

**Protocol for Monitoring Springs at Ozark National Scenic Riverways, Missouri.
Heartland I&M Network**

SOP 8: Fish Community Sampling

Version 1.10 (1/30/2020)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.0	1/30/2020	Dodd	Moved Reach Conditions and Weather form to Habitat SOP. Added equipment list. Modified Fish Form. Made SOP more concise. Added QC and certification procedures.	Reach & Weather conditions form more appropriate in Habitat SOP as it relates to the sampling event. Fish form now consistent with modified database.	1.1

The fish sampling protocols described in this SOP present methods for collecting a representative sample of the fish community from a spring-run. Such a sample should contain most, if not all, species in the spring-run at the time of sampling, in numbers proportional to their actual abundance.

Methodology presented below follows the NAWQA protocols described in Meador et al. (1993), Walsh and Meador (1998), and Moulton et al. (2002) and those used in Dodd et al. (2018) for the rivers and tributaries at OZAR and Bowles et al. (2008) for OZAR springs. The methods have been modified from NAWQA where appropriate to meet the specific objectives of the National Park Service. Further, only those portions of the NAWQA protocols applicable to sampling fish at the large springs at OZAR are included here. Electrofishing will be the only method of collecting fish in the springs and it is conducted in a single pass of the sampling reach for wadeable reaches. This SOP presents equipment necessary for fish monitoring (Table 1) and techniques for fish sampling and procedures for processing fish.

Table 1. Fish sampling equipment for springs monitoring at OZAR.

General Equipment

Waders
Rain gear
Park radio & charger
GPS unit & park maps
Flagging

Stop watch
Fish Collecting permits
Field guides
Camera
Tool box & volt/ohm meter
Polarized sunglasses & hats

Fish Sampling - Non-wadeable

Boat electrofishing unit
- generator, gas can, & extra oil
- pulse box
- boom and cords
- switch pad
- paddles (2) & boat hook
- Rope & anchor
- Throw bag
Electrofishing gloves
Ear plugs
Outboard motor & gas can (1 per watercraft)
Life jacket (1 per person)
Throwable cushions (1 per watercraft)
Large tub or live well in boat
- bilge pump for aeration

Fish Sampling – Wadeable

Backpack electrofishing unit
- backpack
- anode
- cathode
- batteries & battery charger
Tow barge electrofishing unit
- barge
- generator, gas can,& extra oil
- pulse box
- anodes
- gas can with gas
5 gal. buckets for holding fish
Aerators & batteries
Dip nets (1 per person)
Electrofishing gloves

Fish Processing Equipment

In-stream pen for large fish
Several 5 gal. & 1 gal. buckets to sort fish
Aquarium nets (4-5)
Measuring board
Weighing balance/scale & batteries
Plastic container to hold fish on scale
Fold out wind break boards for scale
Clipboard - pens, pencils, waterproof paper
Fish field sheets on waterproof paper
1 gal. jugs & plastic baggies for preserving
specimens (1 jug per site)
10% buffered formalin (pH = 7)
Tags for jars

II. Electrofishing Methods

Overview

Electrofishing is the use of electricity to capture fish. A high-voltage potential is applied between two or more electrodes that are placed in the water. The voltage potential is created with either direct current or alternating current; only direct current is used in the NAWQA Program and for sampling rivers and tributaries at OZAR (Dodd et al. 2018) and will be used in this protocol. Direct current produces a unidirectional, constant electrical current. Pulsed direct current, a modified direct current, produces a unidirectional electrical current composed of a sequence of cyclic impulses (Meador et al. 1993). The frequency of the pulses produced when using pulsed direct current can be adjusted by the operator.

Procedures for Setting Electrofishing Gear:

