Methods for Monitoring Fish Communities of Buffalo National River and Ozark National Scenic Riverways in the Ozark Plateaus of Arkansas and Missouri, Version 2.0

Standard Operating Procedure (SOP) 5: Fish Community Sampling, Version 1.2

Revision History Log:

Previous version number	Revision date	Author	Changes made	Reason for change	New version number
1.0	3/22/2016	Dodd	Moved field sheets and equipment lists from Protocol Narrative appendices into SOP; updated references for species name changes	Make SOP stand-alone document for field use	1.1
1.0	3/22/2016	Dodd	Updated necessary crew requirements for sampling wadeable and non-wadeable reaches	Enhance quality assurance of data collected and crew safety	1.1
1.1	4/6/2020	Dodd	Updated Fish Community Field Form for changes in database and revised QA/QC section	Reduce error in collecting and entering field data	1.2

This SOP presents methods and equipment needed (see Table 1) for collecting representative fish community samples. Methods closely follow in methodology, wording, and scope the NAWQA protocols described in Moulton *et al*. (2002) and Walsh and Meador (1998). The methods have been modified where appropriate to meet the specific objectives of the NPS to collect representative samples and measure changes in fish communities from streams in BUFF and OZAR. A representative sample contains most, if not all, species in the stream at the time of sampling in numbers proportional to their actual abundance. Each reach contains representative instream channel units (riffles, runs, glides, and pools), substrates, and hydrologic conditions. Many fish species are specialized for specific habitat conditions and their occurrence in the stream is determined largely by the relative abundance of aquatic habitats.

Electrofishing Overview

Electrofishing is viewed as the single most effective method for sampling stream fish communities (Bagenal 1978; Barbour *et al*. 1999) and involves 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 can be created with either direct current (DC) or alternating current (AC) using a pulsator; however, because of less harm to fish from the use of DC, only DC will be used for this fish monitoring program. DC produces a unidirectional, constant electrical current. Pulsed DC is a modified direct current that utilizes a sequence of cyclic impulses to immobilize fish. The frequency of the pulses produced when using pulsed DC can be adjusted by the operator.

Some fish are more susceptible to electrofishing injury, and injury rate can vary by fish species, environmental conditions, and electrofishing techniques. Salmonids (Dalbey *et al*. 1996; Kocovsky *et al*. 1997; Ainslie *et al*. 1998) and larger fish (McMichael *et al*. 1998) are more susceptible to injury than other fish. Pulse frequencies greater than 60 pulses per second (pps) have proven to be more effective in collecting fish but appear to cause spinal injuries, particularly in trout and salmon species (Coffelt Manufacturing, Incorporated, cited in Meador *et al*. 1993). Pulse rates of less than 30 pps have caused low incidence of injury, but are generally ineffective in collecting fish. Therefore, a pulse-frequency range from 30 to 60 pps is generally used to optimize collection effectiveness with a minimum potential for damage to fish. However, Dolan and Miranda (2004) suggested that electrofishing with intermediate to high duty cycles (pulse frequency X pulse duration X 100, as percent) could reduce injury and mortality to warmwater fish. These intermediate to high (36 to 100 percent) duty cycles tested by Dolan and Miranda (2004) had pulse frequencies of 60 to 100 pps and pulse durations of 6 milliseconds or were non-pulsed DC. While fishing, the operator should note the behavior of the fish so that adjustments can be made to the output of the electrofishing unit. To minimize mortality rates, Dolan and Miranda (2004) recommended that the power output and electrode system should be managed to induce narcosis (a state of induced immobility with slack muscles) and prevent tetany (immobility with rigid muscles) of large individuals.

Water conductance also affects the response of the fish to the electrical field and is the single most important limiting abiotic factor in electrofishing effectiveness. Water with low conductance is highly resistant to the flow of electrical current and the electrical field is limited to the immediate area of the electrode. Water with high conductance produces the opposite effect by concentrating a narrow electrical field between the anode and cathode (Meador *et al*. 1993). Water in most streams of BUFF and OZAR has moderate values of specific conductance (typically about 150 to 400 microsiemens per centimeter at 25 degrees Celsius, µS/cm) (Hauck *et al*. 1996; Mott and Luraas 2004). Water in the upper mainstem and some tributaries of the Buffalo River, however, has lower specific conductance (typically about 50 to 150 µS/cm) than water from other areas of the two parks. In low-conductance water, higher voltage is needed to immobilize fish, while in high-conductance water, lower voltage is needed to achieve the same result and to minimize potential damage to the fish. However, conductance will vary from site to site and from year to year; therefore, pre-sampling measurements of conductivity and experimentation with electrofishing settings is necessary.

Water clarity also affects electrofishing success and determines which techniques will be used. In clear streams, fish can see the electrofishing crew or boat. Evasion will be the response to both the electrical field and the presence of the crewmembers. In turbid streams, immobilized fish may be difficult to see resulting in low capture efficiency. At both BUFF and OZAR, water clarity is high and therefore, specific ambush or herding techniques (described within this SOP) will be used to increase capture rate.

