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SOP: FISH SAMPLING AND ANALYSIS

HERRINGTON LAKE, KENTUCKY

RAMBOLL ENVIRON

PROTOCOL FOR SAMPLING FISH FOR TISSUE ANALYSIS

HERRINGTON LAKE, KY

Standard Operating Procedures (SOP) Document Control: Standard Operating Procedure for Collection of Fish for Tissue Residue Analyses		
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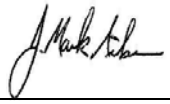
Approved by	Mark Nielsen: 	9/14/2017
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CONTENTS

ATTACHMENTS

ACRONYMS AND ABBREVIATIONS

CAP	Corrective Action Plan
COC	Chain of Custody
KDOW	Kentucky Department for Environmental Protection Division of Water
MS/MSD	Matrix spike/matrix spike duplicate
SOP	Standard operating procedure
USEPA	United States Environmental Protection Agency

1 OVERVIEW

This standard operating procedure (SOP) describes field methods for the collection of fish for the analysis of chemical concentrations in tissue as described in the E.W. Brown Corrective Action Plan (CAP) (Ramboll Environ 2017a). This fish sampling 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). The approaches outlined herein for the collection of fish from Herrington Lake is consistent with the collection of representative samples as described by the United States Environmental Protection Agency (USEPA) Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers (Flotemersch et al. 2006) and Guidance for Assessing Chemical Contamination Data for Use in Fish Consumption Guidelines (USEPA 2000). Because SOPs are already available for the collection of fish in Kentucky for use in water quality assessments, this SOP includes study-specific information specific to the sampling approach for the Herrington Lake CAP.

2 PROCEDURES FOR SAMPLE COLLECTION

This section describes fish sample collection and fish sample handling procedures prior to receipt by the laboratory. Attachments A, B, and C provide supporting information for sample collection and collection methods.

2.1 Fish Sample Collection

- Fish sample collection will include the three target species identified in the CAP: a small home-range fish (bluegill), an upper-trophic-level fish (largemouth bass), and a bottom feeding fish (channel catfish). Eight fish sample collection locations are identified in the CAP Figures 2-1 and 2-2A though 2-2F for the Phase I fish collection effort (Ramboll Environ 2017a). Seven of the eight locations will include sampling of the three target species (bluegill, largemouth bass, and catfish). Bluegill will be the target species for the small area of the HQ Inlet.
- Two samples of each species are planned from each Phase I fish sampling area, as is indicated in Table 2-1 of the CAP. Each individual fish sample will be a composite sample comprised of two to five fish per sample.
- For a subset of fish, fish ovary tissues will also be removed from the fish and analyzed separately. Fish ovary samples will be collected from one largemouth bass and from one catfish sample from each of the Phase I fish sampling stations. This will amount to a total of 14 ovary samples for two species from 7 fish sampling stations.
- Fish sample collection via electroshocking will be conducted to the extent possible and feasible (Attachment C contains the SOP for Procedures for Electrofishing and Seining). If necessary, other fishing methods may include hook-and-line and gill netting.
- Collected target species will be placed into temporary holding containers (“live wells”) until sampling for that area is complete.
- Collected non-target fish (wherever easily identifiable) will be immediately released.
- In select cases where the collected fish species is not obvious (e.g. small sunfishes), the fish will be placed into a live well to await further taxonomic review upon sampling completion at that area.

- Efforts will be made to collect each of the target species in each area using the fishing methods described above. It is noted, however, that any biological sampling is constrained by the presence of the species of interest at the time of sampling. A maximum of 1.5 days of fish sampling effort per station will be expended as part of this Phase I fish sampling effort. This will include multiple efforts with electroshocking equipment and one night of gill nets or set lines. If the target species are not collected within this designated timeframe, alternative fish species from similar trophic feeding levels may be considered. Field decisions will be made based on conditions at the time of sampling.

