# Standard Operating Procedure
## Collection Methods for Fish in Wadeable Streams

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

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# Table of Contents

Procedures........................................................................................................................... 4  
Scope and Applicability ..................................................................................................... 4  
Definitions....................................................................................................................... 4  
Health & Safety Warning ................................................................................................. 5  
Personnel Qualifications / Responsibilities .................................................................... 6  
Equipment and Supplies ................................................................................................. 6  
Methods........................................................................................................................... 7  
  Instrument Calibration ................................................................................................ 8  
  Type of Collections ..................................................................................................... 8  
  Sampling Periods ........................................................................................................ 8  
  Sample Reach .............................................................................................................. 8  
  Sampling Methods ...................................................................................................... 9  
  Sample Processing and Preservation ........................................................................ 11  
Data and Records Management ...................................................................................... 11  
Quality Control and Quality Assurance Section ............................................................... 12  
Reference Section ............................................................................................................. 12  

Procedures

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 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. The Kentucky Index of Biotic Integrity (KIBI) is used to assess stream health by examining fish community structure (Compton et al. 2003). 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 KIBI are outlined in this manual. Collection methods used in large-wadeable and non-wadeable streams (>200 mi.² drainage area) may not provide reliable KIBI results; separate methods and assessment criteria for larger streams and rivers are under development (Compton et al. 2003).

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. The anode is always a probe when backpack electrofishing.

Backpack Electrofisher– unit designed for electrofishing.

Back Pack Electrofishing (BPEF) – electrofishing with a backpack electrofisher.

Cathode – The negative electrode. The cathode can be either a rat tail or probe.

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.

KDOW – Kentucky Division of Water

Netter – The individual who nets the captured fish during electrofishing operations.
Probe – Pole fitted with a metal ring and serves as either the anode or cathode (with two probe method).

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

Sample Point – Latitude and longitude that identifies sampling location. This can be either an existing site location, new site or randomly chosen site.

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

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

**Health & Safety Warning**

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, it must be transported in a secondary leakproof 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. Backpack electrfisher should be refueled in an open area. Care should be taken when pouring gasoline into the electrfisher 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. 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**

(Modified from Barbour et al. 1999)

- Field Datasheet: High or Low Gradient Stream Data Sheet (KDOW 2008) or Waterproof Notebook
- Chain of Custody (KDOW 2009b) if fish crew leader is not retaining fish samples.
- Dipnets (Minimum of 3)
- DC backpack shocker (Gasoline or Battery Power)
- 2 Probes with rings (1 may be equipped with a net ring and shallow net)
- Spare probe and rings
- Rat tail
- Fuel: gasoline and oil or batteries
- Two cycle oil (gasoline powered electrfisher)
- Field guide (e.g. Peterson’s Field Guide Freshwater Fishes)
- Seine (10 and 15 foot)
• Formalin and MSDS sheet
• Fish jars (Various sizes)
• 5 gal bucket
• Waterproof paper for sample labels
• Lineman’s gloves if using non-insulated probes or nets
• Waders and boots (equipped with wading cleats, when necessary)
• Polarized sunglasses
• Copies of field protocols
• Pencils
• Clipboard
• First aid kit
• Global Positioning System (GPS) Unit

Methods

While following these sampling techniques, it is important to keep the sampling reach intact and undisturbed. Field personnel should not walk through 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.

Collectors should be aware of the advantages and shortcomings of seining and electrofishing techniques. KDOW has observed electrofishing to be more effective in streams that have numerous boulders, undercut banks and woody debris. KDOW also has observed that electrofishing tends to be biased toward catostomid and centrarchid members while not fully representing the schools of cyprinids (i.e., Lythrurus and Notropis spp.) in large pools. However, cyprinids can be effectively sampled with a seine in large pool habitats to yield a better representation of their presence in the community (Onorato et al. 1998). Both methods have advantages and disadvantages in different habitat types and species groups. The combination of seining and electrofishing yields better results than one technique independently (Onorato et al. 1998 and Yoder and Smith 1999). Due to habitat variability, a combination of seining and electrofishing is used in wadeable streams. Sampling consists of using a backpack electrofisher unit working in an upstream direction in a side-to-side/bank-to-bank sweeping technique and seine (if appropriate).
**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 (40-60 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) has been used to designate streams into two classes, headwater and wadeable, and a set methodology is outlined for each classification. Headwater streams are streams with a drainage area < 5 mi.². Wadeable streams are streams with a drainage area greater > 10 mi.² up to 200 mi.². However, streams with the drainages of 5-10 mi.² fall within a “gray” area of stream classification and best professional judgment by the sample team shall be used to determine if the stream is headwater or wadeable.