Seining Overview

Although electrofishing is the most effective fish sampling method, it is biased toward collection of large-sized fish (Wiley and Tsai 1983; Dolan and Miranda 2003). Thus, seining is a common collection method often used to complement electrofishing (Bagenal 1978; Nielsen and Johnson 1983). Seining is a highly effective method for sampling small-sized individuals that are less than about 10 cm total length (Bayley and Herendeen 2000).

Wadeable streams and shallower parts of nonwadeable streams are sampled using a "common sense" seine (about 3 x 1.2 m with a 6.44-mm mesh size) or a bag seine, depending on the size of the stream. Use and effectiveness of a particular seine depend on the channel units (for example, riffles, runs, pools), channel size and features, and instream habitats present in the sampling reach. The presence of submerged objects such as woody snags, large cobble, or boulders in a sampling area makes it difficult to collect representative samples. Therefore, the potential for collecting a representative and repeatable sample should be evaluated before seining an area. Seines are most commonly used in wadeable streams with smaller substrates such as gravel or sand with little or no woody debris. In nonwadeable streams, seines are generally used to collect smaller fish along banks or in shallow backwater areas.

A second method of seining is known as kick seining. This method involves shortening the length of a common sense seine by rolling the seine onto the brails. The seine then is placed within a riffle, and the substrate is kicked to dislodge benthic fish species that then are carried by the current into the seine. This technique also is used in combination with backpack or towed barge electrofishing equipment, whereby the crewmember holding the anode will shock down into the seine while kicking the substrate. However, using electrofishing equipment with a seine is an electrofishing method and not strictly a seining technique.

Sampling Methods for Wadeable Streams

Electrofishing

Wadeable streams generally are less than 1.5 m deep, but may contain some areas that are substantially deeper. Backpack and towed electrofishing gears are used for sampling fish in wadeable streams. Backpack electrofishing with a single anode is usually most effective in shallow (less than 1 m), narrow (less than 5 m wide) streams. Towed barge electrofishing gear (multiple anodes) is usually more effective in wide (greater than 5 m) wadeable streams with pools deeper than 1 m. Channel width, depth, and access need to be considered before choosing between backpack and towed electrofishing gear. In some situations, it may be desirable to use a combination of backpack and towed electrofishing gear; for example, the backpack might be used in long, shallow riffles (or in conjunction with riffle kicking) and the towed gear in the deeper sections of the stream.

Electrofishing techniques for wadeable streams require an electrofishing crew consisting of three to six individuals in order to ensure representative and effective collection of fish community data while maintaining crew safety. When using backpack electrofishing gear, three crewmembers are necessary. One crewmember is designated as the operator and the other two collect fish with dip nets (netters). One of the two netters must also carry the holding bucket. With towed gear, five to six crewmembers are necessary. Two persons operate the anodes and one operates the barge and generator. Two or three crewmembers (or netters) use dip nets to collect stunned fish with the third netter assigned to transfer netted fish from dip nets into buckets or into a flow-through holding container (or live cage). For safety reasons, all crewmembers must wear non-breathable waders and low-voltage rubber gloves. Crewmembers should wear polarized sunglasses to enhance their ability to see fish that have been immobilized by the electrical field.

Techniques for collecting samples using either backpack or towed electrofishing gear are generally similar in riffle/pool streams. To obtain a representative sample using single pass electrofishing, thorough sampling along both banks of the stream and in mid-channel areas will be completed at each reach. All types of instream habitat features such as woody snags, undercut banks, macrophyte beds, large boulders, and geomorphic channel units (riffles, pools, runs) should be sampled. This technique requires electrofishing from one edge of the water to the other in a “zig-zag” pattern, while attempting to sample all types of habitat features and channel units within the reach in proportion to their occurrence (Figure 1). In reaches that have substantial and diverse habitat features along the banks (such as woody snags/tree roots, and boulder fields), it may be necessary for the crew to sample along one bank for a distance and then move back downstream and sample critical habitat along the opposite bank.

