (for example, two jacks at the bow for the dip net operator(s), one jack at the boat operator position, and one jack for the supervisory position). Cordless receiver-transmitter units may be used to replace cord-type models. Handles for dip nets should be made of nylon or wood, or glass fiber-wrapped aluminum.

8. A power control box (Figure 3) must be incorporated into the system. It should be located near the boat operator. It should have an instant cutout device so power can be cut immediately to all electrofishing circuits in the event of an emergency.

Crew Composition and Responsibilities

9. In each electrofishing activity, one person must be designated as the *crew supervisor.* This person should be thoroughly trained and qualified to operate all equipment. The crew supervisor is responsible for the electrofishing operation, including enforcement of safety standards.

10. The *boat operator* is responsible for operating the boat safely, for following the directions of the crew supervisor, and for operating the electrical control panel. He or she will respond to the guidance of the dip-netter(s) regarding underwater obstructions. If required, the crew supervisor may also operate the boat.

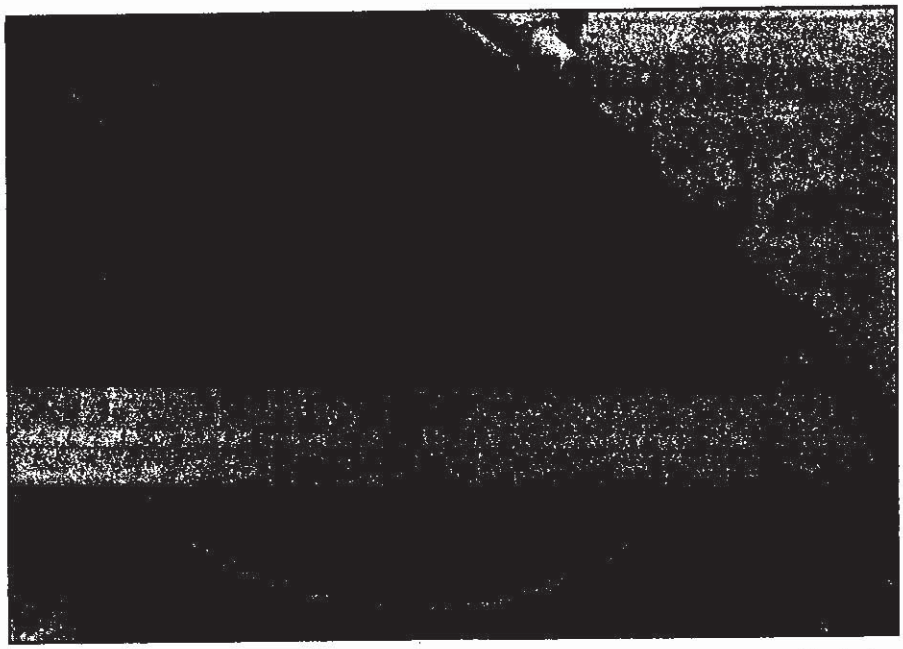

Figure 2. The fish-holding tank is located amidships, adjacent to the dipping area. The isolating screen *(left)* has been pulled aside to show the aerator.

11. The dip-netter(s) will work from the bow of the boat to retrieve fish that have been stunned by the electrical shock. Each dip-netter must be on the lookout for underwater obstructions and immediately advise the boat operator of them. No passengers should be permitted on board during actual shocking projects; exceptions to this rule must be made only by high-level management, if at all.

Figure 3. The power control box, with instant cutout device, should be located near the boat operator, who can then cut power instantly to all electrofishing circuits in an emergency.

Equipment Construction

Generator/alternator

12. The *generator/alternator* (Figure 4) must have enough capacity to provide for all electrical requirements without overloading. Because power and lighting requirements vary widely, no standard can be set for generator size, wiring configuration, and electrical supply voltage.

13. The *battery enclosure* for wet-cell batteries should be acid-proof, nonmetallic, and vented.

14. An *isolating transformer* is required on the output of all generators/alternators.

Voltage and insulation thickness

15. The rated voltage of the insulation of conductors used to deliver output current from the generator/alternator to the pulsator and from the pulsator to the electrodes must exceed the maximum potential voltage of the generator/alternator or pulsator by the next higher rating as shown in Table 1.

Conductor size

16. Conductor size must be approved for rated amperage of equipment as shown in Table 2.

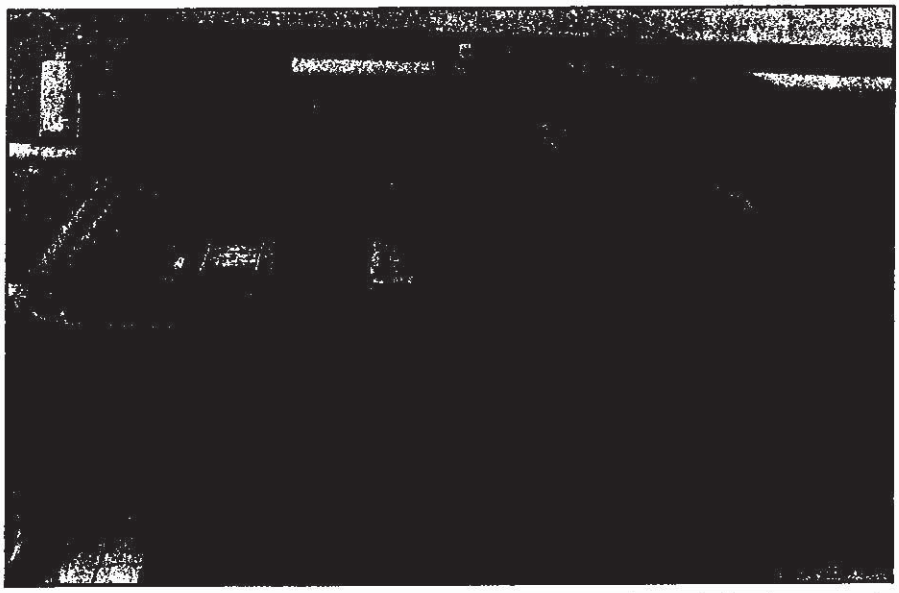

Figure 4. The motor-driven generator/alternator should be safeguarded to keep people away from hot pipes. Exhaust should be kept away from the boat operator.

Table 1. Voltage and Insulation Thickness

Pulsator or Generator/ Alternator	Minimum Insulation Rating of Conductor
0- 249 volts	250 volts
250- 599 volts	600 volts
600- 899 volts	900 volts
900- 12,999 volts	13,000 volts

Table 2. Conductor Size

Amperage	Conductor Size	mm
0-15	14 American Wire Gage	1.628
16-20	12 American Wire Gage	2.053
21-30	10 American Wire Gage	2.568

Conductor type

17. Conductors must be of the stranded type, and insulation of a type recommended for wet locations. All conductors in the boat must be enclosed in raceways, conduit, or liquid-tight flexible conduit. However, when greater flexibility in installation is desired, appropriate heavy-duty rubber-covered cord may be used (Figure 5).

18. **Connections**. If connections are necessary, the rating of the connector (plastic wire nut) must be the same as or greater than that of the wire. Connectors used with flexible cords must be of the locking, water-resistant type. No wire splices are permitted.

19. **Conductor rating.** All conductors within a given raceway or conduit must be rated at or above the maximum voltage of any conductor in the raceway or conduit. For example, if 12-volt lighting conductors are contained within conduit that also carries an 800-volt conductor, then all other conductors must be rated at or above 800 volts.

20. **Junction boxes**. Junction boxes must be made of cast iron, cast aluminum, glass fiber, or plastic. Depending on use, all boxes must be either weatherproof or watertight:

- Boxes with switching equipment must be *weatherproof.* According to the *National Electrical Code (NEC),* Article 100, Section A, this means the boxes must be "so constructed or protected that exposure to the weather will not interfere with successful operation."
- Boxes without switches may be *watertight.* According to the *NEC,* this means the boxes must be "so constructed that moisture will not enter the closure."

21. All junction boxes must be labeled as to voltage and designated by caution labels. To prevent mistakes in using the electricity, use a different type of plugs for each voltage.

Circuit breaker

22. Power output conductors for the generator or alternator must include a circuit breaker or fuse to protect branch circuits. As defined in the *NEC,* a *circuit breaker* is "a device designed to open and close a circuit by a non-automatic means and to open the circuit automatically on the predetermined over-current without injury to itself when properly applied within its rating." The *NEC* definition of a *branch circuit* is "the circuit conductors between the final over-current device protecting the circuit and the load(s)."

23. Circuit breakers or fuses used for protecting branch circuits must be enclosed in a weatherproof enclosure or cabinet that complies with Article 373-2, Section A of the *NEC.* That requirement states:

> In damp or wet locations, cabinets and cut-out boxes of the surface type shall be so placed or equipped so as to prevent moisture or water from entering and accumulating within the cabinet or cut-out box, and shall be mounted so that there is at least ½-inch air space between the enclosure and the wall or other supporting surface. Cabinets or cut-out boxes installed in wet locations shall be weather-proof.

Figure 5. Conductors should be enclosed *(top).* As shown below the junction box, heavy-duty rubber-covered cord can be used for greater flexibility in installation. All conductors must be of a type recommended for wet locations.

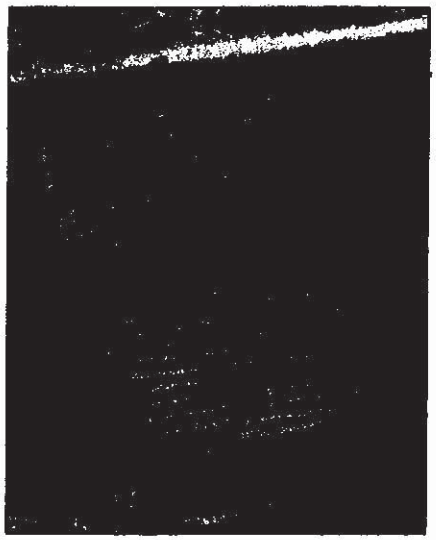

Figure 6. Each dip-netter should have a foot-actuated emergency stop switch (such as this emergency stop pedal) to cut the power from the pulsator or generator.

Figure 7. A steering console should be installed so the operator can see the water around the electrodes and not have to keep turning around to operate the motor.

24. **Central controls.** Unless located otherwise by the manufacturer, all circuit breakers, switches, and controls must be placed in a central control box within easy reach of the boat operator.

25. An instant-stop switch device from power box and outboard motor should be attached to the outboard motor operator. This is designed to assure the operator's safety if he or she falls overboard.

26. Indicator lamps (for example, a neon circuit tester) should be mounted in the power-output circuit to indicate to boat and dip net operators when circuits are energized.

Deadman switch

27. Each dip-netter should have an emergency stop (deadman) switch (Figure 6) that controls the power from the pulsator or generator/alternator. This type of switch requires constant pressure to supply power. If there are two or more dip-netters, each should have a stop switch, electrically connected in series. The boat operator also must have an emergency stop switch connected in series with the netters.

28. Power control circuits—which control the current from the pulsator or generator to the electrodes—may not exceed 24 volts AC or DC.

Electrodes

29. Metal boat hulls may not be used as the cathode (the negative terminal). The anode (positive terminal) and cathode must be electrically insulated from their respective booms (Figure 1).

Grounding and bonding

30. All metal surfaces within the boat, including any electrically conductive cathode and anode booms, must be electrically connected or bonded to the boat hull. In this sense, "connected" means a connection between conductors and a terminal by means of mechanical pressure and without the use of solder. Bonding refers to permanently joining metallic parts to form an electrically conductive part that will assure electrical continuity and the capacity to conduct safely any current likely to be imposed.

31. Grounding, as defined by the *NEC*, is a conducting connection, whether intentional or accidental, between an electrical circuit or equipment and the earth, or to some conducting body that serves in place of the earth.

General lighting requirements

32. The boat should have internal lighting to illuminate walking and work areas. Safety low-voltage direct-current lighting should not exceed 24 volts, with 12 volts preferable. If the lamp is shielded with a non-conductive cage, 110-volt lamps may be used.

Ground-fault circuit interrupters

33. All circuits must be provided with ground-fault circuit interrupters.

Tank and aerator

34. The fish-receiving tank and the aerator (Figure 2) should be located just forward of the amidship position. The tank should be located so that the dip-netter(s) can empty the nets without undue stress, strain, or reaching.

Safety rails and decks

35. Safety rails should be provided around the outside of the dip-netting area (Figure 1) and should be at least 42 in. (1.08 m) high and constructed of heavy-wall steel pipe at least ¾ in. (1.9 cm) in diameter or heavy-wall aluminum pipe 1½ in. (3.8 cm) in diameter. Rails must be designed to withstand lateral pressure of 200 lb-force (900 newtons).

36. Work decks must be covered with nonskid material. They should be sloped to allow drainage.

Boat and electrical control panel console

37. To correct the problem of the operator constantly facing toward the rear to operate the motor, all electrofishing boats should be equipped with a steering console (Figure 7). For larger boats, the control console is best mounted in an elevated position (such as a conning tower), so the operator can see the water around the electrodes.

Exhaust from power source

38. Exhaust from the motor generator/alternator must be piped away from the boat operator. To reduce the potential of burns resulting from contact with the exposed hot pipes, all piping should be enclosed in protective railing or screening. The use of galvanized pipe for exhaust is prohibited because toxic gases may be produced under extreme heating conditions. Mufflers, where applicable, should be used.

Fuel storage

39. Gasoline and diesel fuel must be stored and transported in approved containers. For design requirements, see 29 *CFR* 1910.106.

Warning signs

40. All areas of access or possible access to energized equipment must be equipped with at least two warning signs. Any moving equipment or hot machinery also must be color-coded and labeled with appropriate warning signs.

Safety and General Precautions

Communication

41. The electric power generator can produce sound levels greater than 95 dBA. Together, the generator, the boat engine, and the aerator used to oxygenate the fish-holding tank can produce a noise level that exceeds 100 dBA. Hearing protection is therefore necessary.

42. All personnel should wear communications headsets for communication between positions and for hearing protection. Hearing protection should be provided for all participants in the boat not equipped with communication headsets.

Life vests

43. All occupants of the boat must wear Coast Guard-approved life vests at all times. Life vests must meet the requirements of Type II as a minimum. A Type II vest is an approved device designed to turn an unconscious person in the water from a face-downward position to a vertical or slightly backward face-upward position.

44. Follow U.S. Coast Guard or local applicable fire safety regulations, whichever are more restrictive). As a minimum, each boat must be equipped with at least two 5-lb (2.25 kg) one 10-lb (4.5 kg) type ABC fire extinguisher. These should be mounted in a holder for easy access to the boat operator and away from potential sources of fire.

Dip net

45. The dip net must not be used as an electrode. Net handles must be constructed of a nonconductive material such as nylon or wood. It should be long enough for dip-netters to avoid hand contact with water. Aluminum wrapped in glass fiber may be used.

Clothing and footwear

46. Dip-netters must wear footwear with rubber soles.

47. Dip-netters must either wear long pants or wear hip boots when wearing shorts, swim trunks, or swimsuits.

48. Dip-netters should be provided with rubber gloves. Studies indicate, however, that personnel who wore rubber gloves depended upon them for protection and became less cautious about handling electrical connectors. People should not stick their hands into the water to retrieve lost fish or equipment that had fallen overboard.

Labeling and color-coding of significant hazards

49. Identify and mark with warning signs and color-coding hazards that are specific and significant. For example, although yellow is the code for physical hazards, such as slipping or tripping, it is not helpful to color-code the entire deck.

50. All areas of access or possible access to energized equipment should be color-coded or labeled with appropriate warnings. Exposed moving equipment or hot machinery that cannot be safeguarded and could cause injury if contacted also should be color-coded and/or labeled with appropriate warning signs.