**Sampling Periods**

The sampling index period is mid-March 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.

**Sample Reach**

Each sample reach should consist of two riffles, two runs and two pools, if present. In cases where two riffles, runs, and pools cannot be sampled, one riffle, run and pool sequence is sampled or the recommended reach maximum length is sampled. A minimum reach length is necessary to ensure the collection of representative samples of biological communities, and a maximum reach length is needed to prevent unnecessary sampling and to minimize crew fatigue (and associated reduction of sampling efficiency) (Meador et al. 1993). In order to stay consistent with KDOW (2002, 2008 and 2009a), both headwater and wadeable streams will have a minimum reach length of 100 m. However, the maximum reach length is being extended to 300 m. This increase is to allow longer reaches at sites where macro habitat may not be available in shorter reaches. Meador et al. (1993) estimated a sampling reach length of 300 m for wadeable streams as the maximum length of sampling reach necessary to ensure the collection of a representative sample, yet minimize unnecessary sampling and reduced sampling efficiency as a result of crew fatigue. The maximum reach length for both headwater and
wadeable streams will be 300 m. The time limits of each sampling method should be observed in the sampling reach.

**Sampling Methods**

The sampling crew consists of a minimum of two members with at least one qualified member. One individual operates the electrofishing unit (minimum of 400 watts) while the other(s) work(s) the seine and dip net(s), and carry the bucket used to transport captured fish. The electrofishing operator should also carry a dip net (Barbour et al. 1999) if using one anode. The lower and upper ends of the reach should be associated with a natural in stream barrier such as a riffle, if possible. Best professional judgment of the crew is used to sample available habitat with electrofishing or seining techniques.

Collected fish should be frequently transferred from dip nets and seines to a bucket of water to lessen stress and mortality. In addition, water in the bucket should be changed periodically (warmer water temperatures require more frequent water changes) to reduced stress and mortality of fish.

**Electrofishing**

The electrofishing duration within the sample reach should be a minimum of 600 “shocking” seconds in headwater and wadeable streams to a maximum of 1000 seconds in headwater streams and 1800 seconds in wadeable streams. Electrofishing consists of using a backpack electrofisher unit working in an upstream direction in a side-to-side/bank-to-bank sweeping technique. Crew members with dip nets walk alongside and behind the electrofishing operator to collect stunned fish. One pass of the reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled (Barbour et al. 1999). In addition, some circumstances (e.g. swift and/or deep water) may require the use of a seine (rather than a dip net) and electrofishing. The seine may be set perpendicular to the current (to act as a block net) by two crew members and the electrofishing operator applies current upstream to downstream to the seine. Stunned fish are carried by current into the seine where they are captured. The electrofishing operator may need to dislodge specimens caught in the substrate. In some large wide wadeable streams (streams near the 200 mi² maximum drainage area) with reaches approaching 300m, the duration of BPEF may exceed 1800 seconds if all habitats can not be thoroughly sampled within the time limit. These exceeds of maximum times should be noted and recorded. In addition, at some large sites a BPEF may not provide sufficient power to collect fish. In these circumstances a tote barge or similar electrofisher capable of producing 2,500 watts may be used. Record the time spent electrofishing (in seconds).