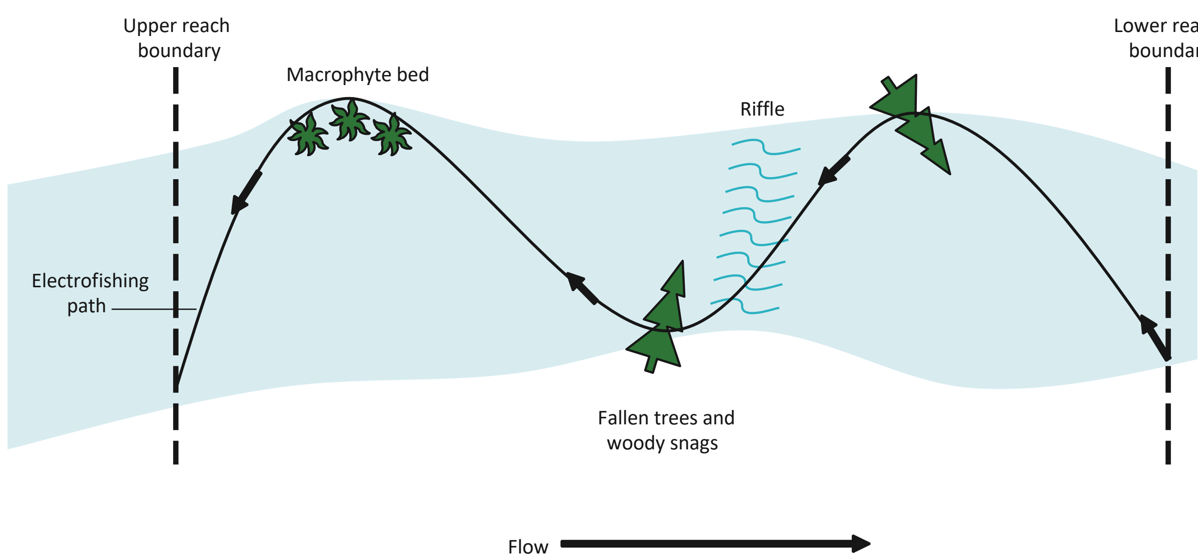

Figure 1. Single pass zig-zag sampling technique used in electrofishing wadeable streams.

The electrofishing crew should begin electrofishing at the downstream boundary of the sampling reach and proceed upstream. Sampling in an upstream direction in wadeable streams is preferred over sampling in a downstream direction for several reasons (Hendricks *et al*. 1980). Disturbance caused by electrofishing crews walking in the stream increases turbidity, thereby greatly reducing visibility and sampling efficiency. Also, sampling in an upstream direction allows stunned fish to drift downstream, thus facilitating their capture by the netters. Generally, the distance that the net is held downstream from the anode increases with current velocity and turbidity. It is also important to have one netter near the anode to collect fish stunned by the anode and an additional netter downstream from the cathode to collect fish that are stunned near the cathode or that float down from the anode.

Different fish species have different adaptations and several different electrofishing techniques may be necessary for efficient sampling. In some situations, applying a continuous electrical current to the water is effective. In other situations, it is more effective to apply current intermittently. Using a continuous electrical current, schooling species can be "herded" into confined spaces where they are more susceptible to capture. Fish generally respond to continuously applied electrical current by attempting to avoid exposure to the electrical field. Thus, continuous application of electricity can result in fish moving just ahead and away from the operator. The operator needs to be aware of this response and take advantage of natural barriers where fish can be herded (such as banks, bars, or shallow riffles) to facilitate their capture by the netters. Upon reaching a barrier, fish will turn and attempt to evade the approaching electrical current. Therefore, netters should be alert when approaching barriers so that fleeing fish can be captured.

Another technique that can be used for capturing fish species that flee the weak edge of the electrical field is for the crew to herd the fish toward the operator while the electrofishing equipment is turned off and to begin electrofishing once fish have come within the electrical field. This approach works well in runs or long pools where a crewmember (or two) moves upstream from the fish by getting out of the stream and walking up the bank. Once ahead of the fish, the crewmember returns to the stream and creates a disturbance in the water that drives the fish downstream toward the operator. The electricity is turned off as the crewmember herds the fish downstream. When the fish are visible and close to the anode, the electricity is turned on to stun them. Use of a small seine as an additional barrier improves the efficiency of this technique.

A third technique, referred to as the ambush technique, can be used for capturing fish species such as black bass and other members of the sunfish family that have affinities for cover, including submerged woody debris, boulders, and bed-rock ledges. These species can be captured by approaching the cover with the electricity turned off and thrusting the anode into the cover while turning on the electricity.

Shallow riffles, cascades, and torrents often are very swift and require different electrofishing techniques than run and pool areas. A useful technique for sampling shallow riffles is to have the operator stand beside the habitat to be sampled and sweep the anode across the riffle from upstream to downstream. Stunned fish will be carried downstream and into dip nets or a seine. This technique minimizes escape and avoidance of the electrical field by benthic fish species such as darters and sculpins, which commonly inhabit riffles. Sculpins and darters do not have swim bladders and, therefore, do not float when stunned, but rather roll along the bottom with the water current. Because they lack a swim bladder and have cryptic coloration, these species may not be seen until the net is examined. Swift riffles, cascades, and torrents with large cobble or boulder substrate can be sampled with similar techniques. For these habitats the operator works the anode downstream through the swift current between rocks and through small plunge pools while a dip net or seine is maintained on the stream bottom and about a meter downstream.