51. **Red.** Red is the color for marking the following critical locations:

- *Fire extinguishers.* Besides identifying the extinguishers themselves, red should also be used on the housing, bulkhead, or support to identify the location of the fire extinguishers.
- *Danger.* Safety cans or other portable containers of flammable liquids should be red, with the name of the contents conspicuously stenciled or painted on the can in yellow.
- *Stop.* Stop buttons or electrical switches used for emergency stopping of equipment should be red.

52. **Yellow.** Yellow is the basic color for designating caution and for marking significant physical hazards, such as striking against, stumbling, falling, tripping, and getting caught in between.

53. **Orange.** Orange designates dangerous equipment: parts of machines and equipment that may cut, crush, shock, or otherwise injure.

54. Labeling and color-coding do not serve as a substitute for physical guarding where necessary.

Hearing protection

55. Through engineering methods, maintain noise levels of the generator within acceptable exposure of 90 dBA for an eight-hour day. Ways to accomplish this include using a hooded and shrouded recreational vehicle-type generator/alternator or installing an insulated, sound-absorbing cover, vented to the outboard side of the boat.

56. If the combined noise level exceeds the 90 dBA permissible exposure limit, crew members should be provided with hearing protection. Communications headsets for primary crew members will meet the requirement. Observers, when authorized, should have hearing protection; the disposable type is permissible.

Training of crew members

57. Everyone who will be operating

electrofishing equipment should be thoroughly trained on each position's responsibilities. Training should include normal procedures as well as emergency procedures. Each crew member should also be trained in first aid and cardio-pulmonary resuscitation (CPR).

58. The electrofishing boat supervisor must have training in basic electrical safety. All crew members must be provided similar training.

Operation checklist

59. Checklists should be developed for all phases of electrofishing operations. These checklists should include procedures from launching to taking the boat out of the water. Procedures for electrical hookup are particularly important and must be included.

60. Checklists for boat, equipment, and operational procedures should be enclosed in a waterproof plastic container. They should be readily available at all times during the electrofishing operation.

Maintenance schedule

61. Because electrofishing equipment is exposed to water, wiring must be periodically checked for corrosion. A schedule for maintenance inspection should be developed. The time intervals depend upon the frequency of use and the severity of the exposure.

Warning

62. Where appropriate, supervisors must provide adequate warning and take positive steps to assure that the public is not exposed to the potential hazards of electrofishing operations. In addition, only a minimum number of employees (or others) may participate in electrofishing operations.

Design

63. General boat design and equipment layout must provide adequate working space to conduct operations. The boat must be kept clean and orderly at all times.

Emergencies

64. The supervisor must provide for emergencies, as appropriate. Examples include submerged logs, sandbars, and a crew member overboard with power on.

Gauges

65. Adequate instrumentation should be provided to monitor the electrical power equipment on the boat.

Refueling

66. To refuel the generator/alternator, turn off all equipment and allow hot surfaces to cool. Fill all tanks before each operation.

Electrofishing Restrictions

Private waters

67. Undertake electrofishing operations on private waters only if the owner or owner's appointed representative is present, or a signed written request has been received from the owner or owner's representative.

Storms

68. Electrofishing should not take place during electrical storms, rain, high winds, or any other conditions considered unsafe by the crew supervisor.

Participants

69. Only qualified personnel may participate in electrofishing operations. Infrequent exceptions to this rule may be made and only with written approval of upper management.

Operations in the vicinity of other vessels

70. Electrofishing operations may not be conducted near other craft. A rule of thumb is to maintain a minimum distance of 100 ft (30 m).

BIBLIOGRAPHY

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American National Standards Institute (ANSI). *Safety Color Code*, ANSI Z535.1-1991. New York: ANSI, 1991.

National Fire Protection Association (NFPA). *National Electrical Code*, NFPA 70. Quincy, MA: NFPA, 1993.

ACKNOWLEDGMENT

This data sheet was prepared by the Public Employee Section of the Industrial Division, National Safety Council.

An alphabetical index of all Occupational Safety & Health Data Sheets (Stock No. 12310-0000) is available from the Council on request.

National Safety Council

STANDARD OPERATING PROCEDURES

SEINING

SOP 1029 – SEINING

Scope: This operating procedure describes the methods to be followed when seining for fish in various waterbodies.

Purpose: The purpose of this procedure is to establish a uniform method of seining to assure quality control in field operations and uniformity among different field teams.

Possible Equipment Needed:

- Collection permit
- Field logbooks
- Seine with leadline and float line
- Poles (two for each seine)
- Chest waders
- Personal floatation devices
- Buckets/livewells
- 10% formalin
- Sample jars
- Scale, fish board, rulers
- GPS
- Clipboards, pens, sharpies, etc.
- Personal field equipment (sunglasses, sunscreen, etc.)

Procedures:

1. Sampling should be conducted at normal seasonal flows at times of high water clarity.

2. Unless a specific component of the fish community is to be assessed, select sites with a variety of habitats in order to assess the broadest extent of the fish community possible. Mesh size will determine the size of fish that will be collected.

3. Establish sample sites with comparable habitats (e.g., riffle/run/pool prevalence, in-stream cover, substrate, depth, etc.) to insure comparability of the fish community data.

4. Establish reference sites appropriate to project goals (e.g., comparison to ecoregion reference sites, or reference sites reflecting general land uses of the site but without any site contaminants of concern). *In-situ* water quality characterization (pH, dissolved oxygen, conductivity, temperature) can also be used to establish comparable reference sites.

5. Document site location with GPS and topographic maps.

6. Place the net in the water, perpendicular to the flow of the river, with the float line at the surface, the leadline at the stream substrate, and a pole at each end. Poles should be at least equal to the height of the net and should be held at a 45° angle away from the direction of movement when pulling the seine.

7. One person should hold each pole on either side of the net. Pull the seine against the current, keeping the poles directly along the bank, and under it if the bank is undercut. The leadline must remain in contact with the bottom to prevent fish from escaping under the net, and the float line must stay on or above the water surface.

8. After a collection is made, both seiners should walk onshore and pull the leadline up immediately. If there is no convenient place to beach the seine, the leadline can be lifted above water by both collectors at the same time. After the net is out of the water, captured fish should immediately be transferred to water-filled containers.

9. Record level of effort and implement similar effort at all sites.

10. Hold all fish in buckets or tubs of site water, using aeration if extended holding is necessary or high numbers of fish are encountered.

11. All fish, excluding larvae, should be collected, enumerated, and identified to the species level using standard taxonomic keys, specific to the region sampled if available. Record total length and wet weight, and enumerate any external lesions, anomalies, and parasites.

12. Specimens that cannot be identified with certainty are preserved in 10% formalin and stored in labeled jars for subsequent laboratory identification. Return all other live fish to the water from which they were collected.

13. If necessitated by project goals, maintain a representative voucher collection (i.e., one specimen of each species collected), excluding threatened and endangered species or other species of special concern. Preserve in formalin. Label all samples to indicate the client/project, site location, date, collectors' names, and sample identification code and/or station numbers for the particular sampling site.

STANDARD OPERATING PROCEDURES

FYKE NET SAMPLING

SOP 1031 – FYKE NET SAMPLING

Scope: This operating procedure describes the methods to be followed when collecting fish with fyke nets in various waterbodies.

Purpose: The purpose of this procedure is to establish a uniform method of fyke net sampling to assure quality control in field operations and uniformity among different field teams.

Possible Equipment Needed:

- Collection permit
- Field logbooks
- Fyke net
- PVC poles or crowbars (4' height, 4 for each net)
- Buoys
- Rope
- Chest waders
- Personal floatation devices
- Buckets/livewells
- 10% formalin
- Sample jars
- Scale, fish board, rulers
- GPS
- Clipboards, pens, sharpies, etc.
- Personal field equipment (sunglasses, sunscreen, etc.)

Procedures:

1. Unless a specific component of the fish community is to be assessed, select sites with a variety of habitats in order to assess the broadest extent of the fish community possible.

2. Establish sample sites with comparable habitats (e.g., riffle/run/pool prevalence, in-stream cover, substrate, depth, etc.) to insure comparability of the fish community data.

3. Establish reference sites appropriate to project goals (e.g., comparison to ecoregion reference sites, or reference sites reflecting general land uses of the site but without any site contaminants of concern). *In-situ* water quality characterization (pH, dissolved oxygen, conductivity, temperature) can also be used to establish comparable reference sites.

4. Document site location with GPS and topographic maps.

5. Depending on water depth, select the appropriate net size. Large nets (~1.0 m x 1.5 m opening) should be used in water greater than 0.75 meters deep. Small nets (~0.5 m x 1.0 m opening) should be used in water less than 0.75 meters deep.

6. Place nets so the opening is facing the shore/vegetation and the funnel is perpendicular to the shore/vegetation.

7. Set wings at a 45 degree angle to the net opening.

8. Confirm that funnels are under water once the net is set.

9. Use rope to attach the buoy to make the net more visible to other boaters.

10. Leave net for 24 to 48 hours before collection.

11. Collect the fish by starting at the open end and simultaneously hold net up while shaking fish down. Successive hoops should then be lifted while still keeping the opening of the net out of the water. This will move fish down to the end of net and keep fish from escaping.

12. Hold all fish in buckets or tubs of site water, using aeration if extended holding is necessary or high numbers of fish are encountered.

13. All fish, excluding larvae, should be collected, enumerated, and identified to the species level using standard taxonomic keys, specific to the region sampled if available. Record total length and wet weight, and enumerate any external lesions, anomalies, and parasites.

14. Specimens that cannot be identified with certainty are preserved in 10% formalin and stored in labeled jars for subsequent laboratory identification. Return all other live fish to the water from which they were collected.

15. If necessitated by project goals, maintain a representative voucher collection (i.e., one specimen of each species collected), excluding threatened and endangered species or

other species of special concern. Preserve in formalin. Label all samples to indicate the client/project, site location, date, collectors' names, and sample identification code and/or station numbers for the particular sampling site.

Attachment D

Kentucky Department for Environmental Protection Division of Water (KDOW) 2017 Standard Operating Procedure (SOP) for Preparation and Homogenization of Fish Tissue Samples

Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

Effective Date: May 11, 2017

Revision Date: May 1, 2017

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Document Control No: DOWSOP0300032

Action By:	Signature	Date
Garrett Stillings, Prepared, SOP Author	Garrett Stillings	5-9-17
Melanie Arnold Reviewed, Monitoring Section Supervisor	Melanie Arnold	5-9-17
Andrea Keatley Approved, Water Quality Branch Manager		5/9/17
Andrea Keatley, Acting Reviewed and Approved, Water Quality Branch QA Coordinator		5/9/17
Lisa Hicks Approved, Division of Water, Quality Assurance Officer	Lisa Hicks	5/11/2017
Peter Goodmann Approved, Division of Water, Director		5/11/2017

Revision History

Date of Revision	Page(s) Revised	Revision Explanation
May 1, 2017	All pages	Sections added include Lyophilization homogenization, dissection of whole bodies and dry weight to wet weight calculations. Revised Fish Tissue Data Sheet and created Lyophilization Data Sheet and Scale Check Log.
July 1, 2014	All pages	Laboratory Procedures for Resection of Fish Fillets and Homogenization of Tissue Samples was separated from preceding document and revised/updated for general content regarding laboratory methods.
March 13, 2008	All pages	**Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky** General Content-Document was re- formatted for maintaining headers, section titles, etc in a consistent style. All references to detailed water chemistry sampling were removed, and a reference inserted directing the reader to the 'Standard Operating Procedures for Sampling and Monitoring Surface Waters for Kentucky', in draft.
July, 2002	All pages	Methods for Assessing Biological Integrity of Surface Waters in Kentucky original document.

Suggested Citation: Kentucky Division of Water (KDOW). 2017. Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples, Version 2.0. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Table of Contents

Procedures

Scope and Applicability

This manual has been developed by the Division of Water as guidance for the uniform and accurate procedures for the preparation and homogenization of tissue samples. The procedures defined herein are required for the preparation and homogenization of tissue samples and QA/QC activities resulting in information used for issuing fish consumption advisories and the biennial Integrated Report to Congress on Water Quality in Kentucky (305[b] and 303[d] Reports). Any data submitted to KDOW for review will undergo QA/QC review and those identified as not following the methods set forth in this document will be flagged as not suitable for issuing fish consumption advisories or for the Integrated Report. These data may be retained in KDOW files for other data purposes.

Fish consumption advisories are jointly issued by the representatives from the Division of Water, Department of Fish and Wildlife Resources and Department for Public Health when contaminants in fish tissue exceed the level considered safe for unlimited human consumption. On December 6, 2004 each agency signed an Interagency Agreement to work together on the issuance of fish consumption advisories. The Interagency Agreement was updated June 24, 2015. The Interagency Agreement outlines the roles of each agency, but does not detail the standard operating procedures concerning how fish consumption advisories should or will be issued. Human health risk-based methodologies, based on previously developed protocols by the Great Lakes Sport Fish Advisory Task Force (GLSFATF 1993) and the U.S. Environmental Protection Agency (EPA 2000a), are used to determine if fish consumption advisories should be issued and what restriction level the advisories recommend. The protocols provide information in greater detail to target populations such as women of childbearing age and children, and recommends the number of fish meals a person may consume to minimize health risks.

Section 305(b) of the Federal Clean Water Act requires Kentucky to monitor, assess and report on the quality of its waters in accordance with Kentucky Water Quality standards. Federal fish tissue based water quality criterion for methylmercury (USEPA 2001) and selenium (USEPA 2016a) have been created and reported in wet weight and dry weight units, respectively. Kentucky has promulgated state specific selenium criterion in 401 KAR 10:031. Surface Water Standards. Kentucky fish tissue resultant information will be used to assess waterbodies for impairment based on Kentucky surface water standards.

Definitions

ESB-Environmental Services Branch
GLSFATF-Great Lakes Sport Fish Advisory Taskforce
KDOW – Kentucky Division of Water
KWADE – Kentucky Water Assessment Data for Environmental Monitoring
SDS –Safety Data Sheet
PPE – Personal Protective Equipment
PTFE – Polytetrafluoroethylene (Teflon)
USEPA-United States Environmental Protection Agency

Health & Safety Policy/Section

Proper PPE shall be worn by all personnel while processing samples and handling chemicals. Refer to the appropriate SDS for the correct PPE while handling chemicals. It is recommended that lab coats also be worn to protect clothing from spillage. Protective eyewear should be worn when the potential exists for particulate, vapor, liquid or foreign objects to become lodged in the eye. When working with chemicals that cause harmful fumes, personnel shall use a fume hood to reduce the threat of inhalation exposure to them and their fellow coworkers.

Toxic or caustic materials must be stored in a chemical storage cabinet. When a chemical spill (e.g. a broken mercury thermometer, broken large containers of acids or preservatives) occurs, the first line supervisor will be notified. The first line supervisor will notify the second line supervisor and the division safety officer. The division safety officer will then notify the department safety officer. Do not attempt to clean-up a chemical spill, if inhalation exposure or skin, throat or eye irritation is a threat. Extreme care shall be taken when processing tissue samples. When cutting frozen fish samples with a band saw or knife, fingers should be kept away from the blade at all times. Care shall be taken when handling and cleaning blenders to reduce the chance of cutting fingers by the blades. Ear protection shall be used when loud machinery will be in use (i.e. processing fish tissue samples using saws or other electronic machinery).

If injury or exposure occurs within the laboratory facilities, then proper first aid attention will be administered by other lab personnel as soon as possible. If the condition is serious, the victim should be transported to a medical facility as soon as possible. For chemical exposures refer to the appropriate SDS for first aid treatment. Safety Data Sheets shall be maintained in a readily accessible location in the lab for each chemical stored or used in the lab. If any exposure occurs while in the laboratory, a 1A1 exposure or injury form needs to be submitted to the Division of Workman's Compensation within 24 hours of exposure or injury.