- A. Prior to sampling, measure ambient conductivity of the water to aid in selecting voltage, frequency, and duty cycle for the electrofishing gear being used. Note: Effectiveness of electrofishing gear is primarily affected by water conductivity.
 - 1. Low conductivity water is resistant to flow, reducing the amount of electrical current traveling through the water.
 - 2. High conductivity water concentrates a narrow electrical field between the electrodes (Meador et al. 1993).
- B. General Rule for Voltage: output of electrofishing gear should be ~ 3,000 watts. Voltage times amperage equals wattage. If the gear is without ammeters, some pre-sampling experimentation is necessary.
 - 1. In low conductivity water, high voltage and low amperage are needed.
 - 2. In high conductivity water, low voltage and high amperage are needed.
- C. General Rule for Frequency: a pulse rate range from 30 to 60 pulses per second (pps) should be used. Frequencies > 60 pulses per second (pps) are effective in collecting fish but can cause injuries, especially to larger fish (Coffelt Manufacturing, Inc., cited in Meador et al. 1993; McMichael et al. 1998). Pulse rates < 30 pps have caused low incidence of injury, but are generally ineffective in collecting fish. Therefore, pulse rate should be set to produce an effective collection of all fish species and sizes while minimizing injury.
- D. General Rule for Duty Cycle: a range of 25% - 100% should be used. Duty cycle is calculated as: pulse frequency X pulse duration X 100. Electrofishing with intermediate to high duty cycles reduces injury and mortality to fish (Dolan and Miranda 2004).

Electrofishing Wadeable Reaches

General Procedures:

- A. Channel width, depth, and access should be considered before choosing between backpack and towed electrofishing methods (Fig. 1).
 1. Backpack electrofishing (with a single anode) should be used in shallow (< 1 m) and narrow (< 5 m wide) reaches. A crew of three is needed.
 2. Towed electrofishing gear (multiple anodes) is more effective in wide (> 5 m) wadeable reaches with pools deeper than 1 m. A crew of 5 is needed.
- B. All crewmembers must wear low voltage rubber gloves and waders to protect them from the electrical current and wear polarized glasses to enhance their ability to see fish.
- C. Techniques for collecting samples using either backpack or towed electrofishing gear are generally similar.



Figure 1. Backpack (left) and towed barge (right) electrofishing gear used in wadeable spring-runs.

Sampling Direction:

- A. Regardless of the gear used, a single electrofishing pass is made in the reach (Fig. 2).
- B. Sampling begins at the downstream boundary of the sampling reach and proceeds upstream. Sampling in an upstream direction in wadeable streams is preferred because disturbance of the streambed by crewmembers increases turbidity and reduces visibility of the stunned fish (Hendricks et al. 1980).
- C. Sampling requires alternating between banks in a “zigzag” pattern in order to cover habitat features as one proceeds upstream (Fig. 2). Using the zigzag pattern, every effort is made to sample all geomorphic channel units and instream habitat features, such as woody snags, undercut banks, macrophyte beds, or large boulders.

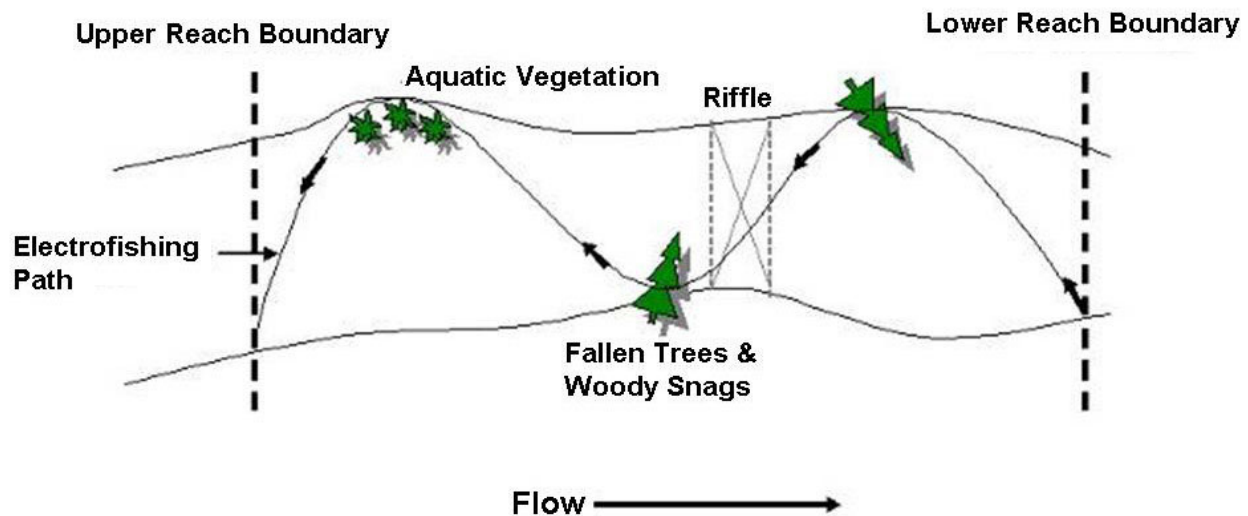


Figure 2. Single pass zigzag sampling technique used in backpack or towed electrofishing for wadeable portions of the spring-run.