While electrofishing riffles, it may also be necessary for the operator to kick the substrate in shallow or slower riffle areas to dislodge benthic species into a seine (Hendricks *et al*. 1980; Matthews 1986; Bramblett and Fausch 1991). Kicking the stream bottom while electrofishing is an effective technique for collecting these species because it involves disturbing the substrate and allowing the water current to carry fish into a common sense seine. Two crewmembers enter the stream below a riffle and hold the seine in a vertical position above the water and perpendicular to the flow at the downstream edge of a riffle. If the riffle is large (wide and long), the crewmembers may enter the riffle at some intermediate point and hold the seine somewhere upstream from the downstream edge. The crewmembers then thrust the brails and lead line of the seine to the stream bottom. The brails are slightly angled downstream so that the flow forms a slight pocket in the seine. It is important that the lead line be on the stream bottom. It may be necessary to remove some rocks from beneath the lead line so that there are no gaps between the seine and the stream bottom. It may also be necessary for the crewmembers that are holding the seine to reach around the front of the brail with one leg and place a foot on the lead line to keep it in close contact with the stream bottom. Upstream from the seine, one or two crewmembers vigorously disturb (or kick) the substrate and electrofish by moving the anode back and forth while moving toward the seine. After reaching the seine, crewmembers lift the seine out of the water by grabbing the lead line. For safety reasons, it is important for all crewmembers to wear low voltage electrofishing gloves and to coordinate turning off the power to the electrofishing equipment before reaching for the lead line.

Seining

Runs and pools of streams can be sampled using a common sense seine or a bag seine, depending on the size (channel width and depth) of the stream. Generally, a common sense seine would be used in reaches where backpack electrofishing gear was used and a bag or common sense seine would be used in reaches where towed barge or boat electrofishing gear was used. Within the reach, specific sites to be seined will most commonly consist of backwaters or small/narrow side channels with smaller substrates and few instream obstacles (woody debris and boulders, for example) and will be based on professional judgment of the HTLN project leader or other staff with experience in fish sampling methods (such as crew leaders).

Seining efficiency can be improved by observing several guidelines. First, submerged objects (woody snags, large cobble, and boulders) make seining difficult, and these areas should be avoided when seining. Electrofishing gear should be used to draw fish out from submerged objects. Second, seining should be completed in a downstream direction, which has been demonstrated to be the most effective seining technique (Hendricks *et al*. 1980); however, seining in a downstream direction may not be effective in swift currents. Third, seining speed should be slightly faster than the stream current; faster seining speeds will push water in front of the seine and force fish away from the seine. Fourth, maintaining contact between the lead line and the bottom of the stream and angling the brails back to keep the net bottom well forward of the float line will minimize the potential for escaping fish. Lastly, when the seine haul is finished, the seine is beached by pivoting the seine and dragging it onto the shore.

Riffle dwelling species (such as darters and sculpins) can be sampled using kick seining without the use of electrofishing gear. This technique can be used when electrofishing gear is not available or when it is restricted because of presence of threatened or endangered species. Similar to the technique of electrofishing while kicking into a seine, kick seining requires two crewmembers to stand at the downstream end of the riffle with a common sense seine while one or two crewmembers kick into the net. Dislodged fish are carried by the swift current into the net.

Sampling Methods for Nonwadeable Streams

The following sections describe electrofishing and seining methods for sampling nonwadeable streams. Nonwadeable streams generally are greater than 1.5 m deep through most of their areas, although wadeable sections can be somewhat common.

Electrofishing

Nonwadeable streams are sampled using electrofishing boats or using boats in addition to towed barge or backpack electrofishing gear in shallow riffle areas. The basic components of the boat electrofishing unit include a generator, pulsator (control box), cathode (usually the boat), and boom (aluminum or PVC tubing used to support electrical cables in front of the boat bow), with attached anodes (cable droppers attached to the end of the boom in front of the boat bow; see Table 1at the end of this SOP). The boat should be large enough to hold all the equipment and provide safe and adequate work space for the crew. Generally, flat-bottomed aluminum hull boats are preferred, because metal hulled boats are easy to ground. To ensure sampling is effective in obtaining representative and quality data, a boat electrofishing crew must consist of an experienced boat operator and one or two experienced netters who collect the fish with long-handled (greater than 3 m) dip nets. Each crewmember will have access to a safety switch which will stop electrical current flow in the event of an emergency. Special training is required to operate this system. The boat operator is required to complete an instructor-led DOI motorboat training and an on-line USFWS electrofishing techniques course. The driver must also be experienced at maneuvering the boat as effectively as possible during electrofishing to allow crewmembers the best opportunity to capture stunned fish. Both the boat operator and the netters are required to read the chapter on electrofishing safety from the USFWS electrofishing techniques course. The netters must have experience (at least one field season) netting fish from the bow of a boat to ensure effective sample collection. As with wadeable electrofishing methods, all crewmembers must wear waders and low-voltage rubber gloves and should wear polarized sunglasses. All crew on the boat must wear Coast Guard approved Type III personal floatation devices (PFD), and one Type IV PFD (throwable) must be in the boat.