Cautions

Several cautions exist with regard to activities and negligence that could possibly cause equipment damage, degradation of the sample and possible invalidation of the results. Potential sources of

contamination include dust, instruments, utensils, work surfaces and containers that may contact the samples. All sample processing (i.e., filleting, removal of other tissue, homogenizing, compositing) will be processed in a clean environment set away from sources of potential contamination. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are a concern, then use of stainless steel in processing should be limited and/or appropriate equipment and rinsate blanks procured. If chromium and nickel are not a concern, the use of high-quality, corrosion resistant stainless steel for sample processing equipment is acceptable. If aluminum is of concern, the tissue samples should be placed on glass cutting boards and in glass or plastic containers. Stainless steel instruments and heavy duty aluminum foil are in use by the KDOW; therefore, if the above parameters are of concern, additional equipment may need to be obtained before processing of tissue samples. Equipment rinsate blanks may be used to evaluate the possibility of contamination (USEPA 2000b).

Personnel Qualifications / Responsibilities

All biologists will meet at least the minimum qualifications for their job classification. In addition, fisheries biologists will be trained in the collection and identification of fish by formal academic instruction. Fisheries biologists that have not had formal academic instruction in collection and identification of fish will be deemed technically competent based on their knowledge, skills and abilities by KDOW management. Taxonomic education will continue with on-the-job training, interaction with experienced taxonomists and continued outside training when education opportunities become available. All laboratory personnel performing sample processing procedures should be trained or supervised by an experienced fisheries biologist in the laboratory procedures for resection of fish fillets and homogenization of tissue samples.

Equipment and Supplies

The following is a list of common equipment and supplies typically employed:

- Heavy duty aluminum foil
- Glass cutting board
- Stainless steel filet knife
- Knife sharpening stone
- High speed stainless steel blender (various sizes)
- Freezer (≤-20 ^{o}C)
- Disposable gloves (powder free)
- Whirl-pack® (24 oz., 6” W x 9“ L)
- Fish Tissue Data Sheets
- Lyophilization Data Sheets
- Taxonomic literature (Appendix A)
- Laboratory detergent (Liquinox®)
- Stainless steel trays
- Freeze dryer
- Precision balance (<=0.01g)

Methods

The following sections describe the laboratory procedures for the preparation and homogenization of fish tissue samples. Samples are collected at designated sites for fish contaminant studies and put on ice in clean coolers for tissue preservation. Individual samples can be laid directly on ice in contact with other samples as long as they are rinsed before resection and there is no risk of puncturing the skin. Small fish that will be processed as whole body samples may be placed in a zip top type bag as a group. Composite samples should only contain fish of the same species. Taxonomic references are listed in Appendix A. All samples will be delivered to the KDOW biological laboratory on ice.

Fish fillets and/or whole body samples will be the tissue types covered in these procedures. To assess methylmercury Kentucky water quality standards and consumption limits, fillets will be used. To assess selenium Kentucky water quality standards, whole body samples will be used.

Initial Sample Processing

All samples must be recorded into the Fish Tissue Data Sheet (Appendix B) upon returning from the field. Information included in the Fish Tissue Data Sheet includes waterbody sampled, collection date and time (EST), location on waterbody sampled, basin where the waterbody is located, SiteID of location sampled, county of locations sampled, coordinates of location sampled, collection method, and collector's names and any notes that should be included with the sample. Data fields such as date and time of resection of fish fillets (if applicable) and laboratory personnel who performed each task should also be included. Other fish tissue data sheet field definitions are described below.

- **Sample ID:** assigned by current year and in consecutive order.
- **Sample Method:** Individual or a composite sample.
- **Tissue Type:** Use abbreviations RF=Right Fillet; LF=Left Fillet; BF=Both Fillets; WB=Whole Body.
- **Species:** Species of the sample collected.
- **Length:** Length of the sample collected in millimeters.
- **Weight:** Weight of the sample collected in grams.
- **Sex:** Sex of sample collected.
- **Age:** Age of sample collected.
- **Aging Method: O**=Otolith; **S**=Scale; **F**=Fin; **S**=Spine

Cleaning of Work Utensils

Equipment will be cleaned following USEPA (2000b) for both organic and metals analysis between the processing of each sample. Glass and stainless steel knives/utensils/parts should be cleaned thoroughly with a detergent solution, rinsed with tap water, rinsed with pesticide grade acetone or isopropanol and then rinsed with metal/organic- free de-ionized water.

Tissue Preparation

All samples will remain on ice until tissue preparation can begin. Tissue preparation should occur within 48 hours of collection (USEPA 2000b). If tissue preparation cannot be performed within 48 hours of collection in the biological laboratory, tissue preparation will be performed in the field. If tissue preparations are performed in the field, a clean area will be set up away from sources of exhaust and areas where gasoline or grease are used to help reduce the potential for surface and airborne contamination of the samples. A notation will be made on the Fish Tissue Data Sheet of the location of tissue preparation. Care must be taken to avoid contaminating tissues with material released from inadvertent puncture of internal organs. If the tissue is contaminated by materials released from the inadvertent puncture of the internal organs during resection, the tissue will be rinsed in deionized water.

Prior to tissue preparation, hands will be washed and rinsed thoroughly in tap water, followed by deionized water. Powder-free gloves are to be worn when handling the samples. A protective glove may be worn under a powder-free disposable glove to help prevent cutting injuries while resecting fillets. Knives with stainless steel blades will be used in the resection of fillets. Specimens will be prepared on glass cutting boards or on cutting boards covered with heavy duty aluminum foil that is changed after each sample. Only parts of the specimen that will not be sent to the analytical laboratory for analysis should come in contact with aluminum foil.

NOTE: Changing cutting boards with heavy duty aluminum foil after each use does not require the cutting board to be cleaned between each sample.

Resection of Fish Fillets

Target fillet (or composite) weight is >50 g wet weight. Only fillets from the right side of each fish will be used as part of the qualifying individual sample. If the target weight is not met, the left fillet(s) should be removed and added to the sample. If the sample still does not meet target weight after combining both right and left fillets, personal communication with the qualified analytical laboratory that will be analyzing the tissue samples should commence to identify if the sample can be sufficiently analyzed. Qualifying composite samples are described below and will only be composed of right fillets or both fillets from each sample. Fillets will be processed as boneless skin-on/off (per study plan) and will include all flesh and fatty deposits from the nape to the caudal fin and from the dorsal fin of the back down to venter including the belly flap area of the fish. If skin-on fillet is required, each fish will be scaled prior to the resection of the fillet and rinsed in de-ionized water after scaling. Any bones should be removed from the fillet if present after resection.

NOTE: KDOW has observed that a skin-on fillet often is difficult to homogenize and skin-off fillets are preferred.

When the fillet is removed it should be placed on a clean glass cutting board and not on aluminum foil. In order to facilitate homogenization, fillet(s) should be cut into small pieces (≤ 1 in) and placed inside a certified clean container. The recommended sample container is a sterile whirlpak®, but can be any certified clean container that can be freeze dried without damage or weight loss. The certified clean container should be weighed to the nearest 0.01g before storing the fillet inside. The certified clean container with the fillet should then be weighed (nearest 0.01g wet weight). If the samples volume is too large to be placed in one whirlpak® or approved clean container, the sample can be divided and placed in multiple containers creating a subsample. These data (subsample number and container weights) will be recorded on the Lyophilization Data Sheet (Appendix C).

Sample information will be written on the outside of the container with a waterproof marker. If aging structures or organism sex is required for a project, collect this information after resection of fillets has occurred.

Dissection of Whole Body Samples

Dissection of whole body samples will be processed using of all body parts, bones, body liquids and scales. It should also include all stomach contents. Whole body samples should be diced into small pieces (≤ 1 inch or as small as it can be safely dissected) with stainless steel saw blades and/or knives on clean glass cutting boards. Care must be taken when dissecting large specimens because it can be difficult to slice through bones and scales. The recommended sample container is a sterile whirlpak®, but can be any certified clean container that can be freeze dried without damage or weight loss. The certified clean container should be weighed to the nearest 0.01g before storing the whole body sample inside. The certified clean container with the whole body sample should then be weighed (nearest 0.01g wet weight). If the samples volume is too large to be placed in one whirlpak® or approved clean container, the sample can be divided and placed in multiple containers creating a subsample. These data (subsample number and container weights) will be recorded on the Lyophilization Data Sheet (Appendix C). Sample information will be written on the outside of the container with a waterproof marker.

Qualifying Composite Samples

Individual samples are preferred over composite samples. Individual samples provide a direct measure of the range and variability of contaminants in the target fish population. Composite samples can be utilized when both the right and left fillets of an individual sample weighs <50 g wet weight, whole body samples or when it is cost-prohibitive to analyze individual samples. Qualifying composite samples must adhere to a set of guidelines:

1.) All tissue in the composite must be the same species.
2.) Right fillets or both fillets should only be used unless it's a whole body sample.
3.) All tissue in the composite must be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length of the largest individual (USEPA 2000b).

It should be noted in the Fish Tissue Data Sheet which individuals make up the composite sample. Sample details such as the length and weight can be averaged to describe the composite sample.

Preservation

Once samples are received from the field and resection of fillets has occurred, samples are placed in the laboratory freezer and stored at ≤-20 ^{o}C until samples can be lyophilized.

Lyophilization and Homogenization

Lyophilization should occur in an appropriate amount of time to allow the analytical laboratory to analyze the samples within six months of collection. A detailed illustration of lyophilization procedures are shown in Appendix D. Personnel should use powder-free gloves when handling samples. Frozen samples inside the certified clean containers (i.e. whirlpak®) will be placed inside the freeze dryer for lyophilization. The certified clean containers will be left slightly open for the evacuation of moisture. Care must be taken when opening the containers as to not allow any contamination or sample loss, including frozen moisture to occur. The freeze dry cycle will consist of a nine hour freeze and seven hour drying time. The process may be changed based on the amount of moisture in each sample cycle. If reporting in wet weight, the percent moisture will be recorded before removing the sample from the lyophilized container. See the "Dry Weight to Wet Weight Conversion" section for the percent moisture calculation. After the percent moisture is calculated and recorded, the sample should be transferred into a stainless steel blender for homogenization. A fillet sample with >75% moisture loss and a whole body sample with >70% moisture loss is normally sufficient for homogenization.

NOTE: Wet weight samples should not be lyophilized after blender homogenization to ensure the correct percent moisture calculation.

Depending on the samples volume, the appropriate blender cup size should be used. Most fillets can be homogenized in the small blender cups (32 ounces) while whole body samples will likely require the large blender cups (1 gallon). If subsamples were created, they should all be combined for homogenization. Wet weight samples should not be lyophilized after blender homogenization to ensure correct percent moisture calculation. The sample should be blended until no obvious non-homogenized masses are visible stirring with a clean stainless steel spoon for verification. After homogenization, the sample will be placed back into the original container for processing and storage. Excess sample tissue can be discarded after homogenization if a sufficient sample weight has been attained.

When reporting in dry weight (i.e. selenium analysis), percent moisture is not needed; therefore samples need to be measured to a constant weight. It may take several lyophilization cycles including a cycle(s) AFTER homogenization. When there is no weight loss and the sample weights remain constant, the sample is completely dry.

NOTE: It is imperative that as much moisture be evacuated from the sample as possible.

To ensure that a record of the sample weights for the calculation of moisture loss is recorded, a Lyophilization Data Sheet (Appendix C) should be used. The Lyophilization Data Sheet field definitions are described below.

- **Sample ID:** ID number designated for each sample (individual or composite) and is assigned by year and in consecutive order.
- **Subsample Number:** Should read # of specified subsample of # of containers used for the sample.
- **Container Weight:** Weight of empty sample container with seal removed in grams.
- **Sample Wet Weight:** Wet weight of the sample subtracting the container weight in grams.
- **Reporting Type Goal:** Wet weight (i.e. mercury) or Dry weight (i.e. selenium).
- **Cycle Weights:** Sample weight + container weight after lyophilization cycle.
- **% Moisture:** The results of the calculation: [(Wet Weight–Dry Weight)/ Wet Weight].

Transferring Homogenized Sample to Receiving Vessel and Storage

Homogenized samples can be sealed in the original container. An additional zip-top freezer bag will be added around the original container to ensure no moisture is lost or added during storage. At this point, all homogenates will be stored at -20^{o}C until processed for analysis in the analytical laboratory.

Dry Weight to Wet Weight Conversion

When the Reports of Analysis are delivered from the Environmental Services Branch laboratory, all samples that have been lyophilized will be reported in dry weight and converted to wet weight for the Integrated Report and consumption advisories unless dry weight is recommended (i.e. selenium). The conversion from dry weight to wet weight requires obtaining the percent moisture of the sample being analyzed (USEPA 2016b). To calculate percent moisture, samples must be weighed before and after freeze drying. Samples will be weighed on a scale to the nearest 0.01g. Percent moisture should be included on the Chain of Custody and presented with the official lab Report of Analysis. The conversion formulas and additional information, which includes justification are found in Appendix E.

Quality Control and Quality Assurance

Delivery to the Analytical Laboratory

Samples will be delivered to the appropriate analytical laboratory following KDOW (2009). A chain of custody will be assigned with the samples (Appendix C). Analysis of samples should occur within six months of sample collection.

Balance Calibration Checks

All samples should be weighed on a balance that is properly calibrated and of adequate accuracy and precision (USEPA 2000b). Balance checks should be recorded at the beginning of each weighing session using the reference weights 200 g, 100 g and 50 g. For the calculation of percent moisture, the acceptable tolerance between reference weights and the balance readings is ± 0.1 grams. Care must be taken to avoid balance interferences. Reference weight handling and standardization procedures are found in ESB 2015. Balance calibration and corrective actions for out-of-control data will follow procedures outlined in ESB 2015. If the instrument fails to meet accuracy specifications after re-calibration, the balance will be tagged "Out of Service" until repair or replacement of the balance has occurred. A Balance Check Log is available in Appendix G.

Replicate (Splits) and Rinsate Blanks

Replicate (split) samples will be collected by submitting two independent samples of homogenized tissue from the same sample to the analytical laboratory if required by the study plan.

Rinsate blanks are a de-ionized water sample collected by rinsing the equipment that typically comes in contact with the tissue during homogenization. The equipment should be cleaned prior to rinsing using the protocols described in the above section "Cleaning of Work Utensils". The sample will be collected in appropriate bottles and submitted for analysis if required by the study plan.

Data Storage, Entry and Verification

All field and laboratory data will be recorded on the Fish Tissue Data Sheet (Appendix B) and Lyophilization Data Sheet (Appendix F) then digitized to the appropriate project folder. Results from the analytical laboratory should be filed in the project's e-files and recorded into KWADE according to KDOW (2015). The project coordinator will be responsible for reviewing the received data for accuracy and resolve any corrective actions if needed.