2.2 Fish Sample Documentation and Handling Prior to Laboratory

- Sample collection efforts will be documented using data collection forms provided in Attachment A (Part 1) and chain of custody (COC) forms as provided in Attachment A (Part 2). All field activities will be documented as detailed in the Herrington Lake Quality Assurance Project Plan (Ramboll Environ 2017b).
- Individual fish weight and length will be recorded and fish identified for each composite sample will be clearly labeled. A composited, whole body sample, will contain two to five same-species individuals having a minimum length of at least 75% of the longest individual member (KDOW 2014).
- Whole fish samples will be wrapped in aluminum foil with the dull side against the sample, placed into a Ziploc bag, and labelled with project name, sample identification, sample date and time, and the analyses requested. The sample will then be double bagged to preserve the labels from water damage.
- The double-bagged samples will immediately 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 lab.
- Remaining target species in the live wells will then be released back into the water at the sampling location.

3 SAMPLE VOLUMES, CONTAINERS, AND HOLD TIMES

Samples will be processed and analyzed in the lab within 30 days of collection (KDOW 2014). Fish preparation will be conducted in a laboratory environment, and processing will be conducted in accordance with the standard operating procedures for preparation and homogenization of fish tissue samples (KDOW 2017), described further in Section 8 of this SOP. The table below provides information on the fish tissue analysis that will be conducted for the CAP, the analytical methods, minimum sample volumes needed for the analysis, the required containers and preservatives and the sample hold times.

Chemicals of Interest	Analytical Methods	Minimum Volumes Required(a)	Container	Hold Times
Metals: arsenic, cadmium, lead, zinc, iron, boron, and magnesium	USEPA 6020A	5 grams	Wrap in Aluminum Foil, double wrap in Ziploc bags and Freeze	1 Year
Mercury	USEPA 7471B	5 grams		
Methylmercury	USEPA 1630M	5 grams		
Selenium	USEPA 7742	5 grams		
% Lipids	Lab SOP	5 grams		

(a) The listed masses are minimum requested. Extra mass is needed for QC samples and reruns.

4 SAMPLE NOMENCLATURE

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

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

Within the CAP, the LHL, UHL, and MHL transects are further numbered as Transect 1, 2, 3, for each portion of the lake (e.g., LHL-1, LHL-2).

Quality assurance samples for fish tissue samples will be labeled as follows:

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

The following sample identification convention for the discrete fish tissue samples will be followed using the prefix “FWB” and “FF”

- “FWB” – Fish whole body
- “FF” – Fish fillet

Each discrete sample will use the following general identification convention:

- [sample matrix code][discrete sampling number][sample date]

An example discrete fish sample identification number is as follows:

- **F-001 - LHL1-170912** – indicates the fish sample number 1 (F-001) collected from Lower Herrington Lake Transect 1 on September 12, 2017 (170912).
- The nomenclature for duplicate samples will include the matrix (F) and transect number (LHL1) but not the exact sample location within that transect (blind duplicate) such as: **F – LHL1—170912-DUP.**

5 HANDLING, PACKING, AND SHIPPING

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

- Samples will be placed immediately on ice[1] upon collection and will be stored until samples can be frozen (within 12 hours of collection). Samples must remain frozen through shipment to lab.
- Samples will be labeled using nomenclature that follows typical nomenclature guidelines described in Section 4.
- Samples will be wrapped in aluminum foil (dull side against the sample), double wrapped / bagged in Ziploc bags and labeled with water-proof labels.
- Samples will be maintained via COC until shipment via overnight express to the analytical laboratory as deemed appropriate to meet hold times described in Section 3.

6 DATA RECORDING AND MANAGEMENT

Field notes will include data collection forms as indicated in Attachment A (Part 1 and Part 2). Field notes will be recorded during sampling activities and, at a minimum, will include the following:

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

7 QUALITY ASSURANCE/QUALITY CONTROL

One quality assurance/quality control sample will be collected (from a location to be determined in the field based on sample availability) for a duplicate and for MS/MSD analysis. Data validation will be performed in accordance with Section 2.4.1 of the CAP.