Sampling with an electrofishing boat begins at the upstream boundary of the sampling reach and proceeds downstream by maneuvering the boat along one shoreline and mid-channel areas in a zig-zag pattern (the opposite shoreline and mid-channel areas will be sampled in a second pass, again proceeding upstream to downstream; Figure 2). Position the boat 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. The electrofishing boat is operated at a speed equal to or slightly greater than the streamflow velocity. Periodically, the boat should be slowed to less than the speed of streamflow so that drifting fish may be more easily observed. Sampling is conducted in a downstream direction because fish are usually oriented upstream (into the direction of flow) and will either swim into the approaching electrical field or turn to escape downstream. In turning to escape downstream, fish orient themselves perpendicular to the electrical field, thereby exposing a greater surface area of the fish to the electrical field that renders them more susceptible to capture. Also, when fish are stunned, they are carried downstream by the flow, providing greater opportunity for capture.

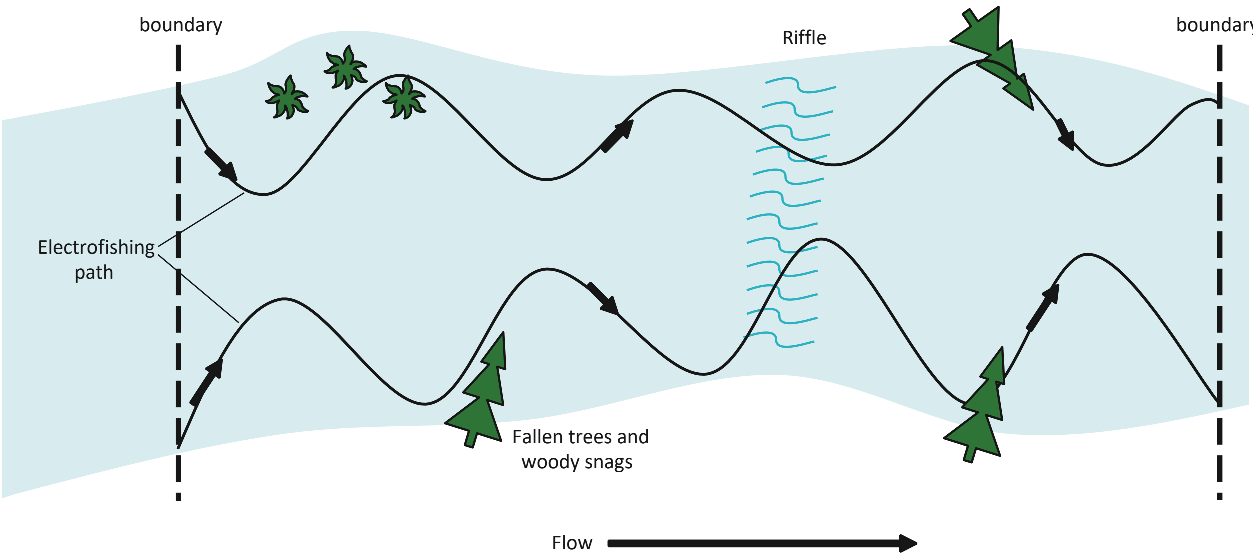

Figure 2. Double pass zig-zag sampling technique used in boat electrofishing for nonwadeable streams.

Intermittent application of electrical current while drifting downstream generally should be used for large, nonwadeable streams. In areas with clear or shallow water and submerged structure, intermittent application of current may be more effective than continuous application. Fish are approached with the current off. When the anodes are in position near the fish, the current is applied. Instream habitat features, such as woody snags and fallen trees, are sampled by maneuvering the boat close to the habitat feature with the electrical current off. As the anode is placed near the habitat feature, the electrical field is applied and the boat is backed away from the habitat feature. This will cause the fish to be pulled away from the habitat feature to facilitate their capture. When boat electrofishing, the duration for applying electrical current should be increased at submerged structures. Fish located in deeper water (2 to 3 m) may require 5 to 10 seconds of current before a response is observed. This duration increases as water temperature decreases. Captured fish are placed into a live well or large aerated tub on the boat and processed after completion of the first electrofishing pass, if necessary. Fish data collected by boat electrofishing in the main channel will be kept separate on the field forms (Figure 3) from fish data collected by boat in backwaters.

Nonwadeable streams of the Ozark Plateaus often are wadeable in large parts of the sampling reach. For example, a stream may be nonwadeable in some areas because of large, long pools but wadeable in riffles, runs, and along pool margins. In such situations, a towed barge or a backpack electrofisher should be used in the wadeable parts of the stream. In wadeable riffles, electrofishing while kicking into a seine is the most effective technique for collecting benthic riffle species. This technique for nonwadeable streams is similar to that described above for wadeable streams. For nonwadeable streams, electrofishing will be the predominate method used in the main channel. In situations where fish are collected from a reach using multiple types of electrofishing gear or collected from multiple channel types (main channel, side channel, backwater), samples for each gear and channel type are processed separately with crewmember initials, electrofishing effort, and fish data (lengths, weights, and counts) recorded on separate field forms (Figure 3). Similar to wadeable streams, samples collected from backwater or side channel types are processed separately regardless of gear type used. For analysis, all data collected with electrofishing gear will be combined for the entire reach.