References

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Appendix B. Fish Tissue Data Sheet

FISH TISSUE DATA SHEET-*example*

Waterbody: *Cave Run Lake*	**Collection Date:** *05/29/2017* **Start Time:** *1200* **End Time:** *1400*
Location: *Near Banger Ramp*	**Basin:** *Licking*
Site ID: *DOW05036025*	**County:** *Rowan*
Coordinates (Latitude/Longitude): *38.04375 -83.43882*	**Collection Method:** *Large Boat Electrofisher*
Tissue Preparation Location: ☐Field ☒ Lab	**Collectors:** *Garrett Stillings, Rodney Pierce and Robert Johnson*
Notes: *Lesions were found on Field Sample ID: 17-001*	

Sample ID (Year-Number; Ex. 17-001; 17-002)	Sample Method (Individual or Composite)	Tissue type (RF,LF, BF,WB)	Species	Length (mm)	Weight (g)	Sex	Aging Method*	Age
17-001	*Individual*	*RF*	*Largemouth Bass*	*507*	*1975*	*F*	*O*	*9*
17-002	*Individual*	*BF*	*Channel Catfish*	*414*	*700*	*M*	*S*	*5*
17-003	*Composite*	*RF*	*Bluegill*	*182*	*125*	*NR*	*NR*	*NR*
17-003	*Composite*	*RF*	*Bluegill*	*167*	*100*	*NR*	*NR*	*NR*
17-003	*Composite*	*RF*	*Bluegill*	*168*	*115*	*NR*	*NR*	*NR*
17-003	*Composite*	*RF*	*Bluegill*	*147*	*60*	*NR*	*NR*	*NR*
17-003	*Composite*	*RF*	*Bluegill*	*171*	*105*	*NR*	*NR*	*NR*
17-004	*Composite*	*WB*	*Creek Chub*	*50*	*40*	*NR*	*NR*	*NR*
17-004	*Composite*	*WB*	*Creek Chub*	*60*	*50*	*NR*	*NR*	*NR*

Resection of fillets or sample dissection by: *Garrett Stillings and Rodney Pierce*
Resection Date: *05/30/2017*
Resection Start Time: *1015*
Resection End Time: *1130*

***Aging Method:** **O**=Otolith; **S**=Scale; **F**=Fin; **S**=Spine
RF=Right Fillet; **LF**=Right Fillet; **BF**=Both Fillets; **WB**=Whole Body; **NR**=Not Recorded

Appendix C. Lyophilization Data Sheet

Lyophilization Data Sheet – example

Sample Details							Sample Weight + Container Weight After Lyophilization Cycle					
			A	B	C							
Sample ID	**Date/Time**	**Subsample Number (ex. 1 of 2; 2 of 2)**	**Container Weight (g)**	**Container Weight + Sample WW (g)**	**Sample WW (g) (A - B)**	**Reporting Type Goal (WW or DW)**	**Cycle #1 Weight (g) Date:5/10/16 Time: 1015**	**Cycle #2 Weight (g) Date:5/11/16 Time:1200**	**Cycle #3 Weight (g) Date:5/12/16 Time:1030**	**Cycle #4 Weight (g) Date:5/13/16 Time:1100**	**Cycle #5 Weight (g) Date:5/14/16 Time:1115**	**Cycle #6 Weight (g) Date: Time:**
16-010	*4/15/16 1015*	*1 of 1*	*6.61*	*72.71*	*66.10*	*WW*	*65.24* ☐	*54.87* ☐	*24.87* ☒	- ☐	- ☐	- ☐
16-011	*4/15/16 1030*	*1 of 2*	*6.59*	*197.25*	*190.66*	*WW*	*125.65* ☐	*98.33* ☐	*57.33* ☐	*57.01* ☒	- ☐	- ☐
16-011	*4/15/16 1045*	*2 of 2*	*6.65*	*152.91*	*146.26*	*WW*	*100.37* ☐	*93.35* ☐	*82.35* ☐	*70.26* ☐	*69.23* ☒	- ☐
16-012	*4/15/16 1100*	*1 of 3*	*6.63*	*40.21*	*33.58*	*DW*	*20.53* ☐	*15.36* ☐	*15.30* ☒	- ☐	- ☐	- ☐
16-012	*4/15/16 1115*	*2 of 3*	*6.65*	*25.13*	*18.48*	*DW*	*16.35* ☐	*10.89* ☐	*8.56* ☒	- ☐	- ☐	- ☐
16-012	*4/15/16 1130*	*3 of 3*	*6.64*	*55.23*	*48.59*	*DW*	*35.45* ☐	20.79 ☐	15.47 ☒	- ☐	- ☐	- ☐

WW=Wet Weight; **DW**=Dry Weight ☒=Final Cycle Weight ☐ Check if the cycle was the final lyophilization cycle and the sample was homogenized. Subsamples should be composited before homogenization.

Use This Section Only if Reporting Type is WW.					
	W	**X**	**Y**	**Z**	
Sample ID	**Sample Weight + Container Weight (g)** (∑ Final Cycle weights with the same Sample ID)	**Container Weight (g)** (∑ **A** with the same Sample ID)	**Sample WW (g)** (∑ **C** with the same Sample ID)	**Sample DW (g)** (**W** – **X**)	**% Moisture** ((**Y** –**Z**)/**Y**) x 100
16-010	*24.87*	*6.61*	*66.10*	*18.26*	***72.38***
16-011	*126.24*	*13.24*	*336.92*	*113.00*	***66.46***

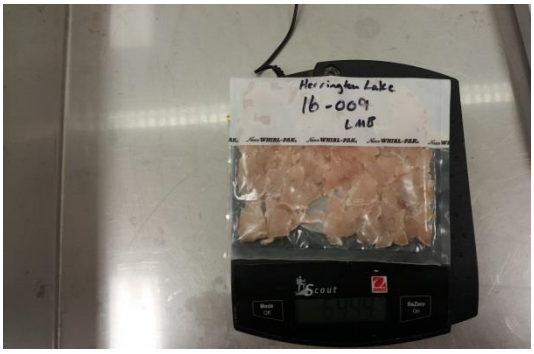
Use This Section Only if Reporting Type is DW and Samples with the Same Sample IDs have been Composited and Homogenized. Reporting Type DW does not require % Moisture.				
	Sample Weight + Container Weight After Lyophilization Cycle			
Sample ID	**Cycle #1 Weight (g) Date:5/15/16 Time:1030**	**Cycle #2 Weight (g) Date:5/16/16 Time:1045**	**Cycle #3 Weight (g) Date:5/17/16 Time:1300**	**Cycle #4 Weight (g) Date: Time:**
16-012	*35.32*	*34.66**	*34.66**	-

*Samples should be lyophilized until a consistent weight is measured.

Appendix D. Lyophilization Procedures

1. Weigh a clean opened whirlpak® to record **Container Weight (g)**.

2. Insert diced fish fillet/whole body into the opened whirlpak® and weigh to record **Container Weight + Sample Wet Weight (g)**.



3. Arrange whirlpaks® on trays and place into freeze dryer.

4. Verify if the freeze dryer settings and vacuum pump oil levels are correct for the lyophilization process. Read freeze dryer instruction guide for more information.

5. When cycle is finished, weigh whirlpaks® and calculate percent moisture. Multiple cycles may be needed. ≥70% of moisture loss is normally sufficient for homogenization of fish tissue. If reporting in dry weight additional lyophilization cycles are needed after homogenization until weights remain constant.

Appendix E. Wet/Dry Weight Conversion Information

In 2016, the Kentucky Division of Water elected to homogenize fish tissue samples by lyophilization, also known as freeze drying, instead of liquid nitrogen or dry ice homogenization. It was concluded that lyophilization would simplify and accelerate the sample homogenization process and limit chances of contamination. There were specific observations where the process of homogenizing samples that were frozen with liquid nitrogen/dry ice had damaged blender blades, introducing contaminants in the sample. Lyophilized samples can be homogenized easily in a blender with no risk of damaging the blender cups or blades.

From personal communication with the Environmental Services Branch staff, lyophilization aids in the analytical analysis of samples. Dry samples make digestion easier because it pre-concentrates elemental compositions by eliminating water while digestion reagents are not diluted. In an extraction when the sample is not homogenized thoroughly the solvent cannot reach all parts of the sample, which can create inconsistent results. Additionally, there is a significant reduction in solvent usage for primary extraction since the volume/mass of the sample will effectively be reduced by > $1/5^{th}$.

Samples homogenized by lyophilization will be reported in dry weight from the Environmental Services Branch and can be converted to wet weight by the user for fish consumption advisories and Integrated Reports. The conversion from dry weight to wet weight requires obtaining the percent moisture of the sample being analyzed (USEPA 2016b). To calculate the percent moisture, samples must be weighed before and after lyophilization. Samples will be weighed on a balance at least to the nearest 0.01 grams. When converting between weights, the least number of significant figures in any number of the conversion determines the number of significant figures in the result. The conversion formulas are described below.

Dry Weight to Wet Weight Conversion Formulas

Wet Weight = Total sample weight before lyophilization

Dry Weight = Total sample weight after lyophilization

% Moisture = [(Wet Weight - Dry Weight)/Wet Weight] x 100

Wet Weight Concentration = Dry Weight Concentration x [1 - (% Moisture/100)]

Dry Weight Concentration = Wet Weight Concentration / [1 - (% Moisture/100)]

Examples:

***14-112** -- [(35.32-6.58)/35.32]x100=81.37=% Moisture*
1.870 x [1– (81.37/100)]=0.348=WW Concentration

***14-125** -- [(28.73-6.24)/28.73]x100=78.28=% Moisture*
0.262 x [1– (78.28/100)]=0.059=WW Concentration

Field ID	Dry Weight (g)	Wet Weight (g)	% Moisture	DW Hg Concentration (mg/kg)	WW Hg Concentration (mg/kg)	WW Hg (mg/kg) from past runs in 2014
14-112	6.58	35.32	81.37	1.87	0.35	0.36
14-125	6.24	28.73	78.28	0.26	0.06	0.06

Appendix F: Chain of Custody

Chain of Custody Record

Program Code: A20

Coordinator:

County	Field ID	Sample Identification	% Moisture Removed	Collection Method	Date	Container 1	Container 2	Container 3
				☐ Composite ☐ Grab		☐ LAB Report #	☐	☐
				☐ Composite ☐ Grab		☐ LAB Report #	☐	☐
				☐ Composite ☐ Grab		☐ LAB Report #	☐	☐

Analysis Requested: Program Code: A20
Sample Matrix: ☐ Tissue-Fillet ☐ Tissue-Whole Body
Sample Type: ☐ Dry Weight ☐ Wet Weight

Container 1: Shipment Temp:
Container 2:
Container 3:
Samples Collected By:

Relinquished by: _____ Date: _____ Received by: _____ Date: _____

Representing: _____ Time: _____ Representing: _____ Time: _____

Appendix G. Scale Check Log

Balance Check Log

Weighing Session Date/Time	Reference Weight Balance Readings			Check if Recalibrated
	200 g	100 g	50 g	
				☐
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SURFACE WATER SAMPLING SOP ATTACHMENTS

Attachment E. Water Quality and Vegetation Field Data Sheet for Herrington Lake

Attachment F. Herrington Lake Surface Water Quality and Dissolved Oxygen Profile Data Collection Sheet.

WATER QUALITY AND VEGETATION FIELD DATA SHEET FOR HERRINGTON LAKE

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET #	AGENCY	
INVESTIGATORS		
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

WEATHER CONDITIONS	**Now**	**Past 24 hours**	**Has there been a heavy rain in the last 7 days?** ❑ Yes ❑ No
	❑ storm (heavy rain)	❑	**Air Temperature**_____^{0} C
	❑ rain (steady rain)	❑	
	❑ showers (intermittent)	❑	**Other**_____
	____%❑ %cloud cover	❑____%	
	❑ clear/sunny	❑	
SITE LOCATION/MAP	**Draw a map of the site and indicate the areas sampled (or attach a photograph)**		
STREAM CHARACTERIZATION	**Stream Subsystem** ❑ Perennial ❑ Intermittent ❑ Tidal		**Stream Type** ❑ Coldwater ❑ Warmwater
	Stream Origin ❑ Glacial ❑ Spring-fed ❑ Non-glacial montane ❑ Mixture of origins ❑ Swamp and bog ❑ Other_____		**Catchment Area**_____km^2

Source: Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition - Form 1

WATER QUALITY AND VEGETATION FIELD DATA SHEET

WATERSHED FEATURES

Predominant Surrounding Landuse
❑ Forest ❑ Commercial
❑ Field/Pasture ❑ Industrial
❑ Agricultural ❑ Other _____
❑ Residential

Local Watershed NPS Pollution
❑ No evidence ❑ Some potential sources
❑ Obvious sources

Local Watershed Erosion
❑ None ❑ Moderate ❑ Heavy

RIPARIAN VEGETATION (18 meter buffer)

Indicate the dominant type and record the dominant species present
❑ Trees ❑ Shrubs ❑ Grasses ❑ Herbaceous

dominant species present _____

INSTREAM FEATURES

Estimated Reach Length _____m

Estimated Stream Width _____m

Sampling Reach Area _____m^2

Area in km^2 (m^2x1000) _____km^2

Estimated Stream Depth _____m

Surface Velocity (at thalweg) _____m/sec

Canopy Cover
❑ Partly open ❑ Partly shaded ❑ Shaded

High Water Mark _____m

Proportion of Reach Represented by Stream Morphology Types
❑ Riffle_____% ❑ Run_____%
❑ Pool_____%

Channelized ❑ Yes ❑ No

Dam Present ❑ Yes ❑ 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
❑ Rooted emergent ❑ Rooted submergent ❑ Rooted floating ❑ Free floating
❑ Floating Algae ❑ Attached Algae

dominant species present _____

Portion of the reach with aquatic vegetation _____%

WATER QUALITY

Temperature_____^{0} C

Specific Conductance_____

Dissolved Oxygen _____

pH _____

Turbidity _____

WQ Instrument Used _____

Water Odors
❑ Normal/None ❑ Sewage
❑ Petroleum ❑ Chemical
❑ Fishy ❑ Other_____

Water Surface Oils
❑ Slick ❑ Sheen ❑ Globs ❑ Flecks
❑ None ❑ Other_____

Turbidity (if not measured)
❑ Clear ❑ Slightly turbid ❑ Turbid
❑ Opaque ❑ Stained ❑ Other_____

SEDIMENT/ SUBSTRATE

Odors
❑ Normal ❑ Sewage ❑ Petroleum
❑ Chemical ❑ Anaerobic ❑ None
❑ Other_____

Oils
❑ Absent ❑ Slight ❑ Moderate ❑ Profuse

Deposits
❑ Sludge ❑ Sawdust ❑ Paper fiber ❑ Sand
❑ Relict shells ❑ Other_____

Looking at stones which are not deeply embedded, are the undersides black in color?
❑ Yes ❑ 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)				

Source: Appendix A-1: Habitat Assessment and Physicochemical Characterization Field Data Sheets - Form 1

Herrington Lake Surface Water Quality and Dissolved Oxygen Profile Data Collection Sheet

Herrington Lake Transect Location		
GPS Coordinates (or where they can be found if collected electronically)		
Investigators:		**Date:**
		Time:
Temperature/Depth/Oxygen Probe Used		
Probe Calibration Date		
Secchi Disk Depth		

Depth (feet)	DO (mg/L)	Temp (oC or oF)	Stratification Layer (if known)	Was Surface Water Collected? (Y,N)	Sample ID (if collected)	Observer Notes, if any

Notes

DO	Dissolved oxygen
Temp	Temperature
^{o}C	Degrees Celcius
^{o}F	Degrees Fahrenheit
mg/L	milligrams per liter
Y/N	Yes or No

APPENDIX A2: STANDARD OPERATING PROCEDURES FOR BENTHIC COMMUNITY ASSESSMENT IN HERRINGTON LAKE

Prepared for
Kentucky Utilities Company

Document type
Standard Operating Procedure (SOP)

Date
August 2021

APPENDIX A2: SOP FOR PERFORMANCE MONITORING - BENTHIC COMMUNITY ASSESSMENT

E.W. BROWN STATION, HERRINGTON LAKE, MERCER COUNTY, KENTUCKY

DOCUMENT DEVELOPMENT AND APPROVAL

TITLE AND APPROVAL SHEET

Action By	Signature	Date
Reviewed by: Katrina Leigh, Ramboll		July 15, 2021
Reviewed by: Mary Sorensen, Ramboll		July 30, 2021

CONTENTS

TABLES

ATTACHMENTS

ACRONYMS

BCA	benthic invertebrate community assessment
EPT	mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera)
GPS	global positioning system
HBI	Hilsenhoff Biotic Index
HD	Hester Dendy
HI	Hardin's Inlet
ID	Identification
KDOW	Kentucky Department for Environmental Protection, Division of Water
LBII	Lake Bioassessment Integrity Index
LCI	lower Curds Inlet
LHL	Lower Herrington Lake
MCI	middle Curds Inlet
NCDEM	North Carolina Division of Environmental Management
NMACI	non-wadeable macroinvertebrate assemblage condition index
RAO	remedial action objective
RBP	Rapid Bioassessment Protocol
SOP	Standard Operating Procedure
RIVPACS	river invertebrate prediction and classification system score
SOP	Standard operating procedure
SRAA	Supplemental Remediation Alternatives Assessment
UCI	upper Curds Inlet
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

1.OVERVIEW

This standard operating procedure (SOP) describes the field methods for the collection of sediment-dwelling (benthic) invertebrates for a benthic community assessment (BCA), as described in the Supplemental Remediation Alternatives Assessment (SRAA) Report. This benthic community analysis SOP has been developed to address the site-specific Remedial Action Objective 3 (RAO 3): "Demonstrate the diversity and abundance of the Curds Inlet sediment-dwelling invertebrate community, considering the presence of selenium in sediment, and arsenic in sediment pore water, and iron in sediment pore water." The benthic community assessment to evaluate RAO 3 is anticipated to be conducted in the spring or summer of 2022, subject to the Cabinet's approval. Process water that formerly flowed to Curds Inlet via Outfall BRN001 is now treated on-site prior to discharge into Herrington Lake through the high rate multi-port diffuser at Outfall BRN006. This operational change has substantially reduced the contaminant loading and water flow in Curds Inlet. The benthic invertebrate community assessment will address the structure, composition, abundance, and diversity of the benthic macroinvertebrate community in Curds Inlet as compared to one or more reference areas with similar habitat within Herrington Lake.