8 LABORATORY SAMPLE PREPARATION

Fish preparation will occur at the laboratory. Fish analyses will include both fillet and whole body tissues. These will be based on composite samples (i.e., 2 to 5 fish per sample). In order to limit the number of fish needed for analyses, selected individual fish will be filleted, and the fillet and corresponding carcass will be submitted separately for analysis, allowing for the mathematical estimation of the whole body chemical tissue concentration. This will satisfy sample volume

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

requirements, reduce the number of fish required for collection, and allow the analyses to inform both the human health and ecological risk assessments.

- Two fish samples for each species will be collected from each of the Phase I fish collection areas identified in the CAP (Table 2-1). The fish samples will be comprised of a composite of two to five fish.
- Each fish sample will be dissected so that the fillet and the carcass are analyzed separately. Weights must be recorded so that the values in whole fish can be mathematically calculated in wet and dry weight. A subset of the samples (7 bass and 7 catfish) will also include analysis of ovary samples. Information must be provided for the ovary portions as well, so that the whole body fish estimate can be mathematically calculated.
- Each fillet sample will be the right fillet with skin on and with belly flap (per the KDOW 2017 description below):

  "Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples" (KDOW 2017, attached to this Request for Proposal) indicates: "*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."*
- Remaining carcass (i.e., all parts of the composite sample not included in the fillet sample described in the previous bullet).

The laboratory will conduct fish freeze-dry homogenization per the KDOW 2017 SOP.

- The handling of the fish must also be in compliance 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 D)
  - Specifically, this includes a process call lyophilization (freeze drying). "*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.*"
- All results for fish tissue must be reported as weight and also as dry weight.

9 REFERENCES

Flotemersch, J. E., J. B. Stribling, and M. J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. EPA 600-R-06-127. US Environmental Protection Agency, Cincinnati, Ohio.

Kentucky Department for Environmental Protection Division of Water. 2017. Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples. Commonwealth of Kentucky Energy and Environment Cabinet.. Revision No. 2.0. Document Control No: DOWSOP0300032.

Kentucky Department for Environmental Protection Division of Water. 2016. Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-Wadeable Streams and Rivers. Commonwealth of Kentucky Energy and Environment Cabinet. Revision No. 1.0. Document Control No: DOWSOP03041.

Kentucky Department for Environmental Protection Division of Water. 2014. Methods for the Collection of Selenium Residue in Fish Tissue Used to Determine KPDES Permit Compliance. Commonwealth of Kentucky Energy and Environment Cabinet. Revision No. 1.0. Document Control No: DOWSOP03031.

Ramboll Environ 2017a. Herrington Lake E.W. Brown Corrective Action Plan. Submitted to the Kentucky Division of Water Agreed Order No. DOW – 17001.

Ramboll Environ 2017b. Herrington Lake Quality Assurance Project Plan for the E.W. Brown Corrective Action Plan. Prepared for the Kentucky Utilities Company. September 2017.

USEPA. 2000. Guidance for Assessing Chemical Contamination Data for Use in Fish Consumption Guidelines. Available at: https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf.

E.W. BROWN FISH SAMPLING SOP ATTACHMENTS

Attachment A. Example Fish Data Collection Sheets from KDOW

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 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

Example Fish Data Collection Sheet from KDOW 2014 Methods for the Collection of Selenium Residue in Fish Tissue Used to Determine KPDES Permit Compliance

SELENIUM FISH TISSUE
CHAIN-OF-CUSTODY

Station #: _____ **Date: _____**

Stream / Location: _____ Time: _____

KPDES Permit#: _____

County: _____ **Lat/Long Upstream Reach: _____**

Lat/Long Downstream Reach:_____

Outfall #: _____ **Duplicate/Replicate (circle one): yes no**

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

Fish #	Genus	Species	Length (mm)	Comments
001				
002				
003				
004				
005				
006				
007				

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: _____

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

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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
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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					
18					
19					
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23					
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.