Seining

Nonwadeable streams of the Ozark Plateaus often have wadeable riffles, runs, margins of pools, and backwaters that can be sampled using a bag seine or a common sense seine. Seining techniques for run and pool habitats are the same as those described in the section of this SOP describing wadeable streams. However, with nonwadeable streams, excessive water depth and flow can be additional factors (in addition to substrate) that adversely affect the use of the seine. In some reaches, it may be that only electrofishing techniques can be used effectively in main channel areas because of swift current in run and riffle habitats and depths in pools. Therefore, seining methods will be used primarily in backwater areas. Location of seining sites will be based on: (1) the HTLN project or crew lead’s judgment on effectiveness of this gear type to collect a representative sample and (2) safety to the crewmembers. Fish from seine hauls should be processed and analyzed separately from those collected with electrofishing gear. If seines are used in several channel types (main channel, backwater, side channel), fish from these areas will be processed separately in the field. However, for analysis, data collected using seining methods (regardless of channel type) will be combined to examine trends in fish communities at each reach.

Sample Processing Procedures

In the field, samples collected with different gear (backpack, towed barge, boat, or seine) and in different channel types (main channel, side channel, or backwater) will be processed separately. For each gear used in each channel type at a reach (for example, backpack in main channel, towed barge in main channel, seine in backwater, or backpack in backwater), fish data will be entered on separate field forms and the channel unit sampled, gear type and time sampled will be recorded (Figure 3).

Riffle samples that are collected by kicking into a seine while electrofishing are considered to be electrofishing samples and, therefore, can be combined in the field with samples collected in the same channel type using the same gear. For example, if backpack electrofishing was used in run and pool habitats in the main channel of a stream and backpack electrofishing with kicking into a seine was used in the riffles, then samples from all three habitats are considered to be from the same channel type (main channel) and, therefore, samples are combined in the field. However, if a towed barge was used in main channel run and pool habitat and a backpack with kicking into a seine was used in riffles, then samples from the towed barge must be recorded separately from those collected with the backpack. In the field, samples from backwater areas and isolated side pools will always be kept separate even when sampled with the same gear type used on the main channel of the stream. For seining, the approximate time (effort), number, and length (meters) of seine hauls should be recorded on the field sheets. During field processing, fish collected by seining should be kept separate from fish collected using electrofishing methods. For example, if both backpack electrofishing and seining are used to sample backwaters within a reach, then these samples must be kept separate because of differences in efficiencies of the gear types.

For analysis, all electrofishing data will be combined for the entire reach (main channel and backwater and side channel) and all seine data will be combined for a reach. However, there may be specific monitoring questions that would require analyzing the data by gear or channel type; therefore, it is important to process the data separately in the field.

The goal of processing fish samples in the field is to collect information on taxonomic identification, abundance, and size structure with minimal harm to specimens that will be released alive back into the stream. All captured fish are placed in a live cage, aerated buckets, a boat live well, or other suitable containers for subsequent processing and an effort is made to minimize stress or death to specimens. Multiple, portable aerated containers for holding and separating each species is needed. Regardless of the effort made to minimize handling and stress to fish, some mortality will occur. However, minimizing mortality involves recognizing which species are sensitive to handling and prolonged confinement and processing them first. Any fish that are released before the reach is completely sampled should be held in an instream net pen to prevent resampling the same fish.

Crewmembers should be thoroughly familiar with the fish species in their study area. Identifications are made by the project leader or certified crew leader (see below for certification process) who are familiar with the fish species commonly found in the study area. The primary references used for identification will be *Fishes of Arkansas* (Robison and Buchanan 1988) and *Fishes of Missouri* (Pflieger 1997). Taxonomic nomenclature and common names follow that established by the American Fisheries Society's Committee on Names of Fishes (Page *et al*. 2013). An attempt is made in the field to identify all fish to the species level. Walsh and Meador (1998) can be consulted for additional guidance regarding taxonomic identification.

Some species of fish (such as minnow genera *Notropis* and *Cyprinella*, and stonerollers *Campostoma anomalum*, *C. oligolepis*) are similar and confirmatory characteristics are either internal (such as pharyngeal teeth) or require exact counts of meristic characteristics (such as the number of lateral line scales or anal fin rays). These species cannot be accurately identified in the field. Accordingly, specimens that cannot be positively identified in the field are preserved, labeled, and identified in the laboratory, and a specimen of each species deposited in a reference collection. Because of the potentially large number of problematic *Campostoma* individuals occurring at some sites, these two species will only be identified to the genus level. All preserved specimens will be added to the data sheets once identified in the laboratory.

Some identifiable specimens also will be preserved and deposited in a reference collection to aid in instructing crewmembers in fish identification prior to the field season and to provide some future assurances about field identifications. In general, some individuals of all non-game species should be preserved, at the discretion of the project leader. Federally listed threatened and endangered species should not be preserved; other species of concern may be protected by State guidelines or regulations. Walsh and Meador (1998) provide guidance and criteria for the selection of specimens for a reference collection.