The methods and descriptions described in this SOP are consistent with the guidance:

- Kentucky Department for Environmental Protection, Division of Water (KDOW) SOPs for invertebrate sample collection, identification, and analysis (2003, 2011, 2015a, 2015b, 2016, 2018);
- United States Environmental Protection Agency (USEPA) Rapid Bioassessment protocols, first and second editions (USEPA 1989, 1999);
- USEPA Concepts and Approaches for the Bioassessment of Non-Wadeable Streams and Rivers (2006);
- USEPA literature about bioassessment lake indices (2001, 2009); and
- USEPA Lake and Reservoir Assessment and Biocriteria (1998).

Because the KDOW SOPs referenced above were developed for shallow, wadeable, rocky streams and rivers, some variances from the KDOW guidance are described in this document to address the specific habitat of Herrington Lake.

The remainder of this SOP describes:

- Sampling collection procedures (Section 2)
- Sample identification nomenclature (Section 3)
- Handling, packing, and shipping (Section 4)
- Data recording and management (Section 5)
- Laboratory analysis (Section 6)
- Benthic community metrics (Section 7)
- Quality assurance/quality control (Section 8)
- References (Section 9)

2.SAMPLE COLLECTION PROCEEDURES

The following section describes the procedures for the collection of benthic invertebrates from Herrington Lake for the benthic community assessment. The benthic macroinvertebrate community can be a relatively sensitive indicator of ecological impacts. However, the benthic macroinvertebrate community can be sensitive to other stressors and alterations in habitat, such as water flow, dissolved oxygen, presence or absence of rocky substrate, type and quality of riparian vegetation, and water depth.

Benthic invertebrate sampling will follow KDOW and USEPA guidance on benthic macroinvertebrate community analysis (KDOW 2003, 2011, 2015; USEPA 1989, 1999, 2009, 2001, 2006) as closely as practical. However, the KDOW guidance was developed for use in wadeable streams and rivers, primarily using nets, and the collected data are compared to the 95th percentile of the Kentucky reference data set (which is taken from wadeable streams and rivers). Because the sampling areas in Herrington Lake are not wadeable, artificial solid-substrate samplers will be deployed from a boat for this benthic community assessment.

Prior to the estimated sampler deployment, weather and information from United States Geological Survey (USGS) stream gauges upstream (USGS 03285000 Dix River Near Danville, Ky) and downstream (USGS 03286000 Herrington Lake Near Burgin, Ky – this gauge is at the dam) of the sampling areas will be tracked. The upstream Dix River gauge tracks precipitation, discharge, and gauge height. The downstream Herrington Lake gauge only tracks reservoir water surface elevation. The purpose of this tracking is to identify weather or water depth changes that may potentially affect the invertebrate community prior to sampling. This information will be tracked during the deployment time as well.

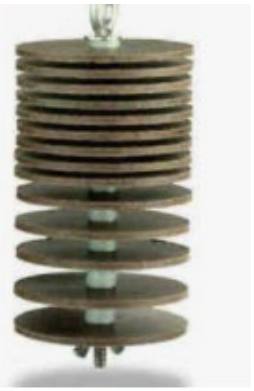
Sampler deployment will occur in spring (March to April). The timing of the deployment and retrieval will be sensitive to temperature, storm events, the spring thermocline overturn (which generally occurs in April), and change of the reservoir water height from winter pool to summer pool. Once the samplers have been deployed for six weeks, consistent with KDOW (2015) guidance, samplers will be retrieved and shipped to the identified taxonomic laboratory.

Prior to deployment, a description of each benthic macroinvertebrate sampling location and a quantitative evaluation of habitat quality will be recorded on field forms (Attachment A). The information obtained will provide support for the comparison of macroinvertebrate communities between sampling locations. Digital photos will be taken to document the surrounding habitat, water condition, and samplers at the time of deployment and retrieval of samplers.

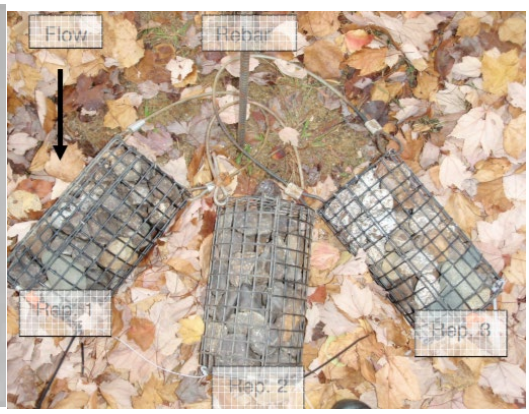
Surface water quality information, using a multi-meter, secchi depth, and global positioning system (GPS) coordinates (horizontal and vertical datums) will be collected at each sampling location at the time of both deployment and retrieval of samplers. Water quality parameters collected will include pH, dissolved oxygen, temperature, conductivity, and turbidity. Water quality measurements will be taken at depths where the samplers will be deployed. Equipment will be calibrated and used according to manufacturer specifications.

Benthic community assessment sampling will use either Hester-Dendy multiplate artificial solid-substrate samplers or rock basket artificial substrate samplers.

Example images of Hester Dendy artificial substrate samplers are provided below.



Example of rock baskets are provided below.



Hester-Dendy samplers consist of a series of up to 9 round hardboard plates that are approximately 3 inches in diameter, separated by spacers and fastened together through their centers to a threaded eyebolt. Kentucky (2015) specifies use of 3 multi-plate samplers for wadeable streams, consistent with the Hester-Dendy sampler identified above. Rock baskets consist of wire mesh filled with either limestone rock or porcelain spheres approximately three inches in diameter (KDOW 2015).

At each sampling location, the selected artificial substrate will be positioned and secured approximately 3 to 6 inches above the sediment surface to be elevated enough to avoid getting the sampler filled with sediment, but close enough to the sediment surface to attract sediment-dwelling organisms. For Hester-Dendy samplers, the arrays will be secured to a cinderblock anchor and lowered into position from a boat using rope. Similarly, rock basket samplers will also be lowered into position from a boat using rope, but they will be free-standing (i.e., not attached to a cinder block). Floats will be attached to the suspension ropes to facilitate identification and retrieval of the samplers.

Artificial substrates will be deployed in triplicate sets per location. Samplers will be deployed for a period of six weeks (KDOW 2015), at which time they will be collected. Because all samples will be collected in triplicate, no field duplicates are necessary for this sampling.

Retrieved samplers will be placed into plastic containers. The containers will be filled to approximately 10% of the volume with ethanol and sealed tight. The containers will then be gently rotated and inverted to ensure that the ethanol has been evenly distributed within the sampler. The container will then be opened and filled completely with deionized water, resulting in a 10% ethanol solution.

3.SAMPLE IDENTIFICATION NOMENCLATURE

The sample identifications will include:

A four-character code indicating the sample matrix. In this case, the code will be benthic community assessment (BCA). This keeps the samples discrete from past and potential future benthic macroinvertebrate sampling to determine tissue concentrations.

A three-character sample number. These will start from 001.

A two-character replicate identifier. This will include an "R" for replicate, and the number of the replicate (1, 2, or 3).

A two-character sampling method identifier. Hester Dendy samples will be "HD" and rock basket samples will be "RB."

A two- to four-character location identifier, such as.

- Upper Curds Inlet - UCI
- Middle Curds Inlet - MCI
- Lower Curds Inlet – LCI
- Hardin Inlet – HI
- Lower Herrington Lake 1 – LHL1
- Lower Herrington Lake 6 – LHL6

An eight-character date. The sample date is the retrieval date. Four digits for the year, two for the month, and two for the day.

The second replicate of the third Hester Dendy sampling location in Middle Curds Inlet retrieved on May 17, 2021 would be: BCA003R2(HD)-MCI-20210517

4.HANDLING, PACKAGING, AND SHIPPING

The BCA samples will be handled, packed, and shipped as follows:

- The retrieved samplers will be placed in plastic containers with ethanol as described in Section 2.2.
- Each sample will be labelled with an identification number (ID) (according to the nomenclature described in Section 3), site name, date, and the collector's initials. The samples will be labelled both internally, with a paper label written in pencil placed inside the container, and externally, written on the container (not the lid) in permanent marker (KDOW 2015).
- Each container will be placed into a zip-top plastic bag and sealed.
- Because samples are preserved in ethanol, ice is not typically needed for preservation. However, ice may be added as a cautionary measure, at the discretion of the Field Team Leader, if sampling occurs during particularly warm periods.
- Samples will be maintained via chain-of-custody until shipment via overnight express to the taxonomy laboratory.

5.DATA RECORDING AND MANAGEMENT

Field notes will include data collection forms as indicated in Attachment A. Field notes will be recorded during sampling activities, such as the following:

- Names of field crew and oversight personnel
- Sample location
- Date, time, and duration of sampling
- General weather conditions
- Substrate characterization – visual notes if available
- Water quality parameters (measured)
- Sample information (including matrix, sampling method, sample ID, sample date and time)
- Habitat description at sampling location
- Digital image number (when images are required/captured)
- Instrument calibration summary

6.LABORATORY ANALYSIS

The laboratory processing and taxonomic identification will be conducted by a commercial taxonomic laboratory, such as Normandeau Associates or Pennington & Associates. The taxonomy will be identified following the Kentucky Guidance, Benthic Macroinvertebrate Processing and Identification (KDOW 2018). In brief, this guidance indicates that when the samples arrive at the taxonomic laboratory, the ethanol will be replaced with 75% fresh ethanol. The invertebrates are removed from the container by pouring out the ethanol and/or gently rinsing the sampling device over a 600-micrometer sieve. The invertebrates will be transferred to a counting tray to be picked. Ideally, 300 organisms will be picked from the sample. If there are more than 300 organisms the guidance has full details on how to properly subsample the organisms (the "300 pick method", KDOW 2018).

The laboratory will identify the organisms to species, or the lowest practical level of identification as indicated by the Kentucky guidance (KDOW 2018). After identification, the identified and unidentified organisms should be stored (separately) in 75% ethanol.

Taxonomic identification data will be used to calculate metrics, as described in Section 7.

7.BENTHIC COMMUNITY METRICS

Benthic community assessment metrics are developed based on the taxonomic identification described in Section 6. Various benthic community metrics are identified by Kentucky and USEPA for wadeable streams and by USEPA and USGS for non-wadeable streams (KDOW 2003, USEPA, 1989, 1999, 2009, 2001, 2006). Metrics are quantitative summaries of benthic community characteristics such as richness, tolerance to disturbance, and composition. The samples collected from Herrington Lake will be assessed using both the KDOW guidance (KDOW 2003) and the USEPA rapid bioassessment guidance (KDOW 2003, USEPA 1989, 1999).

A summary of metrics that will be considered and the associated assessment approaches is provided below. These include both wadeable and non-wadeable habitat types. However, the non-wadeable habitat types are most comparable to Herrington Lake. This section discusses:

- Kentucky metrics and scoring approach for wadeable streams (Section 7.1)
- USEPA metrics and scoring approach for wadeable streams (Section 7.2)
- USEPA metrics and scoring approach for non-wadeable streams (Section 7.3)
- The metrics and scoring approach that will be used for the evaluation of the Herrington Lake benthic community to address RAO 3.

7.1 Kentucky Metrics and Scoring Approach for Wadeable Streams

The Kentucky guidance (KDOW 2003) is based on seven community metrics as described below.

The benthic communities in Curds Inlet and other areas of Herrington lake will differ from those reflected in the Kentucky statewide reference data set because of the significant differences in habitat between wadeable streams and Herrington Lake. KDOW (2003) states that reference datasets for large rivers are under development. KDOW does not have reference datasets for lake habitats. As will be seen in Sections 7.2, these Kentucky metrics are similar to the USEPA metrics recommended for wadeable and non-wadeable habitats. These seven metrics are used in the Kentucky guidance (KDOW 2003). Table 7-1 provides the Kentucky state reference values, and the metric scoring values.

- **Taxa Richness (Number of Genera)**. This refers to the total number of genus-level taxa present in the sample. If organisms can only be identified at a less specific taxon, then they can be counted at the genus level if no other representatives of that group are present (all nematodes are classified as "nematodes" and counted as one taxon.) This metric decreases in response to stress, habitat diversity, or habitat quality.
- **Number of Ephemeroptera, Plecoptera, Trichoptera (EPT) Genera**. Mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) are generally pollution sensitive and this metric declines in response to stress.
- **Kentucky Modified Hilsenhoff Biotic Index (HBI)**. The HBI is a summary metric that indicates the degree of pollution-tolerance in the community sampled. The Kentucky guidance uses a mix of standard tolerance values (Hilsenhoff, 1987), North Carolina Division of Environmental Management (NCDEM 2001) tolerance values, and their own tolerance values (KDOW, 2003). The Kentucky values are regionally modified for streams in the state. This metric increases in response to stress.
- **Modified Percent EPT Individuals**. In the Kentucky guidance (KDOW 2003), this index is modified by exclusion of the caddisfly genus Cheumatopsyche which can be dominant in some stressed environments. This metric decreases in response to stress.

- **Percent Ephemeroptera individuals**. Mayflies are considered intolerant of metals, high conductivity, brines, and dissolved solids. Many mayflies require well-oxygenated, cool water in shallow streams with high flow and abundant rocky substrate. This habitat is not the habitat of concern in Herrington Lake. This metric decreases in response to stress.
- **Percent Chironomidae + Oligochaeta individuals**. Flies (Chironomids) and worms (oligochaetes) are generally considered pollution tolerant organisms. This metric increases in response to stress.
- **Percent Primary Clingers**. This metric quantifies the organisms that inhabit rocky, silt-free substrate, typically in flowing streams and rivers systems. This habit is unlikely to be found in the sampling areas in Herrington Lake. This value is largely determined by the presence of this substrate, not necessarily chemical stressors.