Electrofishing Techniques:

- A. Continuous application of electrical current and herding of fish by the operator is used in open run habitats. Fish generally respond to continuous electrical current by avoiding exposure to the electrical field, resulting in fish moving just ahead and away from the operator. The operator uses the current to herd the fish into natural barriers such as banks, bars, or shallow riffles to facilitate capture.
- B. Intermittent application of electrical current and herding fish to the operator can be used in runs or long pools in narrow reaches. This technique requires a crewmember (or two) to enter the reach upstream of the operator. The crewmember(s) moves downstream toward the operator, creating a disturbance and driving fish downstream. The electricity is turned on once the fish are visible and close to the anode.
- C. To sample shallow riffles, the operator sweeps the anode across the riffle from upstream to downstream while walking across the riffle. Crewmembers with nets are positioned downstream of the operator to collect stunned fish.
- D. The ambush technique should be used when a reach has complex instream habitat such as woody debris, boulder fields, etc. Using this technique, the operator approaches the habitat feature with the electrical current off. In quick succession, the anode is thrust close to the habitat feature, the current is turned on, and the anode is withdrawn from the feature. This produces galvanotaxis, where the current forces the fish to swim out from the habitat feature and towards the anode (Meador et al. 1993).

Electrofishing Nonwadeable Reaches

Procedures:

- A. Nonwadeable reaches are sampled using an electrofishing boat (Fig. 3) with supplemental use of backpack or towed electrofishing gear in wadeable areas.
- B. A boat crew consists of a driver and one or two persons who collect the fish with dip nets.
- C. For safety, all crewmembers must wear low voltage gloves, waders, and life jackets. Polarized sunglasses should also be worn.



Figure 3. Boat electrofishing gear used in nonwadeable reaches.

Sampling Direction and Speed

- A. Two separate electrofishing passes, one along each bank, are made in the reach (Fig. 4).
- B. Sampling begins at the upstream boundary of the sampling reach and proceeds downstream by maneuvering the boat along one shoreline. Sampling is conducted downstream because when fish are stunned they are carried downstream by the flow, providing greater opportunity for capture by netters standing on the bow of the boat.
- C. A zigzag pattern between the bank and center channel is used to collect fish from all instream habitats (Fig. 4). Once one side is complete, the other bank is sampled.
- D. The electrofishing boat is operated at a speed equal to or slightly greater than the current velocity. The boat should be positioned so the bow is angled downstream and

toward the bank. This allows the boat operator to reverse direction (generally upstream and away from the banks) and not pass over stunned fish.