**Seining**

Habitats not effectively sampled by electrofishing (e.g. pool habitat and swift runs) are sampled after electrofishing by seining. Seine hauls are generally preformed in a downstream direction (Etnier and Starnes 1993, Jenkins and Burkhead 1993 and Hendricks et al. 1980). 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. When the seine haul is finished, the seine is beached by dragging it onto the shore. 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. If the seine cannot be beached, then in one motion, the seine is quickly lifted out of the water and carried onto shore. However, certain situations may dictate the use of other seining techniques (i.e. kick seining technique or specific habitat seining). Kick seining involves two crewmembers holding the seine in a position downstream of the area to be sampled. The brails are slightly angled downstream so that the flow forms a pocket in the seine. A third crewmember disturbs (or kicks) the substrate while moving toward the seine. After reaching the seine, crewmembers lift the seine out of the water. Specific habitat seining involves encircle 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. After disturbing the habitat the seine is lifted out of the water. Other seining techniques may be warranted to sample unique situations. During all seining, it is important to keep the lead line of the seine in contact with the stream bottom in order to prevent fish from escaping under the net.

After each seine haul, fish are briefly examined by the fisheries biologist for the species present and then placed in a bucket of water. Large fish are identified, recorded and released immediately after each seine haul. Smaller fish are identified and released or retained as a voucher after all seining has been completed. A seine is used for a minimum of 30 minutes and continues until no new species are collected in three consecutive hauls or until a maximum of 90 minutes of effort is reached. If 30 minutes of effort has been expended and no new species were encountered in the last three hauls, seining may cease if all appropriate habitats in the reach have been sampled. Minimum and maximum times are defined as the start to finish of the seining effort. Record the time spent seining (in minutes start to finish). Seining may not be appropriate in some headwater streams, best professional judgment of the fisheries biologist should be used to determine if seining is appropriate in headwater streams. All wadeable streams should be seined. If conditions prevent a seine from being used in wadeable waters (e.g. too many obstacles in the channel such as downed trees or numerous large boulders), this should be noted.
**Sample Processing and Preservation**

Seining and electrofishing fish should be kept separate. This will result in one jar for electrofishing and one jar for seining for each site.

Fish collections are preserved in the field with a 10%-15% buffered formalin solution. Large Specimens should have a slit made in the abdomen to permit entrance of preservative into the body cavity or injected with a formalin solution. This is particularly important in warm weather to prevent partial decomposition of internal organs. Field containers should be large enough to accommodate the largest specimen without distorting it. Large specimens are identified in the field, recorded and released, unless the specimen(s) represent a significant ichthyological find (e.g., state or drainage record), then they are to be preserved as voucher specimens. Easily identified fish that are collected in large numbers (i.e., *Camptostoma* spp.) are also recorded in the field and released. Young of the year fish should not be retained as voucher or included in field counts.

At least two and up to five specimens of each species released should be kept as vouchers from the sample event. Large specimens not retained in the field should be photographed for voucher. Vouchers (retained specimens or photographs) need to be made for verification. 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.). Federally protected species must be identified, photographed and released immediately.

While at the sampling location, all fish samples will receive a label. The label is placed in the sample jar (labels placed in the jar will be written in No. 2 pencil on waterproof paper). The label will include the site number (if known), stream name, location, county, date sampled, collectors’ initials and collection method. Additional label information may include: shocking seconds, seine time and distance sampled.

**Data and Records Management**

Released fish are counted and recorded in the fisheries biologist’s field notebook or on the field datasheet (KDOW 2008). Photographed fish are recorded with the file number from the camera.

Record the time spent BPEF (in seconds).

Record the time spent seining (in minutes).

Completed High or Low Gradient Stream Data Sheet (KDOW 2008).

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 Section

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. Results will be considered acceptable if the same narrative KIBI rating is attained or if the KIBI score falls within 14 points on both sampling occasions. If this does not occur, all fisheries biologists will meet to assess the issue and take corrective actions which will be documented with other QA files.

Field data must be complete and legible and entered on field data sheet 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.

Reference Section


Compton, M.C., G.J. Pond, and J.F. Brumley. 2003. Development and application of the Kentucky Index of Biotic Integrity (KIBI). Kentucky Department for Environmental Protection, Division of Water, Frankfort, KY.