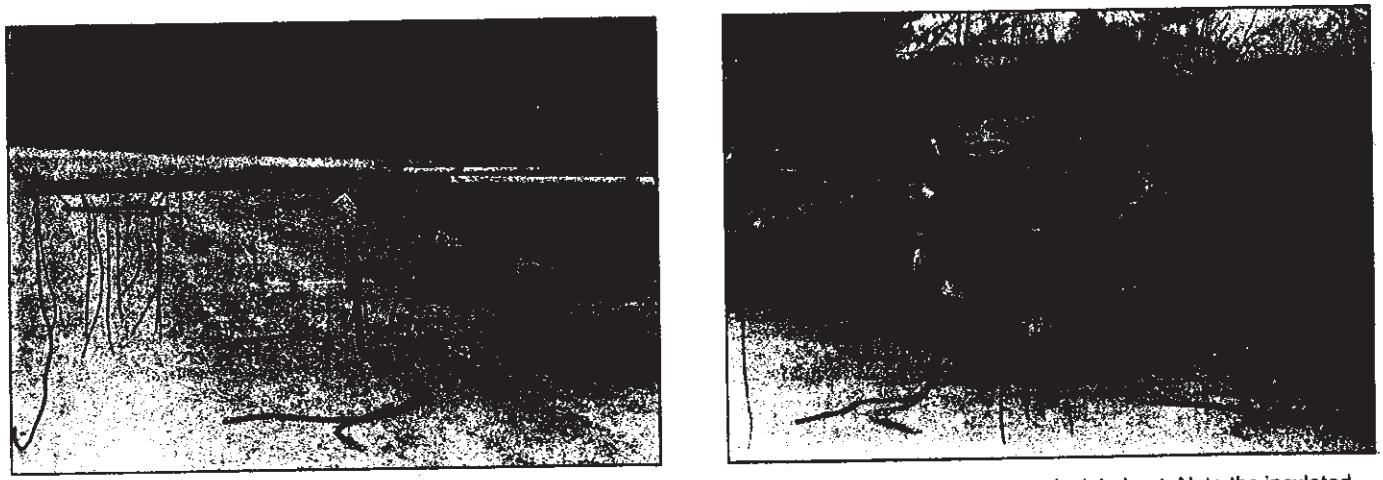

Figure 1. The side view *(left)* and front view *(right)* are of an electrical shocker device mounted on the bow of a johnboat. Note the insulated waist-high safety rails located around the dipping area. (The boat shown here is mounted on a trailer for overland towing to location.)

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.

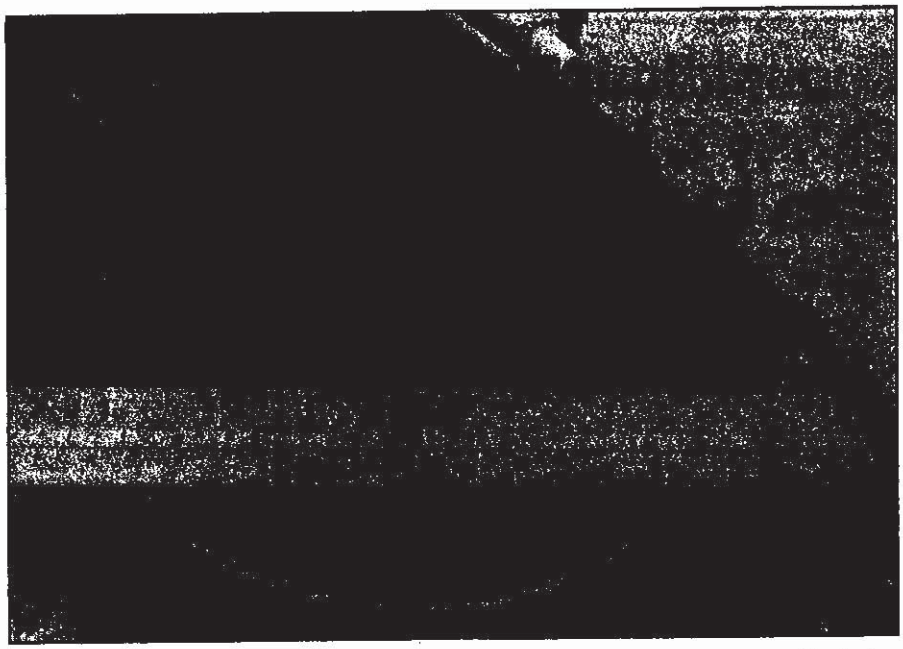

Figure 2. The fish-holding tank is located amidships, adjacent to the dipping area. The isolating screen *(left)* has been pulled aside to show the aerator.