When processing fish specimens whether in the field or laboratory, a group of 30 individuals of each species from a sample reach will be measured (total length) and weighed. Individuals will be selected using a “blind grab” technique. This technique uses a dip net to make a pass through the entire bucket or holding tank to ensure that fish of various sizes are captured with each “grab.” Some species have a large size range with different sizes being collected by specific gears (for example, large sunfish collected with boat and small sunfish collected with backpack). For these species, a blind grab will be taken from samples collected by each gear type and a representative subsample will be measured, keeping data from the gear types separate. For smaller species (those typically smaller than 100 mm; minnows, darters, sculpins, and madtoms, for example), lengths of individuals will be measured and a batch weight will be measured. For species that grow to large sizes (for example, bass, sunfish, catfish, and suckers), each specimen will be measured and weighed individually.

The major steps in processing collections of fish from electrofishing and seining include the following:

1. Sort fish into identifiable and unidentifiable groups, keeping fish collected from each gear type (boat, backpack, barge, and seine) and channel type (main channel, side channel, or backwater) combination separate. Process species of concern (for example, sensitive species or rare species) and game species before other identifiable species.

2. Hold fish in aerated buckets, flow through pen, or live well to minimize stress or death. Do not keep fish out of the water longer than necessary to process them.

3. Identify all species (those that can be identified in the field) and measure total length and weight of 30 individuals for each species for a sample reach. Once 30 fish from a species have been measured, the remaining specimens for that species are counted and recorded as the additional species count.

4. Record data on the fish field data sheet (Figure 3).

    a. Record the Reach ID (see Table 1 in Narrative or in SOP 3 for list of sample reaches).

b. Record the date, gear type used, channel type sampled (main channel, side channel, backwater off of main channel, backwater off of side channel), sampling effort (time in seconds), number of seine hauls and total length of stream seined (if seining without electrofishing gear was used).

c. Record the initials of all crewmembers. Record the person identifying/measuring fish, the person recording the data, and those who operated the electrofishing equipment or seine and those who netted fish.

d. For each individual of a species, record the total length (TL), weight (WT), and any anomalies (Anom = deformities, eroded fins, lesions, tumors, and blackspot ; Smith *et al*. 2002). For larger fish, record individual weights. For smaller fish, batch weigh 30 specimens. Record the additional number of fish collected for each species under "Additional Species Count." For example, if 46 white suckers were collected at a reach, 30 fish would be individually measured and weighed and the remaining 16 would be counted (Additional Species Count = 16). If 135 bluntnose minnows were collected at a reach, 30 would be individually measured (TL) and all 30 weighed in one batch. The remaining 105 specimens would be counted.

5. Preserve selected specimens (those too small or difficult to identify in the field) for identification in the laboratory or for a reference collection. Specimens will be preserved in 10 percent buffered formalin. For specimens larger than 100 mm, a small incision along the side of the body should be made to allow the formalin to penetrate the body cavity. For each reach, all unidentified specimens collected from the same gear type (backpack, towed barge, boat, or seine) and channel type (main channel, side channel or backwater) can be preserved in a single jar with a label that contains the reach sampled, date, gear type, habitat type, and sampling effort (Figure 4). All preserved specimens will be identified at the HTLN Aquatic Program Laboratory at Missouri State University in Springfield, Missouri. Specimens preserved for laboratory identification (or for a reference collection) should be noted on the fish community field form (Figure 3) by putting a check mark in the "Vchrd" column. Any comments should be noted in the "Cmts" column.

Quality Assurance/Quality Control Procedures

A number of procedures can be implemented to ensure the representativeness and accuracy of data used to describe fish communities of BUFF and OZAR. These procedures must be considered in relation to the objectives of the sampling. They include procedures related to collection of fish specimens and accurate taxonomic identification.

Quality Assurance (QA) Procedures:

QA include all activities and skills of staff members that ensure data collection is high quality and prevents inconsistencies. Fish samples should be collected as consistently as possible from year to year by a consistent and experienced crew. For collection of fish data, all crew members will review collecting methods described in this SOP.

Consistency is maximized by using the same crew from year to year and at all sites sampled within a single field season. The use of inexperienced crewmembers will decrease the efficiency and consistency of the sampling effort (Hardin and Connor 1992). Thus, two critical elements to collect quality data and maintain consistency across years are: 1) an adequate number of **experienced** crew members and 2) the consistency in the team conducting the fish sampling. To maintain data quality consistentcy and efficiency while keeping the crew safe, a crew of three to six people are needed for fish sampling, depending on the gear used and size of the sample site. The project lead and field crew leads are to have extensive experience (at least 2-3 field seasons) in boat operation and electrofishing procedures. Project and crew leads will be the only staff allowed to operate the boat during electrofishing and 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.

At least half of the crew must be experienced (i.e. one field season of experience) with electrofishing procedures and gears. For backpack electrofishing, at least one of the three crewmembers must have completed the USFWS electrofishing training and be experienced in proper techniques. For towed barge sampling, at least two crewmembers must have completed USFWS electrofishing training and have experience in proper techniques. For boat electrofishing, at least one person on the boat must have completed USFWS electrofishing training and have experience with the equipment. 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.