Under the Kentucky guidance, these metrics for the assessment site are compared to the 95th percentile values for headwater or wadeable streams in the statewide reference dataset. Table 7-1 provides the Kentucky state reference values, and the metric scoring values. KDOW (2013) states that the regional reference dataset was established based on samples from 106 wadeable reference sites (summer samples) and 92 headwater reference sites (spring samples). These data were collected over a five-year period between 1998 and 2003. KDOW (2003) states that reference datasets for large rivers are under development. KDOW does not have reference datasets for lake habitats.

Given the difference noted above, in the habitat in the sampling areas in Herrington Lake and the habitat in the Kentucky reference data set, the sampling areas in Herrington Lake will not be directly comparable to the KDOW reference dataset. However, as discussed below, the KDOW metrics can be used to compare Curds Inlet locations to the Herrington Lake reference areas that will be sampled at the same time as Curds Inlet. Therefore, the Kentucky metrics and the Kentucky approach will be considered as part of the monitoring effort.

7.2 USEPA Metrics and Scoring Approach for Wadeable Streams

USEPA guidance (1989, 1999)[1] identifies a variety of metrics for assessment of benthic communities in wadeable streams. The USEPA Rapid Bioassessment Protocols (first edition) includes an eight-metric evaluation to compare stations to an analogous site with similar habitat that is not within site influence (USEPA 1989). The benefit of this USEPA approach is that scoring is done with metrics from impacted sampling stations compared to upstream reference sites with similar habitat. The final score is expressed as a percent of the comparison site (rather than a percent of a state-wide ideal) and provides a useful way to determine if the benthic macroinvertebrate community is altered due to site influences (USEPA 1989).

The USEPA metrics and associated scoring process are presented on Table 7-2.

- **Taxa richness**. This is the same metric identify by Kentucky.
- **HBI**. This is similar to the Kentucky modified HBI.
- **Ratio of scrapers to filterer-collectors**. This abundance ratio gives insight into the balance between organisms that scrape periphyton (particularly diatoms) off hard surfaces versus those that feed on particulate organic matter. The response to stress is variable.
- **Ratio of EPT to Chironomid abundances**. Generally, EPT are thought to be pollution intolerant and chironomids are thought to be pollution tolerant. This metric declines in response to stress.
- **Percent contribution of dominant taxon**. A community dominated by one or a few species is indicative of stress. This metric increases in response to stress.

[1] Also cited as Plafkin et al. (1989) and Barbour et al. (1999).

- **EPT Index**. This is the presence of mayflies, stoneflies, and caddisflies, similar to that provided by Kentucky guidance, but does not exclude the genus of caddisflies excluded by Kentucky in this metric.
- **Community Loss Index**. This metric measures the loss of species compared to a reference area, and generally increases in response to stress.
- **Ratio of shredders/total organisms**. In general, insects that shred vegetation that has fallen into the water using their tearing mouthparts are sensitive to disturbance. They may be more exposed to constituents that may adhere or partition into organic material in streams that some other organisms. This metric decrease in response to stress.

7.3 USEPA Metrics and Scoring Approaches for Non-Wadeable Waters

Three lake assessment multi-metric approaches available from USEPA are described below.

Non-Wadeable Macroinvertebrate Condition Index (NMACI)

USEPA (2009)[2] identifies an integrated multi-metric scoring approach for lakes which is the average of seven metric scores and is expressed the non-wadeable macroinvertebrate assemblage condition index (NMACI). The NMACI is comprised of the following metrics with scoring indicated on Table 7-3:

-
- Diptera taxa richness
- EPT taxa richness
- Percent Coleoptera taxa
- Percent Oligochaete and leech taxa
- Percent Collector-filterer individuals
- Predator taxa richness
- Percent Burrower taxa
- Tolerant taxa richness (HBI value 6 or greater)
- Percent Facultative individuals (HBI value 4-6)

The average of these metrics is the NMACI score. In scores above 60% are generally consistent with reference conditions and scores less than 40% are typical of stressed conditions. Scores between 40 and 60% are not directly classifiable as either reference or stressed communities. The NMACI has been demonstrated to show a strong response to disturbance when compared to regional reference sites. NMACI scores were highest for the federally protected St. Croix River and lowest for the Illinois River. There are no NMACI values available for Kentucky lakes. USEPA concluded that this approach provides a valuable frame of reference for the potential of large river/lake benthic communities to aid management and restoration efforts.

Lake Bioassessment Integrity Index (LBII)

USEPA (2001)[3] identifies an integrated multi-metric scoring approach which is referred to as the lake bioassessment integrity index (LBII), using the metrics as listed on Table 7-4A. The LBII is comprised of the following metrics with scoring indicated on Table 7-4B:

-

[2] Also cited as Blocksom and Johnson (2009).

[3] Also cited as Lewis et al (2001).

- Hilsenhoff Biotic Index
- Taxa richness
- Relative abundance
- Percent intolerant taxa
- Percent oligochaetes
- Percent noninsects
- Percent chironomidae
- Percent dominant taxon
- Community loss index
- Community similarity index
- Trophic condition index
- Dominant-incommon-5

River Invertebrate Prediction and Classification System (RIVPACS)

USEPA (2006)[4] identifies concepts and approaches for the bioassessment of non-wadeable streams and rivers. This guidance refers to the regional reference site approach which compares impacted areas to similar reference areas within a watershed, which is called the river invertebrate prediction and classification system score (RIVPACS). This approach does not pre-define the metrics but rather suggests that the metrics to be used should be those that that best discriminate between reference and study sites for a specific region. The particular metrics that "best discriminate" for a specific assessment may be determined after taxonomic identification is complete and the community composition of the reference areas are better understood. This USEPA guidance includes a list of expected taxa the assessment site based on its physical similarity to reference sites and the taxonomic composition of those reference sites. The list of expected taxa is, in essence, the average taxonomic composition of reference sites weighted toward those most physically similar to the test site. This approach identifies categories of metrics to be considered but inclusion of all metrics is not required. Metric categories identified[5] are richness metrics, community composition metrics, tolerance measures, and trophic/habitat measures, as follows.

- Richness metrics:
  - Taxa richness
  - EPT taxa richness
  - Ephemeroptera taxa richness
  - Plecoptera taxa richness
  - Trichoptera taxa richness
- Community composition metrics:
  - EPT %
  - Mayfly %
  - Chironomidae %
- Tolerance Metrics
  - Number of intolerant taxa
  - Tolerant organism %
  - HBI

[4] Also cited as Flotemersch et al. (2006).

[5] Table 8-1 from Flotemersch et al (2006).

  - Dominant taxon %
- Trophic/Habitat Measures
  - Number of clingers
  - Clinger %
  - Filterers %
  - Scrapers %
  - Ratio of Filterers to Total Organisms.

USEPA (2006) refers to the USEPA 1989, 1999 multi-metric scoring approach already discussed and presented in Table 7-2 for use in comparison of the metrics from impacted areas to the appropriate reference areas.

7.4 Approach for Herrington Lake Monitoring

The artificial substrate samplers will be deployed in three areas within remedial Footprint 3, as well as one reference area, according to the following considerations:

- In Curds Inlet, the samplers will be located in the areas with the highest measured selenium in sediment as well as areas with the highest measured arsenic and iron in sediment pore water, as identified in Figure 4-3 of the SRAA Report. Three samplers will be deployed at each sampling area.
- The actual placement of the individual samplers will be based on results obtained from a sub-bottom profile and side-scan sonar, which will identify sediment thickness and potential debris in Curds Inlet that will influence the placement of the samplers. The sub-bottom profile and side scan sonar imagery approach is described in Appendix A3.
- Either Hester-Dendy or rock baskets will be used. 9-Plate Hester-Dendy samplers will be the preferred sampling devices if they can be reliably deployed and retrieved without entanglement in debris at the sediment surface. The presence of debris observed via the sub-bottom profiling and side-scan sonar will indicate that the Hester-Dendy devices cannot be deployed and retrieved without likely entanglement, and therefore, for such locations, rock baskets will be used. The same artificial substrate samplers will be used in Curds Inlet and the reference area(s).

The USEPA metrics and scoring approach for non-wadeable streams (2006) will be used to evaluate the benthic community in Curds Inlet for RAO 3. The final list of metrics most appropriate to Herrington Lake will be identified based on the benthic community observed at the Herrington Lake reference area(s) selected for the assessment. The USEPA LMACI and NBII will also be calculated in accordance with USEPA 2009 and 2001, with comparisons made to the reference area(s) selected for Herrington Lake. In accordance with scoring presented in Table 7-2, multi-metric scoring and indices (LMACI and NBII) will be considered for Curds Inlet compared to Herrington Lake reference area(s) for RAO 3, as follows:

- Conditions greater than or equal to 80% in Curds Inlet relative to reference area(s) in Herrington Lake will be considered comparable;
- Conditions between 50 and 79% in Curds Inlet relative to reference area(s) in Herrington Lake will be considered slightly impaired;
- Conditions between 21 and 49% in Curds Inlet relative to reference area(s) in Herrington Lake will be considered moderately impaired; and,
- Conditions less than 20% in Curds Inlet relative to reference area(s) in Herrington Lake will be considered severely impaired.

8.QUALITY ASSURANCE/QUALITY CONTROL

Three sampler devices will be deployed at each sampling location and each device will be evaluated as an individual sample, with the results for each sampling location thereafter aggregated for comparison to the reference area(s). The selected taxonomic laboratory will adhere to established internal quality assurance/quality control procedures, with supporting re-identification of organisms at a rate of 5% by a second taxonomist. The taxonomic laboratory will submit a copy of its quality assurance/quality control procedures with its report.

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FIGURES

TABLES

ATTACHMENT A
FIELD FORMS

TABLES

Table 7-1. Description and Scoring of Kentucky Metrics for Wadeable Streams
EW Brown Station
Bethic SOP

Metric	Description	Scoring Formula	95th or 5th Percentile State Reference Value
Taxa Richness (genus level)	Number of genera in the sample	# Genera / 95th percentile value x 100	74
EPT Genera	Number of mayfly, stonefly, and caddisfly genera in the sample	# EPT Genera / 95th percentile value x 100	30
Kentucky Modified Hilsenhoff Biotic Index	Where: $\sum \frac{x_i \times t_i}{n}$ xi=The number of organisms within species (maximum 25) ti=The tolerance value of species n=Total number of organisms in sample	(10 - modified HBI) / (10 - 5th percentile value) x 100	3.11
Modified % EPT individuals	Number of mayflies, stoneflies, and caddisflies (but not from the order Cheumatopsyche) in the sample divided by the total number of organisms in the sample	% EPT individuals / 95th percentile value x 100	74
% Chironomids+ Oligochaete Individuals	Number of non-biting midges and worms in the sample divided by the total number of organisms in the sample	(100 - % Chironomid+Oligochaete individuals) / 5th percentile value x 100	1.0
% Clinger Individuals	Number of individuals that cling to hard substrate in the sample divided by the total number of organisms in the sample	% Clinger Individuals / 95th percentile value x 100	74

Notes:
From Kentucky Department for Environmental Protection Division of Water. 2003. KY Macroinvertebrate Bioassessment Index. https://eec.ky.gov/Environmental-Protection/Water/QA/Pages/default.aspx

EPT Ephemeroptera (mayflies), plecoptera (stoneflies), and trichoptera (caddisflies)
HBI Hilsenhoff Biotic Index

Table 7-2. Description and Scoring of USEPA Metrics for Wadeable Streams
EW Brown Station, Herrington Lake
Bethic SOP

Benthic Macroinvertebrate Metric	Formula
A. Taxa Richness	Number of different taxa or species
B. Hilsenhoff Biotic Index	Where: $\sum \frac{x_i \times t_i}{n}$ xi=The number of organisms within species ti=The tolerance value of species (2) n=Total number of organisms in sample
C. Ratio of Scrapers/ Filterers-Collectors	Simple ratio of the number of individuals from two feeding types
D. Ratio of EPT and Chironomidae Abundances	Simple ratio of the number of mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Trichoptera) individuals vs Chironomidae individuals
E. Percent Contribution of Dominant Taxon	Percent of the total sample made up by the most numerous taxon
F. EPT Index	Number of species of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera)
G. Community Loss Index	(Number of species in the reference - number of species common to both samples)/ number of species in the non-reference
H. Ratio of Shredders/Total	Simple ratio of the two feeding types

Benthic Macroinvertebrate Metric (1)	Biological Condition Scoring Criteria (1)			
	6	4	2	0
A. Taxa Richness (2)	> 80 %	60-80 %	40-60 %	< 40 %
B. Biotic Index (modified) (3)	> 85 %	70-85 %	50-70 %	< 50 %
C. Ratio of Scrapers/Filterers Collectors (2,4)	> 50 %	35-50 %	20-35 %	< 20 %
D. Ratio of EPT and Chironomidae Abundances (2)	> 75 %	50-75 %	25-50 %	< 25 %
E. Percent Contribution of Dominant Taxon (5)	< 20 %	20-30 %	30-40 %	> 40 %
F. EPT Index (2)	> 90 %	80-90 %	70-80 %	< 70 %
G. Community Loss Index (6)	< 0.5	0.5-1.5	1.5-4.0	> 4.0
H. Ratio of Shredders/Total (2,4)	> 50 %	35-50 %	20-35 %	< 20 %

Table 7-2. Description and Scoring of USEPA Metrics for Wadeable Streams
EW Brown
Bethic SOP

Comparison to Reference Score (7)	Biological Condition Category (7)	Support Status (7)
> 80 %	Non- impaired	Comparable to a reference station (upstream location).
51-79 %	Slightly impaired	Community structure less than expected compared to the reference station. Composition (species richness) lower than expected due to loss of some intolerant forms. Percent contribution of tolerant forms increases.
21-50%	Moderately impaired	Fewer species due to loss of most intolerant forms. Reduction in EPT Index.
< 20%	Severely impaired	Few species present. If high densities of organisms, then dominated by one or two taxa.

Notes:

(1) USEPA Rapid Bioassessment Protocols III (USEPA 1989)
(2) Score is a ratio of study site to reference site x 100.
(3) Score is a ratio of reference site to study site x 100.
(4) Determination of Functional Feeding Group is independent of taxonomic grouping.
(5) Scoring criteria evaluate actual percent contribution, not percent comparability to the reference station.
(6) Range of values obtained. A comparison to the reference station is incorporated in this index.
(7) Percentage values obtained that are intermediate to the above ranges will require subjective judgement as to the correct placement. Use of the habitat assessment and physiochemical data may be necessary to aid in the decision process.

Adapted from USEPA. 1989. Rapid Bioassesment Protocols for Use in Streams and Rivers, Benthic Macroinvertebrates and Fish, first edition. EPA/440/4-89/001.

Table 7-3. USEPA Non-Wadeable Macroinvertebrate Assemblage Condition Index (a)

EW Brown

Bethic SOP

Thresholds and Formulae for Scoring Metrics Included in the NMACI			
Metric	**Ceiling (95th percentile)**	**Floor (5th percentile)**	**Scoring formulae**
Diptera taxa richness	24	9	[(X - 9)/15] * 100
EPT taxa richness	17	2	[(X - 2)/15] * 100
% Coleoptera taxa	13.3	0	[(X)/13.3] * 100
% Oligochaete and leech taxa	11.5	1.8	[(11.5 - X)/9.7] * 100
% Collector-filterer individuals	29.6	0.7	[(X - 0.7)/28.9]*100
Predator taxa richness	16	3	[(X - 3)/13] * 100
% Burrower taxa	29	7.5	[(29 - X)/21.5] * 100
Tolerant taxa richness	15	6	[(X - 6)/9] * 100
% Facultative individuals	66.2	8.7	[(X - 8.7)/57.5] * 100

Notes:

The final metric is the average of the individual metric scores.