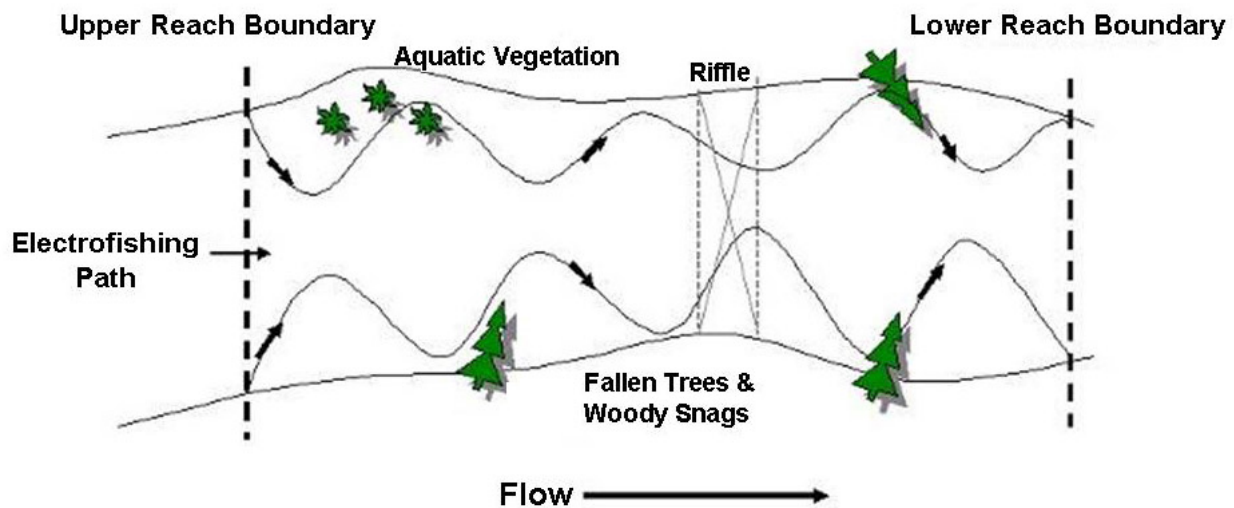


Figure 4. Double pass zigzag sampling technique used in boat electrofishing for non-wadeable portions of the spring-run.

Electrofishing Techniques

1. Continuous application of current is used for most nonwadeable reaches.
2. Intermittent application of current may be more effective in areas with clear or shallow water. Fish are approached with the current off. When the anodes are in position near the fish, the current is applied.
3. Ambush technique (similar to that in wadeable reaches) is used for sampling instream habitat features along the shoreline. The boat is maneuvered close to the habitat feature with the electrical current off. As the anode is placed near the habitat feature, the electrical field is generated and the boat is backed away from the habitat feature.

III. Sample Processing

The goal of processing fish in the field is to collect information on taxonomic identification, length, weight, abundance, and the presence of external anomalies with minimal harm to specimens that will be released alive back into the spring-run. Many species are too small or difficult to identify in the field. These specimens must be preserved and brought back to the laboratory for processing.

Procedures:

- A. If different types of gear are used within a reach, fish samples from different gear types are kept separate.

1. Record the spring name, date, gear used, sampling effort (time spent electrofishing).
 2. Record the three letter initials of all crewmembers. Record the person identifying/measuring fish, the person recording the data, and those who operated the electrofishing equipment and netted fish.
- B. Hold fish in a manner consistent with minimizing stress or death. Captured fish are placed in a live cage or aerated holding tanks or buckets during processing to reduce stress and mortality. Regardless of the effort made to minimize handling and stress to fish, some mortality will occur.
- C. Sort fish into identifiable and unidentifiable groups. Identifications are made to species level by a crewmember that is familiar with the fish species commonly found in the study area and has been certified by the Fisheries Biologist. Taxonomic nomenclature follows that established by the American Fisheries Society's Committee on Common and Scientific Names of Fishes (Page et al. 2013). Process threatened and endangered (T&E) or sensitive fish species before other identifiable species.
- D. Obtain a subsample of 30 individuals of each identifiable species for measurement.
1. A “blind grab” technique is used where a dip net is used to make a pass through the entire bucket or holding tank to ensure fish of various sizes within that species are captured with each “grab”.
 2. For species that have a large size range with different sizes being collected by specific gear (e.g., large sunfish collected with boat and small sunfish collected with backpack), a blind grab will be taken from samples collected by each gear type and a representative subsample will be measured.
- E. Measure and record total length (Fig. 5) and weight of 30 individuals for each and record any anomalies on the field sheet (Fig 6).
1. Length measurements are determined by using a measuring board consisting of a linear metric scale on a flat wooden or plastic base with a stop at the zero point. Total length is taken from tip of snout (with mouth closed) to end of caudal fin (while depressed).
 2. Weight measurements are obtained by using portable electronic or hanging scales.
 3. A batch weight will be recorded for smaller species (e.g., minnows, darters, sculpins, and madtoms). Individual weights will be recorded for species that obtain large sizes (e.g., bass, sunfish, catfish, suckers).