As boats and canoes are used to access the sample sites, all crew members will be trained on boat operation and safety by the project lead or field crew leads. In order to operate a boat operation when electrofishing *is not being conducted*, the crewmember must be MOCC certified or have a MOCC certified crewmember in the boat.

To ensure data collected is high quality, at least two people on the crew must have competent data recording and fish measuring abilities. This will ensure that the numerous fish measurements and counts will be taken and recorded accurately. To train new crewmembers, the project or crew leads will explain and demonstrate how to measure fish and record data on the field forms and will check the new crewmembers repeatedly during fish processing.

Ideally, all sites will be sampled during similar biologic and hydrologic conditions (during base flow conditions, if possible) because doing so will reduce variability associated with fish movements (for example, those related to spawning) and lower sampling efficiency (because of higher stages, higher velocities, and higher turbidity). For this reason, the sampling period for BUFF is early summer (late May to late June) and autumn (September to late October) for OZAR. To the extent possible, sample gear used and sampling effort (generally, sampling time) should remain consistent at each site from year to year (see Table 1 in Narrative or in SOP 3 for sampling effort at each site). However, site specific conditions and safety considerations dictate sampling period and equipment used; therefore, implementation of all desired practices may not always be feasible at a reach.

Quality Control and Certification Procedures:

Prior to the field season, the field crew leads (including the project lead) need to refamiliarize themselves with the fish fauna by examining preserved specimens located in the reference collection and reviewing fish species lists within BUFF (see Appendix 1in Protocol Narrative) and OZAR (see Appendix 2 in Protocol Narrative) or expected to occur in the area.

The project lead for fish monitoring will have taxonmomic 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. those that have 1-2 years of fish identification experience or have taken an Ichthyology class) and inexperienced staff who will assist with fish identifications. Field crew leads responsible for fish identifications down to the species level will have completed an Ichthyology course and be certified by the QA. To obtain certification, crew leads will independently identify a subset of 50% of the fish collected from at least three mainstem and two tributary sites within both parks. Mainstem and tributary sites selected for certification will of high species diversity. A 90% agreement between crew leads and QA officer is required for certification. Only the project lead or certified crew leads will be allowed to make final determinations of identifications to the species level. If a species is identified that is not within the range of southern Missouri and northern Arkansas, the specimen will be preserved and sent to a taxonomic expert. If a species is identified as not being collected previously at the park but is within it's native range, the specimen will be identified by at least two different certified crew members for verification. 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.

Table 1. Fish sampling equipment for BUFF and OZAR.

General Equipment
Waders
Rain gear
Polarized sunglasses & hats
GPS unit & park maps
Flagging

Stop watch
Collecting permits
Field guides
Camera
Tool box & volt/ohm meter
Park radio & charger
Cell phone
Directions to nearest hospital
First aid kit
Bug spray & sunscreen
Water or sports drink

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

Seine
5 gal. buckets for holding fish
Aerators & batteries
Dip nets (1 per person)
Electrofishing gloves

Fish Sampling - Non-wadeable
Boat electrofishing unit
- generator, gas can, & extra oil
- pulse box & cradle
- boom and cords
- switch pad
- paddles (2) & boat hook
- Rope & anchor
- Throw bag

Electrofishing gloves
Ear plugs
2 canoes with 2 paddles each
Outboard motors & gas cans (1 per watercraft)
Marine grease for jet motor
Life jacket (1 per person)
Throwable cushions (1 per watercraft)
Large tub or live well in boat
- bilge pump for aeration

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
2 folding chairs
1 fold out awning
1 gal. jugs & plastic baggies for preserving specimens (1 jug per site)
10% buffered formalin (pH = 7)
Tags for jars

Fish Community Data – River Parks page ____ of ____

Reach ID: _____________ Date:___________ IDed by:_________ Recorder:__________

Gear: *Backpack Barge Boat Seine* Channel Type: *Main Channel Side Channel Backwater on Main Backwater on Side*

Effort (sec.): ________ No. Seine Hauls: ________ Seine Reach Length (m): ________ Crew: *Shocker(s):*___________
Boat/Barge: ___________
*Netter(s):*______________
*Seine:*______________

Anomalies: D = deformities, E = eroded fins, L = Lesions, T = tumors, B = Blackspot
Vchrd = check if specimen preserved. Cmts = comments

Species						Species						Species					
TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts
Additional Species Count:						Additional Species Count:						Additional Species Count:					

Species						Species						Species					
TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts
Additional Species Count:						Additional Species Count:						Additional Species Count:					

Species						Species						Species					
TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts
Additional Species Count:						Additional Species Count:						Additional Species Count:					

Species						Species						Species					
TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts	TL (mm)	Wt (g)	Anom	Vchrd	Batch ID	Cmts
Additional Species Count:						Additional Species Count:						Additional Species Count:					

Figure 3. Fish community sampling field sheet.

Reach ID: Date: Gear: Effort (sec): Seine Reach Length: No. Seine Hauls: Channel Unit:

Figure 4. Label for preserving specimens in field.

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