EPT Ephemeroptera (mayflies), plecoptera (stoneflies), and trichoptera (caddisflies)

NMACI Non-wadeable Macroinvertebrate Assemblage Condition Index

(a) From Table 3 in Blocksom, K. A., & Johnson, B. R. 2009. Development of a regional macroinvertebrate index for large river bioassessment. Ecological Indicators, 9(2), 313–328. doi:10.1016/j.ecolind.2008.05.005

Table 7-4A. USEPA Lake Bioassessment Integrity Index
EW Brown
Bethic SOP

Metric	Description
Hilsenhoff Biotic Index	$\sum \frac{x_i \times t_i}{n}$ Where: xi=The number of organisms within species (from USEPA 1999) ti=The tolerance value of species n=Total number of organisms in sample
Taxa richness	Total number of distinct taxa in the sample
Relative abundance	Total number of individuals in the sample ÷ total number of distinct taxa in the sample
Percent intolerant taxa	(Number of intolerant taxa/all taxa) x 100
Percent oligochaetes	(Number of oligochaete taxa/all taxa) x 100
Percent noninsects	(Number of non-insect taxa/all taxa) x 100
Percent chironomidae	(Number of chironomidae individuals/all taxa) x 100
Percent dominant taxon	(Number of individuals in the dominant taxon/total individuals in the sample) x 100
Community loss index	Community Loss Index = d - a / e a = number of taxa common to both stations d = total number of taxa present at a reference station e = total number of taxa present at the station of comparison
Community similarity index	Community Similarity Index = 2 C / A + B A = total number of taxa at reference station B = total number of taxa at comparison station C = number of taxa common to both stations
Trophic condition index	Trophic Condition Index = C x (1/2 n0 + n1 + 2 n2 + 3 n3)/ N n0 = number of individuals in group 0 (intolerant species) n1 = number of individuals in group 1 (tolerant of minor enrichment) n2 = number of individuals in group 2 (tolerant of eutrophic conditions) n3 = number of individuals in group 3 (tolerant of gross organic enrichment) N = total number of oligochaete individuals C = a constant that varies according to the number of total individuals of all taxa at the station. < 5 individuals C = 0 5-10 individuals C = 0.25 11-30 individuals C = 0.50 31-100 individuals C = 0.75 >100 individuals C = 1.00
Dominant in common top 5	List 5 dominant taxa at each station and count number common to both

Table 7-4B. USEPA Lake Bioassessment Integrity Index Scoring

EW Brown

Bethic SOP

Metrics	Excellent	Good	Fair	Poor	Very Poor
1. HBI	< 2.0	2.0 - 2.5	2.6 - 3.0	3.1 - 4.0	> 4.0
2. Taxa Richness	> 30	20 - 30	10-19	5-9	< 5
3. Relative Abundance	< 2.0	2.0 - 4.0	4.1 - 10.0	10.1 - 20.0	> 20.0
4. % Intolerant Taxa	> 50	31 - 50	30-Oct	<10, > 0	0
5. % Oligochaetes	< 20	20 - 39	40 - 59	60 - 80	> 80
6. % Non-insects	< 20	20 - 39	40 - 69	70 - 90	> 90
7. % Chironomidae	> 50	30 - 50	20 - 29	10-19	< 10
8. % Dominant Taxon	< 20	20 - 30	31 - 50	51 - 80	> 80
9. Community Loss Index	< 0.5	0.5 - 1.0	1.1 - 2.0	2.1 - 5.0	> 5.0
10. Community Similarity Index	> 0.94	0.70 - 0.94	0.50 - 0.69	0.20 - 0.49	< 0.20
11. Trophic condition index	< 0.6	0.6 - 1.0	1.1 - 1.5	1.6 - 2.5	> 2.5
12. Dominant in common top 5	4,5	3	2	1	0

Excellent = 1 point
Good = 2 points
Fair = 3 points
Poor = 4 points
Very Poor = 5 points

Total the points for the sample
Lake Bioassessment Integrity Index Score

< 21	Excellent
21 - 31	Good
32 - 38	Fair
39 - 48	Poor
> 48	Very Poor

Notes:

(a) Adapted from Lewis, Philip A.; Klemm, Donald J.; Thoeny, William T. (2001). Perspectives On Use Of A Multimetric Lake Bioassessment Integrity Index Using Benthic Macroinvertebrates. Northeastern Naturalist, 8(2), 233–246. doi:10.1656/1092-6194(2001)008[0233:pouoam]2.0.co;2

ATTACHMENT A

HABITAT ASSESSMENT FORMS AND TAXONOMIC ID FORMS

Low Gradient Bioassessment Stream Visit Sheet

STREAM NAME: | **LOCATION:**

STATION #: | **COUNTY:** | **PROGRAM:** **PROJECT:**

INVESTIGATORS:

DATE: | **TIME (24hr)** **Start:** **Finish:**

Verify Site LAT/LONG vs GPS ☐YES ☐NO ☐N/A

	Station	Reach Downstream	Reach Upstream
LAT			
LONG			

CANOPY COVER::
- ☐ Fully Exposed (0-25%)
- ☐ Partially Exposed (25-50%)
- ☐ Partially Shaded (50-75%)
- ☐ Fully Shaded (75-100%)

STREAM TYPE:
- ☐ Perennial
- ☐ Ephemeral
- ☐ Intermittent

WEATHER
Has there been a scouring rain in the last 14 days? ☐ Yes ☐ No

Now	Past 24 hours
☐	☐ Heavy rain
☐	☐ Steady rain
☐	☐ Intermittent showers
☐	☐ Clear/sunny
☐	☐ Cloudy

LOCAL WATERSHED FEATUREES (Predominant Surrounding Land Use):
- ☐ Surface Mining
- ☐ Deep Mining
- ☐ Oil Wells
- ☐ Land Disposal
- ☐ Residential
- ☐ Construction
- ☐ Commercial
- ☐ Industrial
- ☐ Row Crops
- ☐ Forest
- ☐ Pasture/Grazing
- ☐ Silviculture
- ☐ Urban Runoff/Storm Sewers

INSTREAM FEATURES
Stream Width _____ ft
Maximum Depth _____ ft
Reach Length _____m

Riffle/Run/Pool Sequence (No. Sampled in Reach)

_____Riffle _____Run _____Pool

HYDRAULIC STRUCTURES
- ☐ Dams
- ☐ Bridge Abutments
- ☐ Island
- ☐ Waterfalls
- ☐ Other:

STREAM FLOW
- ☐ Dry
- ☐ Pooled
- ☐ Low
- ☐ High
- ☐ Normal

RIPARIAN VEGETATION
Dominate Type:
☐ Trees ☐ Herbaceous
☐ Grasses ☐ Shrubs
Number of strata ____ Dom. Tree/Shrub Taxa

CHANNEL ALTERATIONS
- ☐ Dredging
- ☐ Channelization (☐Full ☐Partial)

P-CHEM Instrument Used: _____ Date Calibrated: _____

Temp(°C)_____ D.O. (mg/l)_____ %Saturation_____ pH(S.U.)_____ Cond._____ Turb._____

Sample Collection Verification

Algae	Sample: ☐ QualMHC ☐ Other ☐ Visual Assessment	Lead Collector:
Fish	☐BPEF ☐ Seine ☐ Other Time: BPEF_____ Seine_____	Lead Collector:
Habitat	☐ RBP ☐ Substrate ☐ Other:	Lead Collector:
Invertebrates	☐ 1m² ☐ Qual ☐ Other:	Lead Collector:
	☐ 20 Jab (#Jabs: Cobble_____ Snags_____ Veg. Banks_____ Sand_____ Macrophytes_____ Other _____)	
Tissue:	No. of Samples collected_____ Sp:	Lead Collector:
Water Chem	☐ Acid/Alk ☐ Bulk ☐ Nutrients ☐ Metals ☐ Low Hg	Lead Collector:
	☐ Herbicides ☐ Pesticides ☐ Ortho P ☐ Other:	

Duplicate Samples Taken:

Substrate Characterization

Substrate ☐Est. ☐P.C.	**Riffle_____%**	**Run_____%**	**Pool_____%**	**Reach Total**
Silt/Clay (<0.06 mm)				
Sand (0.06 – 2 mm)				
Gravel (2-64 mm)				
Cobble (64 – 256 mm)				
Boulders (>256 mm)				
Bedrock				

NOTES/COMMENTS:

SITE NOT SAMPLED:

☐ Land owner denial ☐ Dry ☐Too deep/Impounded

☐ Site not found/Secluded ☐Unsafe

☐ Other (indicate under comments)

RBP Low Gradient Habitat

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
1. Epifaunal Substrate/ Available Cover **Score**	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new and transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
2. Pool Substrate Characterization **Score**	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
4. Sediment Deposition **Score**	Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
5. Channel Flow Status **Score**	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
6. Channel Alteration **Score**	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (>20 yr.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. In stream habitat greatly altered or removed entirely.
7. Channel Sinuosity **Score**	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 2 to 1 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
Left/Right Bank	10 9	8 7 6	5 4 3	2 1 0
8. Bank Stability LB RB	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
9. Vegetative Protection LB RB	More than 90% of the stream bank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the stream bank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the stream bank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the stream bank surfaces covered by vegetation; disruption of stream bank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
10. Riparian Vegetative Zone Width LB RB	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.

Total Score

NOTES/COMMENTS:

Source: Kentucky Department of Environmental Protection, Division of Water. 2015.

HERRINGTON LAKE HABITAT ASSESSMENT FIELD DATA SHEET - NON WADEABLE STREAMS

Adapted From Wilhelm et al. 2005. "Habitat Assessment of Non-Wadeable Rivers in Michigan" Env. Manag. 36(4):592–609

LOCATION ID		**GIS INFORMATION**		
FORM COMPLETED BY		**DATE**		
		TIME		
	Excellent	**Good**	**Fair**	**Poor**
1. Riparian Width	Mean riparian width >24 m	Mean riparian width 17.5-24 m	Mean riparian width 10-17.5 m	Mean riparian width <10 m
Σ	25 24 23 22 21 20	19 18 17 16 15 14 13	12 11 10 9 8 7 6	5 4 3 2 1 0
2. Large Woody Debris (LWD)	Greater than 200 pieces of LWD	Between 100 and 200 pieces of LWD	Between 50 and 100 pieces of LWD	Fewer than 50 pieces of LWD
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 6 2 1 0
3. Aquatic vegetation	>25% aquatic vegetation	15-25% coverage of aquatic vegetation	5-15% coverage of aquatic vegetation	<5% coverage of aquatic vegetation
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 6 2 1 0
4. Bottom deposition	0-5% of the bottom affected by deposition and sedimentation	5-25% of the bottom affected by depostion and sedimentation	25-50% of the bottom affected by deposition and sedimentation	>50% of the bottom affected by deposition and sedimentation
Σ	10 9	8 7 6	5 4 3	2 1 0
5. Bank stability	Banks stable, <5% of banks show evidence of erosion	Moderately stable, 5-30% of reach has areas of erosion	Moderately unstable, 30-60% of reach with erosion	Unstable banks, >60% of reach shows erosion scars
Σ	10 9	8 7 6	5 4 3	2 1 0
6. Thalweg substrate	>60% gravel or larger substrate	35-60% gravel or larger substrate	15-35% gravel or larger substrate	<15% gravel or larger substrate
Σ	10 9	8 7 6	5 4 3	2 1 0
7. Off-chanel habitat	>5 off-channel habitats	4-5 off-channel habitats	2-3 off-channel habitats	<2 off-channel habitats
Σ	5	4 3	2 1	0

TOTAL SCORE OUT OF 100:

NOTES:

Source: Kentucky Department of Environmental Protection, Division of Water. 2015.

HERRINGTON LAKE HABITAT ASSESSMENT FIELD DATA SHEET - LOW GRADIENT STREAMS

Adapted from USEPA Rapid Bioassessment Protocols (USEPA, 1999: EPA 81-B-99-002)

LOCATION ID	**GIS INFORMATION**
FORM COMPLETED BY	**DATE**
	TIME

	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate & Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (I.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Pool Substrate Character - ization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Vari-ability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization , i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankment or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HERRINGTON LAKE HABITAT ASSESSMENT FIELD DATA SHEET - LOW GRADIENT STREAMS

Adapted from USEPA Rapid Bioassessment Protocols (USEPA, 1999: EPA 81-B-99-002)

LOCATION ID	**GIS INFORMATION**
FORM COMPLETED BY	**DATE**
	TIME

	Optimal	Suboptimal	Marginal	Poor
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 2 to 1 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
Σ	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
ΣLB	Left Bank 10 9	8 7 6	5 4 3	2 1 0
ΣRB	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 cm or less in average stubble height.
ΣLB	Left Bank 10 9	8 7 6	5 4 3	2 1 0
ΣRB	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width	Width of riparian zone >18m; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 m; human activities have impacted zone only minimally.	Width of riparian zone 6-12 m; human activities have impacted zone a great deal.	Width of riparian zone <6m: little or no riparian vegetation due to human activities.
ΣLB	Left Bank 10 9	8 7 6	5 4 3	2 1 0
ΣRB	Right Bank 10 9	8 7 6	5 4 3	2 1 0

TOTAL SCORE OUT OF 100:

Notes:

Appendix A. Benthic Macroinvertebrate Laboratory Bench Sheet

KDOW Benthic Macroinvertebrate Laboratory Bench Sheet

Cover Page

Station Information and Collection Information

Program:		Project:			Trip:				
Locale Name:		Location Desc:							
Station Name:		County:		Latitude:		Long.:		Act. Date /Time:	
Bioregion:		Stream Type:		Catchment Area (mi2):		Fld Col. Meth.:		Primary Collector	

Processing

Pick By (Date):	
Processing Type:	
Processing Method:	
Replicate	
Total Picked:	
Quant. Midges Picked:	
Quant. Midges Subsampled:	
Midge % Subsample:	
# Quant Slides:	
MH Midges subsampled:	
# Multi slides:	
Level 1 Squares:	
Level 2 Squares:	
Level 3 Squares:	
% Sub Sample:	
Original TNI:	

Analysis

ID By (Date):	
Analysis:	
Taxonomic level:	
# Organisms ID'd:	
Quant Midges ID'd:	
MH Midges ID'd	
Use Alt Taxa Traits:	N
Taxa Traits Version	1-Jan-2017

MBI

Metric	Raw Value	Scaled	Ref. Cond.?
Genus Taxa Richness			
Genus EPT Richness			
mHBI			
m%EPT			
%Ephem			
% Chiro & Oligo			
% Clinger			
MBI-W			

Modified MBI

Metric	Raw Value	Scaled	Ref. Cond.?
m % Clinger			
mMBI			

O / E Results

Total # of Expected Taxa ID'd:		Ref. Cond.?
O/E Index:		

Supplementary Metrics

Metric	Raw Value	Scaled	Ref. Cond.?
Genus Clinger Richness			
% Intolerant			
Genus Intolerant Richness			
% Tolerant			
% 5 Dominant			
% Hydropsychidae			
% Shredder			
% Predator			
Genus Shredder Richness			
Genus Predator Richness			
% Nut. Tolerant Taxa			
Hilsenhoff Biotic Index		N/A	N/A
% Non-insect		N/A	N/A
# Exhibiting Ref. Condition			
% Reference			

Activity Notes

Processing and ID Notes

Analyst's Assessment Notes

Data Review and Upload Information

Initial Data Review:		Upload:	

Appendix A. Benthic Macroinvertebrate Laboratory Bench Sheet, cont'd

Project:		Station Name:		Taxa List Page 1
Trip:		Locale Name:		
Collection Date:		Collector:		Identified By:

Final ID	SQ	MH	Incl.	Stage	Notes

Appendix A. Benthic Macroinvertebrate Laboratory Bench Sheet, cont'd

Project:		Station:		Slides List Page 1
Trip:		Locale:		
Collection Date:		Collector:	Identified By:	

Final ID	ID'd	SQ	MH	Slide Locations

Appendix B. Slide Bench Sheet

Kentucky Division of Water Bench Sheet - Slides					Page 1
Stream Name:			Station ID:		
Coll. 1:	Date:	ID By:		Date:	
Final ID	**Slide Locations**	**ID'd**	**Quant**	**MH**	**Comments / Tax. QC**

Appendix D. KDOW Benthic Macroinvertebrate Taxonomic Level of Effort

All macroinvertebrates must be identified to the lowest determinable level based on current taxonomic references and resources available. The table below provides a general standardized level of effort that is required for mature and well preserved specimens. Generally, it is required to identify organisms to at least the genus level. Due to taxonomic limitations, some groups cannot be identified to the genus or species level and therefore should be taken to the level specified below. For all taxonomic groups, if the level can easily go lower, for example monotypic genera, or if only one genus or species is known to occur in a certain geographic area, then these specimens should be identified at the lowest possible taxonomic level.