4. Record anomalies and presence of black spot (*Neascus* spp.), a fish parasite, for each fish measured. Anomalies are externally visible skin or subcutaneous disorders or parasites (Ohio Environmental Protection Agency 1987). They include deformities, eroded fins, lesions, and tumors, collectively referred to as "DELT anomalies" (Sanders et al. 1999). Other external anomalies, such as anchor worm (*Lernaea* spp.), and popeye disease should also be noted in the comments.
 5. Once 30 fish of a species have been measured, the remaining specimens for that species are counted and recorded under "Additional Species Count". For example, if 46 white suckers were collected at a reach, 30 fish would be measured and weighed and the remaining 16 would be counted (*i.e.*, Additional Species Count = 16).
- G. Preserve selected specimens for identification in the laboratory (those too small or difficult to identify in the field) or for a reference collection. Specimens are preserved in 10 percent buffered formalin.
1. Make a small incision along the body for specimens larger than 100 mm. This allows formalin to penetrate the body cavity.
 2. All unidentified specimens collected with the same gear type can be preserved in a single jar with a label that contains the spring name, date, gear type, and sampling effort. Labels should be on water proof paper and written in pencil
 3. All preserved specimens will be identified at the HTLN Aquatic Program laboratory at Missouri State University using a microscope and the *Fishes of Missouri* identification key (Pflieger 1997). Specimens not used for a reference collection will be disposed of after one year.



Figure 5. Total length measurements for a fish.

IV. QA/QC

Quality Assurance Procedures:

QA include all activities and skills of staff members that ensure data collection is high quality and prevents inconsistencies. QA processes for fish data collection are:

- A. For collection of fish data, all crew members will review collecting methods described in this SOP.
- B. Two critical elements to collect quality data and maintain consistency across years is: an adequate number of **experienced** crew members and the consistency in the team conducting the fish sampling.
 1. To maintain data quality consistency and efficiency while keeping the crew safe, a crew of four to six people are needed for fish sampling, depending on the gear used and size of the spring.
 2. The project lead and field crew leads are to have extensive experience (at least 2-3 field seasons) in boat operation and electrofishing procedures. Crew leads must have completed the U.S. Fish and Wildlife Service (USFWS) electrofishing course (Principles and Techniques of Electrofishing) and be certified in the Department of Interior (DOI) Motorboat Operators (MOCC) Practical course. An on-line refresher MOCC course is required every 5 years to remain certified in motorboat operations of a government vessel.
 3. At least half of the crew (2-3 persons) must be experienced (i.e. one field season of experience) with electrofishing procedures and gears. Crew members who are inexperienced with electrofishing gear will undergo training by the project lead or field crew leads prior to the field season to learn safety features and proper sampling techniques of the equipment.
 4. All crew members will be trained on boat operation and safety by the project lead or field crew leads.
 5. At least two people on the crew must have competent data recording abilities. This will ensure that the numerous fish measurements and counts will be recorded accurately. Prior to recording data, the project or crew leads will explain and demonstrate to new crewmembers how to record data on the field forms and will check the data recording during fish processing.

Quality Control and Certification Procedures:

- A. Prior to the field season, the field crew leads (including the project lead/fisheries biologist) need to refamiliarize themselves with the fish fauna by examining preserved specimens located in the reference collection and reviewing fish species lists of the springs.

- B. The project lead for fish monitoring (typically the fisheries biologist) will have taxonomic expertise, having completed an Ichthyology course and having 3 or more years of fish identification experience. The project lead will be the QA officer for both experienced staff (i.e. have 1-2 years of fish identification experience or have taken an Ichthyology class) and inexperienced staff who will assist with fish identifications.
- C. Field crew leads responsible for fish identifications down to the species level will have completed an Ichthyology course and be certified by the QA officer (i.e., project lead).
 - 1. To obtain certification, crew leads will independently identify a subset of at least 50% of the fish collected at a spring from at least two springs with high species diversity (Round, Pulltite, Phillips, or Alley). A 90% agreement between crew leads and QA officer is required for certification.
 - 2. If crew leads have been certified based on procedures described in the fish sampling SOP of the HTLN River Fish Monitoring Protocol for BUFF and OZAR (Dodd et al. 2018), this certification is valid for identification of springs samples.
 - 3. Only the project lead or certified crew leads will be allowed to make final determinations of identifications to the species level.
 - 4. If a species is identified that is not within the range of southern Missouri, the specimen will be preserved and sent to a taxonomic expert. If a species is identified as not being collected previously at OZAR but is within its native range, the specimen will be identified by at least two different certified crew members for verification.
- D. In the field, the project lead or crew leads will train other crewmembers in coarse identification of specimens, separating them into families or sub-family groups (such as sunfish, sculpins, minnows, and darters) to aid in initial sorting and processing of fish.

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