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.

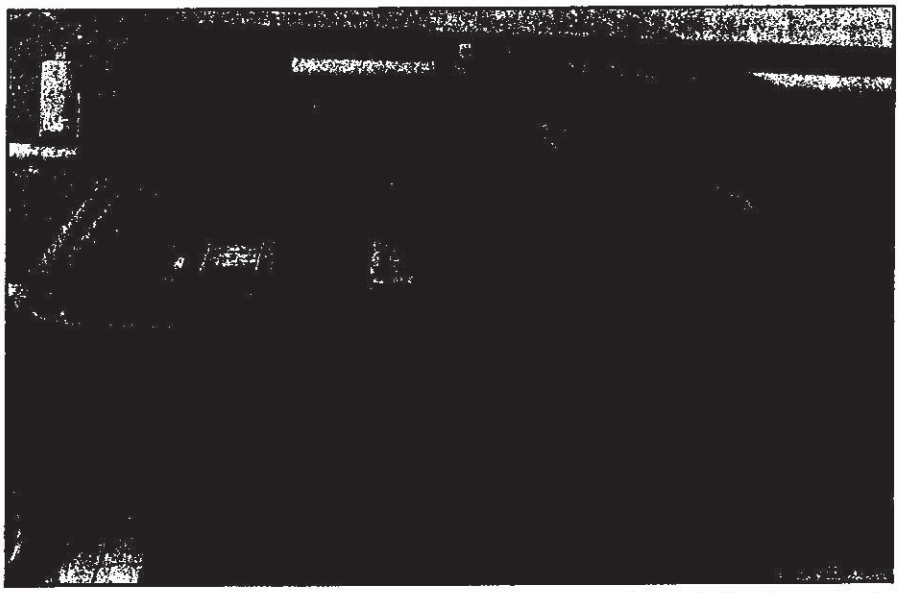

Figure 4. The motor-driven generator/alternator should be safeguarded to keep people away from hot pipes. Exhaust should be kept away from the boat operator.

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.

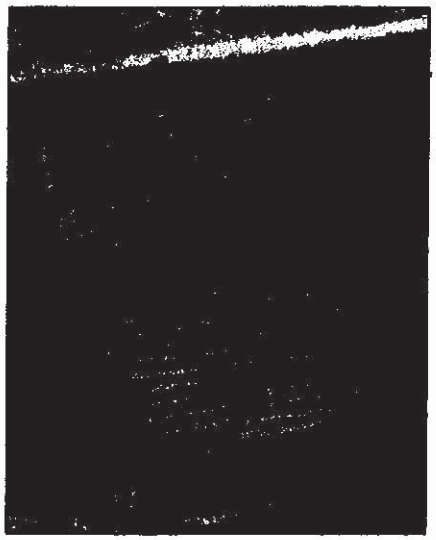

Figure 6. Each dip-netter should have a foot-actuated emergency stop switch (such as this emergency stop pedal) to cut the power from the pulsator or generator.

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).

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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

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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
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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.

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Appendix A. Suggested Taxonomic 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***

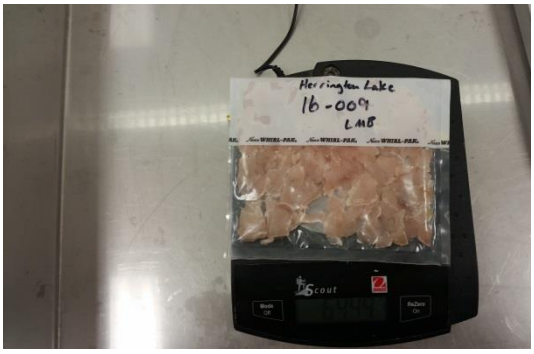
Use This Section Only if Reporting Type is DW and Samples with the Same Sample IDs have been Composited and Homogenized. Reporting Type DW does not require % Moisture.				
	Sample Weight + Container Weight After Lyophilization Cycle			
Sample ID	**Cycle #1 Weight (g) Date:5/15/16 Time:1030**	**Cycle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