Phylum	Class	Order	Family	Taxonomic Resolution
Annelida				
	Oligochaeta			Family
			Hirudinidae	Family
Arthropoda				
	Insecta			
		Coleoptera		Genus/Species
		Diptera		Genus/species, except the following families:
			Dolichopodidae	Family
			Phoridae	Family
			Scathophagidae	Family
			Syrphidae	Family
		Ephemeroptera		Genus/Species
		Hemiptera		Genus/Species
		Lepidoptera		Genus/Species
		Megaloptera		Genus/Species
		Odonata		Genus/Species
		Plecoptera		Genus/Species
		Trichoptera		Genus/Species-except Hydropsyche where morosa group (formerly Ceratopsyche) **MUST** be identified as Hydropsyche morosa gp.
	Crustacea			
		Amphipoda		Genus/Species
		Decapoda		Genus/Species
		Isopoda		Genus/Species
	Arachnida	Trombidiformes		Hydracarina (unranked)
Cnidaria				Genus/Species
Ectoprocta				Genus/Species
Mollusca				
	Bivalvia			Genus/Species
	Gastropoda			Genus/Species
Nematomorpha				Genus/Species
Nemertea				Genus/Species
Porifera				Genus/Species

APPENDIX A3: STANDARD OPERATING PROCEDURES FOR SUB-BOTTOM PROFILE AND SIDE SCAN SONAR STUDY IN CURDS INLET

Prepared for

Kentucky Utilities Company

Document type

Standard Operating Procedure (SOP)

Date

August 2021

APPENDIX A3

SOP FOR PERFORMANCE MONITORING:

SUB-BOTTOM PROFILE, SIDE SCAN SONAR AND MULTIBEAM HYDROGRAPHIC /BATHYMETRIC SURVEY OF CURDS INLET

E.W. BROWN STATION, HERRINGTON LAKE, MERCER COUNTY, KENTUCKY

RAMBOLL

Document Development and Approval

Title and Approval Sheet

Action By	Signature	Date
Reviewed by: Mary Sorensen, Ramboll Environ		July 30, 2021
Approved by: Mark Nielsen, Ramboll Environ		July 30, 2021

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Acronyms and Abbreviations

BIST	Built In self-test
Cabinet	Kentucky Energy and Environment Cabinet
CAP	Corrective Action Plan
DGPS	Differential Global Positioning System
DOP	High dilution of precision
DXF	AutoCAD Drawing file format
e.g.	Example
GPS	Global Positioning System
KDOW	Kentucky Department for Environmental Protection, Division of Water
NAD	North American Datum
NAVD	North American Vertical Datum
NOAA	National Ocean and Atmospheric Administration
NOS	NOAA National Ocean Service
QC	Quality Control
RAO	Remedial Action Objective
RTK	Real time kinematic
OCS	Office of Coastal Survey
SOP	Standard operating procedure
SRAA	Supplemental Remedial Alternatives Assessment
TIN	Triangulated Irregular Network
TPU	Total Propagated Uncertainty
USACE	United States Army Core of Engineers

1 OVERVIEW

This standard operating procedure (SOP) describes the proposed sub-bottom and side-scan sonar bathymetric survey data collection methods designed to produce detailed maps of the Curds Inlet bottom and substrates to guide placement and deployment of the sediment-dwelling invertebrate artificial substrate samplers within Curds Inlet as part of a benthic community assessment. The benthic community assessment is provided as part of the Supplemental Remedial Alternatives Assessment (SRAA) intended to address Remedial Action Objective 3 (RAO 3, SRAA Appendix A2 [Ramboll 2021]) by comparing diversity and abundance of Curds Inlet sediment-dwelling invertebrate communities to reference locations in Herrington Lake. The sonar surveys will provide 3-dimensional views of Curds Inlet to inform placement of the benthic community assessment samplers near sediment deposits and to avoid entanglement in subsurface debris. The surveys would be completed prior to the benthic community assessment deployment phase. Subject to the Kentucky Department for Environmental Protection Division of Water Cabinet approval, the benthic community assessment for RAO 3 is anticipated to be completed in spring or summer 2022.

2 OBJECTIVE

The goal of the sub-bottom profiling is to provide detailed and accurate data, including the identification of areas with deeper sediment and the locations of bottom and buried objects, and subsurface geologic stratigraphy. The goal of the multi-beam bathymetry survey is to collect hydrographic data to achieve a precise and contemporary view of the bottom hypsography of Curds Inlet to also aid in the placement and deployment of the artificial substrates for the benthic community assessment. The survey methods will be consistent with US Army Corps of Engineers Hydrographic Survey minimum performance standards and/or other site-specific (if applicable) guideline and methods.

3 MATERIALS AND EQUIPMENT

This section describes the proposed sounding equipment to be used for the Curds Inlet surveys. Final equipment choices will be made prior to commencing the surveys to address local environmental conditions at time of sampling.

- R2Sonic 2022 or equivalent survey-grade multibeam fathometer and manual
- SyQwest Stratabox (or equivalent) survey-grade sub-bottom profiler
- GPS system (RTK or DGPS) which includes at a minimum: receiver, antenna, power cable, data cable, antenna cable, GPS antenna mount
- Bar, Line, or Secchi disc for calibration of fathometer

4 SURVEY PROCEDURES AND DATA CAPTURE METHODS

This section describes the procedures for the collection of sonar data to ensure efficient and effective deployment and retrieval of the benthic community assessment artificial substrates.

4.1 Survey Preparation

For the sub-bottom and multi-beam side scan surveys, horizontal coordinate system and lane spacing will be determined to set up the survey control software.

Prior to commencement of survey work, Ramboll will complete and prepare the appropriate survey documentation pertaining to completing the Curds Inlet surveys, including, but not limited to, determining the exact survey areas and extents, desired lane spacing and coverage, survey area access, contact information, any helpful previous surveys results (See Ramboll 2018a for Navionics bathymetric data map figures), and any expected physical or operational hazards and/or hindrances. In general, parallel lines of equal spacing are usually sufficient to collect the required data. However, the spacing between parallel lines will vary depending on the desired coverage or scale of the site. Adjustments to transect spacing will be in made real time to account for depth changes to ensure coverage overlap.

4.2 Survey Control and Precision

A suitable survey vessel will be chosen based on the above factors and on Ramboll's knowledge of Herrington Lake. The proper sensor/towfish towing or mounting point on the vessel will be installed and/or configured based on the sensor/towfish that will be used (Figure 1). Satellite geometry and availability will be reviewed prior to each day of data acquisition. The hydrographer in charge will determine if there will be excessive periods of GPS outages or periods of high dilution of precision and note such occurrences in the daily field notes.

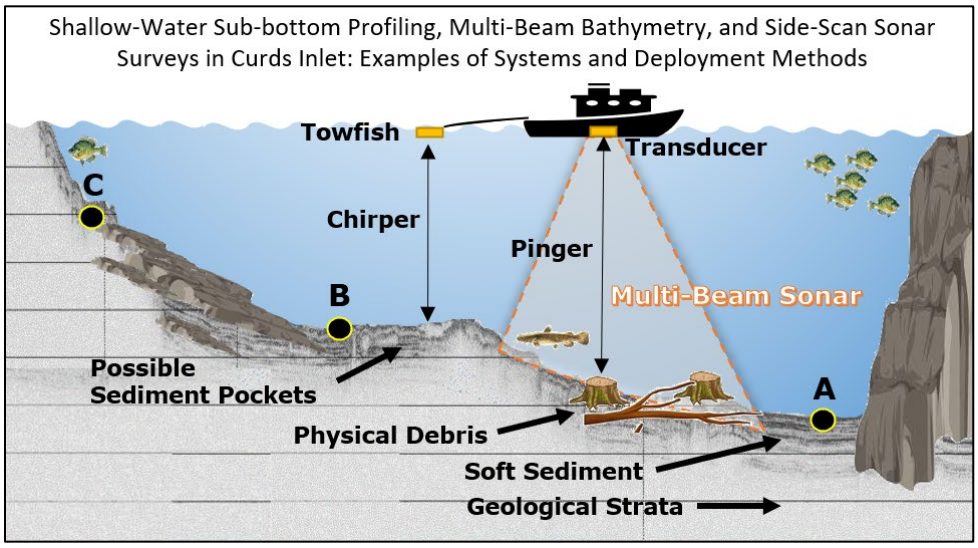

Figure 1. Example Illustration of Sub-bottom Profiling, Multi-Beam Bathymetry, and Side-Scan Sonar Survey and Deployment Methods and Systems

The horizontal or vertical controls used for Real-time Kinematic (RTK) GPS-enabled mapping projects will use benchmarks for the E.W. Brown Station or a licensed surveyor. Work will be relative to NAD 83 as the horizontal input coordinate system and NAVD 88 in the vertical plane unless otherwise noted. A minimum of five satellites will be used for all measurements to resolve vertical and horizontal positions. The monument name and general location will be recorded in the daily field notes.

4.3 Vessel and Survey Navigation in Curds Inlet

Curds Inlet is a relatively narrow inlet, situated in a general north-south direction. Navigation of a larger vessel in Upper Curds Inlet may prove challenging due to lack of room to maneuver and shallow water depths. Modifications to vessel navigation, and navigation and surveying software parameters will be made by the hydrographer to fit survey conditions at time of sampling.

Modifiable survey and vessel parameters may include (but are not limited to):

- Heave filter mode and average heave period
- GPS antenna attitude constraints
- RTK baseline search mode
- Waterline adjustments due to vessel loading changes and or water type
- DGPS link baud rate (i.e., radio or cellular communications)

Any changes made to navigation software parameters will be recorded in the daily field notes and a copy of the parameters will also be stored electronically for future reference.

4.4 Bottom Profile and Bathymetric Data Collection

Hydrographic data, field notes, and echosounder system setup that will be used are described below.

4.4.1 Hydrographic Data Collection

Data collection methods reference guidelines and general hydrographic surveying technique recommendations that will be used are those from the US Army Corps of Engineers (USACE) and the National Oceanic and Atmospheric Administration (NOAA), as follows:

- USACE 2001 Hydrographic Surveying Manual; EM 1110-2-1003
- NOAA OCS 2014 Field Procedures Manual
- International Hydrographic Organization Publication C-13 Manual on Hydrography

4.4.2 Field Notes

Detailed field notes will be recorded daily and supplemented with more information as needed at the end of the survey. The following information, at a minimum, will be included in the survey notes for each survey:

- Base station location, if used
- Survey area, calendar date and day
- Personnel/operators
- General weather and sea state

- Additional comments as outlined in this SOP
- Local environmental factors and visual notes regarding physical barriers and potential other environmental factors that may influence the collected survey data

4.4.3 Echosounder System Setup

Before each survey or when resuming an earlier survey, the hydrographer will run the echosounder processing unit built in self-test (BIST) to confirm proper operation. Multibeam acquisition software runtime parameters will be modified at time of survey as necessary to fit local survey conditions.

Modified parameters may include (but are not limited to):

- Sonar head sector coverage
- Beam spacing and width
- Bottom tracking mode
- Survey depth range
- Ping rate

4.5 Sound Velocity Profiles

Sound velocity profiles (SVP) may be collected twice or more within a day. Additional casts may be required when noticeable changes in water properties are observed. Changes may be due to the following: wind shifts, precipitation events, and proximity to swash, inlet, or other water surface water feature. For each sound velocity dip, the time, location, and chosen file name will be recorded in the survey notes. Recorded SVPs will be uploaded into the runtime parameters of the multibeam acquisition software and noted in the daily field notes.

4.6 Waterline/Sound Velocity QC

Changes in weight of the vessel due to loading may alter the position of the waterline. A lead-line test will be conducted at the dock at the start of a new survey project and throughout the rest of the survey as deemed necessary by the hydrographer. Results of the lead-line test will be compared to the nadir beam depth after a current SVP has been uploaded into the system. The result should be less than +/-0.25 m and recorded in the daily field notes.

4.7 Patch Test

A patch test to properly align the multibeam system sensors will be conducted at the commencement of a new survey project and whenever the sonar heads or ancillary sensors (e.g., heave compensation unit or GPS antennas) may have undergone significant change in position or angular orientation on the survey vessel so as to impact the quality of the survey. The patch test will follow the guidelines as outlined in Chapter 12 of the U.S. Army Corps of Engineers Hydrographic Surveying Manual (EM 1110-2-1003 Change 1). Results of the patch test will be analyzed, and any necessary changes incorporated into the following system components:

- Multibeam acquisition software
- Post- processing software
- Navigation software

Changes made to position or angular offsets of will be recorded in the daily field notes.

4.8 Data Acquisition

The hydrographer will monitor data collection in real time for significant static and dynamic artifacts and will attempt to correct as conditions permit in the field. Monitoring data quality also applies to navigation inputs and includes recording any considerable degradation in the quality of the RTK GPS signal or complete loss of communications with the RTK base station if used. The hydrographer will record any changes in GPS signal quality.

4.9 Data Archiving and Processing

At the conclusion of a survey day, raw multibeam data files, sound velocity data files, and other ancillary data will be downloaded from the field system and backed up on a portable hard drive for transfer to a secure and dedicated data server. Detailed daily field notes will be transferred to the person responsible for data post-processing.

Patch test data will be processed first to determine the corrections for system latency and installation roll, pitch, and yaw misalignments. These corrections will be applied to the survey data files during processing. Speed of sound casts will also be applied to the data. The Hypack Total Propagated Uncertainty (TPU) editor will be used to determine the maximum allowable beam angle. Individual records will be reviewed, and erroneous data points will be removed through a combination of automatic filters and manual editing. The processed data from the individual records will be imported into a matrix and exported at a predetermined grid size generating a gridded .XYZ file. The .XYZ file will be TIN modeled, and contours will be generated in AutoCAD DXF format.

5 QUALITY ASSURANCE/QUALITY CONTROL

Data collected in the field will be reviewed to ensure the required coverage is achieved. If the survey is not a complete coverage survey with overlap of coverage between adjacent lines, repeat data will be collected over a small portion of the data collected each day. A comparison will be made within the cells of the overlapped data to ensure data repeatability. General, hardware, and environmental factors which are used by the TPU editor will be recorded. The TPU editor will indicate the widest beam width allowed to meet the required quality objective.

6 DATA DELIVERABLES

Processed data will be output as both .XYZ files and AutoCAD .DXF contours that will be used for mapping sediment deposits and underwater debris. This mapping will be used for the placement of the benthic community assessment samplers in Curds Inlet.

7 REFERENCES

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