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

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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 A. Suggested Taxonomic References

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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		Right Bank	
		Released	DELT	Released	DELT
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
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17					
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22					
23					
24					
25					
26					